

# *CYP2C19* variant mitigates Alzheimer disease pathophysiology in vivo and postmortem

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## Abstract

### Objective

To verify whether *CYP* polymorphisms are associated with amyloid- $\beta$  ( $A\beta$ ) pathology across the spectrum of clinical Alzheimer disease using in vivo and postmortem data from 2 independent cohorts.

### Methods

A candidate-gene approach tested the association between 5 genes (28 single nucleotide polymorphisms) and  $A\beta$  load measured in vivo by the global [ $^{18}\text{F}$ ]florbetapir PET standardized uptake value ratio (SUVR) in 338 Alzheimer's Disease Neuroimaging Initiative participants. Significant results were then tested using plasma  $A\beta$  and CSF  $A\beta$  and  $A\beta$ /phosphorylated tau ( $A\beta$ /p-tau) ratio in the same cohort. The significant association was also generalized to postmortem  $A\beta$  load measurement in the Rush Religious Orders Study/Memory and Aging Project cohorts. In addition, global cognition was used as a phenotype in the analysis in both cohorts.

### Results

Analysis of  $A\beta$  PET identified a variant in the *CYP2C19* gene (rs4388808;  $p = 0.0006$ ), in which carriers of the minor allele (MA) had a lower global SUVR. A voxel-wise analysis revealed that the variant is associated with a lower  $A\beta$  load in the frontal, inferior temporal, and posterior cingulate cortices. MA carriers also had higher CSF  $A\beta$  ( $p = 0.003$ ) and  $A\beta$ /p-tau ratio ( $p = 0.02$ ) but had no association with  $A\beta$  plasma levels. In postmortem brains, MA carriers had a lower  $A\beta$  load ( $p = 0.03$ ). Global cognition was higher in MA carriers, which was found to be mediated by  $A\beta$ .

### Conclusions

Together, these findings point to an association between *CYP2C19* polymorphism and  $A\beta$  pathology, suggesting a protective effect of the MA of rs4388808. Despite the several possibilities in which *CYP2C19* affects brain  $A\beta$ , the biological mechanism by which this genetic variation may act as a protective factor merits further investigation.

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## Glossary

**AA** = arachidonic acid; **A $\beta$**  = amyloid- $\beta$ ; **AD** = Alzheimer disease; **ADNI** = AD Neuroimaging Initiative; **APP** = amyloid precursor protein; **CDR** = clinical dementia rating; **CN** = cognitively normal; **CYP** = cytochrome P450; **EET** = epoxyeicosatrienoic acid; **LD** = linkage disequilibrium; **MA** = minor allele; **MAF** = minor allele frequency; **MAP** = Memory and Aging Project; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **PHF** = paired helical filament; **p-tau** = phosphorylated tau; **ROS** = Religious Orders Study; **SNP** = single nucleotide polymorphism; **SUVR** = standardized uptake value ratio.

In sporadic Alzheimer disease (AD), accumulation of amyloid- $\beta$  (A $\beta$ ) seems to be associated with reduced A $\beta$  clearance, but little is known about the biochemical pathways underlying cerebral A $\beta$  metabolism.

Cytochrome P450 (CYP) is a family of enzymes known to metabolize several endogenous and exogenous substrates. Besides metabolizing drugs, brain CYPs are also involved in the modulation of blood flow, metabolism of fatty acids, cholesterol, and neurotransmitters, and mobilization of intracellular calcium,<sup>1–3</sup> all pathways that have been somehow linked to AD and/or amyloid metabolism. In addition, studies have shown that CYP proteins and their genetic variants are associated with the brain immune response<sup>2</sup> and neurodegenerative diseases.<sup>2,4,5</sup>

Although previous evidence shows CYP expression in areas affected by AD such as the amygdala, frontal and temporal cortices, and the hippocampus,<sup>6,7</sup> there are only a few reports documenting a link between CYPs and AD pathophysiology. In one study, the overexpression of human amyloid precursor protein (APP) on the Tg2576 mice model was associated with elevated CYP hepatic function, while their renal counterparts were depressed.<sup>8</sup> In addition, another study presented evidence that A $\beta$  stimulates NADPH-cytochrome P450 reductase (POR), a CYP inducer, in APP transgenic mice and human AD brains, which may affect the redox status.<sup>9</sup>

In line with the evidence that A $\beta$  can stimulate metabolic cascades in a similar fashion as foreign compounds,<sup>9,10</sup> and knowing that brain CYP could potentially affect amyloid metabolism also through other mechanisms, we tested the hypothesis that variants in CYP genes could be associated with A $\beta$  and, consequently, have an impact in cognitive performance. We examined the association of CYP variants with biomarkers of A $\beta$  load in vivo and postmortem, as further specified.

## Methods

### Study participants

Discovery analysis data were obtained from the AD Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). For this study, cognitively normal (CN) individuals had a Mini-Mental State examination (MMSE) score of 24 or higher and a clinical dementia rating (CDR) of 0. The

operational criteria adopted for mild cognitive impairment (MCI) were participants who had an MMSE score equal to or greater than 24, a CDR of 0.5, subjective memory concern, objective memory loss measured by education-adjusted scores on delayed recall of 1 paragraph from Wechsler Memory Scale Logical Memory II ( $\geq 16$  years:  $\leq 8$ ; 8–15 years:  $\leq 4$ ; and 0–7 years:  $\leq 2$ ), and essentially normal activities of daily living. Participants with AD were individuals with an MMSE score lower or equal to 26, CDR higher than 0.5, who met the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for probable AD. All individuals had absence of any other neuropsychiatric disorders. The ADNI inclusion/exclusion criteria are described in detail at adni-info.org.

Our report also used data from the Religious Orders Study (ROS) and Rush Memory and Aging Project (MAP), 2 longitudinal clinical pathologic cohort studies of aging and dementia.<sup>11,12</sup> All participants enrolled without known dementia and agreed to annual clinical and neuropsychological assessments and brain donations after death. The clinical diagnosis of dementia and AD followed the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association recommendations. The MCI diagnosis is given to participants who were rated as impaired based on MMSE and on the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neuropsychological measures but who were not found to have dementia by the examining neurologist. Our study includes only MCI participants with 1 impaired domain and no other cause of cognitive impairment. Notably, the studies are conducted by the same team of investigators and share a large common core of testing batteries, which allow for combined analyses.

The discovery sample consisted of a subset of ADNI participants who had [<sup>18</sup>F]florbetapir PET, genetic data, and CSF data available. The findings obtained with the discovery sample were then generalized to the Rush-ROS/MAP participants who died at age 90 years or younger.

### Standard protocol approvals, registrations, and patient consents

This secondary analysis study followed all institutional review board regulations, which are detailed in the supplementary methods.

## Phenotypes

From the ADNI, we obtained demographics, neurocognitive scores, CSF, and plasmatic data available after passing rigorous quality control. The preprocessed imaging and genetic data underwent additional processing and quality control before the statistical analysis in our study as described below. From Rush, all the data used in this project to perform statistical analyses were already processed and quality controlled with very rigorous criteria.

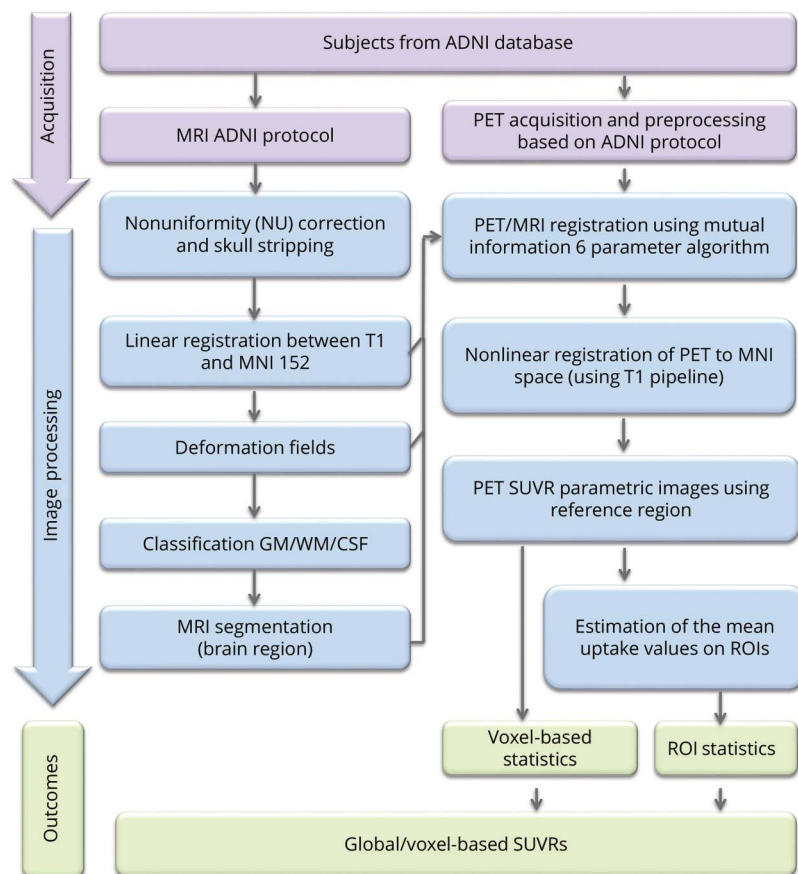
In the ADNI cohort, the brain A $\beta$  load was estimated using the [ $^{18}$ F]florbetapir PET standardized uptake value ratio (SUVR, details are provided in figure 1 and e-methods). Separately, A $\beta$  and hyperphosphorylated tau (p-tau) levels were measured in the CSF and plasma. These data were obtained by the Biomarkers Consortium CSF, and the description of the methodology regarding the sample acquisition, processing, and analysis is available at the ADNI website ([adni.loni.usc.edu/data-samples/bio-specimen-data/](http://adni.loni.usc.edu/data-samples/bio-specimen-data/)).

In the Rush-ROS/MAP cohort, postmortem data of the A $\beta$  load and paired helical filament (PHF) tau tangle density were obtained from 8 brain regions using immunohistochemistry.<sup>13</sup>

Cognitive function was evaluated in both the ADNI and Rush-ROS/MAP. For the ADNI, the global cognition scores were developed by making a z-score of the sum of the memory and the executive function composite scores. In the Rush-ROS/MAP, the global cognitive scores were based on 17 cognitive performance tests, and we included only participants who underwent autopsies; thus, the last valid cognitive score proximate to death was used for this analysis (for further details, see e-Methods, <http://links.lww.com/NXG/A20>).

We used gene expression data to examine cis expression quantitative trait loci (eQTL). For the ADNI, total RNA was obtained from the peripheral blood—at the same visit when the imaging data were obtained—and, after quality control, was hybridized to the Affymetrix Human Genome U219 array plate as described.<sup>14</sup> The normalized and quality-controlled expression data<sup>14</sup> were used. For the Rush-ROS/MAP, RNA was extracted from the gray matter of dorsolateral prefrontal cortex blocks using the miRNeasy Mini Kit (Qiagen, Venlo, Netherlands) and the RNase-free DNase Set (Qiagen). These samples were quantified and qualified as described<sup>15</sup> and then sequenced on the Illumina HiSeq with 101 bp paired-end reads. The normalized and quality-controlled expression data were used. In both analyses, the RNA integrity measurement was used as a covariate.

**Figure 1** [ $^{18}$ F]florbetapir standardized uptake value ratio analytical method



Flowchart showing acquisition methods (purple), image processing (blue), and outcomes (green). ADNI = Alzheimer's Disease Neuroimaging Initiative; GM = gray matter; ROI = region of interest; SUVR = standardized uptake value ratio; WM = white matter.

## Genotyping, imputation, and gene selection

We interrogated single nucleotide polymorphisms (SNPs) from the *CYP* genes of the 5—probably—most important metabolizers: *CYP3A4*, *CYP2D6*, *CYP2C9*, *CYP1A2*, and *CYP2C19*.<sup>3,16–18</sup> Methods involved in DNA processing are detailed in the supplementary methods section (<http://links.lww.com/NXG/A20>). Using PLINK,<sup>19</sup> the SNPs within these genes were recoded based on the dominant model with respect to the MAs, and the ones in high linkage disequilibrium (LD) ( $r^2 > 0.8$ ) were removed from the analysis to avoid unnecessary testing. A total of 28 SNPs were used in the initial step of the association analysis.

For Rush-ROS/MAP cohorts, DNA was extracted, genotyped, imputed, and quality controlled according to the procedures described in e-Methods (<http://links.lww.com/NXG/A20>).

## Statistical analysis

The statistical tests were performed in R statistical software,<sup>20</sup> where linear regression models were used to test the association between the genotypes and the phenotypes in both cohorts, separately. All statistical models included the covariates of age, sex, and *APOE-ε4* carriage status, except for the models in which global cognition was the outcome measure, where the *APOE-ε4* status was replaced by years of education. The initial analysis—using the global [<sup>18</sup>F]florbetapir SUVR as a phenotype—was performed to interrogate a total of 28 SNPs (table e-1, <http://links.lww.com/NXG/A19>). To correct for multiple testing, the statistical significance of the SNP discovery was set at  $p < 0.0017$  using a Bonferroni correction for 28 tests and a type I error  $\alpha = 0.05$ . The significant SNPs were further tested for associations with other phenotypes in ADNI and Rush-ROS/MAP cohorts. To test for group differences in the diagnostic status, we added diagnosis as a covariate in the regression models and also tested the interaction between the diagnostic status and desired independent variable. Effect size calculations (Cohen  $d$ ) were also used.

A voxel-based analysis was performed using the RMINC imaging tool,<sup>21</sup> where parametric images were obtained contrasting the [<sup>18</sup>F]florbetapir SUVR between the genotype groups of the SNP found in the discovery analysis. After random field theory correction for multiple comparisons, the  $t$  value threshold of significance was  $\leq -3.1$  ( $p \leq 0.001$ ).

Mediation analysis was used to examine whether the SNP associations with cognition were mediated through the effect of A $\beta$ . To test this hypothesis, first, the association of *CYP* SNPs with global cognitive performance was evaluated using linear regression models. Then, if the association was significant, the measure of A $\beta$  was added to this model to check whether the SNP associations with cognition were attenuated because of A $\beta$ .

## Results

Demographics and general information of the ADNI and Rush-ROS/MAP cohorts are presented in table 1. After

imaging and genetic quality control, a total of 338 ADNI participants were studied, including 186 CN, 105 single- or multiple-domain amnesic MCI, and 47 AD. A total of 738 Rush-ROS/MAP participants were analyzed, including 301 CN, 179 amnesic MCI, and 258 AD. As expected, in both cohorts, CN individuals had higher MMSE scores and fewer were *APOE-ε4* carriers as compared to both MCI and AD. There was no difference in sex or years of formal education between diagnostic groups in both cohorts (details are provided in table e-2, <http://links.lww.com/NXG/A19>).

## *CYP2C19* polymorphism is associated with the global [<sup>18</sup>F]florbetapir SUVR in the ADNI cohort

The discovery analysis tested the association of 28 SNPs from *CYP* genes with the global [<sup>18</sup>F]florbetapir SUVR. The analysis unveiled an association of an SNP (rs4388808) in the *CYP2C19* gene ( $t = -3.43$ ;  $p = 0.0006$ ) (figure 2A). The minor allele frequency (MAF) of rs4388808<sup>G</sup> in the ADNI cohort was 0.19, and the minor allele (MA) carriers displayed less [<sup>18</sup>F]florbetapir binding as compared to the noncarriers. It is important that the association remained significant when adjusting the model for diagnosis. In addition, no interaction was found between the SNP and diagnostic status. The estimated effect size of rs4388808 in the whole sample was Cohen  $d = 0.36$ . Of interest, the effect size was greater for the subset of *APOE-ε4* carriers (Cohen  $d = 0.51$ ) as compared to *APOE-ε4* noncarriers (Cohen  $d = 0.33$ ).

## MA carriers of the *CYP2C19* polymorphism have less A $\beta$ in AD-related regions in the ADNI cohort

To identify the brain areas responsible for the global difference observed in [<sup>18</sup>F]florbetapir uptake revealed in the discovery analysis, we performed a voxel-wise analysis. The analysis showed that carriers of rs4388808<sup>G</sup> had less [<sup>18</sup>F]florbetapir binding in the frontal, posterior cingulate, and inferior temporal cortices (figure 3), with the voxels in these regions presenting  $p$  values equal to or less than 0.001.

## Findings with brain imaging are corroborated by CSF data in the ADNI cohort

Because of the well-known relationship between brain and CSF levels of A $\beta$ , we tested the association between rs4388808 and CSF A $\beta$ . Because not all participants in the ADNI sample had CSF data, we performed the analysis in a subset of 260 individuals. As expected, MA carriers presented more CSF A $\beta$  than noncarriers ( $t = 2.94$ ;  $p = 0.003$ ) (figure 2B). The *CYP2C19* polymorphism was associated with the A $\beta$ /p-tau ratio ( $t = 2.29$ ;  $p = 0.02$ ), and higher ratios were found in carriers of rs4388808<sup>G</sup> (figure 2C). No association was found using p-tau as a single outcome measure ( $t = -1.24$ ;  $p = 0.21$ ).

## Association between the *CYP2C19* polymorphism and A $\beta$ is exclusive to the A $\beta$ brain levels in the ADNI cohort

To examine whether the *CYP2C19* polymorphism is also associated with A $\beta$  levels outside the brain, we tested the



**Table 1** Demographic and key characteristics of the samples

|                              | ADNI                       |                            |                            | Rush-ROS/MAP               |                            |                            |
|------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                              | CN                         | MCI                        | AD                         | CN                         | MCI                        | AD                         |
| <b>No. of participants</b>   | 186                        | 105                        | 47                         | 301                        | 179                        | 258                        |
| <b>Males, n (%)</b>          | 91 (48.9)                  | 64 (61)                    | 26 (55.3)                  | 182 (60.5)                 | 101 (56.4)                 | 154 (59.7)                 |
| <b>Age, y, mean (SD)</b>     | 75.70 (6.63)               | 74.29 (8.12)               | 75.67 (7.37)               | 83.7 (5.19) <sup>a3</sup>  | 85.26 (4.35)               | 85.95 (3.81)               |
| <b>APOE-ε4 (%)</b>           | 52 (28) <sup>a1</sup>      | 57 (54.3) <sup>a1</sup>    | 30 (63.8) <sup>a1</sup>    | 49 (16.4) <sup>a4</sup>    | 50 (27.9) <sup>a4</sup>    | 112 (43.4) <sup>a4</sup>   |
| <b>MMSE, mean (SD)</b>       | 29.09 (1.14) <sup>a2</sup> | 27.24 (1.95) <sup>a2</sup> | 20.34 (3.66) <sup>a2</sup> | 28.69 (1.42) <sup>a5</sup> | 27.91 (1.83) <sup>a5</sup> | 25.49 (5.17) <sup>a5</sup> |
| <b>Education, y</b>          | 16.41 (2.75)               | 16.67 (2.68)               | 16.13 (2.47)               | 16.53 (3.74)               | 16.34 (3.47)               | 16.66 (3.94)               |
| <b>MA, n (%)<sup>b</sup></b> | 67 (19)                    | 36 (10)                    | 17 (5)                     | 87 (11)                    | 52 (7)                     | 63 (8.5)                   |

Abbreviations: AD = Alzheimer disease; ADNI = AD Neuroimaging Initiative; CN = cognitively normal; MA = minor allele; MAP = Memory and Aging Project; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; ROS = Religious Orders Study.

<sup>a</sup> Statistically different from the other groups of the same sample; <sup>1</sup> $p = 2.3 \times 10^{-7}$ , <sup>2</sup> $p = 2 \times 10^{-16}$ , <sup>3</sup> $p = 9.2 \times 10^{-8}$ , <sup>4</sup> $p = 1.7 \times 10^{-11}$ , and <sup>5</sup> $p = 2 \times 10^{-16}$ .

<sup>b</sup> MA (rs4388808<sup>G</sup>) percentage relative to its major sample (ADNI or Rush-ROS/MAP).

association of rs4388808 with plasmatic levels of A $\beta$  in a subsample of 113 participants. We did not find an association between the SNP and free ( $t = -0.60$ ;  $p = 0.54$ ) or total ( $t = 0.49$ ;  $p = 0.62$ ) plasmatic A $\beta_{1-42}$  levels (figure 2D), indicating that the findings reported above are specific to the CNS.

### Results from the ADNI cohort were generalized to the Rush-ROS/MAP cohort

We next generalized the findings to postmortem indices of the A $\beta$  load and to neurofibrillary tangles in Rush-ROS/MAP cohorts. From the 8 brain regions where the A $\beta$  load was measured, we selected 6 regions (angular gyrus, anterior cingulate, entorhinal cortex, and mesial and inferior temporal cortices) and calculated an average per subject. These 6 regions were chosen to match with the brain areas where we detected differences between genotype groups in the voxel-wise analysis. When testing the association between the average of the A $\beta$  load and the *CYP2C19* polymorphism, we found that rs4388808<sup>G</sup> (MAF = 0.15) carriers had a lower A $\beta$  load than noncarriers ( $t = -2.15$ ;  $p = 0.03$ ) (figure 2E), consistent with findings from the ADNI cohort. The difference between rs4388808<sup>G</sup> carriers and noncarriers in PHF tau tangle density did not reach statistical significance ( $t = -1.65$ ;  $p = 0.09$ ).

### MA carriers of the *CYP2C19* polymorphism had better cognitive performance than noncarriers in both cohorts

To examine whether the *CYP2C19* polymorphism affects the cognitive performance, we used composite measures of global cognition as the outcome in linear regression models. In the ADNI cohort, there was a clear tendency to an association with the *CYP2C19* polymorphism ( $t = 1.92$ ;  $p = 0.05$ ) (figure 2F), while in Rush-ROS/MAP cohorts, the association was such that rs4388808<sup>G</sup> carriers had a higher cognition than noncarriers ( $t = 2.08$ ;  $p = 0.03$ ) (figure 2G).

### The effect of the *CYP2C19* polymorphism on cognition is mediated by A $\beta$

A mediation analysis was used to determine whether the *CYP2C19* polymorphism was directly or indirectly associated with cognitive performance. We retested the association between the *CYP2C19* polymorphism and cognition, but now adding the measures of [<sup>18</sup>F]florbetapir or A $\beta$  load in the model. Neither of the previous genotype-phenotype associations remained significant, indicating that the observed effect of the genotype was mediated by A $\beta$ .

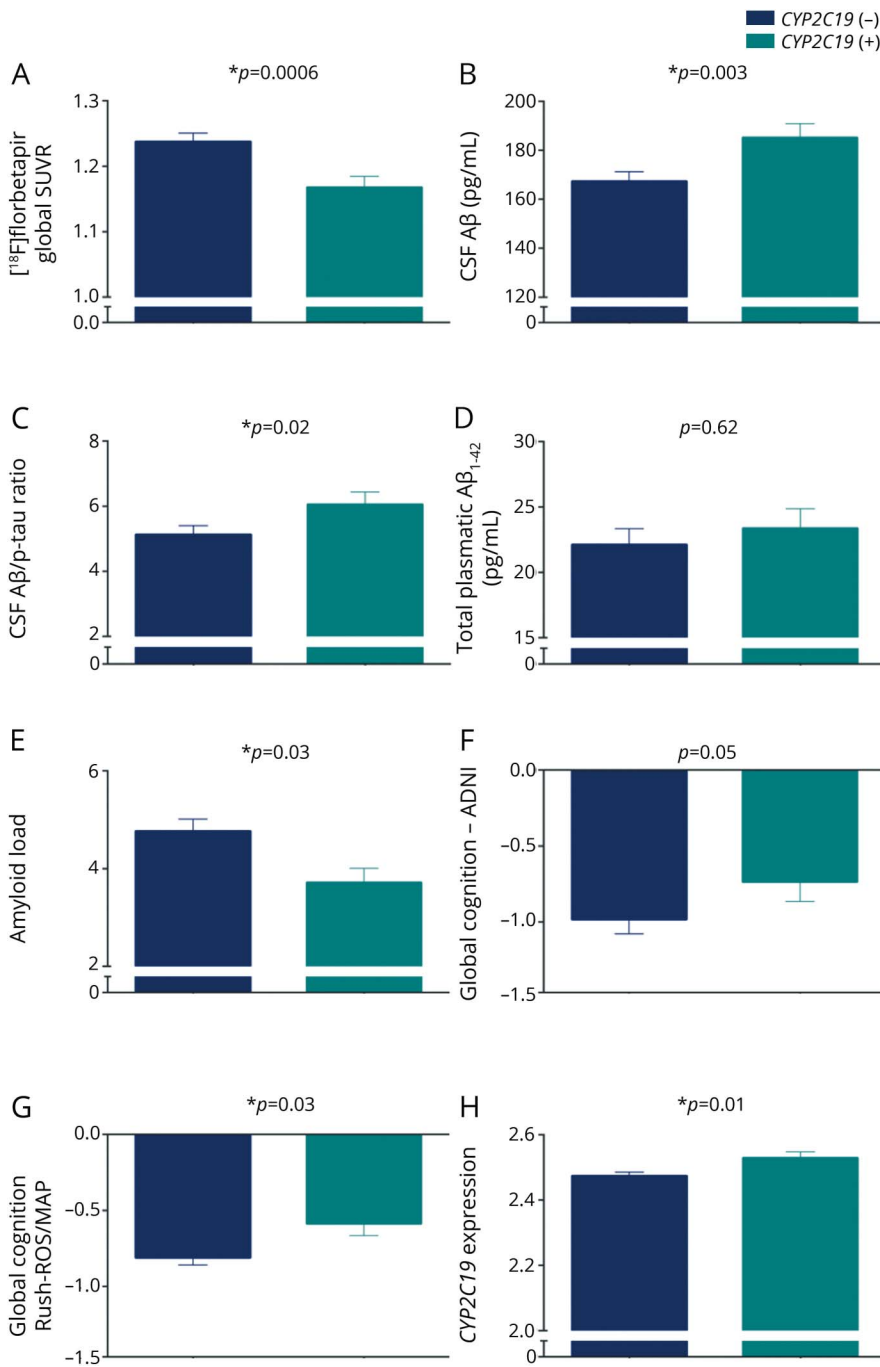
### Association between rs4388808 and gene expression of *CYP2C19* suggests that the SNP is functional

To check whether the polymorphism has an effect in the gene expression, we tested the association between rs4388808 and *CYP2C19* RNA levels. In the ADNI cohort, with 304 participants for this analysis, we found a cis eQTL such that MA carriers displayed higher RNA levels in the blood ( $t = 2.38$ ;  $p = 0.01$ ) (figure 2H). In Rush-ROS/MAP cohorts, very low levels of *CYP2C19* expression were detected in postmortem brains, and the data did not provide consistent results (data not shown) as found in the blood expression data from the ADNI.

## Discussion

In the present study, we found an association between the *CYP2C19* polymorphism and A $\beta$  burden across the spectrum of AD, in which carriers of rs4388808<sup>G</sup> presented a less A $\beta$  load and downstream cognitive impairment. This association was initially detected using the [<sup>18</sup>F]florbetapir SUVR and posteriorly confirmed with CSF A $\beta$  in the ADNI cohort. No association was found between the polymorphism and diagnostic groups, suggesting that the SNP is associated with an amyloid load rather than with the disease status. Effect size

**Figure 2** Comparison between noncarriers (–) and carriers (+) of the minor allele of rs4388808 (*CYP2C19*)

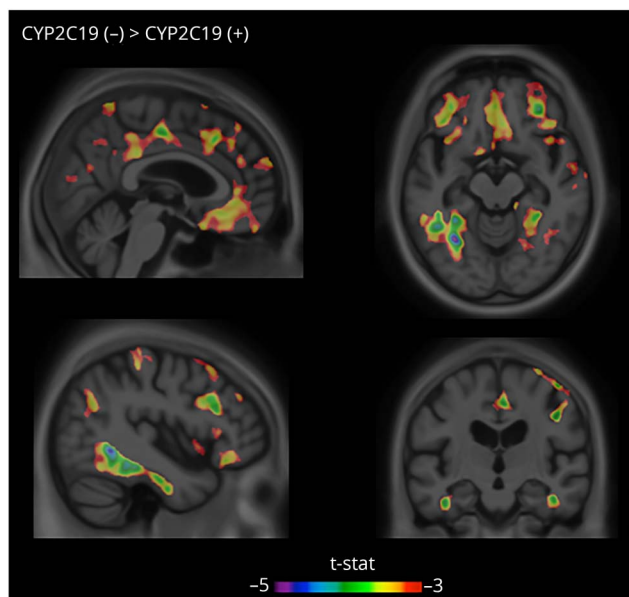


In the ADNI cohort, a difference was observed in the brain Aβ load (A), CSF Aβ levels (B), and CSF Aβ/p-tau ratio (C), but no difference was detected in plasmatic levels of Aβ (D). Results were generalized to postmortem data of Rush-ROS/MAP cohorts, where a concordant pattern was observed in the Aβ load (percentage area occupied by amyloid) (E). A tendency toward significance in global cognitive performance (F) was found in the ADNI cohort, while in the Rush-ROS/MAP, it was significant (G). *CYP2C19* expression levels were also different between noncarriers (–) and carriers (+) of the minor allele of rs4388808 (H) in the ADNI cohort. All linear models were adjusted for age, sex, and APOE-ε4 carriage status, with the exception of the models using global cognition and gene expression, which were adjusted for age, sex, and years of education or RNA integrity, respectively. Aβ = amyloid-β; ADNI = Alzheimer's Disease Neuroimaging Initiative; MAP = Memory and Aging Project; p-tau = phosphorylated tau; ROS = Religious Orders Study; SUVR = standardized uptake value ratio.

analysis suggests that the beneficial effects of the polymorphism are larger in *APOE-ε4* carriers, as compared to noncarriers or the whole sample, in which the effect is similar to what has been described for a *BCHE* gene polymorphism.<sup>22</sup> Subsequently, this association was generalized to postmortem data from the Rush-ROS/MAP cohort. Finally, both cohorts showed a protective association between the MA of *CYP2C19* and cognition. It is important that subsequent mediation analysis suggested that the effects of *CYP2C19* on cognition were mediated by Aβ.

Overall, these results support the notion that the MA of *CYP2C19* (rs4388808) is a protective variant against Aβ and downstream cognitive impairment. Of interest, voxel-wise analysis in in vivo participants revealed that the MA was associated with reduced Aβ burden in AD-related regions in the frontal, inferior temporal, and posterior cingulate cortices.<sup>23</sup> In addition, the *CYP2C19* SNP was associated with the CSF Aβ/p-tau ratio but not with plasmatic Aβ. Beyond the fact that the Aβ/p-tau ratio is postulated to better represent neuritic plaques than the single CSF Aβ information,<sup>24</sup> these results

**Figure 3** T-statistical parametric maps showing differences between noncarriers (-) and carriers (+) of the minor allele of rs4388808 (*CYP2C19*)



T-statistical parametric maps superimposed on average structural MRI show brain regions with lower standardized uptake value ratio (SUVR) values in minor allele carriers (*CYP2C19* (+)) of the polymorphism of *CYP2C19*. Statistical differences overlap with brain regions vulnerable to Alzheimer disease pathophysiology, such as the posterior cingulate, frontal, and temporal cortices.

provide further evidence that the polymorphism affects the brain accumulation of neuritic plaques in a tissue- and disease-specific manner.<sup>24</sup> In both cohorts, the referred polymorphism was not associated with PHF tau or single CSF p-tau, agreeing with the framework that A $\beta$  and tau have independent upstream triggers.<sup>25</sup> In line with overall significant results, the *CYP2C19* polymorphism also presented a protective effect in global cognition, being supported by previous studies that linked A $\beta$  pathology to cognitive changes.<sup>13</sup> Indeed, the mediation analysis supported that the association between the genetic factor and cognitive abnormalities is likely due to the upstream accumulation of A $\beta$ .

Levels of the *CYP2C19* protein are associated with the rs4388808 polymorphism,<sup>26</sup> which is an intronic variant of the *CYP2C19* gene. Indeed, we found a similar association showing that MA carriers expressed higher levels of *CYP2C19* when compared with noncarriers, suggesting that the SNP affects the expression of its gene. To corroborate this finding, we retrieved the SNPs that were previously excluded because of high LD and verified that none was associated with *CYP2C19* expression in the blood (data not shown), reinforcing the idea that rs4388808 is functional. This association could not be detected in post-mortem data probably because of the low *CYP* expression or because of its limited detection by the method used.<sup>7</sup>

Despite the SNP not being widely studied to provide information about the association with other phenotypes, other

polymorphisms in the gene have been correlated with metabolic variability, atherosclerosis, and behavioral traits.<sup>27–29</sup> *CYP2C19* expression has been detected in several brain regions<sup>6,7</sup>; however, its function in the CNS has not been fully elucidated. Some reports have suggested that, besides its role at metabolizing exogenous substrates, *CYP2C19* also participates in other important biological cascades, such as the metabolism of serotonin and sexual hormones<sup>1</sup> and the metabolism of the arachidonic acid (AA), where it functions as an epoxygenase.<sup>30</sup>

The biochemical mechanism underlying the association of *CYP2C19* with A $\beta$  remains speculative. A study has shown that A $\beta$  is able to act as a foreign body and trigger POR, activating CYPs to initiate catabolic reactions.<sup>9</sup> In turn, *CYP2C19* is involved in the catalysis of estradiol.<sup>31</sup> Because several studies have reported the beneficial effects of estrogens on AD—demonstrating that these steroids are able to act as anti-inflammatory compounds<sup>32</sup> and even inhibit A $\beta$  production<sup>33</sup>—one could think that MA carriers of rs4388808 have a reduced expression of this CYP in the brain or that they have an increased structural incompatibility between *CYP2C19* and estradiol. Consequently, MA carriers could present a reduction in the catabolism of estrogen and a reduction in the A $\beta$  load. Alternatively, the A $\beta$  load would be affected by the levels of epoxyeicosatrienoic acids (EETs) produced via the metabolism of AA by CYP epoxygenases. In the brain, studies have shown that EETs have beneficial effects at regulating blood flow, cortical angiogenesis, and at promoting anti-inflammatory reactions (for review, see reference 2). By contrast, when AA is metabolized via other cascades, the protective effect of EETs is lost, and there is a possibility of induction of A $\beta$  production and/or accumulation.<sup>34</sup> In addition, it was demonstrated that A $\beta$  is able to reduce epoxygenase activity and consequently decrease EET production,<sup>35</sup> probably leading to more susceptibility to neuronal damage (for review, see reference 36). In this scenario, it would be possible that the MA of rs4388808 increases the expression of *CYP2C19*, leading to an “A $\beta$ -resistant” epoxygenase metabolism of AA, with a maintained production of EETs and a more protective phenotype against amyloidosis.

It is also plausible to think, however, that the association described here between the *CYP2C19* polymorphism and A $\beta$  is indirect, being mediated by the effect of a drug. *CYP2C19* is known to metabolize several drugs,<sup>37</sup> including medications to treat depression, which has been mentioned as a risk factor for AD (for review, see reference 38). Citalopram, for example, is an antidepressant metabolized by *CYP2C19* that has been shown to decrease A $\beta$  production.<sup>39</sup> Thus, one may think that the polymorphism rs4388808 is then associated with an improved action of citalopram, leading to the decreased brain A $\beta$  burden, rather than associated with A $\beta$  metabolism itself. Because it is difficult to track all the medications the study participants have taken before been enrolled, it is unfeasible at the moment to check whether this association holds true.

The interpretation of the results should take into consideration a few limitations. The use of the 2 cohorts does not allow us to detect and measure A $\beta$  pathology with the same methodology, inserting in the analysis some variability specific to the method used. However, despite being measured with different techniques, both the imaging radiotracer and the antibody used in the brain tissue are expected to bind to the fibrillar form of A $\beta$ .<sup>13</sup> Similarly, the cognitive composite scores from the 2 cohorts have some differences that should be considered. There are also some intrinsic characteristics of the samples that have to be taken into account: (1) the inclusion criteria of each study, (2) the average age difference between the studies, and (3) and the disease stage in which A $\beta$  measures were performed—at the end or during the disease process. Sample size restrictions impose some degree of caution when interpreting findings, including, for example, the lack of association between the genotype and plasmatic levels of A $\beta$ , or cognition, in the ADNI cohort. In addition, both cohorts are mostly composed of non-Latino Caucasians, limiting the extrapolation of the present findings to other population groups. Longitudinal analysis together with functional genomics and biochemistry experiments, not performed here, would also be necessary to determine the effect of the SNP in the protein function as well as to support any theoretical framework proposed in the Discussion section.

Results obtained from the 2 independent cohorts provide compelling evidences linking the *CYP2C19* polymorphism and A $\beta$  pathology, suggesting that the MA of rs4388808 confers protective effects against A $\beta$  accumulation in the brain and its downstream cognitive consequences. These results could have implication for anti-amyloid clinical trial designs, as preclinical rs4388808<sup>G</sup> would present a protective factor against the amyloid load. Therefore, the biological mechanism by which the genetic variation would alter A $\beta$  build up and clearance merits further investigation.

### Author contributions

Andréa L. Benedet: study concept, design, analysis and interpretation of data, composition of figures, and drafting the manuscript.

Lei Yu: analysis and interpretation of data and drafting the manuscript. Aurélie Labbe: supervision of statistical analysis and critical review of the manuscript for intellectual content. Sulantha Mathotaarachchi, Tharick A. Pascoal, Monica Shin, and Min-Su Kang: processing of image data and drafting the manuscript, study concept, design, and drafting the manuscript. Serge Gauthier, Guy A. Rouleau, and Judes Poirier: critical review of the manuscript for intellectual content. David A. Bennett: drafting the manuscript and critical review of the manuscript for intellectual content. Pedro Rosa-Neto: study concept, design, study supervision, and critical review of the manuscript for intellectual content.

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