Neurobiology of Aging 81 (2019) 234–243

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Association of PPP2R1A with Alzheimer's disease and specific cognitive domains

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ARTICLE INFO

Article history:
Received 26 February 2019
Received in revised form 19 June 2019
Accepted 23 June 2019
Available online 2 July 2019

Keywords:
Alzheimer's disease
Genome-wide association study
Quebec Founder Population
APOE4
PPP2R1A
Visuospatial performance

ABSTRACT

In an attempt to identify novel genetic variants associated with sporadic Alzheimer's disease (AD), a genome-wide association study was performed on a population isolate from Eastern Canada, referred to as the Québec Founder Population (QFP). In the QFP cohort, the rs10406151 C variant on chromosome 19 is associated with higher AD risk and younger age at AD onset in *APOE4*− individuals. After surveying the region surrounding this intergenic polymorphism for brain cis-eQTL associations in BRAINEAC, we identified *PPP2R1A* as the most likely target gene modulated by the rs10406151 C variant. PPP2R1A mRNA and protein levels are elevated in multiple regions from QFP autopsy-confirmed AD brains when compared with age-matched controls. Using an independent cohort of cognitively normal individuals with a parental history of AD, we found that the rs10406151 C variant is significantly associated with lower visuospatial and constructional performances. The association of the rs10406151 C variant with AD risk appears to involve brain *PPP2R1A* gene expression alterations. However, the exact pathological pathway by which this variant modulates AD remains elusive.

1. Introduction

Even though age is the major risk factor for Alzheimer's disease (AD), genetic factors account from 60% to 80% in AD variance according to twin studies (Gatz et al., 2006). Genome-wide association studies (GWAS)-identified single-nucleotide polymorphisms (SNPs) for AD (including the *APOE4* locus) only explain 30.62 % of the global genetic variance in this disease (Ridge et al., 2016), pointing out its complexity. Moreover, none of the loci identified by these variants are directly involved in the phosphorylation of Tau (Lambert et al., 2013), the molecular process underlying the formation of neurofibrillary tangles (Ihara et al., 1986). Additional effort is therefore required to identify novel variants associated with AD. A GWAS was therefore performed by our team to identify novel genetic risk factors for the late-onset sporadic form of AD in a population isolate from Eastern Canada, referred to as the Québec Founder Population (QFP) (Hu et al., 2011). This population descends in genetic isolation from a few thousand founders who emigrated from France in the 17th century. The demographic history of the QFP, which is characterized by a population bottleneck, a rapid population expansion, and little admixture, makes it a valuable resource for genetic studies (Tremblay and Vezina, 2010). Population isolates such as this one have been shown to reduce the genetic background noise and to facilitate the detection of weaker population-specific signals when mapping complex traits (Peltonen et al., 2000).

Among the top variants associated with AD, the minor allele (C) of one polymorphism (rs10406151) was in cis-expression quantitative trait loci (cis-eQTL) association with serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform (*PPP2R1A*) messenger ribonucleic acid (mRNA) levels. This gene encodes one of the isoforms of the regulatory subunit A in the protein phosphatase 2A (PP2A) complex. This holoenzyme is one of the main phosphatases believed to regulate the formation of neurofibrillary tangles by dephosphorylating phospho-Tau isomers.

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https://doi.org/10.1016/j.neurobiolaging.2019.06.008

The regulatory subunit A serves as a scaffolding protein, on which the regulatory subunits B compete for binding (Janssens and Goris, 2001). The high diversity of these latter subunits (16 isoforms in total) has many impacts on the whole PP2A complex, such as its targeting to tissues or cellular compartments, its substrate specificity, and its catalytic response to certain agents (Janssens and Goris, 2001). Therefore, it is reasonable to postulate that any significant alterations of PPP2R1A levels or binding to PP2A could attenuate the extent of Tau phosphorylation in the brain and, indirectly, neurofibrillary tangles formation in AD.

These observations prompted us to examine the associations between the rs10406151 C variant, PPP2R1A mRNA, and protein levels, and neurofibrillary tangles densities in the brains of autopsy-confirmed control and AD subjects from the QFP. We also investigated a possible association between this variant, cognition, as well as cerebrospinal fluid (CSF) levels of total Tau and Tau phosphorylated at residue 181 (p(181)Tau) in a cohort of at-risk cognitively normal living subjects with a parental history of AD.

2. Materials and methods

2.1. Subject demographics

2.1.1. Québec Founder Population

751 AD case-control pairs were matched based on their gender, their age, and their region of birth. All living subjects with AD were 65 years and older and diagnosed with probable AD based on the Diagnostic and Statistical Manual of Mental Disorders - fourth edition criteria. The living controls were asymptomatic at the time of their recruitment based on the Mini–Mental State Examination (score adjusted for age and education >26) and the Montréal Cognitive Assessment (MoCA score adjusted for education >26). On the other hand, autopsied AD brains from the Douglas-Bell Canada Brain Bank fulfilled the histopathological National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for a definite diagnosis of AD (Khachaturian, 1985). Tangles and senile plaques densities were measured as described before (Leduc et al., 2009). This study was conformed to the Code of Ethics of the World Medical Association and was approved by the Ethics Board of the Douglas Hospital Research Centre.

2.1.2. Brain eQTL Almanac (BRAINEAC)

Central nervous system tissues originating from 134 control individuals were collected by the Medical Research Council Sudden Death Brain and Tissue Bank, Edinburgh, UK (Millar et al., 2007), and the Sun Health Research Institute, an affiliate of Sun Health Corporation, USA (Beach et al., 2008). All individuals were confirmed to be neuropathologically normal by a consultant neuropathologist using histology performed on sections prepared from paraffin-embedded brain tissue blocks. A detailed description of the samples used in the study, tissue processing, and dissection is provided in the study by Trabzuni et al. (Trabzuni et al., 2011). For additional information, visit www.braineac.org.

2.1.3. International Genomics of Alzheimer's Project (IGAP)

IGAP is a large 2-stage study based on GWAS on individuals of European ancestry. IGAP used genotyped and imputed data on 7,055,881 SNPs to meta-analyze 4 previously published GWAS data sets consisting of 17,008 AD cases and 37,154 controls (The European Alzheimer's disease Initiative, the Alzheimer Disease Genetics Consortium, the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium, and the Genetic and Environmental Risk in AD consortium) (Lambert et al., 2013).

2.1.4. Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) cohort

Participants enrolled in the PREVENT-AD cohort (Breitner et al., 2016) are cognitively normal volunteers with a parental or multiple-sibling history of sporadic AD. They scored ≥23/30 on the education-adjusted Montréal Cognitive Assessment (Nasreddine et al., 2005) and had little if any difficulty with subsequent cognitive testing. Most were 60 years of age or older, but persons aged 55–59 years were eligible if their age was within 15 years of their youngest-affected relative's onset. Each participant and study partner provided written informed consent. All procedures were approved by the McGill University Faculty of Medicine Institutional Review Board. All research complied with ethical principles of the Declaration of Helsinki. We analyzed data collected between September 2011 and August 2017 and archived in PREVENT-AD data release 5.0. More details concerning the demographics of our PREVENT-AD sample can be found in Table 1.

2.2. DNA extraction and genotyping

2.2.1. Québec Founder Population

Deoxyribonucleic acid (DNA) from brain tissues was extracted using the DNeasy Tissue Kit (Qiagen, Hilden, Germany), whereas DNA from blood lymphocytes was extracted from automated DNA extraction (NA-1000; Auto-Gen, Holliston, MA, USA). Genotyping was performed using the HumanHap 550k BeadChip (Illumina, San Diego, CA, USA). SNPs with minor allele frequency below 1% were removed. SNPs and samples with more than 1% of missing values were also dropped. Hardy-Weinberg equilibrium (HWE) was evaluated in the control population, and SNPs that were out of HWE ($-\log(p) > 5$) were removed. Sample sets were checked for genetic outliers and duplicated samples, which were dropped. After these quality control procedures, 491,456 SNPs were kept in the analysis. For additional technical details regarding the original GWAS, please refer to Hu et al. (Hu et al., 2011).

2.2.2. BRAINEAC

Genomic DNA was extracted from subdissected samples of human postmortem brain tissue using Qiagen's DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). All samples were genotyped on the Infinium Omni1-Quad BeadChip (Illumina, San Diego, CA, USA) and on the Immunochip, a custom genotyping array designed for the fine-mapping of autoimmune disorders (Nalls et al., 2011). After standard quality controls, imputation was performed using MaCH (Li et al., 2009, 2010) and minimac (http://genome.sph.umich.edu/wiki/Minimac) using the European panel of the 1000 Genomes Project (March 2012: Integrated Phase I haplotype release version 3, based on the 2010-11 data freeze and 2012-03-14 haplotypes). For additional information, visit www.braineac.org.

Table 1
Demographic characteristics of the PREVENT-AD cohort as a function of the *PPP2R1A* rs10406151 genotypes

rs10406151			p- value
	TT carriers n = 71	TC/CC carriers n = 65	
Age mean ± SEM, years	63.5 ± 0.6	63.1 ± 0.8	0.338
Duration of education mean ± SEM, years	15.6 ± 0.4	15.2 ± 0.4	0.439
Sex n (%) women	55 (77.5 %)	47 (72.3%)	0.488
Apolipoprotein E4 positivity n (%)	28 (39.4%)	24 (36.9%)	0.763

Key: PREVENT-AD, Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease; n, number of subjects; *PPP2R1A*, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; SEM, standard error of the mean.

2.2.3. PREVENT-AD

2.2.3.1. DNA extraction. Automated DNA extraction from buffy coat samples was performed using the QIAsymphony DNA mini kit (Qiagen, Hilden, Germany).

2.2.3.2. rs10406151 genotyping. Genotyping was performed using the Omni2.5-8 BeadChip (Illumina, San Diego, CA, USA). The rs10406151 variant fulfilled our quality standard criteria (minor allele frequency >1%, <1% of missing values, HWE with $-\log(p) < 3$ in the control population), and sample sets were checked for duplicated samples, sex mismatches, and missingness.

*2.2.3.3. Apolipoprotein E (*APOE*) genotyping. APOE* genotype was determined using the PyroMark Q96 pyrosequencer (Qiagen, Hilden, Germany). DNA was amplified using polymerase chain reaction with the following primers: rs429358 forward 5′-ACGGCTGTC CAAGGAGCTG-3′, rs429358 reverse biotinylated 5′-CACCTCGCCGC GGTACTG-3′, rs429358 sequencing 5′-CGGACATGGAGGACG-3′, rs7412 forward 5′-CTCCGCGATGCCGATGAC-3′, rs7412 reverse biotinylated 5′-CCCCGGCCTGGTACACTG-3′, and rs7412 sequencing 5′-CGATGACCTGCAGAAG-3′.

2.3. Gene expression

2.3.1. PPP2R1A and ZNF836 mRNA levels in the BRAINEAC cohort

Total RNA was isolated from human postmortem brain tissues based on the single-step method of RNA isolation (Chomczynski and Sacchi, 1987) using the miRNeasy 96 kit (Qiagen, Hilden, Germany). The quality of total RNA was evaluated by the 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and the RNA 6000 Nano Kit (Agilent, Santa Clara, CA, USA) before processing with the Ambion WT Expression Kit (Thermo Fisher, Waltham, MA, USA) and the Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay (Affymetrix, Santa Clara, CA, USA) and hybridization to the Affymetrix Exon 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA) following the manufacturers' protocols. Further details regarding RNA isolation, quality control, and processing are reported in the study by Trabzuni et al. (Trabzuni et al., 2011). Gene-level expression was estimated for 26 thousand genes by calculating the Winsorized mean (below 10% and above 90%) signal of all probe sets corresponding to each gene. The resulting expression data were adjusted for brain bank, gender, and batch effects in Partek's Genomics Suite v6.6 (Partek Incorporated, Chesterfield, MO, USA).

2.3.2. PPP2R1A *mRNA levels in autopsied brains from the QFP cohort*

Total RNA was extracted from 20 mg of tissues from the frontal cortex, the temporal cortex, or the cerebellum using either one of these devices: QIAsymphony (Qiagen, Hilden, Germany) or Maxwell 16 (Promega, Madison, WI, USA). All RNA samples had $A_{260}/A_{280} \geq 2.0$. To generate complementary DNA (cDNA), RNA samples were reverse-transcribed with SuperScript VILO Master Mix (Thermo Fisher). The TaqMan primers for our *PPP2R1A* gene (Hs00204426_m1) are probed with 6-carboxyfluorescein (FAM) and target the junction between exons 3 and 4. As for the primers for the hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) housekeeping gene, they are probed with VIC and target the junction between exons 6 and 7 (Thermo Fisher). *HPRT1* was chosen as the best housekeeping gene for AD brains among 5 other genes: peptidylprolyl isomerase A (*PPIA*), ubiquitin conjugating enzyme E2 D2 (*UBE2D2*), cyclin-dependent kinase inhibitor 1B (*CDNK1B*), actin beta (*ACTB*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Indeed, it had, with *PPIA* and *UBE2D2*, the best coefficient of correlation with the BestKeeper Index (0.962). According to NormFinder, it also had the lowest stability value (0.034). cDNA from SH-SY5Y neuroblastomas (ATCC, Manassas, VA, USA) was used as the calibrator. The Fast TaqMan quantitative polymerase chain reaction protocol was performed in the QuantStudio 12X Flex instrument (Thermo Fisher). The amplification efficiency for each set of primers was measured from a serially diluted cDNA template. The resulting standard curves contained 5 dilution points spanning 5 orders of magnitude and displayed efficiencies of 92% and 93% for *HPRT1* and *PPP2R1A*, respectively. Data were analyzed using the ExpressionSuite software (Thermo Fisher).

2.4. PPP2R1A protein levels in the autopsied brains from the QFP cohort

100 mg of brain samples (frontal cortex, temporal cortex, and cerebellum) were homogenized mechanically with the Bead Ruptor 24 (Omni International, Tulsa, OK, USA) in ice-cold phosphate-buffered saline with protease inhibitors. After 2 freeze-thaw cycles, the homogenates were centrifuged at 4 °C at 5000 rpm for 5 minutes. The supernatants were used freshly for enzyme-linked immunosorbent assays (ELISAs) targeting human PPP2R1A proteins (Cusabio, College Park, MD, USA). The ELISA protocols were performed according to the manufacturer's instructions. Total protein concentrations were measured with the bicinchoninic acid assay (Thermo Fisher).

2.5. Neuropathological and neuropsychological correlates

2.5.1. Neurofibrillary tangles in autopsied brains from the QFP cohort

The neuropathological staining protocol was performed as previously described (Etienne et al., 1986). Neurofibrillary tangles densities were measured in 6 different brain regions: the cornu ammonis area 1 (CA1), the subiculum, the parasubiculum, the frontal gyrus, the frontal cortex, and the parietal cortex. The detailed methodology used to measure neurofibrillary tangles was published in the following article from Caramelli et al. (Caramelli et al., 1998) and was consistent with the classification of Khachaturian (Khachaturian, 1985).

2.5.2. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) cognitive measurements in the PREVENT-AD cohort

Participants were evaluated with version A of the RBANS. The RBANS is available in both English and Canadian French. It measures 5 domains of cognitive performance (Randolph et al., 1998).

2.5.3. Cerebrospinal fluid measurements of amyloid-beta 1–42 ($A\beta_{1-42}$), p(181)Tau, and total Tau levels in the PREVENT-AD cohort

Lumbar punctures in overnight fasted PREVENT-AD volunteers were performed using the Sprotte 24-gauge atraumatic needle. Samples of 20–30 mL were aliquoted (500 μL) into propylene cryotubes and stored at −80 °C. We used the standardized procedures from the Biomarkers for Alzheimer's and Parkinson's Disease (BIOMARKAPD) consortium (Lelental et al., 2016) to measure CSF concentrations of the AD biomarkers $A\beta_{1-42}$, p(181)Tau, and total Tau with the Innotest ELISA kits (Fujirebio, Ghent, Belgium).

2.6. Statistical analyses

Analyses of the demographics of rs10406151 TT and TC/CC carriers in the PREVENT-AD cohort were performed with independent-samples Mann-Whitney U-tests for age and years of education as well as Pearson χ^2 for sex and *APOE4* positivity. The QFP GWAS data were analyzed with a logistic regression taking into account sex, age, as well as city of birth and using principal components to adjust for possible stratification. The IGAP meta-GWAS

data were taken from the summary of results provided on their website. In all of the analyses, extreme outliers (defined as below the first quartile value −3 interquartile ranges or above third quarter value +3 interquartile ranges) were removed. Associations between age at AD onset and the rs10406151 genotypes in the QFP cohort were analyzed by using between-subjects analyses of covariance (ANCOVAs) with sex and *APOE4* status as covariates for the whole sample and sex for *APOE4*-stratified populations. Correlations of mRNA levels and the rs10406151 genotypes in the BRAINEAC cohort were assessed with Pearson linear regression corrected for age, sex, and postmortem interval for *APOE4*-stratified populations and the same covariates in addition to *APOE4* status for the whole sample. Associations contrasting brain PPP2R1A mRNA/protein levels as a function of AD status were performed by using between-subjects ANCOVAs with age, postmortem interval, sex, and *APOE4* status as covariates. Because neurofibrillary tangle densities values are not normally distributed in our sample, Spearman linear regressions were used to evaluate the association between neurofibrillary tangles densities and the rs10406151 genotypes. A similar approach was applied for correlations between neurofibrillary tangles density and PPP2R1A mRNA/protein levels. In the PREVENT-AD cohort, between-subjects ANCOVAs corrected for age, years of education, and sex for *APOE4*-stratified populations and the same covariates in addition to *APOE4* status for the whole sample were performed between RBANS index scores and the rs10406151 genotypes. Correlations evaluating CSF p(181)Tau, total Tau, p(181)Tau/Aβ_{1-42}, and total Tau/Aβ_{1-42} levels as a function of the rs10406151 genotypes in the PREVENT-AD subjects were analyzed using Pearson linear regressions corrected for age and sex for *APOE4*-stratified populations and the same covariates in addition to *APOE4* status for the whole sample.

3. Results

3.1. Associations of AD risk, age at onset, and PPP2R1A mRNA levels with the rs10406151 C variant

In the QFP cohort, the *PPP2R1A* rs10406151 C variant is associated with a higher risk of developing AD ($p = 5.01 \times 10^{-5}$, odds ratio = 1.39; Fig. 1A). This association is replicated in the IGAP cohort ($p = 0.0208$; IGAP summary statistics are available at this URL address: http://web.pasteur-lille.fr/en/recherche/u744/igap/igap_download.php). In the *APOE4* noncarriers subgroup, rs10406151 CC carriers develop AD approximately 6 years before TT/TC carriers (F[1, 136] = 4.530, $p = 0.035$; TT/TC carriers: mean age at AD onset = 76.1 years; CC carriers: mean age at AD onset = 70.3 years; Fig. 1B). Using the BRAINEAC cohort, we examined the cis-eQTL associations in the genomic region surrounding the rs10406151 C variant on chromosome 19 (Fig. 1C). The average of total *PPP2R1A* mRNA levels across 10 brain regions (frontal cortex, temporal cortex, occipital cortex, hippocampus, thalamus, cerebellum, substantia nigra, putamen, medulla, and white matter) is allele-dose-dependently associated with the rs10406151 genotypes (r[125] = 0.189, $p = 0.033$), with higher transcript levels for C carriers (Fig. 2A). The expression of the *ZNF836* gene, *PPP2R1A*'s genomic neighbor, is statistically not associated with the rs10406151 genotypes (r[128] = 0.071, $p = 0.420$). Further stratification by the *APOE4* genotypes reveals that *PPP2R1A* mRNA levels are significantly associated with the rs10406151 genotypes in *APOE4* noncarriers (r[88] = 0.211, $p = 0.046$; Fig. 2B), but not in *APOE4* carriers (r[33] = 0.258, $p = 0.134$; Fig. 2C). As for the nearby *ZNF836* gene, we could not detect any association between mRNA levels and the rs10406151 genotypes in both *APOE4*-stratified

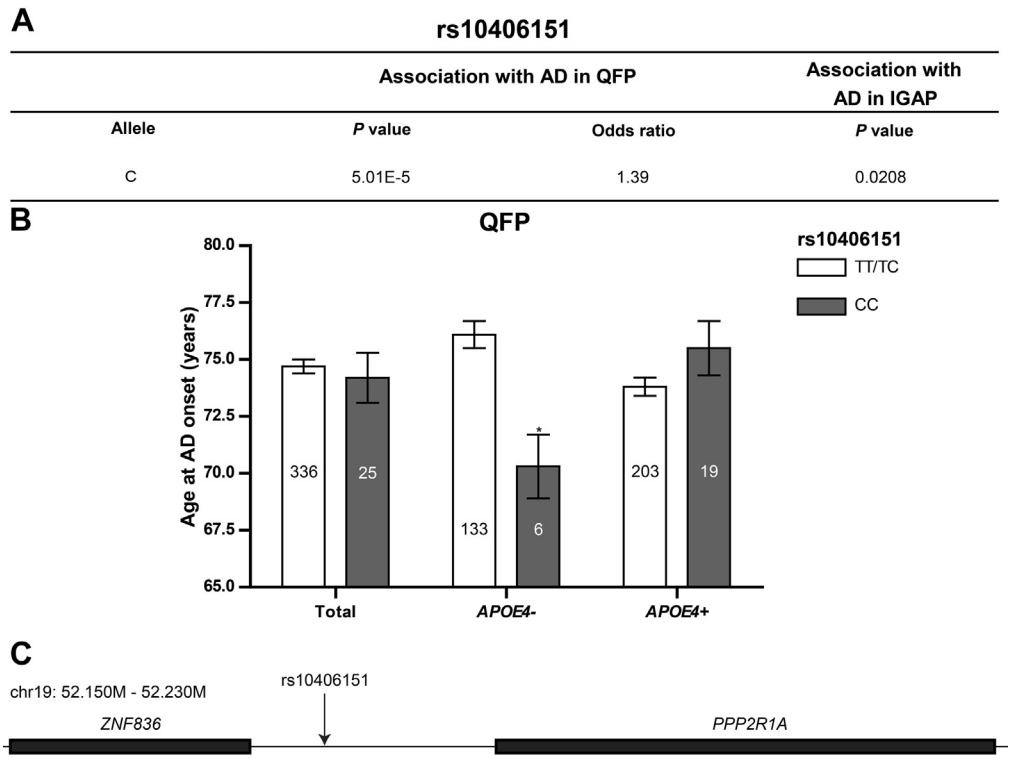

Fig. 1. Higher AD risk and younger age at AD onset associated with the *PPP2R1A* rs10406151 C variant in the QFP cohort. (A) Correlation of the *PPP2R1A* rs10406151 C variant with the disease risk in the QFP and IGAP cohorts; (B) Association, in the QFP cohort, of the *PPP2R1A* rs10406151 C variant with age at AD onset in the whole sample and in *APOE4* noncarriers and carriers. Bars represent mean age at AD onset ±SEM. (C) Region on chromosome 19 surrounding the *PPP2R1A* rs10406151 C variant according to version GRCh38 of the human genome. Abbreviations: AD, Alzheimer's disease; *APOE4,* apolipoprotein E4; GRCh38, Genome Reference Consortium Human Build 38; IGAP, International Genomics of Alzheimer's Project; *PPP2R1A,* serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; PREVENT-AD, Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease; QFP, Québec Founder Population; SEM, standard error of the mean; *ZNF836,* zinc finger protein 836; * $p \leq 0.05$.

subgroups (r[90] = 0.151, p = 0.152 in *APOE4* noncarriers; r [34] = −0.091, p = 0.598 in *APOE4* carriers; Fig. 2B–C).

3.2. Correlations of PPP2R1A mRNA and protein levels with AD in the QFP cohort

In the QFP cohort, *PPP2R1A* mRNA levels are significantly higher in AD cases compared with control subjects in the cerebellum, the frontal cortex, and the temporal cortex (F[1, 99] = 20.278, $p = 1.8 \times 10^{-5}$ in the cerebellum; FC = 1.88; F[1, 125] = 8.722, p = 0.004 in the frontal cortex; FC = 1.64; F[1, 96] = 24.291, $p = 3.5 \times 10^{-6}$ in the temporal cortex; FC = 2.08; Fig. 3A). However, PPP2R1A protein levels are significantly elevated only in the temporal cortex area (F [1, 26] = 3.754, p = 0.064 in the cerebellum; F[1, 32] = 0.047, p = 0.830 in the frontal cortex; F[1, 33] = 5.637, p = 0.024; Fig. 3B).

3.3. Associations of RBANS index scores with the rs10406151 C variant in the PREVENT-AD cohort

In living subjects from the PREVENT-AD cohort, rs10406151 C carriers exhibit lower RBANS visuospatial constructional scores compared with noncarriers (F[1, 130] = 6.704, p = 0.011; Fig. 4). However, there is no significant differences for the other RBANS index scores as a function of the rs10406151 genotypes (F[1, 130] = 0.026, p = 0.872 for immediate memory; F[1, 130] = 0.074, p = 0.787 for language; F[1, 130] = 0.033, p = 0.857 for attention; F[1, 130] = 0.221, p = 0.639 for delayed memory; F[1, 130] = 1.550, p = 0.215 for total score; Fig. 4A). Stratification by the *APOE4* genotypes indicates that, only in noncarriers of the *APOE4* allele, visuospatial constructional scores are significantly lower in subjects bearing the rs10406151 C allele (*APOE4* noncarriers: F[1, 79] = 4.508, p = 0.037; *APOE4* carriers: F[1, 47] = 3.319, p = 0.075; Fig. 4B–C). The associations between the other RBANS index scores and the rs10406151 genotypes remain not statistically significant after *APOE4* genotypes stratification (Fig. 4B and C).

3.4. Correlations of neurofibrillary tangles densities with the rs10406151 genotypes as well as with PPP2R1A mRNA and protein levels in autopsy-confirmed AD brains from the QFP cohort

Using autopsy-confirmed AD brains from the QFP cohort, analyses of the associations between the rs10406151 genotypes and neurofibrillary tangles densities, either in the CA1, the frontal cortex, or the total of the 6 brain regions surveyed (CA1, subiculum, parasubiculum, frontal gyrus, frontal cortex, and parietal cortex),

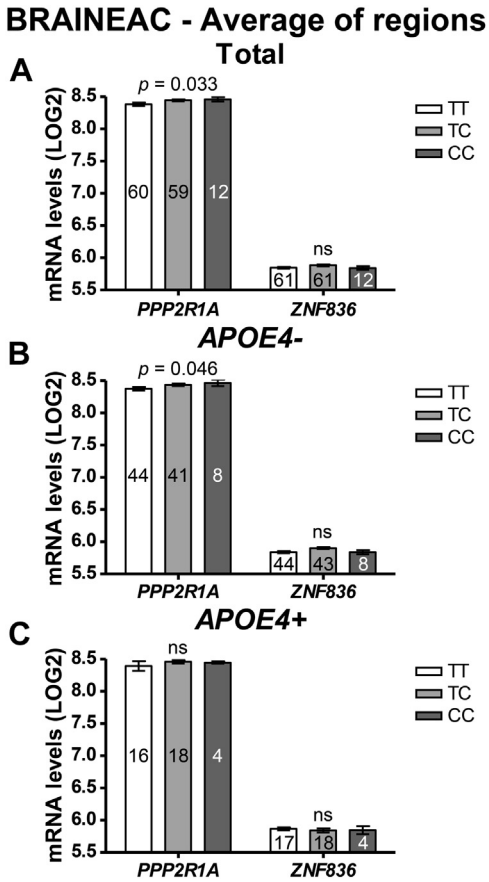

Fig. 2. Cis-eQTL associations for the *PPP2R1A* rs10406151 C variant in the BRAINEAC cohort. Correlations of total mRNA levels for the genes from Fig. 1C (*PPP2R1A* and *ZNF836*) averaged across 10 brain regions (frontal cortex, temporal cortex, occipital cortex, hippocampus, thalamus, cerebellum, substantia nigra, putamen, medulla, and white matter) as a function of the rs10406151 genotypes in (A) the total sample, (B) *APOE4* noncarriers, and (C) *APOE4* carriers. Bars represent mean $\log_2$ expression values ±SEM. Abbreviations: *APOE4,* apolipoprotein E4; BRAINEAC, Brain eQTL Almanac; cis-eQTL, cis-expression quantitative trait loci; mRNA, messenger ribonucleic acid; ns, not significant; *PPP2R1A,* serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; SEM, standard error of the mean; *ZNF836,* zinc finger protein 836.

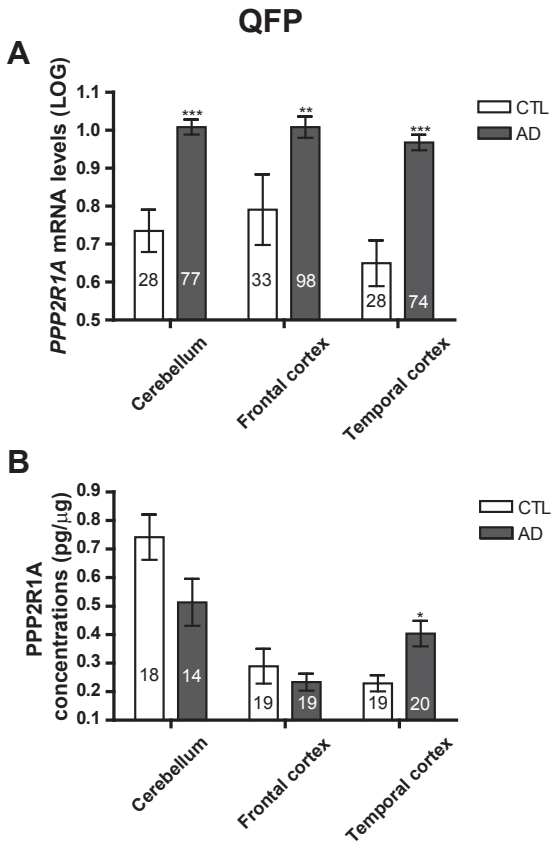

Fig. 3. PPP2R1A mRNA and protein levels as a function of AD status in autopsy-confirmed brains from the QFP cohort. (A) *PPP2R1A* mRNA levels in the cerebellum, the frontal cortex, and the temporal cortex of autopsy-confirmed control and AD subjects from the QFP cohort. Bars represent mean $\log_{10}$RQ + 1 values ±SEM. (B) PPP2R1A protein levels in the cerebellum, the frontal cortex, and the temporal cortex of autopsy-confirmed control and AD subjects from the QFP cohort. Bars represent mean PPP2R1A/total protein level ratios (in pg/µg) ± SEM. Abbreviations: AD, Alzheimer's disease; CTL, control; mRNA, messenger ribonucleic acid; PPP2R1A, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; QFP, Québec Founder Population; RQ, relative quantification; SEM, standard error of the mean; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

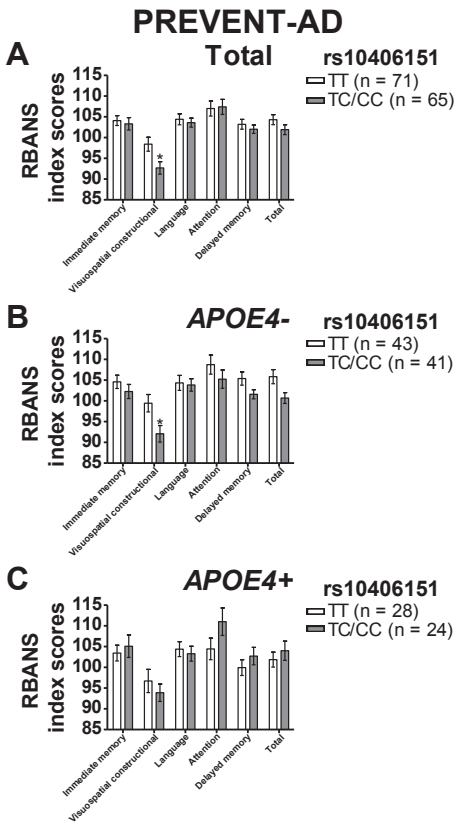

Fig. 4. Correlations of RBANS index scores with the *PPP2R1A* rs10406151 C variant in the PREVENT-AD cohort. The 5 cognitive domains measured in the RBANS (immediate memory, visuospatial constructional ability, language, attention, and delayed memory) as well as the total score were plotted as a function of the rs10406151 genotypes in (A) the total sample, (B) *APOE4* noncarriers, and (C) *APOE4* carriers. Bars represent mean RBANS index scores ±SEM. Abbreviations: *APOE4*, apolipoprotein E4; *PPP2R1A*, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; PREVENT-AD, Presymptomatic Evalutation of Experimental or Novel Treatments for Alzheimer's Disease; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SEM, standard error of the mean; * $p \leq 0.05$.

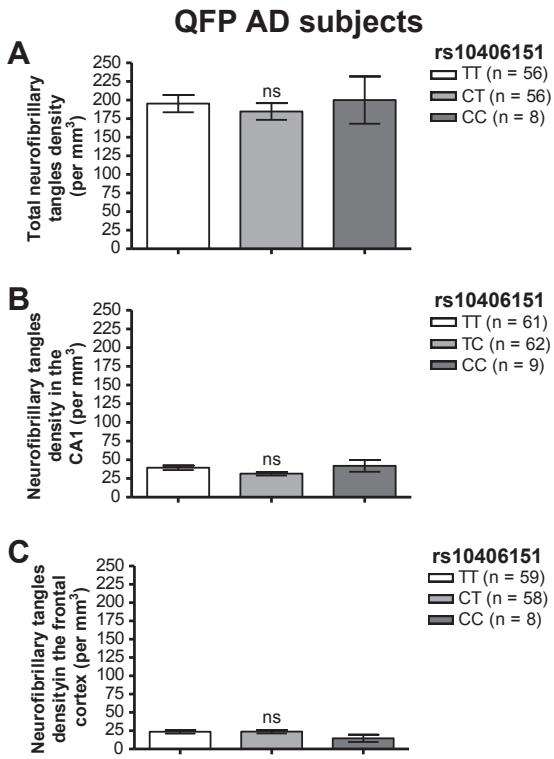

Fig. 5. Associations of neurofibrillary tangles densities with the *PPP2R1A* rs10406151 C variant in autopsy-confirmed AD brains from the QFP cohort. Correlations, in autopsy-confirmed AD subjects, of *PPP2R1A* rs10406151 genotypes with neurofibrillary tangles densities in (A) a total of 6 brain regions (CA1, subiculum, parasubiculum, frontal gyrus, frontal cortex, and parietal cortex); (B) in the CA1 region of the hippocampus; and (C) in the frontal cortex. Bars represent mean neurofibrillary tangle densities ±SEM. Abbreviations: AD, Alzheimer's disease; CA1, cornu ammonis area 1; ns, not significant; *PPP2R1A*, serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; QFP, Québec Founder Population; SEM, standard error of the mean.

display no significant genotype-phenotype correlations ($r[132] = -0.102$, $p = 0.243$ for the CA1; $r[125] = -0.049$, $p = 0.586$ for the frontal cortex; $r[120] = -0.001$, $p = 0.994$ for the total of the 6 regions; Fig. 5). Similarly, in the frontal cortex of autopsy-confirmed AD subjects from the same cohort, no significant association was found between neurofibrillary tangles densities and *PPP2R1A* mRNA levels ($r[62] = -0.073$, $p = 0.574$; Fig. 6A). However, in the same brain region, a significant correlation was observed, in autopsy-confirmed QFP AD brains, between neurofibrillary tangles densities and PPP2R1A protein levels ($r[11] = 0.809$ [$r^2 = 0.654$], $p = 0.003$; Fig. 6B).

3.5. Associations of baseline CSF p(181)Tau and total Tau levels with the rs10406151 genotypes in the PREVENT-AD cohort

Baseline CSF p(181)Tau and total Tau levels in PREVENT-AD subjects are not associated with the rs10406151 genotypes ($r[77] = -0.061$, $p = 0.593$ for p(181)Tau levels; $r[77] = -0.041$, $p = 0.723$ for total Tau levels; Fig. 7A), nor when normalized with $A\beta_{1-42}$ levels ($r[74] = -0.105$, $p = 0.367$ for p(181)Tau/$A\beta_{1-42}$ levels; $r[73] = -0.107$, $p = 0.360$ for total Tau/$A\beta_{1-42}$ levels; Fig. 7B). Stratification by the *APOE4* genotypes does not lead to significant associations (data not shown).

4. Discussion

In a population isolate from Eastern Canada, a polymorphic variant (rs10406151) near the *PPP2R1A* gene on chromosome 19 was found to be significantly associated with AD risk (Fig. 1A). The *PPP2R1A* rs10406151 variant is located 7M bp downstream of the *APOE* locus and is therefore very far from the linkage disequilibrium block in which *APOE* is present (Kulminski et al., 2018). Moreover, the association of the rs10406151 variant with AD risk is replicated in the IGAP study (Fig. 1A). Furthermore, in *APOE4* noncarriers, this polymorphism is associated with earlier onset of AD (Fig. 1B), suggesting a potential pathological involvement. To examine cis-eQTL associations in the genomic region surrounding the rs10406151 C variant (Fig. 1C), we used the BRAINEAC cohort, which by consisting of brains free of any neurodegenerative disorder, allows for the examination of relationships between gene expression levels and genetic variants without any possible disease-related interferences. In this cohort, total *PPP2R1A* mRNA levels averaged across 10 brain regions were found to be significantly associated with the rs10406151 genotypes in an allele dose-dependent manner, with higher transcript levels for C carriers (Fig. 2A). The effect was subsequently found to be specific to *APOE4* noncarriers (Fig. 2B).

The *PPP2R1A* gene encodes the alpha isoform of the regulatory subunit A in the PP2A complex, one of the main Tau phosphatases responsible for the formation of neurofibrillary tangles (Gong et al., 1993). It serves as a scaffolding protein on which the regulatory subunits B compete to target the holoenzyme to specific tissues or

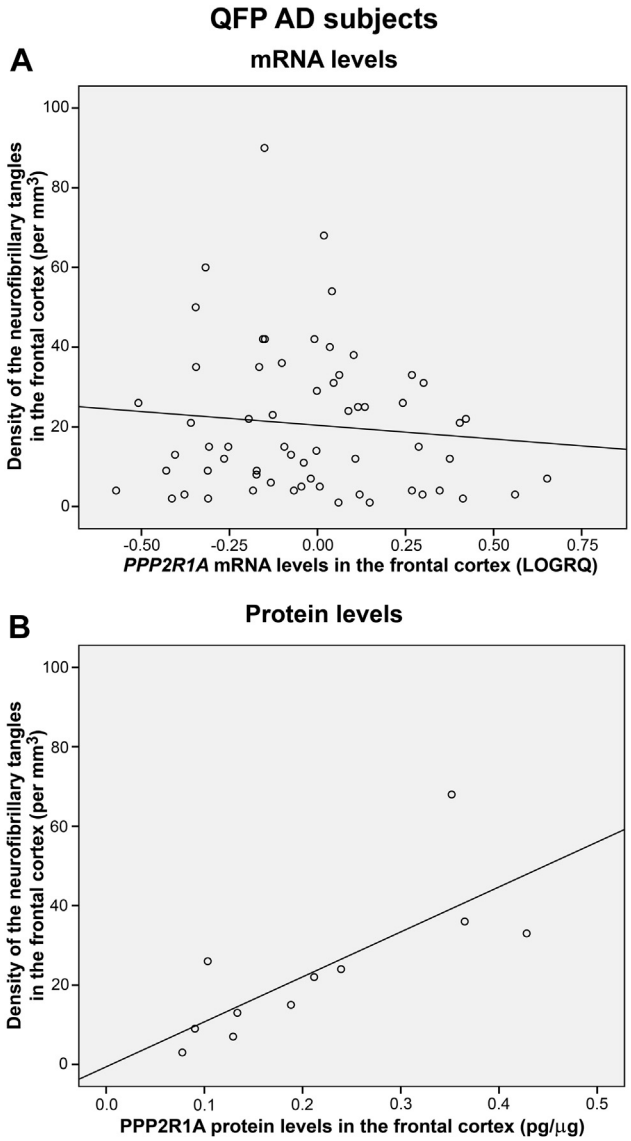

Fig. 6. Associations of neurofibrillary tangles densities with PPP2R1A mRNA and protein levels in the frontal cortex of autopsy-confirmed QFP AD subjects. Linear relationships between neurofibrillary tangles, PPP2R1A (A) mRNA, and (B) protein levels were performed in the frontal cortex of autopsy-confirmed QFP AD cases. *PPP2R1A* mRNA levels are represented as $\log_{10}$RQ values, whereas units for protein levels are pg/μg of total protein. Abbreviations: AD, Alzheimer's disease; mRNA, messenger ribonucleic acid; *PPP2R1A,* serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; QFP, Québec Founder Population; RQ, relative quantification.

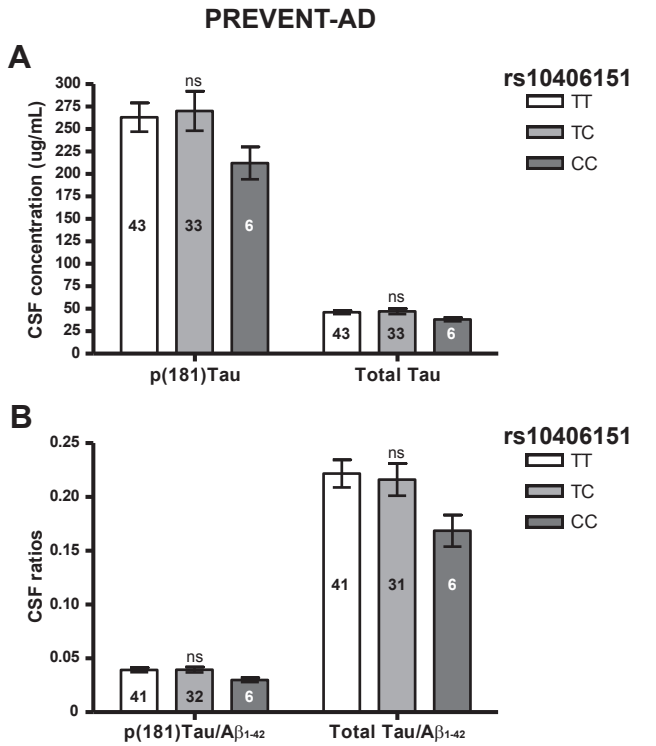

Fig. 7. Correlations of CSF p(181)Tau and total Tau levels as a function of the *PPP2R1A* rs10406151 genotypes in the PREVENT-AD cohort. Correlations, in the PREVENT-AD cohort, of the *PPP2R1A* rs10406151 genotypes with (A) CSF p(181)Tau and total Tau levels as well as with (B) CSF p(181)Tau/Aβ_{1-42} and total Tau/Aβ_{1-42} ratios. Abbreviations: Aβ_{1-42}, amyloid-beta 1–42; CSF, cerebrospinal fluid; ns, not significant; p(181)Tau, Tau phosphorylated at residue 181; *PPP2R1A,* serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform; PREVENT-AD, Presymptomatic Evalutation of Experimental or Novel Treatments for Alzheimer's Disease.

cellular compartments, to alter its substrate specificity, or to modify its catalytic response to certain agents (Janssens and Goris, 2001). Moreover, overexpression of PPP2R1A leads to multinucleated cells, highlighting its role in cell division control (Wera et al., 1995). Nonetheless, cell division activation in postmitotic neurons leads to neuronal death as well as Tau and amyloid pathology in mice (Park et al., 2007). Furthermore, 3 coding mutations (P179L, R182W, and R258H) in the *PPP2R1A* gene have been associated with intellectual disability characterized by corpus callosum agenesis. Although 2 mutations (P179L and R182W) markedly reduce PP2A activity, all mutations reduce the regulatory subunit A binding to the catalytic subunit and to most regulatory subunits B (Houge et al., 2015).

In autopsy-confirmed brains from the QFP cohort, all regions surveyed in our analysis (cerebellum, frontal cortex, and temporal cortex) display significantly elevated *PPP2R1A* mRNA levels in AD subjects versus controls (Fig. 3A). However, only the temporal cortex area displays significantly increased PPP2R1A protein levels in AD (Fig. 3B). In autopsy-confirmed AD brains from the same cohort, we analyzed neurofibrillary tangles densities in a total of 6 regions (CA1, subiculum, parasubiculum, frontal gyrus, frontal cortex, parietal cortex) as well as in the frontal cortex and the CA1 alone. After stratifying these pathological changes by the *PPP2R1A* rs10406151 genotypes, we could not find any direct association between neurofibrillary tangles and this risk allele, failing to support our initial working hypothesis (Fig. 5). However, in the frontal cortex of autopsy-confirmed QFP subjects with AD, despite the fact that neurofibrillary tangles densities do not correlate with *PPP2R1A* mRNA levels (Fig. 6A), they are significantly associated with PPP2R1A protein levels (Fig. 6B). These results suggest a disconnection between PPP2R1A mRNA and protein concentrations in the AD brain, consistent with post-translation modifications, such as methylation, ubiquitination, or phosphorylation, on the PP2A catalytic subunit that affect the composition and the activity of this holoenzyme (Janssens et al., 2008; McConnell et al., 2010). Moreover, a similar discrepancy for PPP2R1A was observed in prostate cancer tissues, where an upregulation was found for mRNA levels (Li et al., 2018), whereas a downregulation was observed for protein levels (Pandey et al., 2013).

The observed association of the rs10406151 CC genotype with reduced age at AD onset in *APOE4* noncarriers suggests a pathophysiological involvement in the formation of neurofibrillary tangles in the years or even the decade preceding the onset of the disease. This possibility prompted us to re-examine this issue in a cohort of cognitively normal individuals with a familial history of AD. Although we did not find an overall effect of the risk allele on

global cognition, we detected lower RBANS visuospatial scores in *PPP2R1A* rs10406151 C carriers (Fig. 4A). The fact that the visuospatial/constructional index score is the only one associated with this variant in presymptomatic participants from PREVENT-AD is in agreement with a longitudinal study on preclinical AD, where visuospatial declines were detected before verbal and memory deficits as well as global cognitive worsening (Johnson et al., 2009). Moreover, similar to age at onset, the association between the rs10406151 variant and lower visuospatial performances was observed, after stratification for the *APOE4* genotypes, only in *APOE4* noncarriers (Fig. 4B,C). The fact that age at onset, *PPP2R1A* mRNA levels, and RBANS visuospatial constructional index scores are all only associated, after *APOE4* stratification, with the rs10406151 C variant in *APOE4* noncarriers can be explained in 2 ways: (1) there could be a direct interaction between the rs10406151 and *APOE4* genotypes or (2) the lower prevalence of the *APOE4* allele may compromise the statistical power required to detect this effect. In the PREVENT-AD cohort, we found no association between the *PPP2R1A* rs10406151 C risk variant and CSF p(181)Tau and total Tau levels, which are believed to act as surrogate markers of tangles formation and neuronal loss, respectively (Hampel et al., 2004; Hesse et al., 2001; Ost et al., 2006; Riemenschneider et al., 2003; Zetterberg et al., 2006). Furthermore, CSF p(181)Tau/Aβ and total Tau/Aβ ratios, which often serve as indicators of disease progression in nonsymptomatic subjects (Steenland et al., 2014), are not associated with the *PPP2R1A* rs10406151 C risk variant (Fig. 7B).

Given the results obtained so far in the presymptomatic "at risk" subjects from PREVENT-AD, in the QFP sample used for the association with AD risk and age at onset, consisting mostly of mild-to-moderate AD clinical cases, and in the end-stage autopsy-confirmed AD brains from the QFP, it appears that the *PPP2R1A* risk allele is associated with the disease pathophysiology at the end of the prodromal stage. It is conceivable that a mechanism other than Tau dephosphorylation may be at play in this pathophysiological process. For example, it was shown that the regulatory subunit A of *PP2A* (which is encoded by both *PPP2R1A* and serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform [*PPP2R1B*] genes) interacts with glycogen synthase kinase 3β (GSK3β) in transgenic mice with a p25-induced overaction of cyclin-dependent kinase 5 (CDK5) (Plattner et al., 2006). Hence, both CDK5 and GSK3β are key neuronal kinases that have been linked to AD pathology through the phosphorylation of Tau (Baumann et al., 1993; Hanger et al., 1992).

In summary, we believe that the *PPP2R1A* rs10406151 C variant increases the AD risk and impairs visuospatial perception in the presymptomatic phase of the disease by increasing *PPP2R1A* mRNA levels, which are elevated in AD brains. Moreover, PPP2R1A protein levels are upregulated and associated with neurofibrillary tangles densities in the brains of AD subjects. However, additional research is needed to investigate with further details the pathological pathway by which PPP2R1A might have an impact on the development of AD and its related cognitive impairments.

Disclosure

The authors have no actual or potential conflicts of interest.

Acknowledgements

This study was supported by the Canadian Institutes of Health Research (grant N° MOP-119321), the Natural Sciences and Engineering Research Council of Canada (grant N° RGPIN-2015–03790), the Fonds Québécois de la Recherche en Santé, the J.-Louis Lévesque Foundation, the Lemaire Family Foundation, and the ICAO Charity Drive. JM and CP were supported by the Centre for Studies on the Prevention of Alzheimer's disease. JM was also supported by the Djavad Mowafaghian Studentship. These funding sources had no involvement in the study design. The authors also wish to thank Mrs Danielle Cécyre and Josée Prud'homme from the Douglas-Bell Canada Brain Bank in Montréal for the generous donation of the brain tissue samples from the QFP cohort.

The authors thank the International Genomics of Alzheimer's Project (IGAP) for providing summary results data for these analyses. The investigators within IGAP contributed to the design and implementation of IGAP and/or provided data, but did not participate in analysis or writing of this report. IGAP was made possible by the generous participation of the control subjects, the patients, and their families. The i–Select chips were funded by the French National Foundation on Alzheimer's disease and related disorders. EADI was supported by the LABEX (Laboratory of Excellence) Investments for the Future program, the DISTALZ grant, Inserm, Institut Pasteur de Lille, Université de Lille 2, and the Lille University Hospital. GERAD was supported by the Medical Research Council (grant N° 503480), Alzheimer's Research UK (grant N° 503176), the Wellcome Trust (grant N° 082604/2/07/Z), and the German Federal Ministry of Education and Research (BMBF): Competence Network Dementia (CND) grants N° 01GI0102, 01GI0711, and 01GI0420. CHARGE was partly supported by the National Institutes of Health/National Institute on Aging (NIH/NIA) grant N° R01 AG033193, the NIA grant N° AG081220, the AGES contract N° N01–AG–12100, the National Heart, Lung, and Blood Institute (NHLBI) grant N° R01 HL105756, the Icelandic Heart Association, the Erasmus Medical Center, and the Erasmus University. ADGC was supported by the Alzheimer's Association grant N° ADGC–10–196728, and the NIH/NIA grants N° U01 AG032984, U24 AG021886, and U01 AG016976.

BRAINEAC was supported by the MRC through the MRC Sudden Death Brain Bank (Colin Smith), the King Faisal Specialist Hospital and Research Centre, Saudi Arabia (Daniah Trabzuni), the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust, the King's College in London, a Project Grant (G0901254 to John Hardy and Michael E. Weale), and a Training Fellowship (G0802462 to Myna Ryten).

Data used in preparation of this article were obtained from the PREVENT-AD program (https://douglas.research.mcgill.ca/stop-ad-centre), data release 5.0 (November 30, 2017). A complete listing of PREVENT-AD Research Group can be found in the PREVENT-AD database: https://preventad.loris.ca/acknowledgements/acknowledgements.php?date=[YYYY-MM-DD]. The investigators of the PREVENT-AD program contributed to the design and implementation of PREVENT-AD and/or provided data, but did not participate in the analysis or writing of this report.

PREVENT-AD was launched in 2011 as a $13.5 million, 7-year public-private partnership using funds provided by McGill University, the Fonds de Recherche du Québec–Santé (FRQS), an unrestricted research grant from Pfizer Canada, the J-Louis Lévesque Foundation, the Douglas Hospital Research Centre and Foundation, the Government of Canada, and the Canada Fund for Innovation. Private sector contributions were facilitated by the Development Office of the McGill University Faculty of Medicine and by the Douglas Hospital Research Centre Foundation (http://www.fondationdouglas.qc.ca/).

The primary goal of PREVENT-AD is to test whether serial determination of multimodal biomarkers of Alzheimer's disease may be measured and used in presymptomatic persons at high risk of subsequent AD dementia to trace the progression of the disease process and to measure effects of any potentially preventive treatment interventions. This work is intended to provide preliminary data regarding the probable efficacy and safety of potential new treatments for prevention of AD dementia.

The founders of the program were John C. S. Breitner (MD, MPH), Judes Poirier (PhD), Pierre Etienne (MD), the Douglas Hospital Research Centre, and the Faculty of Medicine of McGill University (Montréal, QC, Canada). The current Program Director is Judes Poirier (PhD), the Co-Director is Sylvia Villeneuve (PhD), and the Study Coordinator is Jennifer Tremblay-Mercier (MSc). PREVENT-AD is the result of efforts of many other coinvestigators from a range of academic institutions and private corporations as well as extraordinarily dedicated and talented clinical and technical assistant staff, students, and postdoctoral fellows. Subjects being recruited from the greater Montréal area and more distant locations in Québec, the authors would like to take the opportunity to thank the PREVENT-AD volunteers for their extraordinary commitment to this work. For up-to-date information, see https://douglas.research.mcgill.ca/stop-ad-centre.

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