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Featured Article

CDK5RAP2 gene and tau pathophysiology in late-onset sporadic Alzheimer's disease

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Abstract

Introduction: Because currently known Alzheimer's disease (AD) single-nucleotide polymorphisms only account for a small fraction of the genetic variance in this disease, there is a need to identify new variants associated with AD.

Methods: Our team performed a genome-wide association study in the Quebec Founder Population isolate to identify novel protective or risk genetic factors for late-onset sporadic AD and examined the impact of these variants on gene expression and AD pathology.

Results: The rs10984186 variant is associated with an increased risk of developing AD and with a higher CDK5RAP2 mRNA prevalence in the hippocampus. On the other hand, the rs4837766 variant, which is among the best cis-expression quantitative trait loci in the *CDK5RAP2* gene, is associated with lower mild cognitive impairment/AD risk and conversion rate.

Discussion: The rs10984186 risk and rs4837766 protective polymorphic variants of the *CDK5RAP2* gene might act as potent genetic modifiers for AD risk and/or conversion by modulating the expression of this gene.

Keywords: Alzheimer's disease; Genetics; CDK5RAP2; Tau; Neurofibrillary tangles

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, representing between 50% and 56% of all cases [1]. Age being the major risk factor for this disease, the incidence of AD doubles every 5 years starting at the age of 65 years to reach one-third of people aged 85 years and more [1]. In addition to amyloid plaques [2] and neuronal and synaptic loss [3,4], AD pathology is characterized by the accumulation of neurofibrillary tangles (NFTs) in neurons [5]. The burden of neocortical NFTs correlates with memory impairment in sporadic AD [6].

Because currently known AD single-nucleotide polymorphisms only explain 30.62% of the genetic variance in this disease [7], further effort needs to be made to identify new variants associated with AD, especially those implicated in the tau pathophysiology. A genome-wide association study (GWAS) was therefore performed by our team to identify novel genetic risk factors for late-onset sporadic AD in a population isolate from Eastern Canada [8]. The use of these populations with a small number of founders, such as the Quebec Founder Population (QFP), has been shown to reduce the genetic background noise and to allow the

The authors have declared that no conflict of interest exists.

[1]A complete listing of Alzheimer's Disease Neuroimaging Initiative investigators can be found at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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detection of population-specific signals. All the subjects used in the GWAS belonged to the QFP isolate. 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 [9]. The QFP has been well characterized as having reduced genetic heterogeneity for Mendelian diseases [10].

Besides the usual variants in the apolipoprotein E (*APOE*) locus that markedly exceeded genome-wide significance, the strongest hit, rs10984186, in our cohort is in cis-expression quantitative trait loci (cis-eQTL) association with the cyclin-dependent kinase 5 regulatory subunit–associated protein 2 (*CDK5RAP2*) gene, which codes for a protein that binds to the activators of cyclin-dependent kinase 5 (CDK5; p35 or p25) via a domain encoded by exon 34; p25 is believed to facilitate NFT formation and accumulation by overactivating CDK5 [11], one of several kinases regulating tau phosphorylation in the central nervous system [12].

CDK5 is also involved during development as a regulator of cortical lamination [13], fasciculation of thalamocortical and callosal projections [14], as well as neurite outgrowth during neuronal differentiation [15]. These processes are all mediated through binding with p35. As for CDK5RAP2, it is implicated in the proliferation of neuronal progenitors in the developing neocortex by regulating centrosome function and cohesion, as well as mitotic spindle orientation and pole number [16,17]. Homozygous and compound heterozygous mutations resulting in premature terminations of the translation of the *CDK5RAP2* gene have been shown to cause microcephaly 3 [18–21], as well as the Seckel syndrome [22], two medical conditions characterized by reduced head circumferences. Some intronic single-nucleotide polymorphisms located in the *CDK5RAP2* gene are also associated with reduced brain volumes and/or cortical areas in males [23].

In the present study, we examined the pathophysiological effects of risk-associated or protective variants located in the *CDK5RAP2* locus and their impact on the gene expression profiles from multiple cohorts.

2. Methods

2.1. Subject demographics

2.1.1. Quebec Founder Population

All living AD subjects were ≥65 years and diagnosed with probable AD based on the *Diagnostic and Statistical Manual of Mental Disorders-IV* criteria. The living controls (CTLs) were also ≥65 years and were asymptomatic at the time of their recruitment based on the Mini–Mental State Examinations (score adjusted for age and education > 26) and the Montreal Cognitive Assessments (score adjusted for education > 26). Autopsied brains, on the other hand, had to fulfill 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 [24]. Tangle and senile plaque densities were measured as described before [25]. This study was conformed to the Code of Ethics of the World Medical Association and was approved by the Ethics Board of the Douglas Mental Health University Institute Research Center.

2.1.2. Alzheimer's Disease Neuroimaging Initiative

The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort consists of over 1500 adults, aged 55–90 years, who have accepted to participate in multiple research projects. These participants are cognitively normal older individuals, subjects with early or late mild cognitive impairment (MCI), or patients with early AD. Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu).

2.1.3. Brain eQTL Almanac

Central nervous system tissues originating from 134 CTL individuals were collected by the Medical Research Council Sudden Death Brain and Tissue Bank, Edinburgh, UK [26], and the Sun Health Research Institute, an affiliate of Sun Health Corporation, USA [27]. 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 [28]. For additional information, visit www.braineac.org.

2.1.4. International Genomics of Alzheimer's Project

International Genomics of Alzheimer's Project (IGAP) is a large two-stage study based on the GWASs on individuals of European ancestry. IGAP used genotyped and imputed data on 7,055,881 single-nucleotide polymorphisms to meta-analyze four previously published GWAS data sets consisting of 17,008 AD cases and 37,154 CTLs (the European Alzheimer's Disease Initiative, Alzheimer's Disease Genetics Consortium, Cohorts for Heart and Aging Research in Genomic Epidemiology consortium, and Genetic and Environmental Risk in AD consortium) [29].

2.2. DNA extraction and genotyping

2.2.1. Quebec Founder Population

DNA from brain tissues was extracted using the DNeasy Tissue Kit (Qiagen, Hilden, Germany), whereas DNA from blood lymphocytes was extracted using automated DNA extraction (NA-1000; AutoGen, Holliston, MA, USA). Genotyping was performed using the Illumina HumanHap 550k Beadchip (Illumina, San Diego, CA, USA).

2.2.2. Alzheimer's Disease Neuroimaging Initiative

Genotyping information from the ADNI cohort was obtained via the Human 610-Quad Bead Chip (Illumina), as

well as the non-CLIA whole genome sequencing (Illumina). Information about the specific GWAS protocol used to obtain genotyping information can be found in a recent report by Shen et al [30].

2.2.3. Brain eQTL Almanac

Genomic DNA was extracted from subdissected samples of human postmortem brain tissue using Qiagen's DNeasy Blood and Tissue Kit (Qiagen). All samples were genotyped on the Illumina Infinium Omni1-Quad BeadChip (Illumina) and on the Immunochip, a custom genotyping array designed for the fine mapping of autoimmune disorders [31]. After standard quality controls, imputation was performed using MaCH [32,33] 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.

2.3. Neuropathological correlates

2.3.1. Densities of NFTs in the QFP

The neuropathological staining protocol was performed as previously described [34] and was consistent with the classification of Khachaturian [24].

2.3.2. Cerebrospinal fluid Aβ42, total tau, and phospho-tau levels in ADNI

Aliquots of all ADNI GO plus ADNI 2 cerebrospinal fluid (CSF) samples were analyzed using the xMAP Luminex platform (Austin, TX, USA) and Innogenetics/Fujirebio AlzBio3 immunoassay kits (Ghent, Belgium). In addition, 28 newly thawed randomly selected replicate aliquots were tested [35]. For additional information, visit http://adni.loni.usc.edu/methods/biomarker-analysis.

2.3.3. Magnetic resonance imaging analysis of hippocampal volumes in ADNI

Semiautomated hippocampal volumetry assessment was carried out using the commercially available high-dimensional brain mapping tool Surgical Navigation Technologies (Medtronic, Dublin, Republic of Ireland). For additional information, visit http://adni.loni.usc.edu/methods/mri-analysis.

2.4. Gene expression

2.4.1. Quebec Founder Population

Total RNA was extracted from the frontal cortex, temporal cortex, or cerebellum tissues using either one of this device: QIAsymphony (Qiagen) or Maxwell 16 (Promega, Madison, WI, USA). Purity of RNA was estimated using the absorbance values at 260 and 280 nm. To generate complementary DNA, RNA samples were reverse-transcribed with SuperScript VILO Master Mix (Thermo Fisher, Waltham, MA, USA). The TaqMan primers were purchased from Thermo Fisher: Hs01001427_m1 for CDK5RAP2 and 4326321E for HPRT1. Using BestKeeper and NormFinder, HPRT1 was chosen as the best housekeeping gene in the brain for AD among five other genes: peptidylprolyl isomerase A (*PPIA*), ubiquitin-conjugating enzyme E2 D2 (*UBE2D2*), cyclin-dependent kinase inhibitor 1B (*CDNK1B*), actin β (*ACTB*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). All quantitative polymerase chain reaction assays displayed efficiencies ranging from 92% to 109%. Data were analyzed using the ExpressionSuite software (Thermo Fisher).

2.4.2. Alzheimer's Disease Neuroimaging Initiative

Gene expression profiling from blood samples of ADNI participants was contributed by Bristol-Myers Squibb (BMS, New York, NY, USA) and performed at the BMS laboratories for 811 ADNI participants belonging to the whole genome sequencing cohort. The Affymetrix Human Genome U219 Array (Affymetrix, Santa Clara, CA, USA) was used for expression profiling. Additional details can be found on the ADNI website.

2.4.3. Brain eQTL Almanac

Total RNA was isolated from human postmortem brain tissues based on the single-step method of RNA isolation [36] using the miRNeasy 96 kit (Qiagen). The quality of total RNA was evaluated by the 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and RNA 6000 Nano Kit (Agilent) before processing with the Ambion WT Expression Kit (Thermo Fisher) and Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay (Affymetrix) and hybridization to the Affymetrix Exon 1.0 ST Arrays (Affymetrix) following the manufacturers' protocols. Further details regarding RNA isolation, quality control, and processing are reported in the study by Trabzuni et al. [28].

2.5. CDK5RAP2 protein expression in QFP

Brain samples (frontal cortex, temporal cortex, and cerebellum) were homogenized mechanically with the Bead Ruptor 24 (Omni International, Kennesaw, GA, USA) in ice-cold phosphate-buffered saline with protease inhibitors. The Enzyme-Linked Immunosorbent Assay protocols were performed on fresh samples according to the manufacturer's instructions (Cusabio, College Park, MD, USA).

2.6. Statistical methods

The rs10984186 genotypes were contrasted in the ADNI and QFP cohorts using the sex-adjusted logistic regression test in PLINK v1.09. To assess risk level for the rs4837766 polymorphism, a χ^2 for the AA genotype was performed in the ADNI cohort for both genders. Moreover, analysis of the effect of rs4837766 genotypes

on the conversion to MCI/AD was performed using a Mantel-Cox analysis in SPSS (SPSS, version 19; IBM, Armonk, NY, USA). In all the analyses, extreme outliers for each diagnosis group (defined as below the first quartile value − 3 interquartile ranges or above third quarter value + 3 interquartile ranges) were removed. Shapiro-Wilk tests were performed for each analysis to measure the deviation of the data from the normal distribution; and when the P value was below 10^{-5}, nonparametric tests were used.

3. Results

3.1. Identification of a disease risk variant in the CDK5RAP2 locus

The GWAS analysis of the QFP isolate identified a list of genome-wide significant variants located in the well-known *APOE* locus ($49 > -\log[P \text{ values}] > 9$), as well as the novel *CDK5RAP2* rs10984186 A polymorphism (odds ratio = 1.426, $P = 4.23 \times 10^{-7}$, minor allele frequency [CTL/AD] = 0.2581/0.3328; Table 1). Analysis of NFT density in the QFP cohort revealed a significant association with the rs10984186 A risk allele (r[141] = 0.208, $P = .016$, when corrected for age, gender, and diagnosis [CTL/AD]; Table 1). In the ADNI cohort, a significant association was observed between the rs10984186 A allele and disease risk (odds ratio = 1.346, $P = .0462$, minor allele frequency [CTL/AD] = 0.2154/0.2706; Table 1), whereas structural magnetic resonance imaging revealed the reduced hippocampal volume for this allele (r[541] = −0.108, $P = .013$, when corrected for age, gender, and diagnosis [CTL/MCI/AD]; Table 1). However, in the IGAP meta-analysis, the rs10984186 A variant defined by imputation is not significantly associated with disease ($P = .2249$; Table 1). In a Manhattan plot of the QFP GWAS results for the genomic region surrounding the rs10984186 variant (Fig. 1), we clearly observed that the association with AD is restricted to this variant, with little or no involvement of nearby variants in linkage disequilibrium.

3.2. Identification of the cis-eQTL for the rs10984186 A variant in the BRAINEAC cohort

In the Brain eQTL Almanac (BRAINEAC) cohort, where all postmortem human brains are free of neurodegenerative disorders, for the region delimited in Fig. 1, the best eQTL association for the rs10984186 A variant in the hippocampus is with the *CDK5RAP2* gene. Indeed, the rs10984186 A allele is associated with an increased hippocampal *CDK5RAP2* mRNA prevalence (r[122] = 0.243, $P = .008$, when corrected for age, gender, and postmortem interval; Fig. 2).

3.3. Brain CDK5RAP2 gene and protein expression in the QFP cohort

When using age, gender, and postmortem interval as covariates, CDK5RAP2 mRNA prevalence is significantly increased in AD patients compared with CTL subjects in the frontal cortex [F (1, 127) = 7.824, $P = .006$; Fig. 3A], the temporal cortex [F (1, 98) = 48.191, $P = 4.2 \times 10^{-10}$; Fig. 3A], and the cerebellum [F (1, 100) = 17.198, $P = 7.1 \times 10^{-5}$; Fig. 3A]. Moreover, when controlled for age, gender, and postmortem interval, higher CDK5RAP2 protein concentrations were observed in AD versus CTL subjects in the temporal cortex [F (1, 35) = 13.750, $P = 7.2 \times 10^{-4}$; Fig. 3B], but not in the cerebellum [F (1, 10) = 0.957, $P = .351$; Fig. 3B] or the frontal cortex [F (1, 34) = 0.008, $P = .929$; Fig. 3B]. Furthermore, a significant association was observed in the frontal cortex between NFT density and *CDK5RAP2* gene expression ($r_{spearman}$[75] = 0.266, $P = .021$; Fig. 4).

Table 1
Associations between the rs10984186 A variant, AD diagnosis and AD-related neuropathologies

Cohorts										
Quebec Founder Population					ADNI					IGAP
Disease			NFT density		Disease			Hippocampal volume		Disease
P value	Odds ratio	MAF (CTL/AD)	P value	r	P value	Odds ratio	MAF (CTL/AD)	P value	r	P value
4.23×10^{-7}	1.426	0.2581/0.3328	.016	0.208	.0462	1.346	0.2154/0.2706	.013	−0.108	.2249

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; CA1, cornu ammonis area 1; CTL, control; IGAP, International Genomics of Alzheimer's Project; MAF, minor allele frequency; MCI, mild cognitive impairment; NFT, neurofibrillary tangles; QFP, Quebec Founder Population.

NOTE. The association with AD in the QFP was performed on 977 CTLs and 966 AD subjects. NFT density in the QFP represents the sum of tangles in six brain regions: the CA1 region of the hippocampus, the subiculum, the parasubiculum, the fusiform gyrus, the frontal cortex, and the parietal cortex. The association with this total density (per cubic millimeter) was measured on 18 CTLs and 123 AD subjects and was corrected for age, gender, and AD diagnosis (CTL/AD). The association with AD in the ADNI cohort was assessed on 195 CTLs and 340 AD subjects. The association with AD in IGAP was obtained from summary results. The association with the hippocampal volume (in cubic millimeter) in ADNI was assessed on 159 CTLs and 251 MCI and 131 AD subjects and was corrected for age, gender, and AD diagnosis (CTL/MCI/AD).

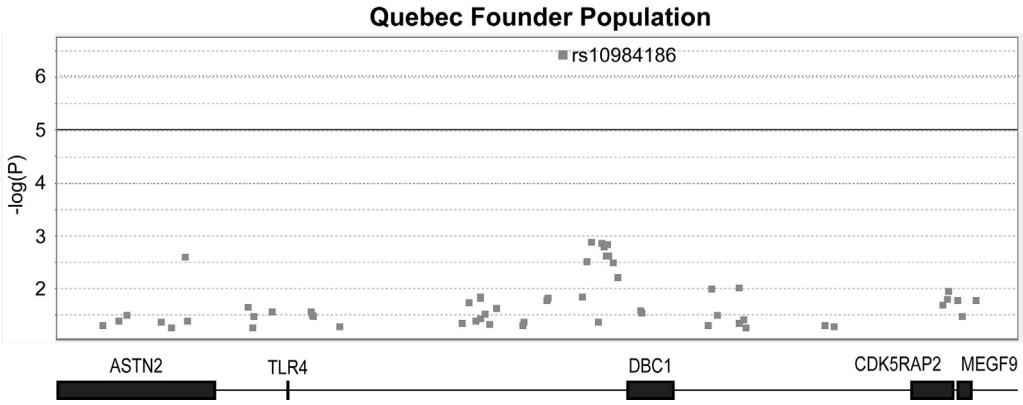


Fig. 1. Manhattan plot representing the region surrounding the rs10984186 polymorphism in the case-control study performed in the QFP. Abbreviations: ASTN2, astrotactin 2; CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit–associated protein 2; DBC1, deleted in bladder cancer protein 1; MEGF9, multiple epidermal growth factor like domains 9; QFP, Quebec Founder Population; TLR4, toll-like receptor 4.

3.4. Disease risk, conversion, and gene expression in carriers of the CDK5RAP2 rs4837766 A variant

In the BRAINEAC cohort, the rs4837766 A variant is among the top variants located in the *CDK5RAP2* gene that are in cis-eQTL association with this gene. When using age, gender and post-mortem interval as covariates, this variant is associated with reduced total CDK5RAP2 mRNA prevalence (r[123] = −0.376, $P = 2.3 \times 10^{-5}$; Fig. 5A). In cognitively intact subjects from the ADNI cohort, the rs4837766 A variant is correlated with a reduction of the baseline CSF phospho-tau (pTau)/Aβ ratio ($r_{Spearman}$[106] = −0.193, $P = .048$; Fig. 5B). Furthermore, stratification for the gender and for the *CDK5RAP2* rs4837766 variant in the ADNI cohort revealed a significant protective effect for female AA carriers characterized by a significant decrease in the risk of developing MCI or AD (χ^2 [1, N = 362] = 6.703, odds ratio = 0.389 [0.186–0.811], $P = .010$; Fig. 5C), as well as a delayed conversion

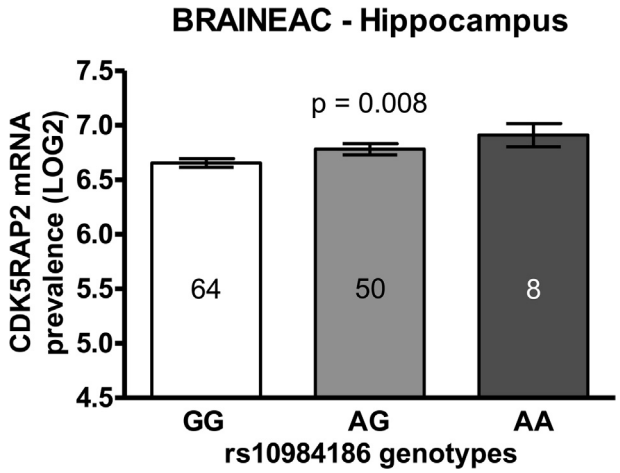


Fig. 2. CDK5RAP2 mRNA prevalence as a function of rs10984186 genotype in the hippocampus of BRAINEAC subjects. This allele-dose association was corrected for age, gender, and postmortem interval. Each bar represents a mean $\log_2$ expression value ± SEM. The number of subjects is indicated on each bar. Probe: 3223531. Abbreviations: BRAINEAC, Brain Expression Quantitative Trait Loci Almanac; CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit–associated protein 2; SEM, standard error of the mean.

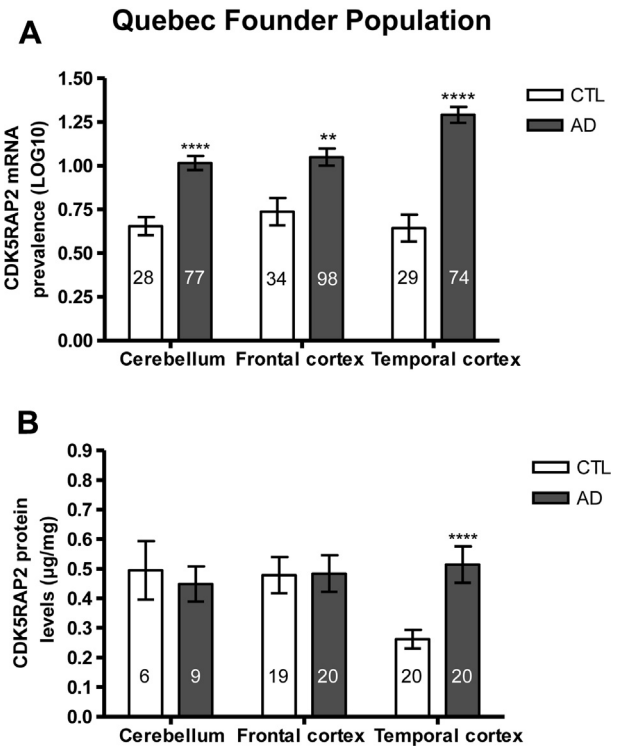


Fig. 3. (A) CDK5RAP2 mRNA prevalence in different brain regions from QFP subjects. Each bar represents a mean regional $\log_{10}$RQ + 1 value ± SEM. (B) CDK5RAP2 protein levels in different brain regions from QFP subjects. Each bar represents a mean CDK5RAP2/total protein level ratio ± SEM. The number of subjects is indicated on each bar. The associations for both gene and protein expression were corrected for age, gender, and post-mortem interval. $^{**}P \leq .01$; $^{****}P \leq .001$. Abbreviations: AD, Alzheimer's disease; CTL, control; CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit–associated protein 2; QFP, Quebec Founder Population; RQ, relative quantification; SEM, standard error of the mean.

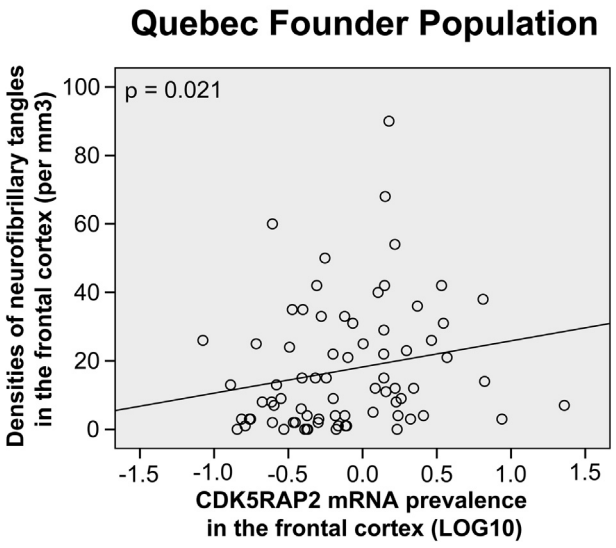


Fig. 4. Neurofibrillary tangle densities as a function of CDK5RAP2 mRNA prevalence in the frontal cortex of QFP control and AD subjects. Abbreviations: CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit–associated protein 2; QFP, Quebec Founder Population; RQ, relative quantification.

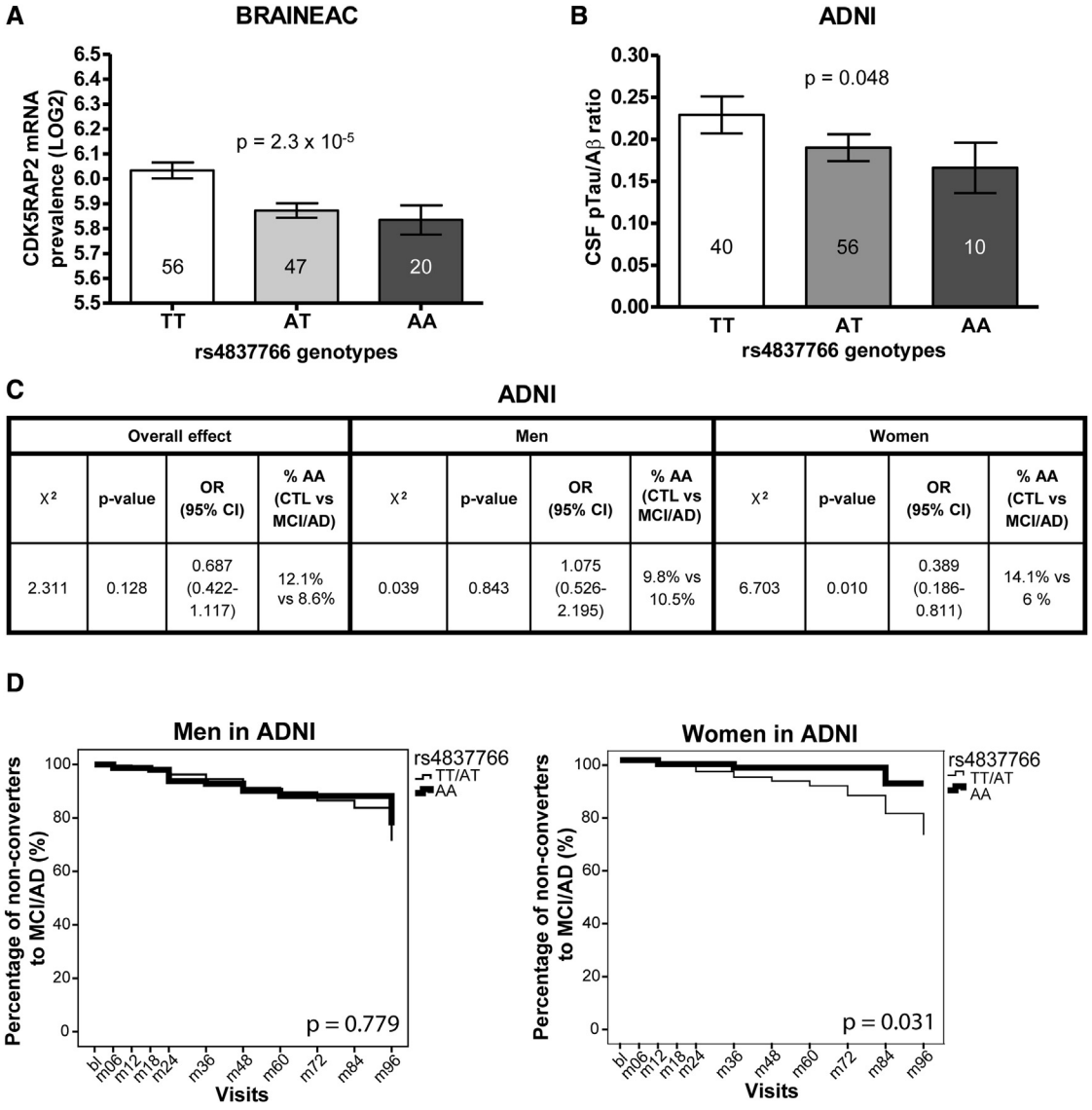
(−69%) of CTL and MCI subjects to MCI/AD some 8 years after initial visit (χ^2 [1, N visits = 2043] = 4.627, $P = .031$; Fig. 5D).

4. Discussion

In AD subjects from the QFP cohort, CDK5RAP2 exhibits a significantly higher gene expression in the frontal cortex, the temporal cortex, and the cerebellum, as well as higher temporal cortical protein levels (Fig. 3). Microarray data from Hokama et al. [37] revealed a similar increase in CDK5RAP2 mRNA levels in both the frontal and temporal cortices of pathologically confirmed AD cases (data not shown). In the QFP cohort, CDK5RAP2 mRNA prevalence in the frontal cortex is quantitatively associated with the NFT density in the same region (Fig. 4), a well-established correlate of memory impairment in symptomatic AD subjects [6]. The relationship between NFTs and *CDK5RAP2* gene expression appears to be driven, at least in part, by the rs10984186 minor (A) allele. Indeed, this variant is significantly associated with disease risk in both the QFP and ADNI cohorts and correlates with NFT burden in the QFP brains (Table 1). This risk variant is also associated with an increased hippocampal *CDK5RAP2* gene expression in healthy subjects from the BRAINEAC cohort (Fig. 2), although not in cortical and cerebellar regions (data not shown), suggesting an enhanced pathophysiological risk in this vulnerable brain area. In ADNI, where the vast majority of AD cases are mild-to-moderate, the rs10984186 risk variant does not affect MCI or AD conversion rate (data not shown) nor baseline CSF pTau levels or pTau/Aβ_{42} ratios (all disease states, data not shown), consistent with a biological process unfolding later in the disease process, most likely when the tangle pathology spreads throughout the brain. We therefore hypothesize that biomarkers of tangles accumulation, such as retention of the ^{18}F-AV-1451 PET ligand, would be significantly affected by the presence of the rs10984186 risk variant in symptomatic carriers. Indeed, this marker is associated with neurodegeneration and cognitive decline in all disease states [38], as opposed to CSF pTau/Aβ_{42} ratios, which are mostly predictors of cognitive decline and conversion to MCI/AD in cognitively intact subjects [39]. The association with disease risk in the QFP was not replicated in the IGAP meta-analysis (Table 1). While a population-specific effect could be invoked to explain the discrepancy with IGAP, the independent replication in the multiethnic ADNI cohort suggests otherwise (Table 1). Alternatively, the fact that the rs10984186 A variant is present on the Illumina beadchips used in the ADNI and QFP analyses but not on most of those in IGAP raises the issue of imputation quality, which is not available in the IGAP public database.

In contrast to the rs10984186 risk variant, the rs4837766 AA genotype, which is located in the *CDK5RAP2* gene, is associated with a decreased risk of developing MCI/AD in women enrolled in the ADNI cohort (Fig. 5C). Stratification for the gender and for the rs4837766 variant in a large sample of CTL and MCI subjects from ADNI revealed a significant protective effect in female AA carriers on MCI/AD conversion some 8 years after initial visit (Fig. 5D). A similar striking reduction in the conversion rate in women was recently described in a multiple-cohort analysis for the protective rs3846662 variant, which is located in the HMG-CoA reductase (*HMGCR*) gene, another variant shown to affect NFT density in multiple brain areas in women [40]. *CDK5RAP2* rs4837766 A variant is significantly associated with reduced levels of CDK5RAP2 mRNA levels (Fig. 5A) in the brain in the BRAINEAC cohort and in circulating lymphocytes in ADNI subjects (Supplementary Fig. 1). Additional stratification by gender in ADNI indicated that the association is significant only in women (Supplementary Fig. 2), which is consistent with the fact that only female AA carriers are protected against AD (Fig. 5C and 5D). In CTL subjects from the ADNI cohort, the rs4837766 A variant is associated with decreased baseline CSF pTau/Aβ_{42} ratios (Fig. 5B). As mentioned previously, pTau/Aβ_{42} ratios are typically used as surrogate markers of cognitive decline and conversion to MCI/AD in cognitively intact healthy subjects. Therefore, CTL subjects bearing one or two copies of the rs4837766 A allele are producing more phosphorylated tau and are more prone to convert to MCI/AD, which is consistent with the previous observations on MCI/AD conversion and risk in women.

In conclusion, we believe that the aforementioned results are consistent with the notion that modulation of *CDK5RAP2* gene expression by the rs10984186 risk variant or the rs4837766 protective polymorphism may regulate the extent of AD pathology by respectively increasing or



Overall effect				Men				Women			
X^2	p-value	OR (95% CI)	% AA (CTL vs MCI/AD)	X^2	p-value	OR (95% CI)	% AA (CTL vs MCI/AD)	X^2	p-value	OR (95% CI)	% AA (CTL vs MCI/AD)
2.311	0.128	0.687 (0.422-1.117)	12.1% vs 8.6%	0.039	0.843	1.075 (0.526-2.195)	9.8% vs 10.5%	6.703	0.010	0.389 (0.186-0.811)	14.1% vs 6 %

Fig. 5. Modulation of MCI/AD risk, conversion to MCI/AD, and *CDK5RAP2* gene expression by the rs4837766 polymorphism. (A) CDK5RAP2 mRNA prevalence as a function of rs4837766 genotypes in the BRAINEAC cohort. Each bar represents a mean $\log_2$ expression value ± SEM. The number of subjects is indicated on each bar. This allele-dose association was corrected for age, gender, and postmortem interval. Probe: t3223425. (B) CSF pTau/Aβ ratios as a function of rs4837766 genotypes in CTLs from the ADNI cohort. Each bar represents a mean CSF pTau/Aβ value ± SEM. The number of subjects is indicated on each bar. (C) Association of the rs4837766 polymorphism with the risk of developing AD in men, women, and the whole population. Men: 400 TT/AT (101 CTL vs. 299 MCI/AD) and 46 AA (11 CTL vs. 35 MCI/AD). Women: 330 TT/AT (110 CTL vs. 220 MCI/AD) and 32 AA (18 CTL vs. 14 MCI/AD). (D) Association of the rs4837766 polymorphism with the conversion to MCI/AD was plotted with Kaplan-Meier curves. Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; bl, baseline; BRAINEAC, Brain Expression Quantitative Trait Loci Almanac; CDK5RAP2, cyclin-dependent kinase 5 regulatory subunit–associated protein 2; CI, confidence interval; CTL, control; CSF, cerebrospinal fluid; m, month; MCI, mild cognitive impairment; OR, odds ratio; pTau, phospho-tau; SEM, standard error of the mean.

decreasing CDK5 activity through the calpain-dependent cleavage of p35. This cleavage leads to an increased production of p25 [41], which has a 5- to 10-fold increased half-life compared with p35. Therefore, p25 overproduction leads to CDK5 overactivation, increases tau phosphorylation, and may facilitate the subsequent accumulation of NFTs [11]. This working model is also consistent with the fact that the rs10984186 A risk variant is associated with marked reductions of baseline hippocampal volumes in subjects from ADNI (Table 1). Indeed, recent evidence with the new radiotracer for NFTs (^{18}F-THK5105) has shown that the temporal signal and the neocortical signal are inversely correlated with the hippocampal volume in living AD patients [42]. The ladder observation is thus quite consistent with the postmortem findings reported previously.

Another potential mechanism at play could be the role of CDK5RAP2 in the cell cycle activation of postmitotic neurons, which normally leads to cell death [43]. In fact,

CDK5RAP2 is required for the attachment of the γ-tubulin ring complex to the centrosomes, therefore contributing to the microtubules nucleation [44]. It also acts in the recruitment of the end binding 1 protein, a facilitator of microtubules polymerization [45,46]. Thus, because microtubules form the mitotic spindle [47], CDK5RAP2 is important in the cell-cycle spindle checkpoint [48].

Altogether, these observations show that both the rs10984186 risk and rs4837766 protective polymorphic variants of the *CDK5RAP2* gene might act as potent genetic modifiers for AD risk and/or conversion by modulating the expression of this gene, particularly for women in the case of the rs4837766 polymorphism. It is conceivable that *CDK5RAP2* gene expression in turn regulates the phosphorylation of tau and, in the symptomatic phase of the disease, modulates the accumulation of NFTs. To our knowledge, these two variants represent the first genetic modulators of the tau pathophysiology to be implicated in AD. Genotyping of these polymorphisms should therefore be performed routinely in the imaging of NFTs, as well as in the clinical trials designed to target the phosphorylation of tau or the accumulation of NFTs.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2017.12.004.

RESEARCH IN CONTEXT

1. Systematic review: Because currently known Alzheimer's disease (AD) single-nucleotide polymorphisms represent only a small fraction of the genetic variance in this disease, our team performed a genome-wide association study on subjects from the Quebec Founder Population isolate to identify new loci associated with the late-onset sporadic AD.
2. Interpretation: The rs10984186 variant is associated with an increased risk of developing AD and a higher CDK5RAP2 mRNA prevalence in the hippocampus. On the other hand, the rs4837766 variant, which is among the best cis-expression quantitative trait loci in the *CDK5RAP2* gene, was found to confer, in women from Alzheimer's Disease Neuroimaging Initiative, decreased mild cognitive impairment/AD risk and conversion rate.
3. Future directions: In this study, two variants located in the *CDK5RAP2* locus were identified, to our knowledge, as the first genetic modulators of the tau pathophysiology to be implicated in AD. Therefore, these variants could be used in the future as novel targets and diagnostic tools for the treatment of AD.

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