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FEATURED ARTICLE

# Characterization of the contactin 5 protein and its risk-associated polymorphic variant throughout the Alzheimer's disease spectrum

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Compliance with ethical standards

Data used in preparation of this article were obtained from the program of PRe-symptomatic EValuation of Novel or Experimental Treatments for Alzheimer's Disease (PREVENT-AD) at the Centre for Studies on Prevention of Alzheimer's Disease (StoP-AD), Douglas Mental Health University Institute Research Center (http://douglas.research.mcgill.ca/stop-ad-centre). A complete listing for the PREVENT-AD Research Group can be found at:https://preventad.loris.ca/acknowledgements/acknowledgements.php?date = 2022-02-21.

## Abstract

**Introduction:** We investigate the *CNTN5* rs1461684 G variant and the contactin 5 protein in sporadic Alzheimer's disease (sAD).

**Methods:** Contactin 5, sAD biomarkers, and synaptic markers were measured in the cerebrospinal fluid (CSF). Amyloid and tau deposition were assessed using positron emission tomography. Contactin 5 protein and mRNA levels were measured in brain tissue.

**Results:** CSF contactin 5 increases progressively in cognitively unimpaired individuals and is decreased in mild cognitive impairment and sAD. CSF contactin 5 correlates with sAD biomarkers and with synaptic markers. The rs1461684 G variant associates with faster disease progression in cognitively unimpaired subjects. Cortical full-length and isoform 3 *CNTN5* mRNAs are decreased in the presence of the G allele and as a function of Consortium to Establish a Registry for Alzheimer's Disease stages.

**Discussion:** The newly identified rs1461684 G variant associates with sAD risk, rate of disease progression, and gene expression. Contactin 5 protein and mRNA are affected particularly in the early stages of the disease

KEYWORDS
Alzheimer's disease, cerebrospinal fluid, cognition, contactin 5, positron emission tomography, scans, synaptic markers, tau pathology

## 1 | INTRODUCTION

Alzheimer's disease (AD) is one of the most important causes of cognitive decline in the elderly population.[1,2] Pathologically, AD is characterized by progressive accumulation of amyloid plaques and neurofibrillary tangles, and by synaptic and neuronal degeneration and loss.[3,4] Several genetic variants have already been identified as common risk factors for sporadic AD (sAD), with the most important one being the ε4 allele of the apolipoprotein E (*APOE*) gene.[5,6] However, it is estimated that the single nucleotide polymorphism (SNP)–based heritability of sAD ranges from 13% to 33%, indicating that a significant part of the risk alleles remains to be identified.[5,7]

The *CNTN5* gene encodes the contactin 5 protein, which is a cell-surface protein with multiple isoforms that belongs to the contactin family. Contactins are a family of cell-adhesion molecules that contain six members: contactin 1 to contactin 6. These proteins play an important role in neurodevelopment through the regulation of neurite outgrowth, neuronal migration, axon guidance, synaptogenesis, myelin formation, neuron—glia interactions, and cell survival.[8,9]

Single nucleotide polymorphisms and copy number variants in the *CNTN5* gene have been associated with increased risk for several neuropsychiatric disorders such as autism spectrum disorder,[10,11] anorexia,[12] and attention deficit hyperactivity disorder.[13] In sAD, a risk-associated polymorphism in the *CNTN5* gene (rs10501927) was reported by Harolds et al.[14] but it failed to reach genome-wide significance (odds ratio [OR]: 1.18; $P = 2.0 \times 10^{-6}$, $n = 11789$). Using a unique population isolate from eastern Canada, we report here the presence of a distinct risk variant (rs1461684 G) in the *CNTN5* gene, which is associated with increased risk for sAD in this cohort, as well as in other heterogenous genetic studies.

In the present work, our objective is to characterize the role of contactin 5 (soluble in the cerebrospinal fluid [CSF] and membrane-bound to neurons in the frontal cortex) and its association with different pathological biomarkers of sAD in both the asymptomatic and symptomatic stages of the disease. In parallel, we examined the newly identified rs1461684 risk variant G throughout the spectrum of sAD. More specifically, we investigate the role of this common polymorphism (minor allele frequency [MAF]: 0.16) in the risk of developing sAD and its effect in the clinical and pathological progression of the disease using four different cohorts.

## 2 | METHODS

This study used four different patient cohorts. All of them received local approval from the research ethics committee or institutional review boards of the participating centers.

### 2.1 | PREVENT-AD

#### 2.1.1 | Study participants

The Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) cohort is composed of cognitively unimpaired individuals over the age of 55, who have a first degree relative diagnosed with sAD.[15] There are 373 active participants (or subset of participants) who are followed annually with clinical and cognitive assessments, blood and CSF biomarkers, structural and functional magnetic resonance imaging (MRI) and brain positron emission tomography (PET) scans to assess amyloid beta (Aß) and tau deposition. Data used in this article were obtained from data release 6.0 (2020, https://openpreventad.loris.ca/).

#### 2.1.2 | CSF

Lumbar punctures were performed in a subset of volunteers ($n = 170$) in the morning after an overnight fast using a Sprotte 24-gauge atraumatic needle. CSF samples were centrifuged within 4 hours, cells and insoluble material were excluded, and samples were aliquoted and stored at −80°C. The AD biomarkers phosphorylated tau (p-tau)181, total tau (t-tau), and $Aß_{42}$ were measured following procedures developed by the BIOMARKAPD consortium,[16] using validated Innotest enzyme-linked immunosorbent assay kits (P(181)-tau Cat.# 81581, T-tau Cat.# 81579, and Aβ42 Cat.# 81583) from Fujirebio. CSF contactin 5 levels were measured using Olink's proximity extension assay and the neurology panel. Synaptic markers were quantified in the CSF using immunoprecipitation followed by mass spectroscopy as described previously.[17–20]

#### 2.1.3 | Neuroimaging acquisition and processing

18F-NAV4694 (Navidea Biopharmaceuticals) was used to quantify Aß accumulation. Scans were performed 40 to 70 minutes after injection in a subgroup of PREVENT-AD subjects ($n = 129$). Flortaucipir ([18F]AV1451) was used to measure tau deposition and scans were acquired 80 to 100 minutes post injection as described previously.[21] Standardized uptake value ratios (SUVRs) were obtained using the cerebellum as a reference region for Aß-PET and the inferior cerebellum gray matter for flortaucipir.[21] AD-related tau deposition was assessed by averaging flortaucipir SUVR in the entorhinal cortex, fusiform, parahippocampal, and lingual gyri.[21,22]

#### 2.1.4 | Alzheimer Progression Score

The composite Alzheimer Progression Score (APS) was developed by our team to map the progression of the disease in the absence of visible cognitive deficits in the PREVENT-AD cohort, for the purpose of prevention drug trials.[23] The APS is a composite that incorporates multimodal imaging, neurosensory, cognitive, and CSF markers, based on an assumption that change in each of these arises from a single underlying latent process (i.e., AD pathogenesis). Its scores are scaled as a standard normal distribution, with higher scores denoting increasing severity. Constituent measures are summarized in greater detail in Leoutsakos et al.[24]

#### 2.1.5 | Genotyping

Automated DNA extraction from buffy coat samples was performed using the QIAsymphony DNA mini kit (Qiagen). Genotyping was performed using the Omni2.5-8 BeadChip (Illumina). PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) was used to filter sex mismatches, filter missingness at sample level (< 5%) and SNP level (< 5%), assess sample heterozygosity, and filter SNPs in Hardy–Weinberg disequilibrium ($P > 0.001$). Only post-imputed SNPs with an info score > 0.7 were kept.

### 2.2 | COMPASS-ND cohort

#### 2.2.1 | Study participants

The Comprehensive Assessment of Neurodegeneration and Dementia (COMPASS-ND) study is enrolling 1650 memory-impaired/concerned subjects from 31 centers across Canada. Participants typically undergo comprehensive baseline evaluation including clinical and neuropsychological assessment, biospecimen collection, polymorphisms mapping, and MRI neuroimaging.[25] Data are made available to investigators in the Canadian Consortium on Neurodegeneration in Aging (CCNA) as well as others through the Longitudinal Online Research and Imaging System (LORIS) database at https://ccna-ccnv.ca/national-platforms/. CSF collection and measurements are performed as described above for the PREVENT-AD cohort. No genotype information is available for this cohort at the moment.

### 2.3 | ROS-MAP

The Religious Orders Study (ROS) was established in 1994 and it includes nuns, priests, and brothers from across the United States.[26] The Rush Memory and Aging Project (MAP) started in 1997 and includes lay people from the state of Illinois. Participants from the cohorts were cognitively normal at enrolment and were followed annually with neuropsychological evaluation and blood test and consented to genotyping and brain donation.[26] *Post mortem* evaluation was performed to assess AD pathology using Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and Braak staging. ROS-MAP datasets and protocols used in the present study are summarized in the supporting information 1.

**RESEARCH IN CONTEXT**

1. **Systematic review**: Our team identified the genetic variant rs1461684 G in the *CNTN5* gene, which is associated with increased risk for sporadic Alzheimer's disease (sAD). Previous reports found that another variant, rs10501927, is associated with structural brain changes in the absence of contactin 5 protein alterations in sAD patients.
2. **Interpretation**: The newly identified genetic variant increases the risk of sAD and appears to play a prominent role in the pre-symptomatic stage of the disease. The characterization of new genetic risk factors can help identify new disease pathways and better diagnostic and therapeutic targets.
3. **Future directions**: The rs1461684 G variant has a minor allele frequency of 0.16, so it would be important to replicate our findings in populations with different allele frequencies. Molecular studies are needed to clarify the role of the *CNTN5* gene and the contactin 5 protein in sAD pathology, especially in the very early stages of the disease when pathology begins to unfold.

#### 2.3.1 | Tandem mass tag proteomic data

Three hundred forty cortical prefrontal brain tissue samples from the community-based aging ROS-MAP cohort were analyzed by a mass spectrometry-based protein quantification approach, using isobaric multiplex tandem mass tags (TMT) as described previously by Ping et al.[27] Briefly, TMT labeling with synchronous precursor selection (SPS)-MS3 for reporter ion quantitation was used to achieve comprehensive global quantitation of 100 mg (wet tissue weight) pre-frontal cortex from healthy controls and sAD cases. In total, 127,321 total unique peptides were identified from >1.5 million peptide spectral matches (PSMs), which mapped to 11,840 unique proteins groups, representing 10,230 gene symbols, which map to ≈65% of the protein coding genes in the brain. Two major isoforms of *CNTN5* expressed in the brain are available in the ROS-MAP dataset: the full length (O94779) wild type variant and the isoform type 3 (O94779-4, missing amino acids 912–1100), which are particularly prevalent in the central nervous system (CNS).

### 2.4 | The QFP cohort

The Quebec Founder Population (QFP) cohort is a population isolate from eastern Canada that descends from 3000 French settlers that founded Nouvelle France in the 17th and 18th centuries.[28] The migration and the isolated nature of settlements created a founder

effect, which resulted in a population with less genetic heterogeneity, large linkage disequilibrium blocks, and low genetic noise, which is highly advantageous for genetic studies,[28,29] especially for genome-wide association study (GWAS) case/control studies in which age, sex, and especially place of birth are used to control the sample's demographic characteristics. Genealogical information for this population, for almost four centuries, is available in the BALSAC database.[30]

### 2.5 | Statistical analyses

Analyses of the demographics were done using analysis of variance (ANOVA) and the significant main effects were decomposed using Tukey's post hoc test. The progression of CSF contactin 5 levels over time was tested using mixed linear model adjusted for sex, age, and *APOE* ε4. The correlation of CSF or plasma contactin 5 with CSF biomarkers, synaptic proteins, and amyloid and tau PET in the PREVENT-AD cohort was assessed using linear regression adjusted for sex, age, and *APOE* ε4. For this analysis we used only data acquired at baseline visit. The effect of the *CNTN5* variant on NAV4694 and flortaucipir SUVR retention in the PREVENT-AD cohort was assessed using linear regression adjusted for sex, age, and *APOE* ε4. ANOVA was used to assess the difference in APS scores and CSF contactin 5 according to *CNTN5* genotype, the difference in CSF contactin 5 according to clinical diagnosis (cognitively unaffected, mild cognitive impairment [MCI], and AD), the levels of contactin 5 isoforms in the prefrontal cortex according to Braak stages and the difference in *CNTN5* mRNA and proteins levels in asymptomatic and MCI subjects according to the number of *CNTN5* rs1461684 G variants; all analyses were adjusted for sex, age, and *APOE* ε4 genotype. Changes in contactin 5 levels in the CSF of controls versus MCI and sAD subjects were decomposed using Tukey's post hoc test. The association between contactin 5 mRNA levels and Braak and CERAD stages in the ROS-MAP cohort was assessed using ordinal logistic regression and adjusted for age, sex, and *APOE* ε4. For this cohort, the exact age is not specified for participants over the age of 90. Because there is a significant number of participants older than 90, age was considered using age groups: < 80, 80 to 84, 85 to 89, > 90.

## 3 | RESULTS

### 3.1 | Demographics

Table 1 summarizes the demographic characteristics of the main cohorts used to analyze the impact of the *CNTN5* variant on CSF protein level (PREVENT-AD) and on cortical protein and mRNA prevalence (ROS-MAP) at different stages of sAD's spectrum. There was no difference in age, sex, education, and *APOE* ε4 status between the *CNTN5* genetic subgroups.

### 3.2 | PREVENT-AD cohort: contactin 5 protein levels in the CSF and plasma

CSF contactin 5 levels were measured in cognitively unimpaired participants from the PREVENT-AD cohort. Longitudinal measures of CSF contactin 5 showed a progressive increase in the levels of this protein over time, with significant alterations at follow-up visits at 12 ($P = 0.025$), 24 ($P = 0.018$), and 48 months ($P = 8 \times 10^{-5}$; Figure 1).

CSF contactin 5 measures were contrasted with sAD pathological biomarkers in the same cognitively unimpaired subjects. A positive correlation was found between CSF contactin 5 and CSF $Aß_{42}$ ($r^2 = 0.26$; $P = 0.00049$), $Aß_{40}$ ($r^2 = 0.13$; $P = 0.002$), t-tau ($r^2 = 0.47$; $P = 1.4 \times 10^{-11}$), and p-tau ($r^2 = 0.51$; $P = 6.6 \times 10^{-12}$; Figure 2). There was a trend toward a positive correlation between CSF contactin 5 and tau deposition in the entorhinal cortex measured by PET ($r^2 = 0.23$; $P = 0.065$) in these asymptomatic subjects.

CSF contactin 5 levels were also positively correlated with synaptic proteins GAP43 ($r^2 = 0.50$; $P = 1.4 \times 10^{-06}$), neurogranin ($r^2 = 0.37$; $P = 0.0004$), Syt1 ($r^2 = 0.43$; $P = 2.7 \times 10^{-05}$), and Snap 25 long ($r^2 = 0.46$; $P = 1.6 \times 10^{-05}$; Figure 2) in cognitively unimpaired subjects from the PREVENT-AD cohort.

No association was found between CSF and plasma levels of contactin 5 protein nor between plasma contactin 5 and CSF sAD biomarkers (not shown).

### 3.3 | Effect of the *CNTN5* rs1461684 risk G variant on clinical progression and on CSF contactin 5 protein levels in the pre-symptomatic PREVENT-AD cohort

Searching for polymorphic variants affecting AD risk level in the QFP, a GWAS was performed in case and control subjects matched for sex, age, and especially place of birth.[31,32] More than two thirds of the GWAS subjects saw their disease status confirmed by a pathologist at autopsy, thus reducing diagnostic uncertainties. Among the top variants found to be associated with AD in the QFP was the minor allele (G) of polymorphism rs1461684 (MAF 0.16), a variant found in intron 1 of the contactin 5 (*CNTN5*) gene. Table 2 summarizes results for the QFP and replication analyses obtained from the International Genomics of Alzheimer's and Alzheimer's Disease Genetics Consortium cohorts. This variant is distinct from the genetic polymorphism reported in the *CNTN5* gene (rs10501927 variant) by Harold et al.'s original UK GWAS.[14] While both *CNTN5* SNPs are in linkage equilibrium (D': 0.21, $r^2$: 0.04, $P = 0.006$), the low $r^2$ and modest D' indicates that the SNPs cannot substitute each other. This is mostly likely due to the low prevalence of the rs10501927 variant found in the QFP.

In the asymptomatic PREVENT-AD cohort, *CNTN5* rs1461684 risk variant G is significantly associated with a much faster rate of progression compared to G-negative subjects as assessed by the APS in the cognitively unimpaired participants (Figure 3A, $P = 0.01$). Figure 3B

**TABLE 1** PREVENT-AD and ROS-MAP cohort demographics

| | PREVENT-AD Cohort | | | | | ROS-MAP Cohort | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *CNTN5* rs1461684 G allele dose | | | | | *CNTN5* rs1461684 G allele dose | | | | |
| | Overall $N = 373$ | 0 $N = 259$ | 1 $N = 103$ | 2 $N = 11$ | *P*-value | Overall $N = 1{,}118$ | 0 $N = 821$ | 1 $N = 269$ | 2 $N = 28$ | *P*-value |
| **Age** | 63.2 ± 5.2 | 63.2 ± 5.2 | 62.9 ± 5.2 | 64 ± 5.3 | **0.77** | 89.0 (84.7–90.0) | 89.0 (84.7–90.0) | 88.9 (84.7–90.0) | 88.3 (83.3–90.0) | **0.3** |
| < 80 | | | | | | 102 (9.1%) | 69 (8.4%) | 30 (11%) | 3 (11%) | |
| 80–84 | | | | | | 196 (18%) | 145 (18%) | 43 (16%) | 8 (29%) | |
| 85–89 | | | | | | 329 (29%) | 240 (29%) | 80 (30%) | 9 (32%) | |
| > 90 | | | | | | 491 (44%) | 367 (45%) | 116 (43%) | 8 (29%) | |
| **Sex** | | | | | **0.64** | | | | | **0.4** |
| Male | 111/373 (30%) | 76/259 (29%) | 33/103 (32%) | 2/11 (18%) | | 377 (34%) | 279 (34%) | 92 (34%) | 6 (21%) | |
| Female | 262/373 (70%) | 183/259 (71%) | 70/103 (68%) | 9/11 (82%) | | 741 (66%) | 542 (66%) | 177 (66%) | 22 (79%) | |
| ***APOE ε4*** | | | | | **0.13** | | | | | **>0.9** |
| *APOE ε4−* | 230/373 (62%) | 168/259 (65%) | 57/103 (55%) | 5/11 (45%) | | 824 (74%) | 603 (74%) | 200 (74%) | 21 (75%) | |
| *APOE ε4+* | 143/373 (38%) | 91/259 (35%) | 46/103 (45%) | 6/11 (55%) | | 293 (26%) | 217 (26%) | 69 (26%) | 7 (25%) | |

Abbreviations: *APOE*, apolipoprotein E; PREVENT-AD, Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease; ROS-MAP, Religious Orders Study Rush Memory and Aging Project.

**TABLE 2** *CNTN5* risk variant in different cohorts

| Cohorts | | | | |
|---|---|---|---|---|
| **GWAS disease association** | **rs1461684/ Chromosome 11:99225748 (GRCh38)** | **Beta/OR** | ***P* values** | **N** |
| **Quebec Founding Population isolate*** | G carriers | .222/1.25 | 0.0001 | 1502 matched for age, sex, and place of birth |
| | GG carriers | 1.18/3.28 | 0.0000002 | |
| **IGAP Stage 1**** | G carriers | 0.0581/1.14 | 0.0025 | 94,497 |
| **IGAP Stage 2**** | G carriers | 0.0487/1.12 | 0.0012 | |
| **ADGC Stage 1***** | G carriers | na | 0.0008 | 15,675 |
| **ADGC Stage 2***** | G carriers | na | 0.0006 | 7096 |

*: Original GWAS description from Hu et al.[32]
**: Derived from Kunkle et al. Note: the IGAP cohort includes a large subset of subjects from France, where the QFP originates.
***: Original GWAS description from Naj et al.
Abbreviations: ADGC, Alzheimer's Disease Genetics Consortium; GWAS, genome-wide association study; IGAP, International Genomics of Alzheimer's; na, not applicable; OR, odds ratio; QFP, Quebec Founder Population.

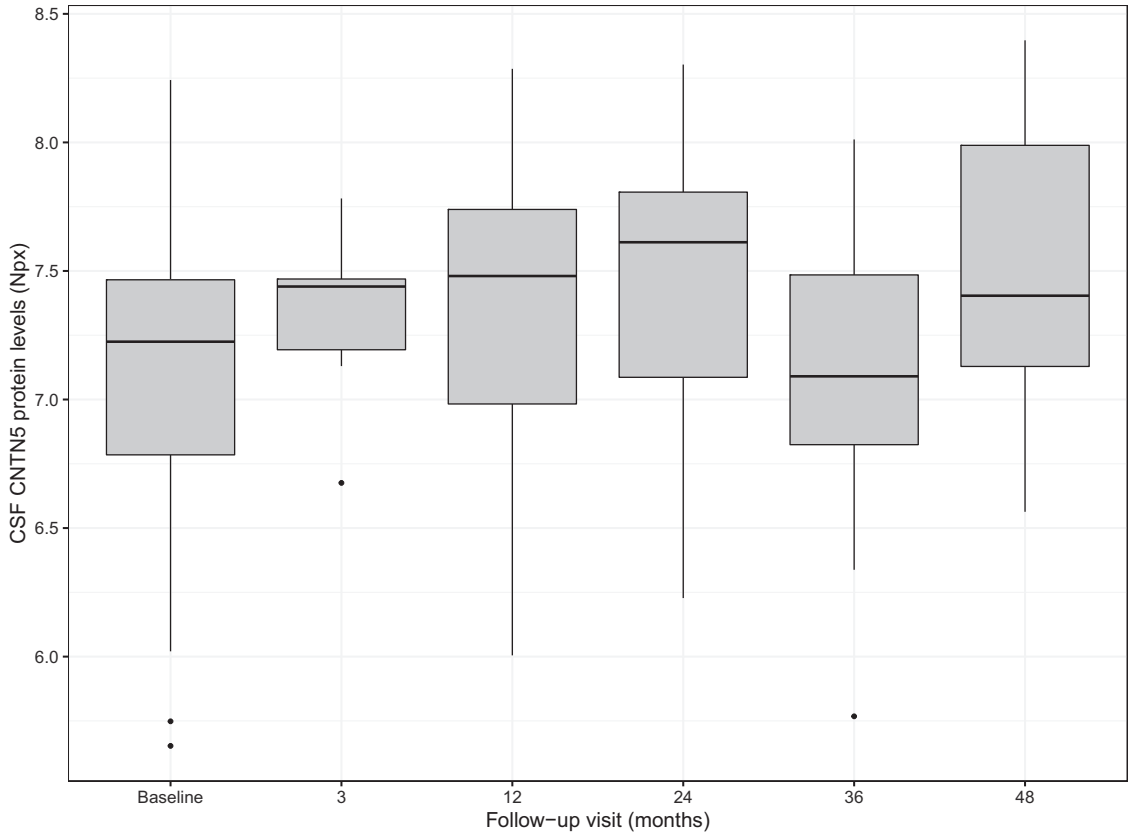



**FIGURE 1** Cerebrospinal fluid (CSF) contactin 5 levels over consecutive assessments in cognitively unimpaired subjects from the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease cohort. Contactin 5 protein was measured in the CSF using Olink's proximity extension assay. Assessments were done at baseline and on follow-up visits. CSF contactin 5 is significantly increased on follow-up visits at 12 ($P = 0.025$, $n = 25$), 24 ($P = 0.018$, $n = 25$), and 48 months ($P = 8e\text{-}5$, $n = 15$) compared to baseline ($n = 33$)

illustrates the CSF contactin 5 protein levels measured as a function of *CNTN5* rs1461684 G allele: no significant difference was detected.

### 3.4 | Effect of the *CNTN5* rs1461684 G variant on PET biomarkers

We investigated the effect of the *CNTN5 rs1461684* G variant on 18F-NAV4694 and flortaucipir PET uptake in the asymptomatic PREVENT-AD cohort and found no significant association with either amyloid or tau deposition in these cognitively unaffected subjects (not shown).

### 3.5 | CSF contactin 5 levels in cognitively unimpaired, mild cognitively impaired, and sAD subjects

Using PREVENT-AD cognitively unimpaired subjects, we contrasted CSF contactin 5 concentrations with subjects from the COMPASS-ND cohort who have received a diagnosis of MCI and sAD. Figure 4 illustrates the modest cross-sectional reduction in CSF contactin 5 levels in MCI and sAD relative to the cognitively unimpaired subjects. Unfortunately, the COMPASS-ND does not have a functional genetic component yet and we were unable to examine the effects of *APOE ε4* or *CNTN5* variants on CSF contactin 5 protein concentrations in these subjects. Of interest, the reduction was significant when the pre-symptomatic stage and MCI were contrasted, but not between MCI and AD stages, consistent with an early time-specific pathophysiological role.

### 3.6 | Contactin 5 protein levels in the prefrontal cortex in the different stages of the AD spectrum

Tissue levels of two contactin 5 protein isoforms were obtained from the ROS-MAP TMT proteomic database, which is described in detail in the Ping et al.[27] Cortical levels of the two major isoforms of contactin 5 expressed in the brain were obtained for some 288 subjects for which we also have corresponding *CNTN5* genotype information, the full length (O94779) wild type variant and the truncated isoform type 3 (O94779-4) protein, which are particularly prevalent in the CNS. Stratification of the two contactin 5 protein variants by Braak stages reveals a modest (but not significant) reduction of cortical contactin 5 levels between stages 0 and the later pathological stages (Figure 5). This is consistent with the CSF *CNTN5* changes reported in living patients

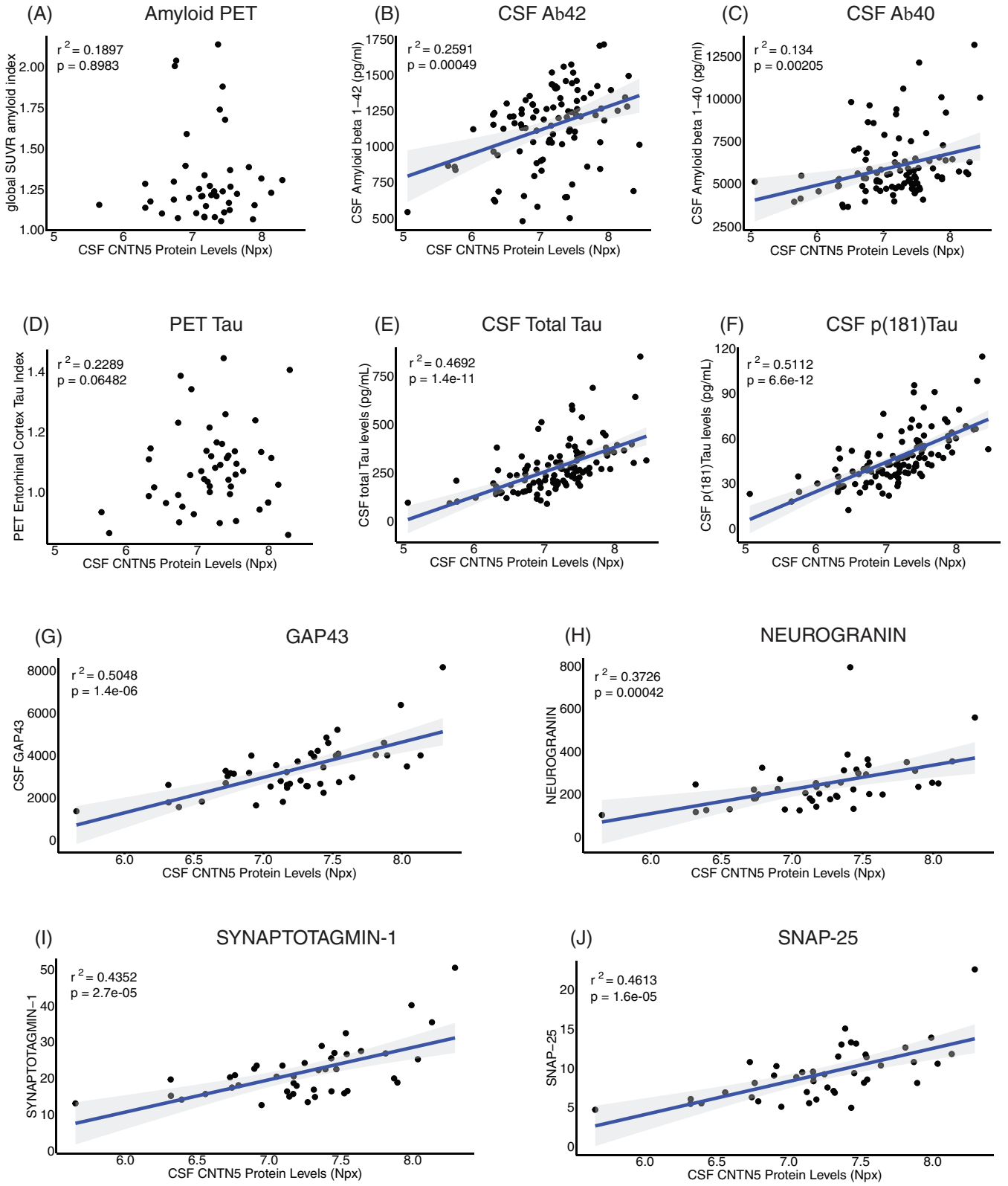



**FIGURE 2** Association between CSF contactin 5 and sAD biomarkers or synaptic proteins in the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease cohort. Contactin 5 protein was measured in the CSF using Olink's proximity extension assay. Global SUVR amyloid index (A) was measured with [18F]NAV4694 PET ($n = 44$). CSF AD biomarkers Aß 1-42 (B; $n = 99$), Aß 1-40 (C; $n = 96$), total tau (E; $n = 113$) and p-tau (F; $n = 113$) were measured by enzyme-linked immunosorbent assay according to the procedures from the BIOMARKAPD consortium of the EU Joint Program in Neurodegenerative Diseases. Tau deposition in the entorhinal cortex (D) was measured with flortaucipir PET ($n = 48$). The synaptic markers GAP43 (G; $n = 45$), neurogranin (H; $n = 45$), synaptotagmin-1 (I; $n = 43$) and SNAP-25 (J; $n = 43$) were quantified using selective reaction monitoring mass spectroscopy. Significant linear regressions are represented with a blue confidence region of

*(Continues)*

**FIGURE 2** (Continued)
the fitted line. R squares and *P* values are shown in the top left corners of each figure. Analyses were adjusted for age, sex and apolipoprotein E ε4. Aß, amyloid beta; AD, Alzheimer's disease; BIOMARKAPD, Biomarkers for Alzheimer's Disease and Parkinson's Disease; CSF, cerebrospinal fluid; PET, positron emission tomography; p-tau, phosphorylated tau; sAD, sporadic Alzheimer's disease; SUVR, standardized uptake value ratio

with emerging cognitive deficits in the COMPASS-ND cohort described above.

Parallel analyses of the mRNA prevalence for the wild type and spliced isoform 3 variants were performed in the ROS-MAP subjects who underwent cortical RNA sequencing profiling. While we did not observe any significant changes across Braak stages (Figure 6A, B), a significant reduction of the isoform 3 variant was observed as a function of CERAD staging ($P = 0.027$, Figure 6D).

Finally, stratification of the *CNTN5* mRNA variants (full length and isoform 3) as a function of rs1461684 G risk variant reveals a strong allele-dependent reduction of the mRNA prevalence of both isoforms in the cortex of asymptomatic and MCI (early stage, $n = 152$) cases (Figure 7A). A concomitant modest reduction (trend only) of the protein concentrations was observed in G allele carriers (Figure 7B). The small sample size of the asymptomatic group ($n = 6$) and of the homozygous G allele carriers ($n = 3$) greatly limits our genomic analysis in the early phase of the disease process in this cohort.

## 4 | DISCUSSION

In the present study, *CNTN5* gene expression and protein (contactin 5) alterations were investigated in both the brain tissue and CSF throughout the sAD spectrum. Contactins represent major proteins involved in neuronal development, formation of dendrites, and synaptic contacts. Indeed, their roles in neuritogenesis, fasciculation of neurons, axonal and dendritic targeting, fine tuning of synapse formation, and synaptic plasticity have been demonstrated in multiple situations.[33,34] Contactins are neural cell adhesion molecules that encode axon-target specificity during the patterning of the developing CNS, and also in response to neuronal injury and damage.[35]

*CNTN5* is specifically implicated in the specification of dendritic arbors. Recent studies further examining the coreceptor function of contactins with the amyloid precursor proteins (APP) have shown that-contactin 4 and contactin 5 can bind to APP and its precusor-like variants (APLP1) when they co-opt their cytoplasmic regions to relay information across the plasma membrane using amyloid-dependent signal transduction pathways.[36,37] These observations suggest a significant interplay between the different contactins and APP metabolism during dendritic remodeling and synaptic formation during neuronal response to injury. This could explain, at least in part, the observed positive correlation between contactin 5, $Aß_{42}$, and $Aß_{40}$ in the CSF in the pre-symptomatic phase of the disease (Figure 2).

Figure 1 shows that cognitively unimpaired elderly subjects with a parental history of AD display a slow but steady longitudinal increase in contactin 5 protein level in the CSF over the course of 4 years. These subjects typically carry a 2- to 3-fold risk of developing AD compared to subjects without a familial history.[38] CSF t-tau and p-tau in these subjects display a significant association with contactin 5 with correlation coefficients of 0.47 and 0.51, respectively. Interestingly, the associations do not translate into significant deposition in the brain when PET scanning was used for total brain amyloid ($P = 0.89$) and entorhinal tau depositions ($P = 0.06$; Figure 2). One possible interpretation for these discrepancies is the fact that CSF amyloid and tau changes precede by several years (sometime a decade) tangle deposition detected by PET imaging.[39] However, the weak association observed between contactin 5 and PET tau in the entorhinal cortex ($P = 0.06$), the brain region typically used for the detection of AD-specific early tau deposition,[40] is certainly consistent with the known spreading of tau pathological cascade. An upcoming second round of PET imaging in these subjects will most likely provide a more definitive answer to this question.

Contactin 5 is known to play an important role in the formation of glutamatergic synapses in the rodent central auditory system during postnatal development and to interact with the E1 domain of APP/APLP1 in the presynaptic compartment.[41] This prompted us to examine the possible association between contactin 5 and well-established soluble biomarkers of synaptic integrity in the CSF, namely GAP43, neurogranin, synaptotagmin-1, and SNAP-25. Figure 2 illustrates the highly significant associations between contactin 5 and all four synaptic biomarkers in the CSF of our cognitively unimpaired subjects: consistent with tau-mediated, contactin 5–associated modulation of synaptic pathology in the pre-symptomatic phase of the disease.

These findings led us to examine the situation later in life, in subjects in which symptoms emerge (MCI) in response to markedly compromised synapses and, later when neuronal damage and cortical atrophy takes a toll (sAD). Cross-sectional analysis of the CSF contactin 5 level in cognitively unimpaired subjects, MCI, and sAD cases reveals a disease-dependent reduction in the COMPASS-ND cohort. The modest but significant reduction of contactin 5 protein levels observed in the CSF ($P < 0.01$) of our living subjects and, in cortical tissue (trends only, ROS-MAP) of autopsied MCI and AD subjects markedly contrast with the t-tau and p-tau alterations observed in the CSF in the asymptomatic (pre-symptomatic ?) stage of the disease in our cognitively unimpaired subjects with a parental history of AD.

Together, these results suggest that the strong association between contactin 5 and tau/synaptic biomarkers prior to the emergence of symptoms serves as an index of early neuronal damage. As synaptic and neuronal structures become more compromised with emerging cognitive symptoms, contactin 5 levels in the brain decrease both in tissues and CSF, presumably in parallel to the ongoing neuronal loss.

As we further examined the pre-symptomatic phase of the disease, the analysis of the rs1461684 G risk variant in the asymptomatic PREVENT-AD cohort led to an interesting finding when used in

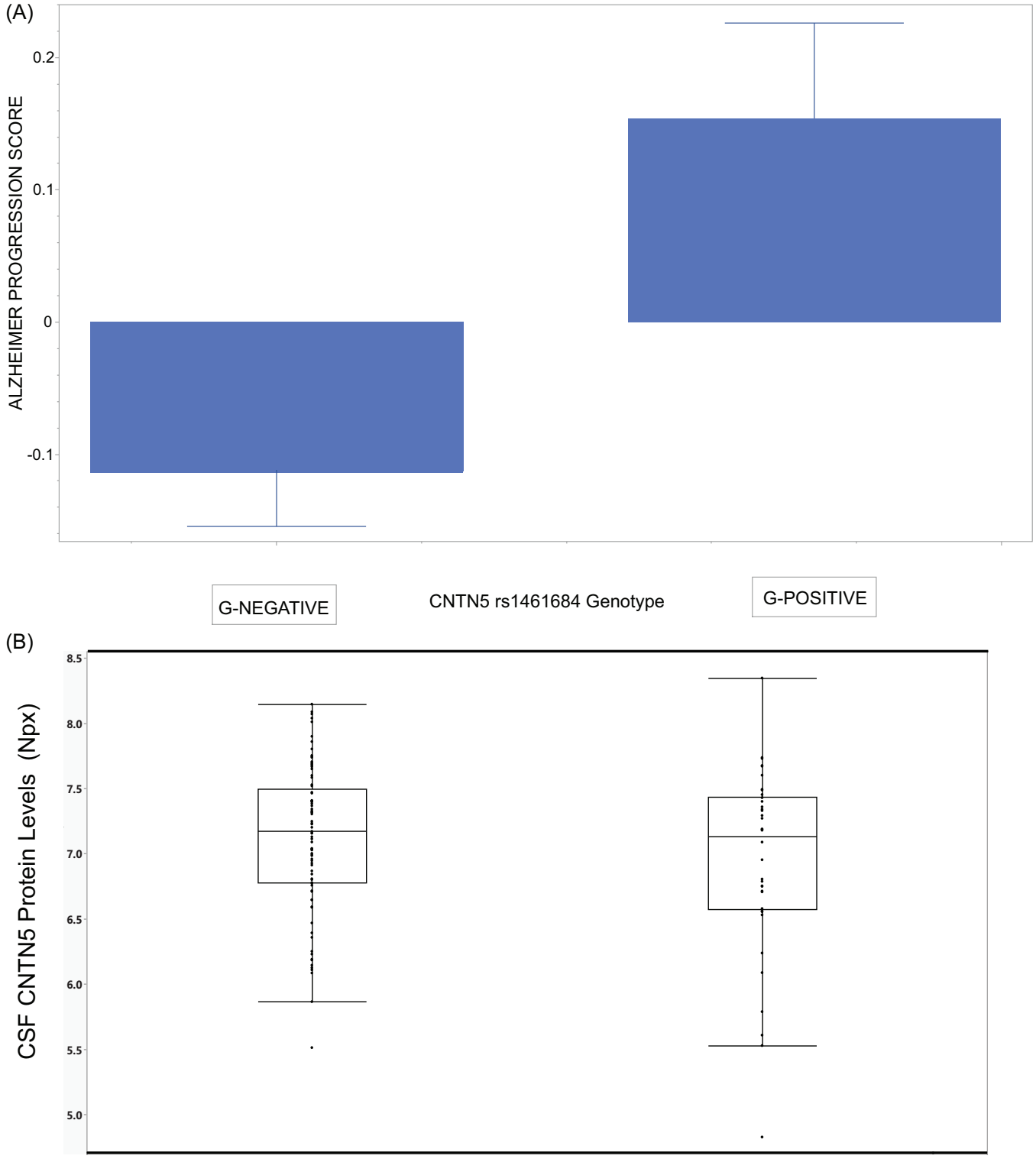



**FIGURE 3** Association between the *CNTN5* rs1461684 G variant and Alzheimer Progression Score in the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) cohort. A, Association between the presence of the rs1461684 G allele and the Alzheimer's disease progression score in the PREVENT-AD cohort ($P = 0.01$, $n = 418$). B, Association between the presence of the rs1461684 G allele on cerebrospinal fluid (CSF) contactin 5 protein levels at baseline (non-significant, $n = 103$). Analyses were adjusted for age and sex

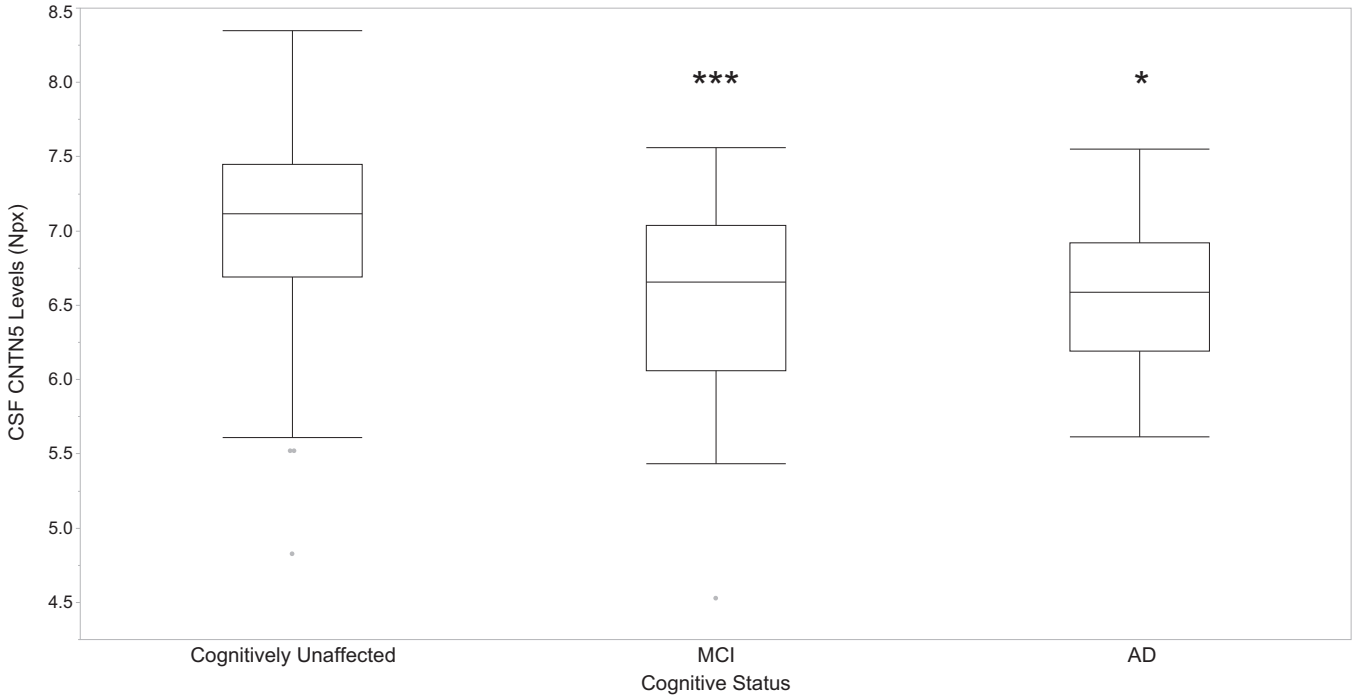



**FIGURE 4** Association between cerebrospinal fluid (CSF) contactin 5 and cognitive status in the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) and Canadian Consortium on Neurodegeneration in Aging (CCNA) cohorts. Contactin 5 protein was assessed using Olink's proximity extension assay in subjects who are cognitively unimpaired (PREVENT-AD cohort: $N = 105$) or diagnosed with mild cognitive impairment (CCNA's MCI: $N = 28$) or Alzheimer's disease (CCNA's AD: $n = 14$). CSF contactin 5 is significantly decreased in MCI ($P < 0.0001$) and AD ($P < 0.02$) relative to cognitively unaffected individuals

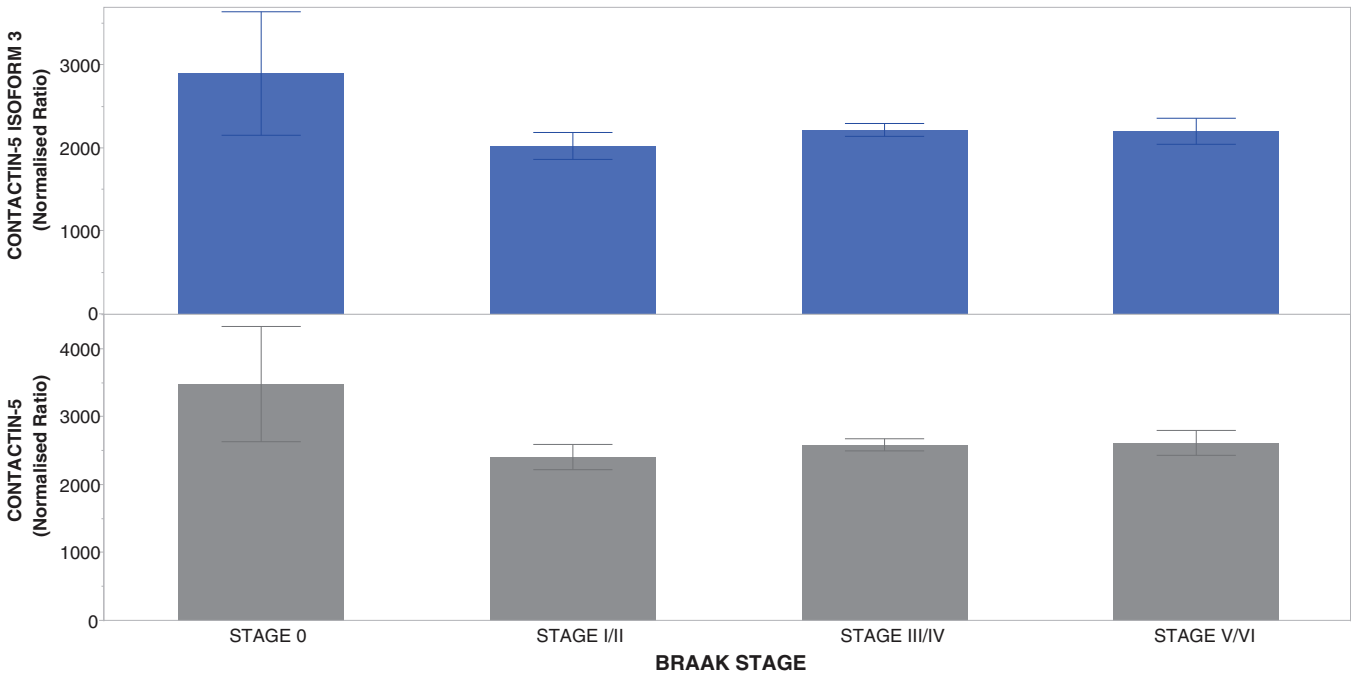



**FIGURE 5** Cortical contactin 5 protein levels at different Braak stages in the Religious Orders Study Rush Memory and Aging Project (ROS-MAP) cohort. Contactin 5 protein was assessed using tandem mass tags proteomic data in subjects from the ROS-MAP cohort. Normalized cortical contactin 5 (full length and isoform 3) ratios are not significantly affected by tau pathology accumulation ($P > 0.05$, $n = 288$)

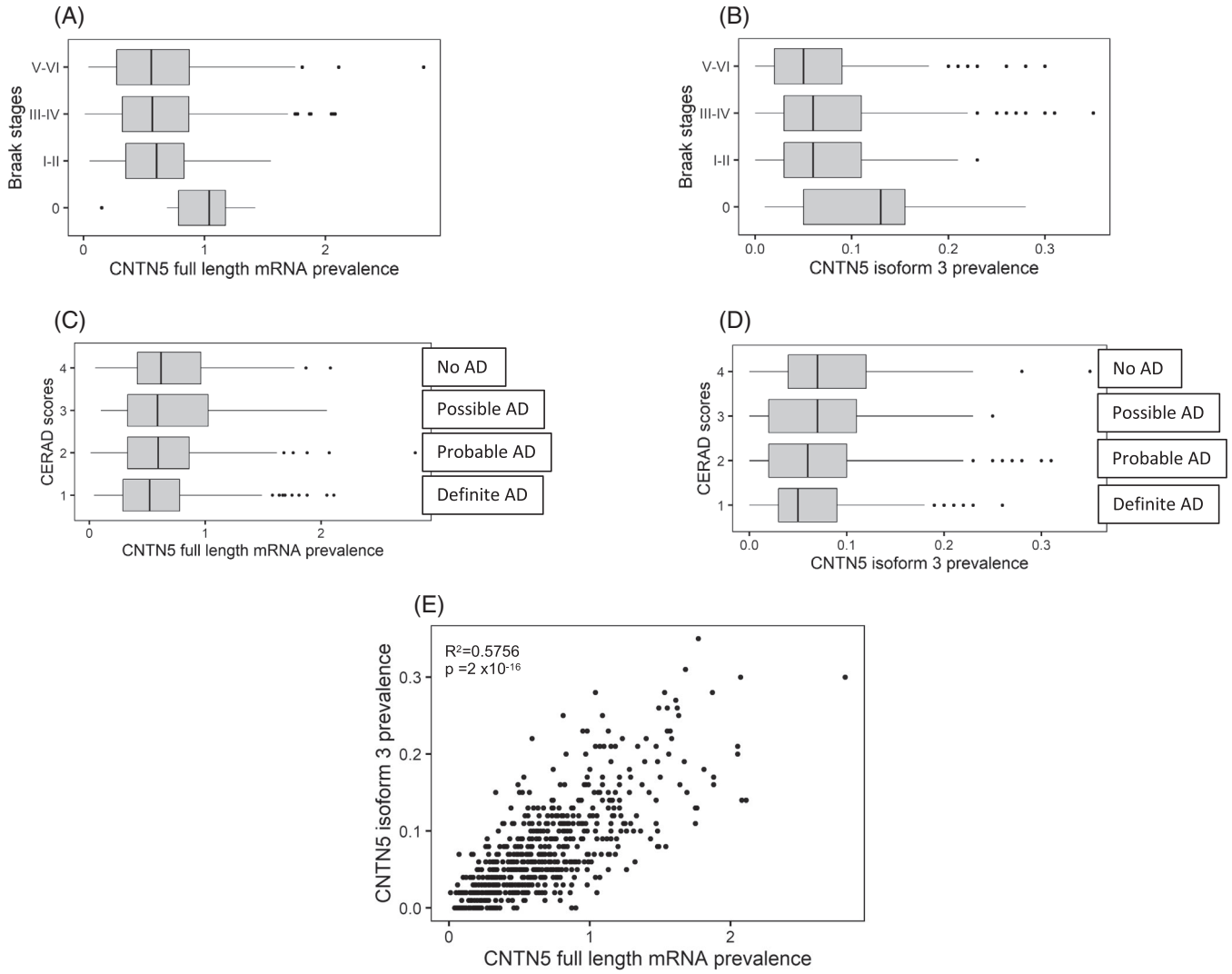
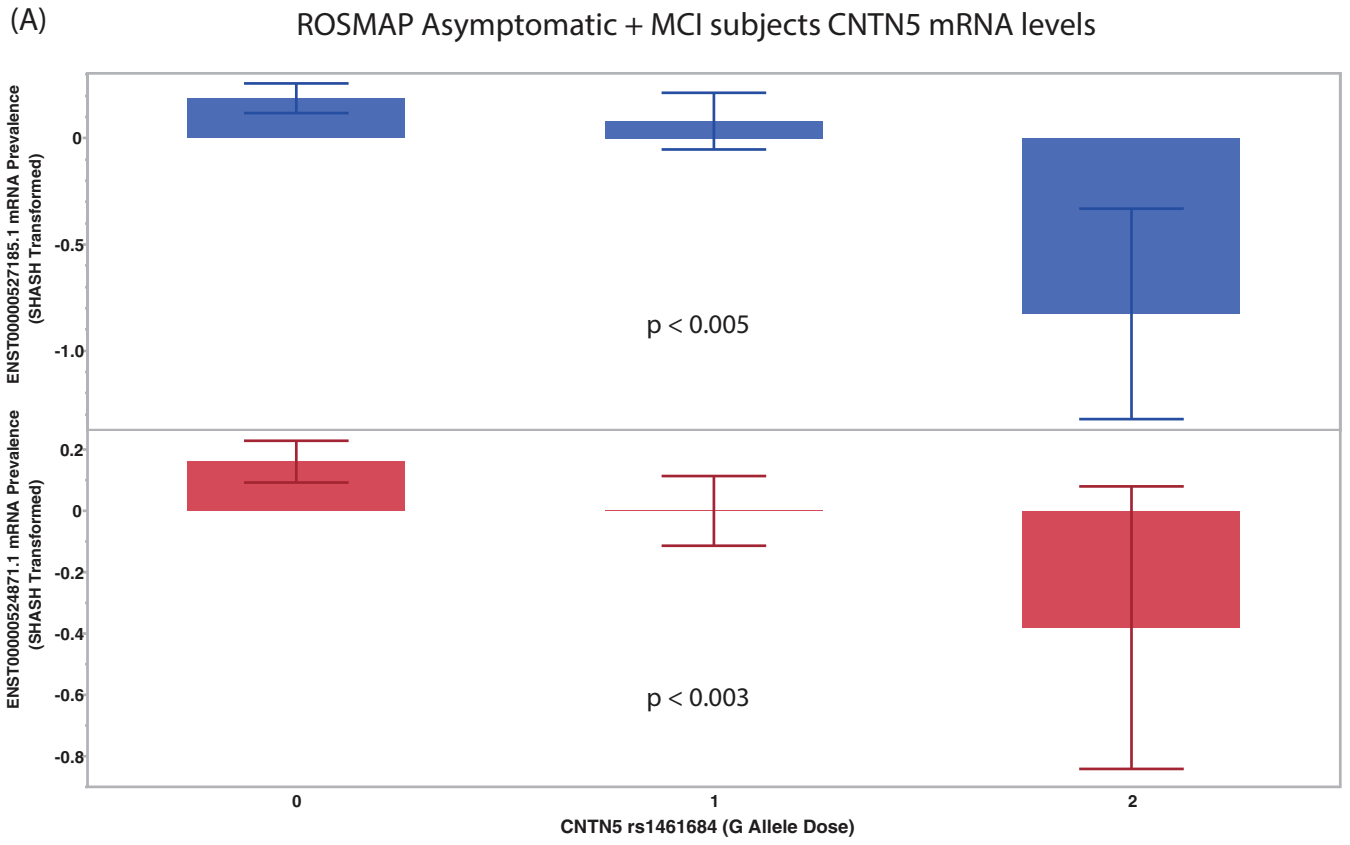



**FIGURE 6** Association between cortical *CNTN5* mRNA splice variants and Alzheimer's disease (AD) pathology (Consortium to Establish a Registry for Alzheimer's Disease [CERAD] and Braak stages) in the Religious Orders Study Rush Memory and Aging Project (ROS-MAP) cohort. *CNTN5* splice variants were obtained from the ROS-MAP RNAseq database. A, There was no association between ENST00000524871.6 full length mRNA and Braak stages ($P = 0.2$, $n = 608$). B, There was no association between ENST00000527185.5 isoform 3 variant prevalence and Braak stages ($P = 0.2$, $n = 608$). C, There was a weak association (trend) between ENST00000524871.6 full length mRNA and CERAD scores ($P = 0.066$, $n = 615$), (D) ENST00000527185.5 isoform 3 variant prevalence is associated with worse CERAD scores ($P = 0.027$, $n = 615$). E, There is a significant association between cortical levels of ENST00000524871.6 full length mRNA and enst00000527185_1 isoform 3 variant (r2 = 0.5756, $P = 2e\text{-}16$). Analyses were adjusted for age, sex, and apolipoprotein E ε4

conjunction with APS to map disease progression in the absence of obvious cognitive deficit (Figure 3). Presence of the G allele was found to be significantly associated with a faster rate of progression ($P = 0.01$) compared to G-negative subjects (Figure 3A), but it does not affect contactin 5 levels in the CSF. So, if the soluble form of contactin 5 found in the CSF is not affected by the presence of the G allele, what about the tissue concentration?

In this context, we used the ROS-MAP cohort to explore the cortical expression of the two major mRNA isoforms of *CNTN5* transcripts found in the CNS, that is, the full length (ENST00000524871.6) and the isoform 3 (ENST00000527185.5), which is truncated at the 3′ end. Figure 6 summarizes the findings. Using CERAD and Braak staging, we examined *CNTN5* gene expression throughout the AD spectrum. *CNTN5* isoform 3 prevalence is associated with worsening on the CERAD scores ($P = 0.027$) but not on the Braak scores ($P = 0.636$; Figure 6B, D). There was a modest association (trend only, $P = 0.066$) between *CNTN5* full length mRNA prevalence and CERAD scores but no correlation with Braak staging ($P = 0.437$; Figure 6A,C).

When mRNA results from early-stage ROS-MAP subjects (asymptomatic + MCI) were pooled and stratified by rs1461684-G risk variant, we observed a strong allele–dose reduction of the CNTN5 isoform 3 (Figure 7A; $P < 0.004$) and full-length mRNA transcripts ($P < 0.005$) in cortical tissues. Using a similar approach to stratify cortical contactin 5 protein levels as a function of the G allele, we only found modest reductions (trends only, full-length $P = 0.07$, isoform 3 $P = 0.10$) of tissue concentrations. As stated above, in contrast to the



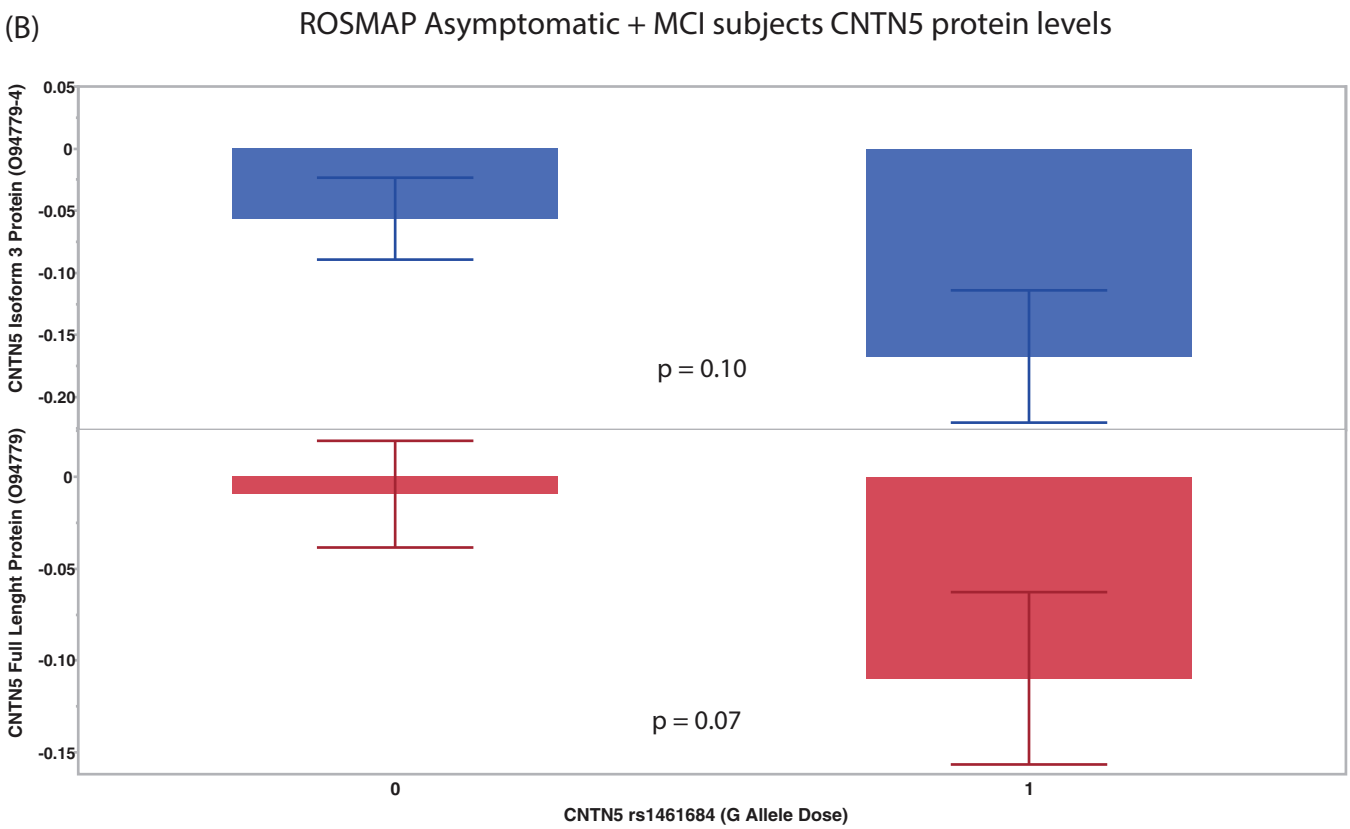




**FIGURE 7** Association between the *CNTN5* rs1461684 G variant and cortical *CNTN5* mRNA and proteins levels in asymptomatic and mild cognitive impairment (MCI) subjects from the Religious Orders Study Rush Memory and Aging Project (ROS-MAP) cohort. A, Association between

*(Continues)*

**FIGURE 7** (Continued)
the presence of the rs1461684 G allele and *CNTN5* mRNA prevalence for the full length (bottom: ENST524871, r2: 0.13, $P < 0.003$, $n = 301$) and isoform 3 (top: ENST524185, r2 = 0.07, $P < 0.005$, $n = 301$) variants. B, Association between the presence of the rs1461684 G allele and contactin 5 protein levels (full length O94779, $P = 0.10$, $n = 103$ and isoform O094779-4, $P = 0.07$). Analyses of variance were adjusted for age, apolipoprotein E ε4, and sex

mRNA dataset, the ROS-MAP proteomic dataset that overlaps with the subjects enrolled simultaneously with the GWAS contains a relatively small number of subjects in the asymptomatic group for which we have rs1461684 genetic information, thus explaining the small sample size.

Together, these results indicate that *CNTN5* gene expression contributes, at least in part, to the observed reduction of brain contactin 5 protein levels when amyloid-associated staging (CERAD) is used to map the disease progression in symptomatic subjects in ROS-MAP. The presence of the G allele contributes to a marked reduction of the *CNTN5* full-length and isoform 3 mRNA variant in cortical tissue.

Isoform 3 lacks amino-acid sequence 912–1100. While speculative, one could suggest that G-allele mediated decrease in isoform 3 mRNA leads to a protein variant of contactin 5 that is missing a portion of the cytoplasmic region, which is required to relay information across the plasma membrane using the amyloid-dependent signal transduction pathways described previously.[36,37] Whether the result is a cause or a consequence of neuronal loss associated with the tau/contactin 5 interaction detected in the extracellular space remains to be elucidated. Additional molecular studies are now planned to examine the role of isoform 3 and its relationships to APP, amyloid, and tau metabolism.

Altogether, these results suggest that *CNTN5* plays a prominent role in the early phase of the disease, in the pre-symptomatic stage, when tangle pathology emerges but amyloid and tau deposition are still limited. The strong association of CSF contactin 5 protein with synaptic markers, especially pre-synaptic ones, is consistent with its neurodevelopmental role in the regulation of dendritic arborization remodeling and synaptic connectivity. We know that the brain is not static in the face of early neuronal loss and that compensatory mechanisms exist to limit the loss of synaptic input and to facilitate dendritic remodeling and synaptic reorganization from intact neuronal circuits. The presence of a relatively common *CNTN5* risk variant that affects this cascade was found to significantly affect the disease progression on the APS scale in the pre-symptomatic phase, *CNTN5* tissue mRNA prevalence in the early stage of the disease, affecting the CERAD scale in symptomatic subjects from ROS-MAP.

## AUTHOR CONTRIBUTIONS

MD, JP, CP, SV, KB, and HZ conceptualized the research. AL, JM, NJA, HZ, and KB performed CSF biomarker measurements, data quality control, and data compilation. CP performed the pan-genomic analysis of DNA samples and risk assessments. MD, JP, CP, JM, AL, SV contributed to data analysis. JP, MD, SV, and CP developed the algorithms for data analysis. MD, JP, CP, NJA, and SV wrote the original manuscript draft. All authors reviewed, edited, and approved the final manuscript.

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## CONFLICTS OF INTEREST

Dr. Zetterberg has served on scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). JP serves as a scientific advisor to the Alzheimer Society of France. KB has served as a consultant or at

advisory boards for: Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Lilly, MagQu, Prothena, Roche Diagnostics, and Siemens Healthineers, with payments made to individual. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), and the National Institute of Health (NIH), USA (grant #1R01AG068398-01). JP and SV have received CIHR project grants awarded to the academic institution. JP has received project grants from the J.L. Levesque Foundation, NSERC and FQRS and PR from Weston Brain Institute, which were paid to academic institution. MD has received research support from the CAPES Foundation. All other authors have nothing to disclose. Author disclosures are available in the supplementary file.

## INSTITUTIONAL REVIEW BOARD STATEMENT

All procedures were approved by the McGill University Faculty of Medicine Institutional Review Board and complied with the ethical principles of the Declaration of Helsinki. In this cohort, 382 individuals were genotyped and selected for evaluation. Each participant and study partner provided written informed consent.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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