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RESEARCH ARTICLE

Blood levels of MCP-1 modulate the genetic risks of Alzheimer's disease mediated by HLA-DRB1 and APOE for Alzheimer's disease

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Abstract

Introduction: C-Reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) are both implicated in the peripheral proinflammatory cascade and blood–brain barrier (BBB) disruption. Since the blood CRP level increases Alzheimer's disease (AD) risk depending on the apolipoprotein E (*APOE*) genotype, we hypothesized that the blood MCP-1 level exerts different effects on the AD risk depending on the genotypes.

Methods: Using multiple regression analyses, data from the Framingham Heart Study (n = 2884) and Alzheimer's Disease Neuroimaging Initiative study (n = 231) were analyzed.

Results: An elevated blood MCP-1 level was associated with AD risk in major histocompatibility complex, Class II, DR beta 1 (*HLA-DRB1*) rs9271192-AC/CC (hazard ratio [HR] = 3.07, 95% confidence interval [CI] = 1.50–6.28, p = 0.002) and in *APOE* ε4 carriers (HR = 3.22, 95% CI = 1.59–6.53, p = 0.001). In contrast, among *HLA-DRB1*

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rs9271192-AA and *APOE* ε4 noncarriers, blood MCP-1 levels were not associated with these phenotypes.

Discussion: Since HLA-DRB1 and APOE are expressed in the BBB, blood MCP-1 released in the peripheral inflammatory cascade may function as a mediator of the effects of *HLA-DRB1* rs9271192-AC/CC and *APOE* ε4 genotypes on AD pathogenesis in the brain via the BBB pathways.

KEYWORDS
Alzheimer's disease, blood, genotypes, MCP-1

1 | BACKGROUND

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a long-term deteriorating process. As a complex disease, not every person carrying AD risk variants develops AD. The AD risk potentially depends on both genetics and internal and external environmental components, such as proinflammatory factors, and their interactive effects on the disease.[1]

Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) is a member of the CC family of chemokines expressed in the blood and brain and is implicated in both inflammatory cell recruitment and blood–brain barrier (BBB) disruption.[2] The main function of MCP-1 as a chemoattractant is to induce the migration of leukocytes, especially monocytes/macrophages, into tissues in the brain and periphery.[3,4] Since peripheral immune cells are present in AD brains,[5] MCP-1 may play a role in transporting peripheral immune cells into the brain, activating microglia in the brain and leading to cognitive decline.[6–12]

Peripheral inflammatory factors are often coexpressed and form an inflammatory cascade. For example, C-reactive protein (CRP) increases the expression of a group of proinflammatory factors, including MCP-1.[13,14] Multiple studies, including our own, found that elevated blood CRP levels increase the risk of AD in apolipoprotein E (*APOE*) ε4 carriers.[15,16] We hypothesized that CRP-linked MCP-1 also differentially affects certain vulnerable genotypes to increase the AD risk. Current genome-wide association studies (GWAS) have identified >30 AD loci, some of which are enriched in inflammatory pathways.[17] In this study, we chose the *APOE* genotype and 10 other common single nucleotide polymorphisms (SNPs) in 10 known AD loci, which are also involved in inflammation, and examined whether the blood levels of MCP-1 exert a different effect on the AD risk depending on the genotypes. Using two different cohorts, we found that elevated blood MCP-1 levels increased the AD risk in patients with one genotype, but not their counterpart genotype, of major histocompatibility complex, Class II, DR beta 1 (*HLA-DRB1* rs9271192),[18–20] and *APOE* ε4.[21]

2 | METHODS

2.1 | Participants

2.1.1 | Framingham Heart Study

The Framingham Heart Study (FHS) is a single-site, multigeneration, community-based, prospective cohort study. This study focused on offspring cohort (Generation 2) participants with available data on genome-wide genotyping and serum MCP-1 measurements and have been rigorously evaluated for cognitive functioning and dementia since 1979.[22] After excluding individuals with dementia at baseline, this study used genetic data and the MCP-1 levels measured in Exam 7 (baseline) and data on the AD/dementia incidence with follow-up until 2019 (Figure S1). Participants were evaluated for AD and other dementia diagnoses as previously described.[15] Informed consent was obtained from all study participants, and the study protocol was approved by the Institutional Review Board of Boston University.

2.1.2 | Alzheimer's Disease Neuroimaging Initiative

We used the data from Alzheimer's Disease Neuroimaging Initiative (ADNI-1), which is a longitudinal multicenter study that was launched in 2003 as a public–private partnership, to validate the findings from the FHS. ADNI-1 was designed to test whether neuroimaging, biological markers in cerebrospinal fluid (CSF) and blood, and clinical and neuropsychological assessments can be combined to predict the diagnosis and progression of AD.[23] Participants underwent longitudinal in-depth neuropsychological evaluations, and cognitively normal (CN), mild cognitive impairment (MCI), and AD were diagnosed. The data on blood MCP-1, CSF Aβ42, total tau (t-Tau), and p-Tau levels were included in this analysis. After excluding self-reported nonwhite subjects and those without MCP-1 measurements, genotype information and AD biomarker measurements, 231 participants in ADNI-1 were included in the analysis (Figure S1, Table S1).

2.2 | Selection of AD-related genes and SNPs

Among more than 30 gene loci associated with the AD risk identified by GWAS,[17] 19 have been reported to be related to inflammation.[18,24–31] We chose to study the SNPs of 10 of these genes based on the following criteria: (1) a minor allele frequency (MAF) greater than 5%; (2) most significant SNP associated with AD; and (3) evidence of replication in independent studies (Table S2). Additionally, we included the *APOE* ε4 genotype status in the analysis since the interaction effect of *APOE* ε4 and MCP-1 levels on AD risk had not been studied previously.

2.3 | Genotype measurements

SNP genotype data for the FHS cohort were previously filtered and imputed using the Trans-Omics for Precision Medicine (TOPMed) Imputation Server. We also imputed genotypes for ADNI participants using the TOPMed reference panel. The imputation quality (r^2) of all 10 SNPs was >0.95. *APOE* genotypes for FHS and ADNI subjects were determined using TaqMan assays for two SNPs: rs7412 and rs429358. Details of the SNPs included in this study are provided in Table S2.

2.4 | Measurement of serum MCP-1 levels

Plasma MCP-1 levels in FHS participants were measured using enzyme-linked immunosorbent assay (ELISA) with a Dade Behring BN100 nephelometer[32] from fasting blood samples that were collected from the antecubital vein at exam 7.[33] In ADNI-1 participants, MCP-1 levels were measured in plasma samples using the Human Discovery MAP Panel and measurement platform.[34] The MCP-1 level was log-transformed in the downstream analysis as a continuous variable. The median MCP-1 level was used to divide participants into low and high MCP-1 groups.

2.5 | Brain MRI

A subset of the FHS offspring participants ($n = 2231$) underwent brain magnetic resonance imaging (MRI) scanning between 03/1999 and 12/2017 (Figure S1), as previously described.[12] Briefly, participants were imaged using a 1.5T MRI (Siemens Medical, Erlangen, Germany) with a three-dimensional T1-weighted coronal spoiled gradient-recalled echo sequence. All images were transferred to and processed by the University of California Davis Medical Center without knowledge of the clinical information. Segmentation and quantification of the total cerebral cranial volume (TCV), total brain volume, frontal lobe (FBV), parietal lobe (PBV), occipital lobe (OBV), temporal lobe (TBV), hippocampal (HPV), and lateral ventricle volumes were performed using semiautomated procedures as previously described.[35] TCV was determined using a convolutional neural network method.[36] Nonlinear coregistration of images to the Desikan-Killiany-Tourville atlas enabled the calculation of regional gray matter volumes.[14] MRI measures were corrected for head size by calculating the percent of these volumes relative to the TCV. The percentage of TCV was log-transformed for normality. Each image set underwent rigorous quality control, including assessments of the original acquisition and image processing quality.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional (e.g., PubMed) sources. A proinflammatory cascade could include C-reactive protein (CRP) and other CRP co-expressed factors including monocyte chemoattractant protein-1 (MCP-1), and both impair the blood–brain barrier (BBB). Since elevated blood CRP increases Alzheimer's disease (AD) risk depending on apolipoprotein E (APOE) genotype, we hypothesized that elevated blood levels of MCP-1 also have a different effect on AD risk depending on the genotypes. These relevant citations are appropriately cited.
2. Interpretation: Since major histocompatibility complex, Class II, DR beta 1 (HLA-DRB1) and APOE are expressed in the BBB, blood MCP-1 may face and function as a mediator via the BBB pathways from HLA-DRB1 rs9271192-AC/CC and APOE ε4 genotypes on AD pathogenesis in the brain.
3. Future directions: As multiple MCP-1 inhibitors are currently being developed and tested in clinical trials for cancer and autoimmune diseases, these medications could be repurposed for AD prevention during peripheral inflammation based on precision genetic background.

2.6 | AD and brain neuropathology

A subset of FHS participants ($n = 105$) donated their brains and data on neuropathology were available. Four routine neuropathology variables were selected, including the Braak stage for neurofibrillary degeneration, Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score for the density of neocortical neuritic plaques, and CERAD semiquantitative score for diffuse plaques and microinfarcts in addition to arteriolosclerosis, atherosclerosis and cerebral amyloid angiopathy (CAA). The neuropathological evaluation was performed by neuropathologists who were blinded to the clinical information.[37] Eighty-three participants had information available on the aforementioned neuropathology variables and were included in the analysis (Figure S1).

Additionally, seven AD-related features were measured using antibody-specific immunostaining, including the Iba1 density and

CD68 expression for microglial activation, Aβ40 and Aβ42 expression for amyloid-specific species, and AT8, p-Tau231, and p-Tau202 expression for tauopathy.[12] Seventy-nine participants were included in the analysis of these features (Figure S1).

2.7 | Statistical analysis

Analyses were performed using the R statistical package (R 3.6.2). Several variables, including sample size, age at baseline, sex, years of education, *APOE* ε4 status, and incident AD status, were summarized as the basic characteristics and stratified by MCP-1 levels. Group differences were assessed using analysis of variance (ANOVA) for normally distributed continuous variables, the Kruskal–Wallis rank sum test for continuous variables with skewed distributions, and the χ^2 test for categorical variables.

Subjects were divided into four groups based on cutoff values for the CRP (8 mg/L)[15] and MCP-1 levels (382 pg/ml, third quartile): CRP-low-MCP-1-low group, CRP-high-MCP-1-low group, CRP-low-MCP-1-high group, and CRP-high-MCP-1-high group (Table S4). Using the CRP-low-MCP-1-low group as a reference, associations between the AD risk and the other three CRP-MCP-1 groups were tested among each genotype with a Cox proportional hazards regression model adjusted for age at baseline, sex, and years of education.

For the analysis of the AD incidence, a Cox proportional hazards regression analysis was initially performed by including the SNP minor allele dosage (0, 1, 2), MCP-1 level (log-transformed), and an interaction term between the SNP and MCP-1 level (log-transformed). The analysis was controlled for several covariates, including age at MCP-1 measurement (baseline), self-reported sex, years of education, and the first five principal components (PCs) of ancestry. Significant results of interaction effects with a Bonferroni-corrected $p < 0.005$ (0.05/11)) were further investigated in stratified analyses of genotypes with Cox proportional hazards regression models. For structural brain MRI data, linear regression models were used with the same adjustments as described above in patients with each genotype. All brain volumes were divided by the total cerebrum cranial volume and multiplied by 100% to adjust for the brain size.

For neuropathology, since the number of autopsy cases was small, we first divided the brain variables into low and high scores and used χ^2 tests to compare the subgroups based on the genotypes and low MCP-1 versus high MCP-1 groups (median as cutoff) in sensitivity analyses: Braak score: 0 to 3 versus 4 to 6; diffuse plaque CERAD score: 0 to 1 versus 2 to 3; neuritic plaque CERAD score: 0 versus 1 to 3; and microinfarcts: yes versus no. For the analysis of the Iba1-density and CD68, Aβ40, Aβ42, AT8, p-Tau231, and p-Tau202 expression levels with skewed distributions, rank-based inverse-normal transformation was applied. Two-sample *t*-tests were then performed to compare the mean MCP-1 levels between the high and low MCP-1 groups for each genotype. The ADNI-1 cohort was used to characterize Aβ and tau biomarkers in CSF using linear regression models adjusted for age at baseline, sex, and CRP levels.

3 | RESULTS

3.1 | Characteristics of the FHS population

The FHS participants ($n = 2884$) included in this study had an average follow-up period of 18.5 years, and 171 (5.93%) of the participants developed AD. Since elevated blood CRP levels increase the risk of AD in *APOE* ε4 carriers,[15,16] we first screened 73 inflammatory proteins in the FHS dataset for the interactive effect with the *APOE* genotype using a Cox proportional hazards regression analysis. We found that similar to the CRP level, only the MCP-1 concentration had an interactive effect with *APOE4* (HR = 3.31, 95% CI = 1.53, 7.18, $p = 0.002$), but was opposite to *APOE2* (HR = 0.20, 95% CI = 0.07, 0.60, $p = 0.004$), on the AD risk. We thus predicted that CRP levels would be linked with MCP-1 levels to increase AD risk in a genotype-dependent pattern and decided to focus on MCP-1 in this study.

Next, subjects were divided into four quartiles based on the blood MCP-1 concentration at baseline (Table 1). Indeed, MCP-1 levels were positively correlated with the CRP concentration. Participants with the lowest MCP-1 concentration (first quantile) were youngest ($p < 0.001$) and had the most years of education ($p < 0.001$) compared to participants with higher MCP-1 quartiles. Sex, *HLA-DRB1* rs9271192, and *APOE* ε4 genotypes did not show differences across MCP-1 quartiles. Although an overall increase in the incidence of AD was observed with increasing blood MCP-1 concentrations, the relationship was not linear in the whole sample (3.6% vs. 6.4% vs. 4.7% vs. 9.0%, $p < 0.001$) (Table 1). When directly testing the associations between the continuous increase in the MCP-1 concentration and AD incidence after adjusting for confounders, no statistically significant relationship was identified (Table S3), suggesting that blood MCP-1 levels differentially affect patients with certain vulnerable genotypes for AD.

3.2 | Associations of blood MCP-1 levels and AD risk among carriers of different genotypes

Next, we performed stratified analysis based on SNPs to test the association between MCP-1 levels and the AD risk using a Cox proportional hazards regression model after adjusting for age at baseline, sex, years of education, and PCs. In addition to the *APOE* genotype, we also studied 10 other genes to investigate whether they exert interactive effects with MCP-1 levels on the AD risk. We found that only SNP rs9271192 located close to *HLA-DRB1* ($p = 0.004$) and the *APOE* ε4 status ($p = 0.002$), but not other AD risk genes, exerted such interactive effects after Bonferroni's correction ($p < 0.005$, 0.05/11) (Table 2). Furthermore, a stratified analysis of genotypes found that an elevated MCP-1 concentration (continuous and log-transformed) (Table S4) was positively associated with the AD risk among *HLA-DRB1* rs9271192-AC/CC carriers (HR = 3.07, 95% CI = 1.50, 6.28, $p = 0.002$) and *APOE* ε4 carriers (HR = 3.22, 95% CI = 1.59, 6.53, $p = 0.001$). This dose-dependent relationship was also observed using different MCP-1 percentile cutoffs (Figure 1A,B). This trend was not observed for the

TABLE 1 Basic characteristics, genotypes, CRP levels, and incidence of AD based on blood MCP-1 quartiles in the FHS cohort

Characteristics	All subjects	First quartile (31.1, 253.1), pg/ml	Second quartile (253.1, 312.3), pg/ml	Third quartile (312.3, 382.9), pg/ml	Fourth quartile (382.9, 2139.8), pg/ml	*p* value[d]
N subjects, No. (%)	2884	721 (25)	721 (25)	721 (25)	721 (25)	NA
Age when measuring MCP-1 levels, mean (SD)	60.64 (9.27)	57.65 (9.22)	60.57 (9.12)	61.52 (8.75)	62.83 (9.22)	<0.001[a]
Follow-up, years, median (Q1–Q3)	18.46 (14.87–19.41)	18.78 (17.31–19.60)	18.50 (15.07–19.44)	18.39 (15.00–19.26)	17.93 (12.52–19.22)	<0.001[b]
Male, No. (%)	1332 (46.19)	316 (43.83)	323 (44.80)	349 (48.40)	344 (47.71)	0.24[c]
Years of education, mean (SD)	13.99 (2.44)	14.31 (2.41)	14.18 (2.56)	13.89 (2.33)	13.57 (2.40)	<0.001[a]
HLA-DRB1 rs9271192 MAF	0.25	0.24	0.25	0.25	0.27	0.40 [c]
APOE ε4[e], No. (%)	573 (20.21)	126 (17.48)	148 (20.53)	144 (19.97)	155 (21.50)	0.31[c]
CRP level, median (Q1–Q3), mg/L	2.15 (1.01–5.13)	1.81 (0.94–4.06)	2.07 (0.96–5.04)	2.25 (1.05–5.28)	2.54 (1.15–5.76)	<0.001[b]
Incidence of AD, No. (%)	171 (5.93)	26 (3.61)	46 (6.38)	34 (4.72)	65 (9.02)	<0.001[c]

Notes: A total of 2884 subjects in the FHS were divided into four quartiles based on blood MCP-1 levels in the analysis. Means (SD) were reported. ANOVA was used to analyze continuous variables, while n (%) with the χ^2 test were used for categorical variables for the MCP-1 quartile comparisons. P values indicating statistical significance are shown.

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; APOE, apolipoprotein E; CRP, C-reactive protein; FHS, Framingham Heart Study; HLA-DRB1, major histocompatibility complex Class II, DR beta 1; IQR, interquartile range; MAF, minor allele frequency; MCP-1, monocyte chemoattractant protein-1; SD, standard deviation.

[a]*p* value from analysis of variance (ANOVA).

[b]*p* value from the rank sum test.

[c]χ^2 test *p* value.

[d]*p* value for the comparison between the four groups.

[e]*APOE ε4* = ε34 + ε44.

TABLE 2 Effects of the interactions between 10 common SNPs (MAF > 5%) and APOE4 and continuous blood MCP-1 levels (log-transformed) on AD risk using the Cox proportional hazards regression model[a] in the FHS cohort

							FHS			
							SNP main effect		Interaction Effect (SNP: MCP-1)	
Genelocus	Chr:Pos[bd ay] (GRCh38)	Major allele	Minor allele	dbSNP ID	Function	MAF	HR (95% CI)	P value	HR (95% CI)	*p* value
CR1	1:207518704	G	A	rs6656401	Intron	0.18	1.02 (0.77–1.36)	0.87	0.69 (0.28–1.66)	0.40
HLA-DRB1	6:32610753	A	C	rs9271192	Intergenic	0.25	1.12 (0.88–1.43)	0.36	**2.97 (1.41–6.26)**	**0.004**
CD2AP	6:47520026	A	G	rs10948363	Intron	0.26	1.19 (0.94–1.50)	0.15	1.29 (0.64–2.59)	0.48
EPHA1	7:143412046	T	C	rs11767557	Intron	0.20	0.80 (0.61–1.07)	0.13	0.41 (0.17–0.96)	0.04
CLU	8:27610169	T	C	rs9331896	Intron	0.41	1.09 (0.88–1.35)	0.44	0.82 (0.46–1.46)	0.51
SPI1	11:47354897	A	G	rs1057233	3' UTR	0.33	1.05 (0.84–1.32)	0.66	1.73 (0.92–3.27)	0.09
MS4A6A	11:60156035	A	G	rs983392	Intergenic	0.41	1.01 (0.81–1.26)	0.92	1.04 (0.54–2.03)	0.90
ADAM10	15:58753575	A	G	rs593742	Intron	0.28	1.09 (0.86–1.38)	0.47	1.11 (0.53–2.34)	0.78
ABCA7	19:1063444	G	A	rs4147929	Intron	0.19	0.85 (0.65–1.12)	0.24	1.42 (0.65–3.13)	0.38
CD33	19:51224706	C	A	rs3865444	5'UTR	0.29	0.81 (0.64–1.03)	0.09	1.69 (0.82–3.45)	0.15
APOE ε4	19:44908822 19:44908684	C T	T C	rs7412 rs429358	Missense Missense	0.20	3.24 (2.32–4.51)	4.20e-12	**4.08 (1.70–9.80)**	**0.002**

Notes: Using the FHS dataset and Cox proportional hazards regression model, 10 SNPs related to AD and inflammation plus *APOE4* were chosen, and their relationships and interactive effects with continuous blood MCP-1 levels on AD risk were examined.

[a]Interaction: SNP dosage * MCP-1 level; adjusted for age, sex, years of education and PCs.

Abbreviations: AD, Alzheimer's disease; ApoE, apolipoprotein E; Chr, chromosome; CI, confidence interval; FHS, Framingham Heart Study; HR, hazard ratio; MAF, minor allele frequency; MCP-1, monocyte chemoattractant protein-1; Pos, position; SNP, single nucleotide polymorphism; UTR, untranslated region.

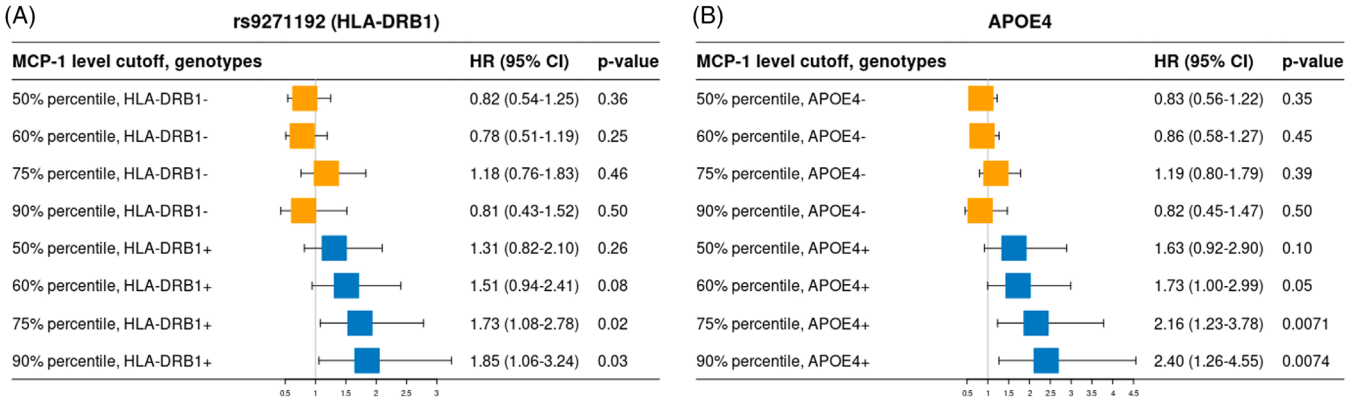

FIGURE 1 Alzheimer's disease (AD) risk based on different genotypes and elevated blood monocyte chemoattractant protein-1 (MCP-1) levels. The data from the Framingham Heart Study (FHS) Gen2 cohorts were used. Participants were divided according to the genotypes: major histocompatibility complex, Class II, DR beta 1- (HLA-DRB1-) = rs9271192 AA, HLA-DRB1+ = rs9271192 AC/CC (**A**), apolipoprotein E (APOE) 4- = ε2ε2/ε2ε3/ε3ε3, and APOE4+ = ε3ε4/ε4ε4 (**B**). Cox proportional hazards regression models were applied to explore the effect of different levels of MCP-1 (groups divided by different cutoffs above the median) on the incidence of AD in individuals with different genotypes after adjusting for age, sex, years of education and principal components (PCs). Hazard ratios (HRs) and *p* values are illustrated. The cutoff MCP-1 levels were as follows: 50% percentile: 312.3 pg/ml; 60% percentile: 336.8 pg/ml; 75% percentile: 382.9 pg/ml; and 90% percentile: 455.2 pg/ml

other counterpart genotypes, *HLA-DRB1* rs9271192 AA carriers and non-*APOE* ε4 carriers. In addition, although peripheral MCP-1 levels significantly affected the AD incidence in both females and males who were *HLA-DRB1* rs9271192-AC/CC carriers and *APOE* ε4 carriers ($p < 0.05$), we observed a larger effect on males than on females (Table S5).

Since MCP-1 and CRP are linked in the inflammatory cascade[13,38] and CRP only increases the AD risk in *APOE* ε4 carriers,[15] we examined the joint effect of CRP and MCP-1 levels and their interaction with *HLA-DRB1* rs9271192 and the *APOE* ε4 status on the AD risk. We divided subjects into four groups based on cutoff values for CRP (8 mg/L[15]) and MCP-1 levels (382 pg/ml, third quartile) (Table S6). Among *HLA-DRB1* rs9271192-AC/CC or *APOE* ε4 carriers, using the group with low levels of both CRP and MCP-1 as a reference group, subjects in the combined CRP-high-MCP-1-high group had the highest risk of developing AD among *HLA-DRB1* rs9271192-AC/CC carriers (HR = 2.75, 95% CI = 1.16–6.56, $p = 0.02$) and *APOE* ε4 carriers (HR = 2.94, 95% CI = 1.00–8.65, $p = 0.05$) compared to those who had only high levels of either CRP or MCP-1 (Table 3).

3.3 | Associations of the interactive effects between blood MCP-1 levels and HLA-DRB1 or APOE genotypes on brain volumes

We further investigated whether the interactions of MCP-1 levels with *HLA-DRB1* rs9271192 and *APOE* ε4 genotypes were associated with brain volumes within groups stratified based on genotype using linear regression models. Consistently, after adjusting for age, sex, years of education and PCs, an elevated blood MCP-1 concentration was negatively associated with the HPV and TBV only in *HLA-DRB1* rs9271192-AC/CC or *APOE* ε4 carriers (Figure 2A–J). Specifically, among carriers of *HLA-DRB1* C alleles compared to AA alleles, increased blood MCP-1 levels were associated with smaller HPV (beta ± SE: −0.009 ± 0.004, $p = 0.05$ for AC; −0.04 ± 0.01, $p = 0.005$, for CC; Figure 2B) and TBV (beta ± SE: −0.25 ± 0.08, $p < 0.001$, for AC; −0.51 ± 0.23, $p = 0.03$, for CC; Figure 2C). Similarly, among *APOE* ε4 carriers, elevated MCP-1 levels were negatively associated with HPV (beta ± SE: −0.024 ± 0.006, $p < 0.001$; Figure 2G) and TBV (beta ± SE: −0.31 ± 0.10, $p = 0.001$; Figure 2H). These associations were not observed among *APOE* ε2 carriers and showed attenuated effect sizes among *APOE* ε3 carriers. We did not observe these relationships for white matter hyperintensities (WMHI) in patients with any genotype (data not shown).

3.4 | Associations of blood MCP-1 levels and brain neuropathology among different genotype carriers

The relationship between blood MCP-1 levels and four neuropathological features was evaluated within each genotype group using the χ^2 test to further investigate the effect of peripheral MCP-1 levels on AD ($n = 83$). As shown in Figure 3, in *HLA-DRB1*+ (i.e., rs9271192-AC/CC) carriers, but not rs9271192-AA carriers, the group with high MCP-1 levels tended to have a higher Braak score (Figure 3A, $p = 0.17$) and was significantly associated with a higher CERAD score (neocortical neuritic plaque) (Figure 3B, $p = 0.02$), a higher CERAD semiquantitative score (diffuse plaques) (Figure 3C, $p = 0.03$), and the presence of microinfarcts (Figure 3D, $p = 0.03$). For *APOE* ε4 carriers, but not non-*APOE* ε4 carriers, a high MCP-1 level was significantly associated with a higher Braak score (Figure 3E, $p = 0.02$) and tended to have higher CERAD scores (Figure 3F) and CERAD semiquantitative scores (Figure 3G) than those with low MCP-1 concentrations, but the differences were not statistically significant.

Seven more brain AD phenotypes, including Aβ and p-Tau ($n = 79$), were also examined. Consistently, as shown in Figure 4, among *HLA-DRB1*+ (rs9271192-AC/CC) carriers, a high MCP-1 was significantly associated with higher Aβ40 (Figure 4C, $p = 0.03$) and Aβ42 (Figure 4D,

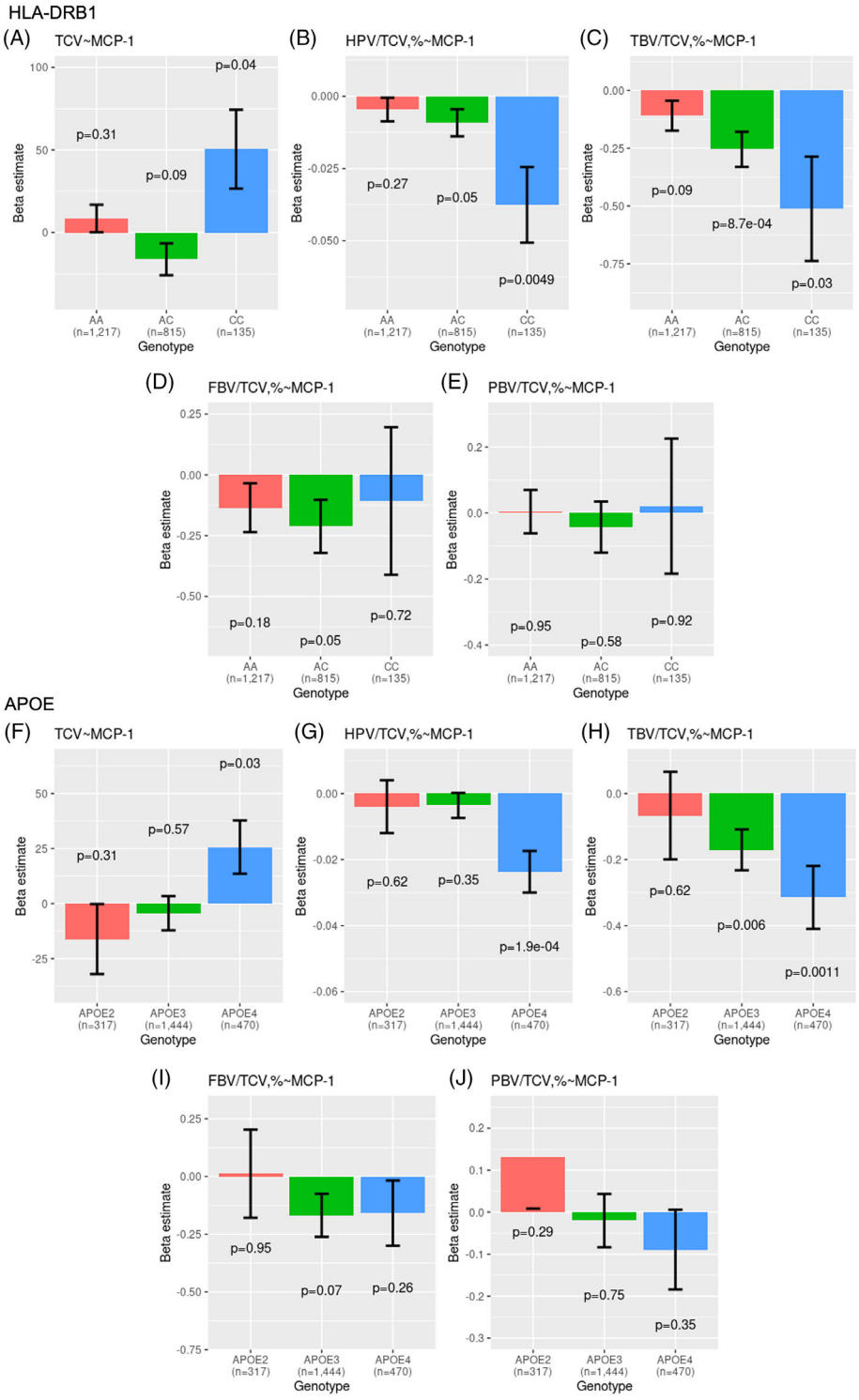

FIGURE 2 Analysis of blood monocyte chemoattractant protein-1 (MCP-1) levels (log-transformed) on structural brain magnetic resonance imaging (MRI), adjusted for age, sex, years of education, and principal components (PCs) in the Framingham Heart Study (FHS) cohort using a linear regression model after stratification by genotype. FHS participants were divided into subgroups based on the genotype of major histocompatibility complex, Class II, DR beta 1 (*HLA-DRB1)* AA versus AC versus CC (A–E) or the genotype of apolipoprotein E (*APOE)* ε2 vs. ε3 versus ε4 (F–J). The brain volumes measured using magnetic resonance imaging (MRI) were analyzed. All other volumes were divided by the total cerebrum cranial volume and multiplied by 100% to adjust for brain size. Linear regression analyses of the relationship between the brain volume and continuous blood MCP-1 concentration (log-transformed) were performed in FHS Gen2 after adjusting for age, sex, years of education and PCs. Bar charts show regression estimates (β) ± SE for the MCP-1 effect on selected structural brain volumes detected using MRI among each of the genotypes with *p* values. Brain volumes: TCV, total cerebrum cranial volume; HPV, hippocampal volume; TBV, temporal lobe brain volume; FBV, frontal lobe brain volume; PBV, parietal lobe brain volume

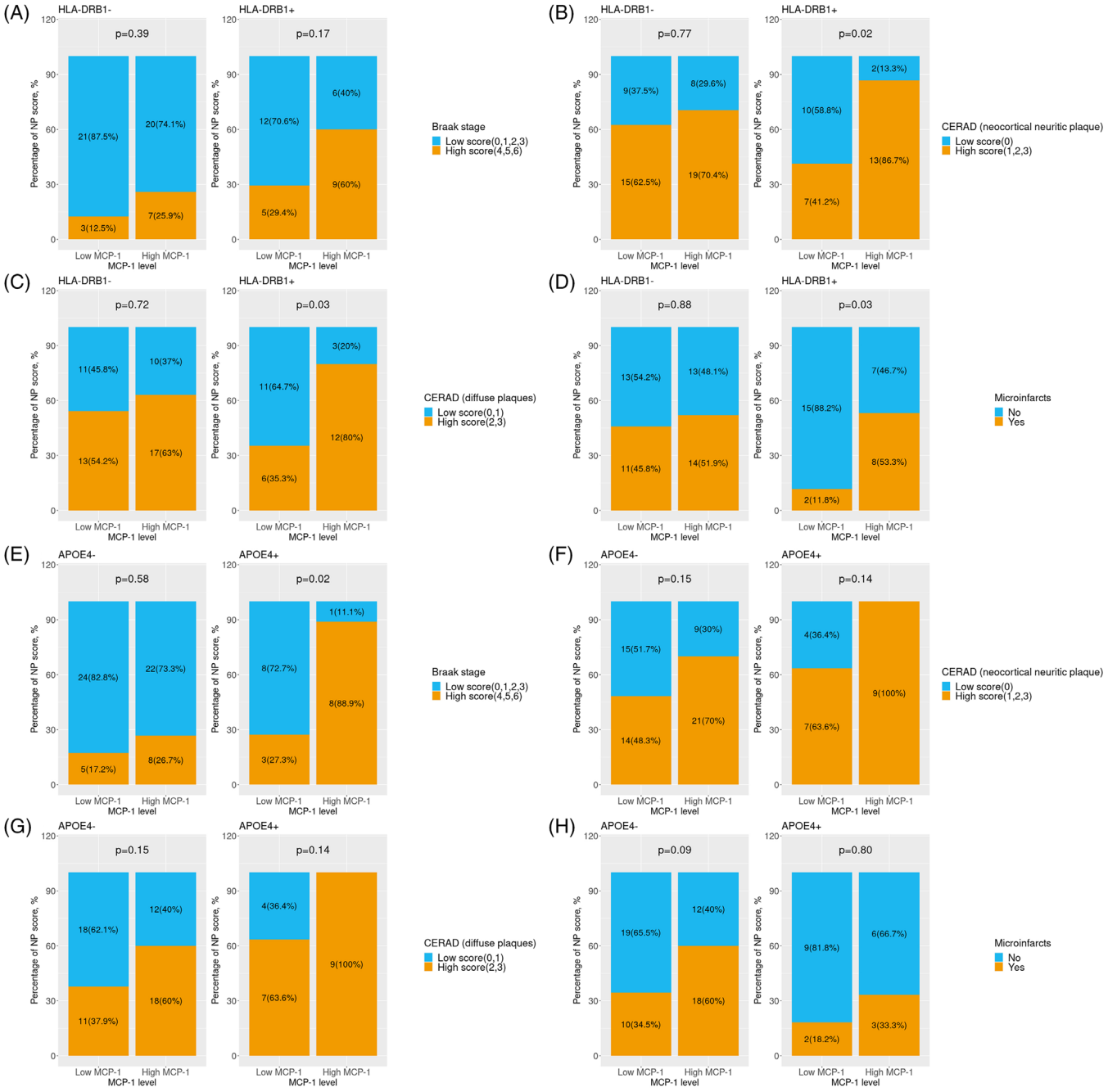

FIGURE 3 Analysis of the effect of blood monocyte chemoattractant protein-1 (MCP-1) levels on brain neuropathology in the Framingham Heart Study (FHS) cohort using the χ^2 test after stratification by genotype. FHS participants with neuropathology data ($n = 83$) were divided into major histocompatibility complex, Class II, DR beta 1- (HLA-DRB1-) = rs9271192 AA versus HLA-DRB1+ = rs9271192 AC+CC genotypes (A–D) or apolipoprotein E (APOE) 4- = nonε4 versus APOE4+ = ε4 genotypes (E–H). Due to a small sample size, brain neuropathology scores were divided into low versus high score groups, including the Braak stage (low = 0, 1, 2, 3 vs. high = 4, 5, 6), Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score (neocortical neuritic plaque, low = 0 vs. high = 1, 2, 3), CERAD semiquantitative score (diffuse plaques, low = 0,1 vs. high = 2, 3), and microinfarcts (no vs. yes). MCP-1 high vs. low groups were based on its median (< median) and high levels (≥ median). Bar charts show the percentage of NP scores groups among MCP-1 level groups for the selected Alzheimer's disease (AD) neuropathology among participants with each of the genotypes along with numbers and χ^2 *p* values

TABLE 3 Interactive effects[a] of MCP-1 and CRP levels on the AD incidence after stratification into different genotypes

		CRP[b] high & MCP-1 low			CRP low & MCP-1 high			CRP high & MCP-1 high		
Gene	Genotypes	N (AD)	HR (95% CI)	*p* value	N (AD)	HR (95% CI)	*p* value	N (AD)	HR (95% CI)	*p* value
HLA-DRB1 rs9271192	AA Reference group[c] (*n* = 1011, AD = 51)	140 (11)	1.20 (0.62–2.33)	0.58	294 (25)	1.19 (0.73–1.94)	0.47	62 (7)	1.27 (0.56–2.88)	0.56
	AC+CC Reference group[c] (*n* = 784, AD = 39)	114 (5)	0.80 (0.31–2.03)	0.64	266 (27)	1.34 (0.81–2.22)	0.26	41 (6)	2.75 (1.16–6.56)	**0.02**
APOE	Non ε4 Reference group[c] (*n* = 1379, AD = 58)	208 (11)	1.10 (0.59–2.05)	0.76	423 (32)	1.10 (0.71–1.69)	0.67	89 (8)	1.57 (0.76–3.23)	0.22
	ε4 Reference group[c] (*n* = 347, AD = 28)	37 (4)	1.02 (0.36–2.93)	0.96	123 (19)	1.88 (1.04–3.43)	**0.04**	10 (4)	2.94 (1.00–8.65)	**0.05**

Notes: FHS participants were divided into subgroups based on the genotype of *HLA-DRB1* AA versus AC versus CC or the genotype of *APOE* nonε4 versus ε4. Four groups were defined by different combinations of MCP-1 and CRP levels based on cutoff values: < or ≥MCP-1: 382 pg/ml (75% percentile); < or ≥CRP: 8 mg/L into the groups of CRP low and MCP-1 low versus CRP high and MCP-1 low versus CRP low & MCP-1 high versus CRP high & MCP-1 high. Reference group: CRP low & MCP-1 low group. The analysis of the effects of blood inflammatory biomarkers* (MCP-1+CRP) on the AD incidence was stratified by genotype and adjusted for age, sex and years of education in the FHS cohort using the Cox proportional hazards regression model.

[a]AD incidences ~ MCP-1&CRP groups + age + sex + years of education, stratified by different genotypes.

[b]CRP, C-reactive protein.

[c]Reference groups: CRP low and MCP-1 low group.

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; CI, confidence interval; FHS, Framingham Heart Study; HLA-DRB1, major histocompatibility complex, Class II, DR beta 1; HR, hazard ratio; MCP-1, monocyte chemoattractant protein-1.

$p = 0.003$) levels and tended to be related to higher levels of different types of p-Tau immunostaining (Figure 4E–G) than those with a low blood MCP-1 concentration. In contrast, these increasing trends were not observed among *HLA-DRB1*- (rs9271192-AA) carriers ($p > 0.05$). For *APOE* ε4 carriers, a high MCP-1 level was significantly associated with higher Aβ40 levels (Figure 4J, $p = 0.025$) and different types of p-Tau immunostaining, that is, AT8 (Figure 4L, $p = 0.032$), pTau202 (Figure 4M, $p = 0.0077$), and pTau231 (Figure 4N, $p = 0.015$), compared to those with a low blood MCP-1 concentration. These relationships were not observed among non-*APOE* ε4 carriers. The Aβ42 level did not reach statistical significance for the comparison (Figure 4K) or for microinfarcts (Figure 3H) within *APOE* ε4 carriers and noncarriers.

In contrast to the typical AD brain pathology, we did not find significant associations of blood MCP-1 levels with the levels of the brain neuroinflammation biomarkers CD68 (Figure 4B and 4I) and Iba1 (Figure 4A and 4H) for participants with either genotype of *HLA-DRB1* or *APOE*. This trend was also not observed for arteriolosclerosis, atherosclerosis or CAA (data not shown).

3.5 | Associations of blood MCP-1 levels and the concentrations of AD biomarkers in CSF among different genotype carriers in the ADNI-1 cohort

We used data from the ADNI-1 cohort to study and validate the relationships in different genotypes by analyzing blood MCP-1 levels and the levels of AD biomarkers in CSF ($n = 231$) to support our findings from the FHS (Table S1). Most likely due to the sample size of the ADNI sample, we did not replicate the interactive effects of either *HLA-DRB1* or *APOE* with MCP-1 levels on AD diagnosis with statistical significance (data not shown). However, consistent with the FHS findings, only *HLA-DRB1* rs9271192-AC/CC carriers with higher blood MCP-1 levels were associated with higher CSF levels of t-Tau ($p = 0.03$) and marginally associated with p-Tau levels ($p = 0.05$) (Table 4). Similar patterns were observed for *APOE* ε4 carriers, but not for *APOE* ε4 noncarriers. *APOE* ε4 carriers with higher MCP-1 levels showed a tendency toward both increased t-Tau and p-Tau levels ($p = 0.08$) (Table 4). Interactions of MCP-1 levels with either *HLA-DRB1* rs9271192 or *APOE* ε4 were not associated with $A\beta_{42}$ levels in CSF.

4 | DISCUSSION

In this study, we found that elevated MCP-1 levels increased the AD risk for carriers of the *HLA-DRB1* rs9271192-C and *APOE* ε4 alleles. Specifically, this risk was higher for carriers with higher blood levels of both CRP and MCP-1. Although MCP-1 is shown to increase BBB permeability by redistributing tight junction proteins and reorganizing the actin cytoskeleton in vascular endothelial cells,[39] our recent preclinical research found that peripheral monomeric CRP levels increased the number of migrated T lymphocytes and monocytes in *APOE* ε4 knock-in mice.[40] Transcytosis of immune cells, especially monocytes and T lymphocytes, across the BBB has been shown to increase neuroinflammation and AD pathology in the brain.[5,41] As both HLA-DRB1 and APOE are expressed in the cerebrovasculature,[42] one possible pathway to enhance AD pathogenesis may be that blood MCP-1

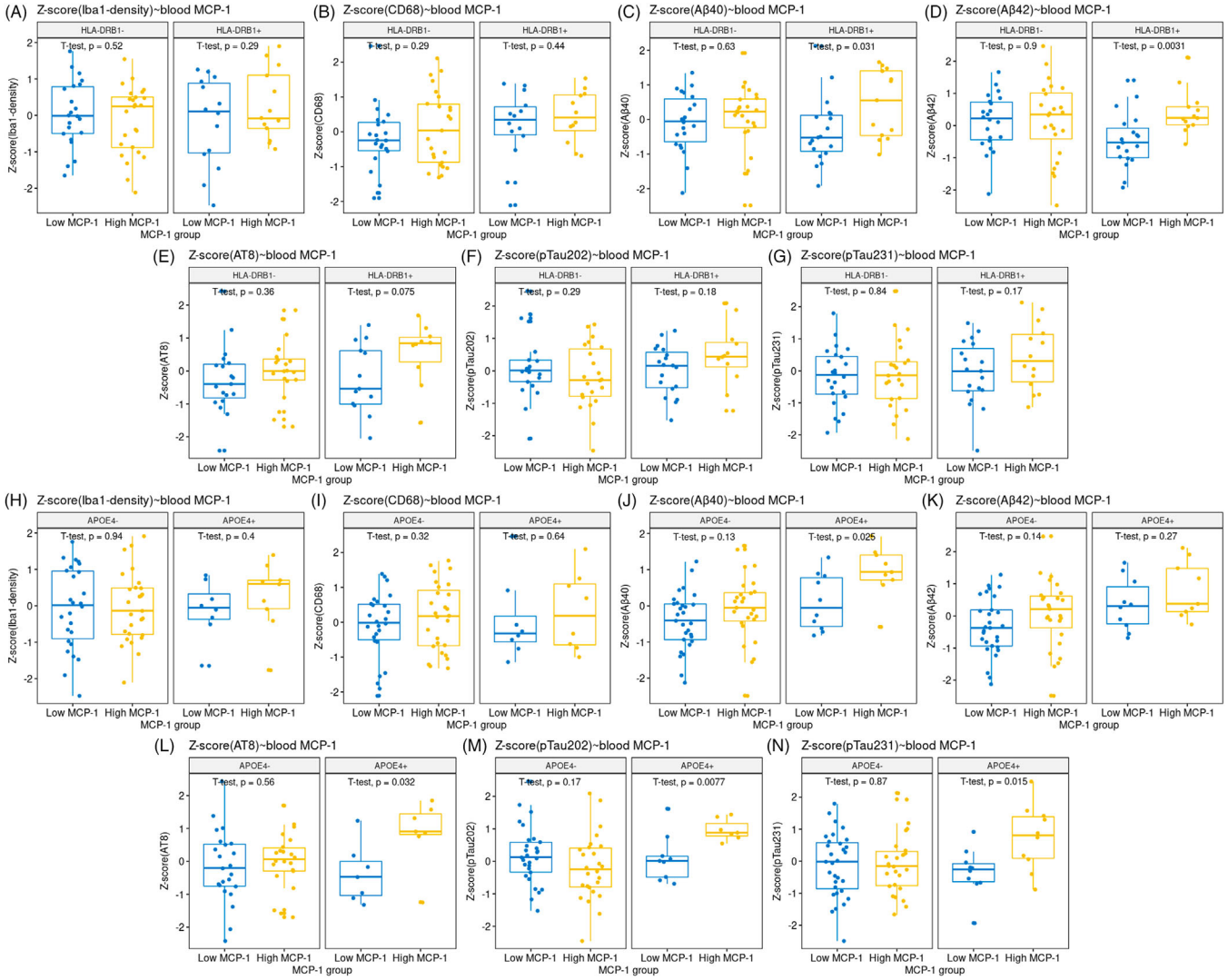

FIGURE 4 Analysis of the effect of blood monocyte chemoattractant protein-1 (MCP-1) levels on brain neuropathology in the Framingham Heart Study (FHS) cohort using a t test after stratification by genotype. FHS participants with continuous neuropathology biomarker data ($n = 79$) were divided into major histocompatibility complex, Class II, DR beta 1- (HLA-DRB1-) = rs9271192 AA versus HLA-DRB1+ = rs9271192 AC+CC genotypes (A–G) or apolipoprotein E (APOE) 4- = nonε4 versus APOE4+ = ε4 genotypes (H–N). Using *t*-tests, brain neuropathology biomarkers, including the Iba1-density (A, H), CD68 (B and I), Aβ40 (C and J), Aβ42 (D and K), AT8 (E and L), p-Tau202 (F, M), and p-Tau231 (G, N) levels, were compared between blood MCP-1 low (< median) versus high (≥median) groups across different genotypes. Boxplots show the Z scores (rank-based inverse normal transformation) for brain neuropathology biomarkers within each genotype along with *p* values calculated using the *t*-test

synergistically works with these two cerebrovascular expressed proteins, HLA-DRB1 and APOE,[6–10,43] by compromising the BBB integrity and inducing the migration of peripheral immune cells into the brain.

Our study suggests that high blood MCP-1 levels are dose-dependently related to the AD risk in participants with the *HLA-DRB1* rs9271192-C and *APOE* ε4 genotypes but not in those with their counterpart genotypes (Table S4 and Figure 1). This finding was supported by the relationships with brain volumes (e.g., temporal lobe and hippocampus) and AD neuropathology in the FHS cohort (Figure 3 and Figure 4), as well as the relationship with CSF t-Tau/p-Tau in the ADNI-1 cohort (Table 4). Previous meta-analyses did not report a significant relationship between blood MCP-1 levels and the risk of developing AD, whereas increased levels of MCP-1 in the CSF have been associated with AD in different studies, when whole samples were used.[44]

HLA-DRB1 plays a key role in the immune system and is expressed in antigen-presenting cells. Its association with AD [19,28] and with AD-related structural brain volumes has been previously reported.[45] The variant rs9271192 of *HLA-DRB1* was found to be associated with its gene expression level in the cerebellum and temporal cortex in both patients with AD and subjects without AD.[46] HLA-DRB1 is related to the immune response and is expressed at high levels in microglia involved in AD.[18] *HLA-DRB1* rs9271192 risk allele carriers may express this gene at higher levels in the cerebrovasculature, leading to enhanced neuroinflammatory responses to inflammatory proteins, such as MCP-1 and an elevated AD risk.

TABLE 4 Effects of blood MCP-1 levels on CSF levels of AD biomarkers in patients with different genotypes of *HLA-DRB1* and *APOE*[a]

		CSF Aβ		CSF t-Tau		CSF p-Tau	
Gene	Genotypes	estimate (SE)	*p* value	estimate (SE)	*p* value	estimate (SE)	*p* value
HLA-DRB1	AA ($n = 129$)	−0.14 (0.26)	0.59	−0.14 (0.20)	0.49	−0.15 (0.23)	0.51
	AC+CC ($n = 113$)	0.02 (0.23)	0.95	0.38 (0.17)	**0.03**	0.41 (0.20)	**0.05**
APOE	Nonε4 ($n = 116$)	0.33 (0.28)	0.23	−0.17 (0.23)	0.46	−0.27 (0.27)	0.32
	ε4 ($n = 126$)	−0.19 (0.16)	0.23	0.27 (0.15)	0.08	0.30 (0.17)	0.08

Notes: ADNI participants were divided into subgroups based on the genotype of *HLA-DRB1* AA versus AC versus CC or the genotype of *APOE* nonε4 versus ε4, and an analysis of the effects of blood MCP-1 levels (log-transformed) on CSF levels of AD biomarkers was performed after stratification by genotype. A linear regression model was used to study the interaction effects between the two genotypes and blood MCP-1 concentration on the levels of AD biomarkers, including Aβ42, total Tau (t-Tau), and phosphorylated Tau (p-Tau), measured in CSF as outcomes after adjustment for age, sex and blood CRP levels in ADNI-1 baseline measurements.

[a]AD biomarkers (baseline) ~ log (MCP-1 baseline) + age + sex + C-reactive protein (CRP), stratified by different genotypes.

[b]Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). Thus, the investigators within the ADNI contributed to the design and implementation of the ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators is available at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; CRP, C-reactive protein; CSF, cerebrospinal fluid; HLA-DRB1, major histocompatibility complex, Class II, DR beta 1; SE, standard error.

We observed the relationship between the blood MCP-1 concentration and the severity of AD pathology, particularly tauopathy, in carriers of the risk alleles *HLA-DRB1* rs9271192 (AC+CC) and *APOE* ε4 (Figures 3 and 4), suggesting that MCP-1 is involved in neurodegeneration. We did not observe a relationship between blood MCP-1 levels and neuroinflammation. On the one hand, peripheral inflammation may predict short-term neuroinflammation in the brain that is attenuated or disappears over time, but we were unable to obtain the brain tissues over time. On the other hand, multiple AD transgenic mouse models expressing either the human APP gene or Tau gene induce neuroinflammation in the brain[47]; AD pathology is strongly associated with neuroinflammation in the human brain.[48] Thus, peripheral inflammation may initially induce or increase the levels of AD pathological proteins in the brain, especially aggregated Aβ and p-Tau,[40] which causes neuroinflammation; thus, peripheral and central inflammation are not directly linked. Notably, p-Tau proteins serving as a seed have been shown to spread and accumulate in the brain over time,[49] which may be another reason why we observed a relationship between blood MCP-1 levels measured years ago and tauopathy in individuals with AD.

This study has a few limitations. The FHS cohort did not undergo lumbar puncture (LP) to obtain CSF levels MCP-1. Most likely, due to the small sample size and age difference in the ADNI cohort, we only observe a statistical tendency to replicate the interactive genotype-MCP-1 effects on AD, although we found that CSF levels of AD biomarkers supported the interactive effects of either *HLA-DRB1* or *APOE* with MCP-1 levels on AD. Since the ADNI study does not include information on the time of AD onset, another factor might be that the survival analysis or Cox proportional hazard regression analysis was not applicable. Future studies are needed to explore the effect of blood MCP-1 levels on AD and AD-related endophenotypes in larger post-mortem cohorts, especially those with multiethnic representation, to assess the generalizability of our findings.

Nevertheless, using two cohorts assessing three aspects, namely, clinical, neuroimaging, and brain pathological biomarkers, our study suggests a likely effect of increased blood MCP-1 levels on AD development in individuals with two AD risk genotypes. As multiple MCP-1 inhibitors are currently being developed and tested in clinical trials for cancer and autoimmune diseases,[50] these medications could be repurposed for AD intervention and prevention in genetic carriers of *HLA-DRB1* rs9271192-AC/CC and *APOE* ε4 with peripheral inflammation.

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CONFLICTS OF INTEREST

None of the authors have conflicts of interest to declare. Author disclosures are available in the supporting information.

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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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