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Levels of Soluble Interleukin 6 Receptor and Asp358Ala Are Associated With Cognitive Performance and Alzheimer Disease Biomarkers

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Abstract

Background and Objectives

Alzheimer disease (AD) is a neurodegenerative disease process manifesting clinically with cognitive impairment and dementia. AD pathology is complex, and in addition to plaques and tangles, neuroinflammation is a consistent feature. Interleukin (IL) 6 is a multifaceted cytokine involved in a plethora of cellular mechanisms including both anti-inflammatory and inflammatory processes. IL6 can signal classically through the membrane-bound receptor or by IL6 trans-signaling forming a complex with the soluble IL6 receptor (sIL6R) and activating membrane-bound glycoprotein 130 on cells not expressing IL6R. IL6 trans-signaling has been demonstrated as the primary mechanism of IL6-mediated events in neurodegenerative processes. In this study, we performed a cross-sectional analysis to investigate whether inheritance of a genetic variation in the *IL6R* gene and associated elevated sIL6R levels in plasma and CSF were associated with cognitive performance.

Methods

We genotyped the *IL6R* rs2228145 nonsynonymous variant ($Asp^{358}Ala$) and assayed IL6 and sIL6R concentrations in paired samples of plasma and CSF obtained from 120 participants with normal cognition, mild cognitive impairment, or probable AD enrolled in the Wake Forest Alzheimer's Disease Research Center's Clinical Core. IL6 rs2228145 genotype and measures of plasma IL6 and sIL6R were assessed for relationships with cognitive status and clinical data, including the Montreal Cognitive Assessment (MoCA), modified Preclinical Alzheimer's Cognitive Composite (mPACC), cognitive domain scores obtained from the Uniform Data Set, and CSF concentrations of $phosphoTau^{T181}$ (pTau181), β-amyloid (Aβ) Aβ40 and Aβ42 concentrations.

Results

We found that inheritance of the *IL6R* Ala^{358} variant and elevated sIL6R levels in plasma and CSF were correlated with lower mPACC, MoCA and memory domain scores, increases in CSF pTau181, and decreases in the CSF Aβ42/40 ratio in both unadjusted and covariate-adjusted statistical models.

Discussion

These data suggest that IL6 trans-signaling and the inheritance of the *IL6R* Ala^{358} variant are related to reduced cognition and greater levels of biomarkers for AD disease pathology. Follow-up prospective studies are necessary, as patients who inherit *IL6R* Ala^{358} may be identified as ideally responsive to IL6 receptor–blocking therapies.

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Glossary

AD = Alzheimer disease; **ADRC** = Alzheimer's Disease Research Center; **Aβ** = β-amyloid; **ALS** = amyotrophic lateral sclerosis; **APOE** = apolipoprotein E; **BMI** = body mass index; **BBB** = blood-brain barrier; **CI** = cognitive impaired; **CN** = cognitively normal; **DSST** = Digit Symbol Substitution Test; **FCSRT** = Free and Cued Selective Reminding Test; **gp130** = glycoprotein 130; **IL** = interleukin; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **MoCA** = Montreal Cognitive Assessment; **mPACC5** = modified Preclinical Alzheimer's Cognitive Composite; **pTau** = phosphorylated tau; **pTau181** = phosphorylated Tau^{T181}; **sIL6R** = soluble IL6 receptor; **UDSv3** = Uniform Data Set Version 3.

Alzheimer disease (AD) is a neurodegenerative disease process characterized primarily by β-amyloid (Aβ) plaques and neurofibrillary tangles of tau protein, manifesting clinically with cognitive impairment and dementia.[1] AD is the most common form of dementia, accounting for an estimated 60%–80% of dementia cases. AD progression is marked through 5 stages, beginning with preclinical through severe dementia.[2] Altered levels of AD biomarkers, such as Aβ and phosphorylated tau (pTau), in the absence of measurable cognitive impairment can indicate the preclinical AD phase, suggesting that underlying disease processes are well under way before overt symptoms appear.[3] For this reason, it has been hypothesized that treatment after symptom onset is too late for substantial beneficial outcomes.[4] Although age is the primary risk factor for AD, identification of other risk factors and modifiers in the early stages of AD may help tailor therapeutic options for patients to delay disease progression.

Cognitive impairment pathology is a complex multifactorial process that, while not fully understood, involves cardio/cerebrovascular, metabolic, and inflammatory processes.[5-7] IL6, a multifunctional cytokine, influences diverse cellular mechanisms, including cell growth, metabolism, differentiation, death, and inflammatory and anti-inflammatory processes with critical influences in the CNS.[8-11] In animal models, blocking IL6 activity or inhibiting downstream Stat3 activation improves cognitive deficits and peripheral glucose intolerance.[12] Furthermore, inhibition of IL6 signaling in animal models has also been shown to decrease amyloid plaque burden in both the cortex and the hippocampus.[13] In humans, increased IL6 levels in serum/plasma and CSF have been reported in patients with AD[12,14] and have been proposed to influence the early stages of amyloid plaque formation and tau hyperphosphorylation.[6,15]

IL6 activity is mediated through 2 different signaling mechanisms: classical IL6 signaling and IL6 trans-signaling.[9] Classical IL6 signaling occurs when IL6 binds membrane-bound IL6 receptor on specific, limited cell populations (e.g., T cells and hepatocytes) and triggers intracellular signaling through membrane-bound glycoprotein 130 (gp130) coreceptor to activate downstream transcription factors such as Stat3. In contrast, IL6 trans-signaling is driven by the presence of extracellular soluble IL6 receptors (sIL6Rs) that are generated by receptor shedding, a process where metalloproteinases cleave IL6 receptors from the cell surface.[7,16] Once cleaved from the cell surface, sIL6R can form an active complex with IL6 and engage membrane-bound coreceptor gp130. Because gp130 is constitutively expressed on all cells, IL6 trans-signaling can activate IL6-dependent cell signaling in cells that do not express IL6 receptor. In the nervous system, IL6 trans-signaling has been proposed to be the major pathologic mechanism of IL6-mediated diseases.[17]

In humans, the level of sIL6R is strongly associated with an *IL6R* coding single nucleotide polymorphism, $Asp^{358}Ala$ (A/C; rs2228145),[18] where the *IL6R* C allele (Ala^{358}) is the minor allele. In humans, the presence of the C allele accounts for >50% of the variation of sIL6R levels.[13,19,20] Most importantly, *IL6R* Ala^{358} variant is frequent in multiple ethnicities, occurring at frequencies of ~10% in African, ~40% in European, and ~50% in Native American ancestries.[18,21] Those inheriting the *IL6R* Ala^{358} variant, referred here as the C* genotype group, exhibit increased concentrations of sIL6R and are at a high risk of localized and systemic IL6 trans-signaling in the presence of elevated IL6.[13,18-22]

The presence of the *IL6R* Ala^{358} variant appears to be a modifier of neurologic disorders, such as amyotrophic lateral sclerosis (ALS) and has been linked as a risk factor for both AD and lower cognitive performance.[19,20,23,24] Therefore, given the potential effects of IL6 trans-signaling on neurologic conditions, AD pathology in murine models, and that the possession of the *IL6R* Ala^{358} variant has been reported to be a potential AD risk factor,[25] we investigated whether levels of sIL6R and the presence of the *IL6R* Ala^{358} variant are associated with cognitive performance and AD pathologic biomarkers phosphorylated Tau^{T181}(pTau181) and Aβ42/40 ratio.[26,27]

Methods

Study Participants

Adults between the ages of 55 and 85 years were recruited into the Clinical Core of the Wake Forest Alzheimer's Disease Research Center (ADRC) from the surrounding community between 2016 and 2020 and underwent standard evaluation in accordance with the National Alzheimer's Coordinating Center protocol for clinical data collection, including clinical examinations, neurocognitive testing, and neuroimaging. Exclusion criteria for this cohort included large vessel stroke (participants with lacunae or small vessel ischemic disease were eligible), other significant neurologic diseases that might affect cognition other than AD, evidence of organ failure, active cancer, uncontrolled clinical depression, psychiatric

Table 1 Study Participant Demographics

	Total	AA	C* genotype group	*p* Value AA vs C*
No. of participants (% female)	118 (64%)	46 (76%)	72 (57%)	0.034
Race				0.002
African American (number; %F)	11 (73%)	9 (89%)	2 (0%)	
Caucasian (number; %F)	107(64%)	37 (73%)	70 (43%)	
Age (y)				0.346
Mean ± SD	69.4 ± 7.7	70.2 ± 7.7	68.8 ± 7.6	
Min-max; 95% CI	55–88; 54–84.8	56–88; 54.8–85.6	55–86; 53.6–85	
Education (y)				0.562
Mean ± SD	16.1 ± 2.3	15.9 ± 1.9	16.2 ± 2.5	
Min-max; 95% CI	12–20; 11.5–20.7	12–20; 12.1–19.7	12–20; 11.2–21.2	
BMI				0.200
Mean ± SD	27.5 ± 4.95	28.2 ± 5.7	27.0 ± 4.4	
Min-max; 95% CI	18.3–41.6; 17.6–37.4	18.3–41.6; 16.8–39.6	19.6–37.7; 18.2–35.8	
Cognitive status (no. of participants; % female)				0.388
Normal	66 (79% F)	28 (82% F)	38 (76% F)	
Impaired	52 (48% F)	18 (67% F)	34 (38% F)	

illness, current use of insulin, history of substance abuse, or heavy alcohol consumption within previous 10 years. Of the 689 participants from the ADRC cohort, 120 consented to both CSF and plasma collection. One hundred eighteen were selected for this study as 2 had incomplete data and could not be included in the adjusted models (Table 1). The subset was disproportionally Caucasian and had higher Montreal Cognitive Assessment (MoCA) scores than the excluded participants (eTable 1, links.lww.com/NXI/A807).

Standard Protocol Approvals, Registrations, and Patient Consents

All research activities were approved by the Wake Forest Institutional Review Board, and written informed consent was obtained for all participants and/or their legally authorized representative.

Determination of *IL6R* Genotype

DNA was genotyped for the *IL6R* Ala^{358} variant (rs2228145) using a validated TaqMan assay for rs2228145, following the manufacturer's instructions (Applied Biosystems, assay ID: C_16170664_10) and a ViiA7 Real-Time PCR system (Applied Biosystems), as previously performed.[19-20]

Determination of Protein Concentrations

Participants who consented to CSF collection completed plasma and lumbar puncture in the morning after an overnight fast. Biofluids were processed as previously described.[28]

CSF levels of Aβ40, Aβ42, and pTau181 were measured using automated chemiluminescent enzyme immunoassays on a Lumipulse G1200 analyzer in the Neuropathology Core of the Wake Forest ADRC. Intra- and inter-assay CVs for all analytes were <6%.

Plasma and CSF IL6 and sIL6R levels were measured with commercial kits (R&D Systems, Q6000B and DR600, respectively) according to the manufacturer's instructions. ELISA plates were read on a Wallac plate reader. Detection limits of assays are reported by the manufacturer to be 0.16 pg/mL for IL6 and 6.5 pg/mL for sIL6R. All measurements for each analyte were above the kit limits of quantification (eTable 2, links.lww.com/NXI/A807).

Cognitive Assessments

Participants completed cognitive testing with the Uniform Data Set Version 3 (UDSv3)[29] test battery, including MoCA, Craft Story, Benson Figure, Number Span, Verbal Fluency, Category Fluency, Trail Making Test, and the Multilingual Naming Test, as well as supplemental tests (Mini-Mental State Examination [MMSE], American National Adult Reading Test, Digit Symbol Substitution Test [DSST], Free and Cued Selective Reminding Test [FCSRT], and the Rey Auditory Verbal Learning Test). MoCA scores were normalized to create z scores based on age, race, sex, and education. Subjective questionnaires assessing mood and perceived change in cognitive symptoms were administered at this visit including the 15-item Geriatric Depression Scale, the Clinical Dementia Rating scale, and the Functional Assessment Questionnaire, which was used to estimate capacity to manage activities of daily living.

UDSv3 cognitive tests scores were normalized to create z scores based on age, race, sex, and education.[29] Z scores were combined to create domain-specific cognitive performance for executive function, memory, language, attention, and visuospatial and phonemic fluency. A modified Preclinical Alzheimer's Cognitive Composite (mPACC5)[30] was created from 5 cognitive tests: the MMSE, FCSRT, Craft Story verbatim recall of the Craft Story, DSST, and category fluency.

Adjudication of cognitive diagnosis by expert panel consensus occurred following review of all available clinical, neuroimaging, and cognitive data in accordance with current National Institute of Aging–Alzheimer's Association guidelines for diagnosis of MCI,[30] AD, and their subtypes.[31] The panel consisted of investigators with extensive experience assessing cognitive status and identifying cognitive impairment in older adults, including neuropsychologists, neurologists, and geriatricians.

Statistical Analysis

For statistical analysis, individuals were classified by cognitive status into 2 groups: (1) cognitively normal (CN) and (2) cognitively impaired (CI; combined mild cognitive impairment [MCI] and AD). To determine potential covariates, cohort demographics were compared between CN and CI groups using binomial logistic regression analysis. IL6 trans-signaling biomarkers were related to continuous outcomes using linear regression analysis. Multiple demographic variables, including sex ($p < 0.001$), age ($p < 0.001$), and education ($p > 0.001$), were all significantly correlated with cognitive status or cognitive performance and were therefore included as basic covariates in subsequent analysis. Race, body mass index (BMI), tobacco, and alcohol use have previously been related to IL6 concentrations in CSF and plasma and were therefore adjusted for in subsequent analysis involving IL6 trans-signaling biomarkers[7] (eTable 3, links.lww.com/NXI/A807). Inheritance of the apolipoprotein E (ApoE)4 gene is the most prominent genetic risk factor for AD.[32] *APOE* genotype data were available for all 120 participants; however, no significant interactions between *APOE* genotype and *IL6R* genotype were found with our measured variables (eTable 4, links.lww.com/NXI/A807). Therefore, *APOE* genotype was not used as a covariate in subsequent analyses. Three models were used for analysis involving cognitive performance and IL6 trans-signaling biomarkers. Model 1 reported unadjusted relationships. Model 2 adjusted for age, race, sex, and education. Model 3 included model 2 adjustments and adjustments for BMI, tobacco, and alcohol use. Analysis involving MoCA and Memory Domain scores only used models 2 and 3 as the data were received as adjusted z scores as previously described. We also evaluated effect modification for the presence of *IL6R* Ala^{358} with cognitive status. Participants inheriting 1 (heterozygous) or 2 (homozygous) copies of *IL6R* Ala^{358} were combined into a single group termed the C* genotype group. Participants homozygous for the *IL6R* Asp^{358} variant were termed the AA group. We examined the ratio of Aβ42/40, as decreases in Aβ42/40 ratio have consistently been demonstrated as a marker for AB aggregation and plaque formation.[26] Unadjusted and model 3 adjusted p and r^2 values are provided in the Results and Figures.

Data Availability

Data not provided in the article because of space limitations will be shared on request of other investigators for purposes of replicating procedures and results.

Results

Participant Characteristics

Paired plasma and CSF (n = 120) and DNA samples were obtained from 66 CN participants, 46 participants with MCI, and 8 participants with AD through the Wake Forest ADRC. To increase statistical power, MCI and AD were combined into the CI group. The demographic and genotype information for each participant included age, sex, race, and education. Cohort data are summarized in Table 1.

Genotyping of the *IL6R* $Asp^{358}Ala$ (rs2228245) polymorphism revealed 57 individuals homozygous for the major (A allele) *IL6R* Asp^{358} variant, 46 heterozygous, and 17 homozygous for the variant (C allele) *IL6R* Ala^{358} variant. The IL6R rs2228245 genotyping data were in Hardy-Weinberg equilibrium and the allele frequencies were consistent other study populations.[18,20]

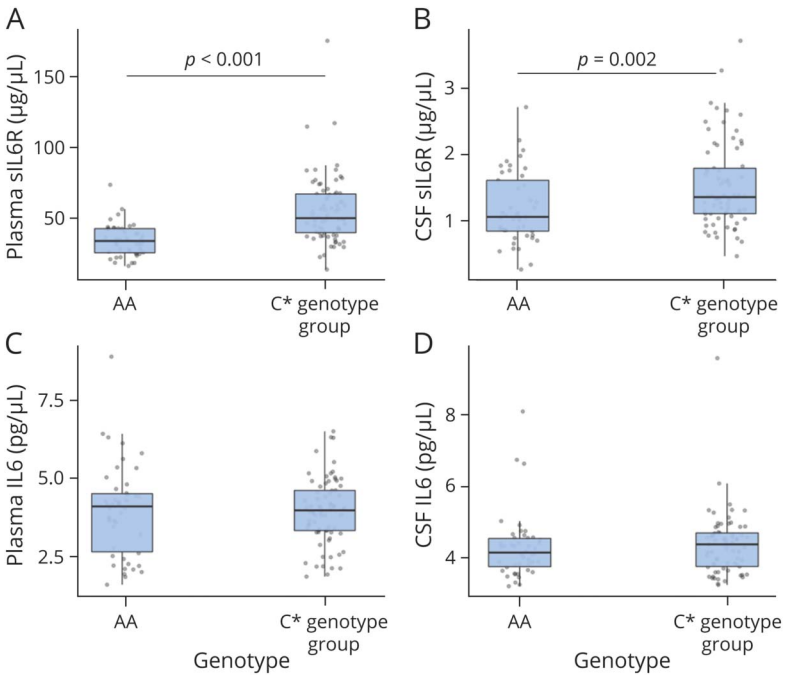
Participants Possessing the *IL6R* Ala^{358} Variant Have Higher Concentrations of sIL6R in CSF and Plasma

Possession of at least 1 copy of the C allele is enough to cause a physiologically significant increase in sIL6R levels and allow for increased IL6 trans-signaling[18] (eTable 2, links.lww.com/NXI/A807). Regardless of cognitive status, the C* genotype group was associated with statistically significant increases in both plasma and CSF sIL6R (Figure 1, A and B), in agreement with previous reports (21-24). There was no association between IL6 levels and IL6R genotype (Figure 1, C and D).

When analyzed against cognitive status, the CI group had increased plasma ($p = 0.034$) and CSF sIL6R levels compared with CN, although the increase in CSF sIL6R levels did not reach statistical significance (Figure 2, A and B). Higher levels of IL6 were also detected in the plasma of CI participants (Figure 2, C and D).

Regardless of cognitive status, individuals within the C* genotype group exhibited increased levels of plasma and CSF sIL6R compared with those in the AA group (Figure 3, A and B). There were no differences in plasma or CSF IL6 cytokine levels between the C* genotype group and AA group across the CI or CN groups (Figure 3, C and D). In the C* genotype group, plasma IL6 was significantly increased in the CI group (Figure 3C). No other associations were found between genotype group and cognitive status for IL6 trans-signaling biomarkers.

Figure 1 Participants in the C* Genotype Group Have Higher Concentrations of sIL6R in CSF and Plasma Indicating Potential for Increased IL6 Trans-signaling

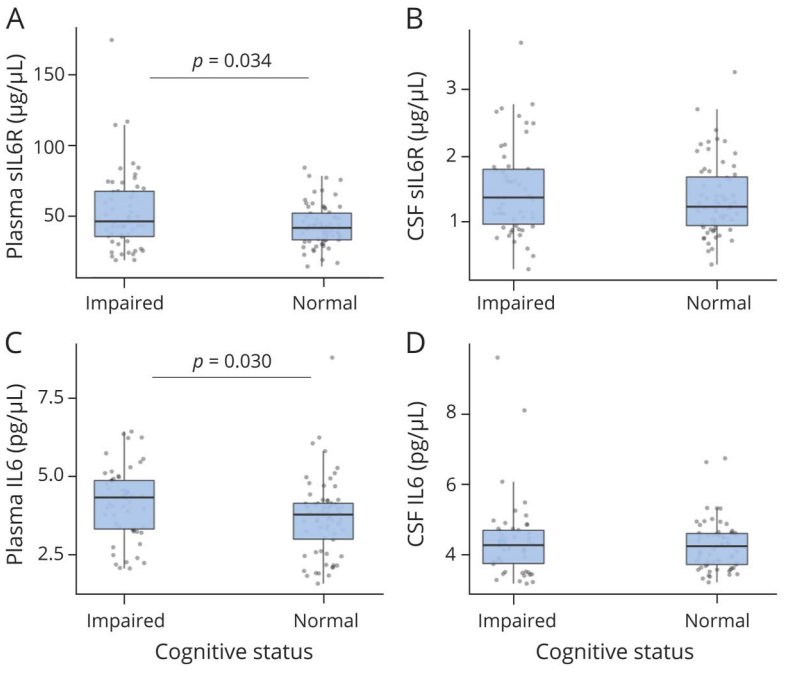


(A) Plasma sIL6R is significantly elevated in those within the C* genotype group (n = 116; coefficient of variation (CV) = 0.47: adj r^2 = 0.181; adj p < 0.001). (B) CSF sIL6R is significantly increased in subjects possessing the *IL6R* Ala^{358} variant (n = 116; CV = 0.44; adj r^2 = 0.044; adj p = 0.013). (C) Plasma IL6 concentrations do not have any significant change in individuals within the C* genotype group (CV = 0.28; n = 74; p = 0.372). (D) CSF IL6 concentrations do not have any significant change in individuals within the C* genotype group (CV = 0.20; n = 69; p = 0.535). Significance was determined using multivariable linear regression adjusting for age, sex, race, BMI, alcohol, and tobacco use. sIL6R = soluble IL6 receptor.

IL6R Ala358 Variant Is Associated With Lower Cognitive Scores

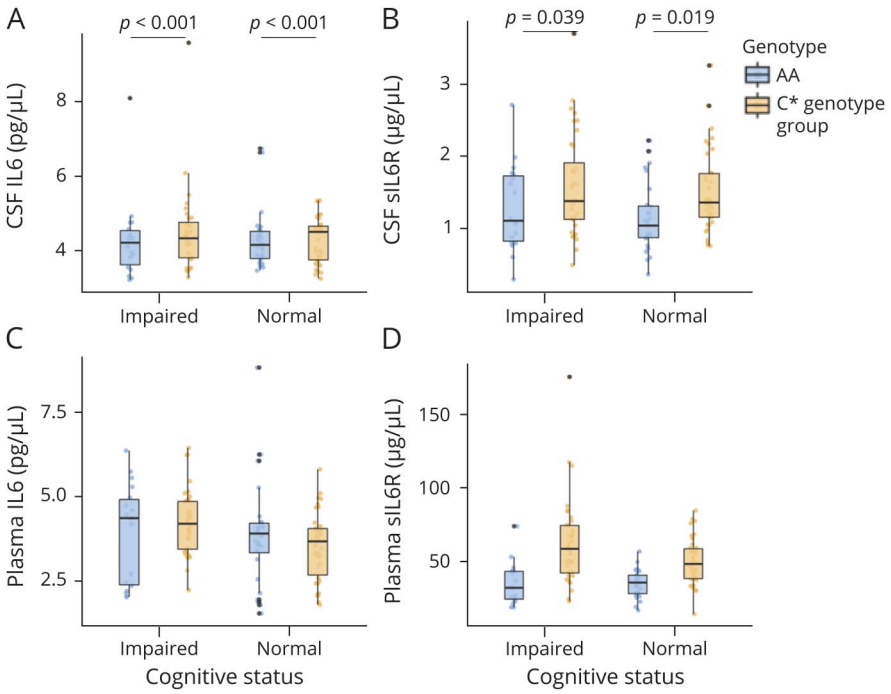
To determine whether the *IL6R* Ala^{358} variant is associated with cognitive performance, we evaluated global cognitive performance with the MoCA z scores and mPAAC, as well as the specific domain scores (Figure 4). Across the entire cohort, participants in the C* genotype group had decreased performance on cognitive performance assays (Figure 4, A–C).

Figure 2 Cognitive Status Appears to Influence IL6 Receptor and Cytokine



(A) Plasma sIL6R is significantly increased in individuals who are cognitively impaired (n = 116; CV = 0.47; adj p = 0.029; adj r^2 = 0.041). (B) CSF sIL6R concentrations appear to be increased in cognitively impaired subjects but is not statistically significant (n = 116; CV = 0.44; p = 0.185; adj p = 0.300; adj r^2 = 0.009). (C) Plasma IL6 concentrations are significantly increased in cognitively impaired subjects (CV = 0.31; n = 116; adj p = 0.029 adj r^2 = 0.040). (D) There were no differences in CSF IL6 concentrations between cognitively impaired and normal individuals (CV = 0.21; n = 118; p = 0.235). Significance was determined using multivariable linear regression adjusting for age, sex, race, BMI, alcohol, and tobacco use. CV = coefficient of variation; sIL6R = soluble IL6 receptor.

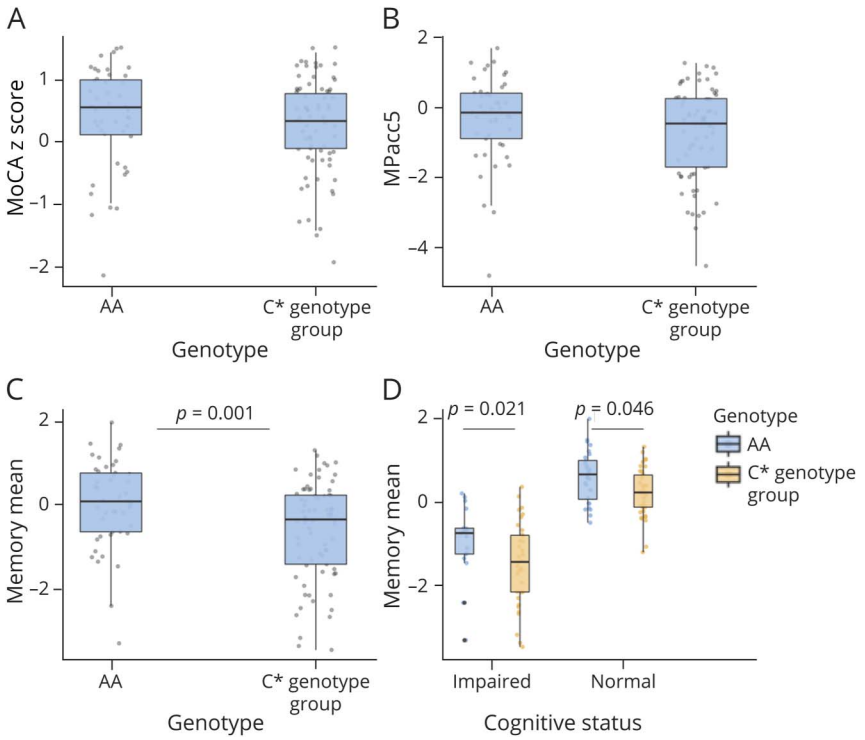
Figure 3 Genotype, Not Cognitive Status, Accounts for Levels of Plasma and CSF IL6R Levels



(A) Plasma sIL6R concentrations are significantly elevated in individuals of the C* genotype group in both the CI (n = 52; CV = 0.43; adj p < 0.001; adj r^2 = 0.301) and CN groups (n = 66; CV = 0.32; p < 0.001; adj p = 0.005; adj r^2 = 0.301). (B) CSF sIL6R concentrations are significantly elevated in individuals in the C* genotype group in both the CI (n = 52; CV = 0.41; adj p = 0.039; adj r^2 = 0.159) and CN groups (n = 66; CV = 0.44; adj p = 0.019; adj r^2 = 0.159). (C) Plasma IL6 concentrations do not significantly change in those with the C* genotype group across both the CI and CN groups (CV = 0.28 n = 74; p = 0.372). In the C* genotype group, plasma IL6 was significantly increased in the CI group (CV = 0.28; n = 74; adj p = 0.013; adj r^2 = 0.318). (D) CSF IL6 concentrations do not significantly change in individuals with the C* genotype group across both the CI and CN groups (CV = 0.20 n = 69; p = 0.535) Significance was determined via binomial logistic regression analysis adjusting for age, sex, race, education, BMI, alcohol, and tobacco use. For multiple categorical outcomes, significance was determined via multinomial logistic regression adjusting for genotype, age, sex, race, education, BMI, alcohol, and tobacco use. CI = cognitive impaired; CN = cognitively normal; CV = coefficient of variation; sIL6R = soluble IL6 receptor.

Individuals in the C* genotype group performed significantly worse on the memory domain (Figure 4C) and lower on global cognitive performance (MoCA and mPAAC), although relationships with global score did not reach statistical significance (Figure 4, A and B). When the entire cohort was analyzed by both cognitive status and being in the C* genotype

Figure 4 Participants in the C* Genotype Group Have Lower Cognitive Performance Scores



(A) Average MoCA z scores appear decreased in individuals within the C* genotype group; however, the decrease is not statistically significant (n = 116; p = 0.215). (B) Average Mpacc5 scores appear to be decreased in those within the C* genotype group: however, the decrease is not statistically significant (n = 114; p = 0.098). (C) Memory domain mean scores are significantly decreased in subjects with the C* genotype group (n = 116; p = 0.001; adj p = 0.013; adj r^2 = 0.043). (D) Memory domain mean scores are significantly decreased in subjects with the C* genotype group in both the CI (n = 51; p = 0.021; adj p = 0.015; adj r^2 = 0.139) and CN (n = 65; CV = 1.6; p = 0.044; adj p = 0.039; adj r^2 = 0.139) groups. CI = cognitive impaired; CN = cognitively normal; CV = coefficient of variation; MoCA = Montreal Cognitive Assessment; mPACC5 = modified Preclinical Alzheimer's Cognitive Composite.

group, lower memory domain scores were observed in both CI and CN (Figure 3D).

Higher Levels of sIL6R Are Correlated With Lower Cognitive Scores

When we looked at sIL6R levels and cognitive performance scores, increased sIL6R levels were correlated with poorer performance in both the global and memory-specific cognitive tests (Figure 5). The correlations were seen in both plasma and CSF values for both global cognitive performance and memory domain tests. The correlation was greater in the memory domain with plasma sIL6R and CSF sIL6R (Figure 5, C and D). Because both plasma and CSF values were correlated with poorer cognitive performance, we examined whether there was a correlation between plasma and CSF levels. Here, we found significant correlations between CSF IL6 and plasma IL6 as well as CSF sIL6R and plasma sIL6R (eFigure 1A, B, links.lww.com/NXI/A807).

Higher Levels of CSF sIL6R Are Correlated With AD Pathologic Biomarkers

In light of our data suggesting that IL6 trans-signaling may be correlated with lower cognitive performance (Figure 4), we next looked at potential IL6 trans-signaling effects on the classic AD pathologies (pTau181 and Aβ42/40 ratio). CSF sIL6R positively correlated with CSF pTau181 concentration and inversely correlated with CSF Aβ42/40 ratio (Figure 6, B and C). There was no correlation between plasma sIL6R and pTau181 or Aβ42/40 ratio (eFigure 2A, B, links.lww.com/NXI/A807).

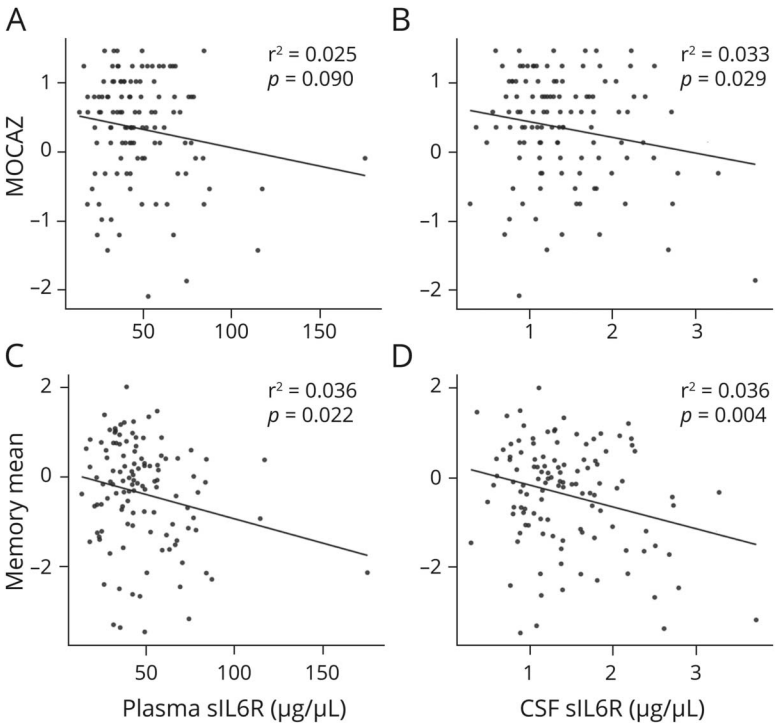
When looking at cognitive performance, individuals in the C* genotype group had significantly worse cognitive performance across the entire cohort and in both the CI and CN groups; however, being in the C* genotype group had no significant effect on pTau181 or AB42/40 ratio (Figure 6, A and B). We found no associations between plasma or CSF IL6 and AD biomarkers (data not shown).

Inheritance of the apolipoprotein E4 gene is the most prominent genetic risk factor for AD.[32] We analyzed APOE genotype for any interaction effects with *IL6R* genotype and measured variables. No interactions between *APOE* genotype and *IL6R* genotype were found with our measured variables (eTable 4, links.lww.com/NXI/A807).

Discussion

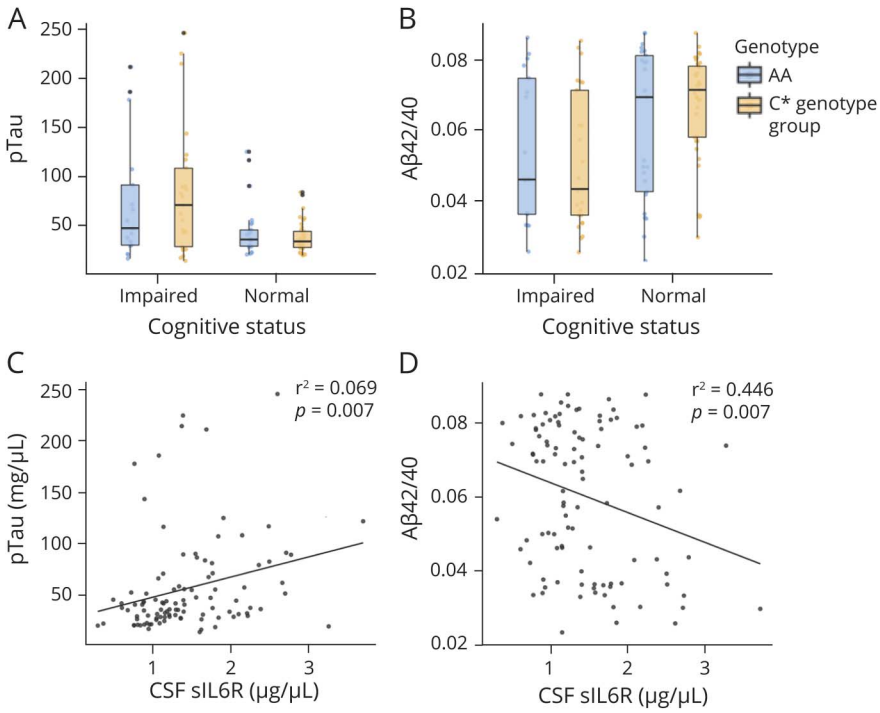
Age-related changes in the brain, MCI, and AD-related forms of dementia are correlated with physiologic changes within and outside of the CNS. To determine whether IL6 trans-signaling and the *IL6R* rs2228145 variant are associated with CI in AD, we examined a cohort of 120 mid- to late-aged adults with normal cognition, MCI, and AD. In the total sample, higher levels of sIL6R were correlated with poorer performance on global and memory-specific cognitive tests. Higher CSF sIL6R was also associated with AD biomarkers, higher pTau181, and lower Aβ42/40 ratio. Of interest, there was no correlation between plasma sIL6R and pTau181 or AB42/40 ratio despite a significant correlation between CSF

Figure 5 Higher sIL6R Values Are Correlated With Lower Cognitive Performance Scores



(A) MoCA z scores have a negative correlation with plasma sIL6R that is not statistically significant. (B) MoCA z scores have a significant negative correlation with CSF sIL6R (n = 114; $p = 0.029$; adj $p = 0.016$ $r^2 = 0.033$). (C and D) Memory domain mean scores have a significant negative correlation with plasma sIL6R (n = 114; $p = 0.022$; adj $p = 0.025$; adj $r^2 = 0.036$) and CSF sIL6R (n = 114; $p = 0.004$; adj $p = 0.020$; adj $r^2 = 0.038$). For both genotype and IL6 trans-signaling biomarker relationships with cognitive performance assays, significance was determined via multivariate linear regression analysis adjusting for age, sex, race, education, BMI, alcohol, and tobacco use. MoCA = Montreal Cognitive Assessment; sIL6R = soluble IL6 receptor.

Figure 6 CSF sIL6R Levels Are Correlated With Pathologic AD Biomarkers



(A and B) Inheritance of IL6R Ala358 did not influence CSF pTau181(n = 102; adj p = 0.474) concentrations or AB42/40 ratio (n = 102; adj p = 0.568) in either the CI (n = 61; adj p = 0.531) or CN group (n = 41; p = 0.991). (C) CSF sIL6R has a significant positive correlation with CSF pTau181 (n = 102; CV = 0.80; p = 0.007; adj p = 0.046; adj r^2 = 0.035). (D) CSF sIL6R has a significant negative correlation with CSF AB42/40 ratio (n = 102; CV = 0.33; p = 0.007; adj p = 0.023; adj r^2 = 0.044). The correlation between CSF sIL6R and CSF pTau181 and CSF AB42/40 ratio universally affects the cohort the same regardless of cognitive status or being in the C* genotype group. Significance was determined via multivariate linear regression analysis adjusting for age, sex, race, education, BMI, alcohol, and tobacco use. CI = cognitive impaired; CN = cognitively normal; CV = coefficient of variation; sIL6R = soluble IL6 receptor.

and plasma sIL6R concentrations. Therefore, the correlation with pTau, AB42/40 ratio, and CSF sIL6R levels may indicate that increased IL6 trans-signaling in the CNS may influence AD pathology.

IL6R Ala^{358} variant was associated with increased sIL6R concentrations in CSF and plasma, and elevated CSF sIL6R concentrations were correlated with AD biomarkers. However, possession of the *IL6R* Ala^{358} variant was not directly correlated to changes in AD biomarkers in this cohort. Overall, these results are in agreement with a previous larger study suggesting that subjects in the C* genotype group have an increased risk of AD[25] or may modify disease progression as proposed in asthma and ALS.[13,18]

We did not see elevations in IL6 concentrations across the C* genotype group in the CSF for either CI or CN groups. IL6 trans-signaling occurs when IL6 and sIL6R form an extracellular complex and activate IL6 pathways through membrane-bound gp130 in cells that normally do not express IL6 receptors. In the current study, elevated sIL6R was correlated with worse CI symptoms/markers (Figures 4 and 5). Cytokine effects occur at a cellular or microenvironment level. As such, the availability of IL6 from local glial cells may be sufficient to initiate IL6 trans-signaling to promote cellular processes contributing to cognitive impairment. In a disease such as AD that is hypothesized to evolve over decades, overall levels of IL6 cytokine may not accumulate to detection in CSF but may be sufficient to promote local pathogenesis. In a previous study[18] investigating the potential effects of IL6 trans-signaling and the *IL6R* Ala^{358} variant in ALS, a disease that appears to progress much more rapidly, patients with ALS in the C* genotype group had increased CSF IL6 and sIL6R levels. In our ADRC cohort, the majority of participants were evaluated as CN or MCI, with a very small number reaching the criteria for AD diagnosis. As such, it is likely that local increases in IL6 levels have not accumulated to sufficient levels to be measured in a global assay of CSF IL6.

IL6 trans-signaling is also reported to influence blood-brain barrier (BBB) permeability, although the specific transport mechanisms for IL6 across the BBB have not been thoroughly investigated.[11] Here, we found a significant association between plasma and CSF levels of both IL6 and sIL6R (eFigure 1A, B, links.lww.com/NXI/A807) that was not observed in our previous ALS study.[19] BBB permeability has been shown to increase with age,[33,34] and the cohort in our current study represents older individuals. The early phase of BBB integrity loss is suggested to be directly linked to the progression of AD through its interactions with Aβ and tau.[26,35-37] BBB dysfunction leads to failure of Aβ clearance and increased tau hyperphosphorylation. The negative feedback loop between amyloid accumulation, tau phosphorylation, and BBB function has been hypothesized to contribute to the onset of AD.[38] Thus, BBB dysfunction, due to increased age or increased IL6 trans-signaling, appears to be playing a role in cognitive impairment and dementia, allowing systemic increases in IL6 trans-signaling components to also contribute to CNS pathology.

It is tempting to speculate why increases in sIL6R may be associated with worse global cognition scores and AD biomarkers. Increased IL6 trans-signaling made possible by higher levels of sIL6R could increase local glial activation promoting inflammation.[19] It is possible that IL6 trans-signaling is working on top of classical IL6 signaling to cause increased glial priming and exacerbating a potentially neurotoxic response from microglia and/or astrocytes in the CNS. This could be contributing to the chronic neurotoxic inflammation seen in neurodegenerative diseases.[37,39] This chronic glial activation may contribute to synaptic loss that is thought to be an early event in disease pathology.[3]

Overall, the possession of the *IL6R* Ala^{358} variant and potential increased IL6 trans-signaling appear to be associated with worse AD symptoms and biomarkers. Limitations of this study prevented investigation of the mechanism linking IL6 trans-signaling effects to CI; however, they should be prioritized to further direct future studies on IL6 trans-signaling effects on AD. Knowledge that a specific subgroup of patients such as those possessing the *IL6R* Ala^{358} variant will have increased disease severity opens the door for personalized treatments of early-stage AD. Future studies can assess whether sIL6R and the *IL6R* Ala^{358} variant are beneficial biomarkers to predict which patients with AD may benefit from sIL6R-blocking strategies.

As a correlational study, there were several significant limitations. As a regional cohort of participants recruited to a single ADRC, there may be limits of applicability to national population demographics (Table 1). The COVID-19 pandemic limited collections of paired CSF and plasma samples across multiple time points during this study, limiting our ability to assess IL6 trans-signaling effects on progression of cognitive impairment at the time of this publication. The sample size is modest for a genetic association study and prevented us from examining relevant subsets of the cohort. Homozygotes and heterozygotes for the *IL6R* Ala^{358} variant and patients with CI and AD had to be combined to maintain statistical power. When separating IL6 trans-signaling effects between CI and CN individuals, we found some significant changes in slopes between the 2 groups; however, when analyzing the regression of each line individually, there was insufficient statistical power to maintain significance due to the sample size. Future plans include evaluation of a larger sample set with longitudinal assessments to more fully assess relationships between IL6R genotype, cognitive status, and progression of MCI to AD.

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