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CCL23: A CHEMOKINE ASSOCIATED WITH PROGRESSION FROM MILD COGNITIVE IMPAIRMENT TO ALZHEIMER'S DISEASE

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Abstract

CCL23 is a chemokine implicated in inflammation and host defense responses. It has been recently associated with acquired brain damage and stroke outcomes. In this study, we reported the role of CCL23 in Alzheimer's disease (AD). We evaluated the levels of CCL23 in 659 individuals: cognitively normal, mild cognitive impaired (MCI) and AD patients. Two cross-sectional (study 1, n=53; study 2, n=200) and two longitudinal (study 3, n=74; study 4, n=332) studies were analyzed

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6.DECLARATION OF INTEREST

The Neurovascular Research Laboratory (VHIR) filed a patent on the "Use of CCL23 as brain damage marker(PCT/EP2013/050411). DA participated in advisory boards for Fujirebio-Europe, Roche, and received speaker honoraria from Fujirebio-Europe, Nutricia and Krka Farmacéutica S.L. JFo participated in consultancy agreements for Merck, Novartis and AC Inmune. AL participated in consultancy agreements for Nutricia, Novartis, Eli-Lilly, Fujirebio-Europe and Otsuka. He is also the inventor of a patent on synaptic markers. CT participated in advisory boards for Roche, received nonfinancial support in the form of research consumables from ADxNeurosciences and Euroimmun, and performed contract research or received grants from Probiodrug, Biogen, Esai, Toyama, Janssen Prevention Center, Boehringer, AxonNeurosciences, EIP Farma, PeopleBio, and Roche. OH receives research support from Alector. CC receives research support from: Biogen, EISAI, Alector and Parabon; he is a member of the advisory board for ADx Healthcare and Vivid Genomics.

separately. CCL23 levels in the blood and/or CSF of each study were measured by immunoassays. Globally, our results suggest a predictive role of CCL23 protein levels both in the plasma in study 3 (hazard ratio (HR)=2.5 (confidence interval (CI) 95%: 1.2–5.3), p=0.02) and in the CSF in study 4 (HR=3.05 (CI 95%: 1.02–5), p=0.04) in cases of MCI that progress to AD. Moreover, we observed that the *APOE ε4* allele was associated with higher levels of CCL23 in study 2 (470.33 pg/mL (interquartile range (IQR): 303.33–597.76) vs. 377.94 pg/mL (IQR: 267.16–529.19), p=0.01) (*APOE* genotypes were available in studies 2 and 4). Together, these findings support the role of CCL23 in neuroinflammation in the early stages of AD, suggesting that CCL23 might be a candidate blood biomarker for MCI to AD progression.

Keywords

Alzheimer's disease; cognitive dysfunction; biomarkers; chemokines; early diagnosis

1. INTRODUCTION

Chemokines are a family of cytokines that are involved in chemotaxis. They are characterized by their small size and the presence of 4 cysteine residues in conserved locations. These molecules are classified into the following 4 subfamilies depending on the motif formed by the 2 cysteines closest to the N terminal: CXC, CC, C3XC and XC. The CC chemokine family is the largest subfamily, and CC chemokines can attract monocytes, eosinophils, basophils, T lymphocytes, natural killer (NK) cells, and dendritic cells [1].

One member of the CC subfamily is CC chemokine ligand 23 (CCL23; also known as chemokine β8–1 (Ckβ8–1), myeloid progenitor inhibitory factor 1 (MPIF-1) and macrophage inflammatory protein 3 (MIP-3)), which has been associated with inflammatory and host defense responses. It is mainly expressed by macrophages in the lung, liver, and pancreas [2]. CCL23 has chemotactic activity in T lymphocytes, monocytes and neutrophils, and binds uniquely to CC receptor 1 (CCR1) [3]. It inhibits the production and release of polymorphonuclear cells (PMNs) and monocytes in bone marrow [4], and is also involved in inflammatory responses by stimulating the production of pro-inflammatory cytokines and adhesion molecules [5]. In this regard, CCL23 levels have been found to be elevated in several inflammatory diseases, such as atherosclerosis, systemic sclerosis and rheumatoid arthritis [6–8].

Recently, our group has described a strong relationship between CCL23 and acquired brain lesions [9]. In this study, higher baseline CCL23 blood levels were found in patients with acute ischemic stroke (IS) and stroke-mimicking conditions, especially in those with underlying brain damage such as tumors or traumatic brain injury (TBI), than in healthy controls. This study proposed CCL23 as a biomarker for the diagnosis of acute inflammatory responses to brain damage. However, it is still unclear whether circulating CCL23 levels are also related to chronic brain damage related conditions, such as neurodegenerative diseases.

Alzheimer's disease (AD) is the most common neurodegenerative disease, accounting for 50–60% of all dementia cases. It is characterized by neuronal loss with the accumulation of

amyloid-β peptide (Aβ), the formation of Aβ plaques, and the deposition of phosphorylated tau (P-tau), which composes neurofibrillary tangles (NFTs) [10].

Multiple studies have indicated that AD pathology starts 15–20 years before clinical onset. For this reason, it is important to identify and validate biomarkers that can be used to identify patients in the presymptomatic stage and to develop disease-targeting therapies [11,12]. Neuroimaging (hippocampal volumetry, FDG PET, and amyloid PET) and cerebrospinal fluid (CSF) biomarkers (Aβ42, tau and P-tau) are being progressively implemented in clinical practice and clinical trials to provide evidence of the underlying pathophysiology and facilitate an early diagnosis [13,14]. However, the use of neuroimaging and CSF in clinical settings has several limitations; the former is highly costly, and the latter can only be obtained by an invasive technique [15]. During the past decade, some efforts have focused on researching blood-based biomarkers because their detection requires minimally invasive techniques, is reproducible and has a low cost [16]. One of the most promising blood candidates is plasma neurofilament light (NfL), which has been shown to be useful for tracking neurodegeneration in AD in a large cohort of patients [17].

In this study, we aimed to examine the role of CCL23 in AD. We evaluated the association of plasma and CSF CCL23 protein levels with AD in two cross-sectional studies and the predictive value of this protein in two longitudinal studies.

2. METHODS

2.1 Study design

This study was a multicenter study performed in four different medical centers across three countries (Spain, the Netherlands and the USA). The study protocols were approved at each of the participating centers, and all participants or relatives gave informed consent in agreement with the Declaration of Helsinki.

2.2 Subjects

Clinical information and biological specimens were collected from each participant. The details of each study are summarized in Table 1 and are more extensively reported in the supplemental data.

Diagnosis -—The clinical diagnosis of AD or dementia Alzheimer's type (DAT) was based on the criteria of the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [18] in studies 1 and 4. In studies 2 and 3, the revised criteria from the National Institute on Aging-Alzheimer's Association (NIA-AA) were used to diagnose AD and mild cognitive impairment (MCI) [19,20]. Furthermore, in study 2, patients with MCI were categorized as amnestic MCI (aMCI) because their memory domain was impaired.

Study 1 -—Forty-seven participants (36 AD patients and 11 cognitively unimpaired controls) were recruited from Vall d'Hebron Hospital, Barcelona, Spain. These patients were part of a larger cohort described previously by Montañola et al. (2016). Cognitive

impairment was rated using the Mini-Mental State Examination (MMSE), which is a 30-point test in which lower scores mean severe cognitive impairment [22].

Study 2 -—Two hundred subjects from the SPIN (Sant Pau Initiative on Neurodegeneration) cohort at the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain (Alcolea, under review), were enrolled. The patients had a diagnosis of AD dementia, MCI or other dementias (frontotemporal dementia or Lewy body dementia). Plasma and CSF samples were collected and processed using previously published methods [23,24]. MMSE scores and Clinical Dementia Rating (CDR) scores were available. Apolipoprotein E (*APOE*) genotyping was also carried out in these patients using previously published methods [23].

Study 3 -—Seventy-four MCI patients from at the Alzheimer Center Amsterdam, Amsterdam UMC, the Netherlands, were enrolled. All patients were followed for a median of 2.6 years (interquartile Range (IQR): 2–3.1). These patients were part of a larger cohort described by Duits et al. (2015), who also described the sample collection and cohort characteristics. MMSE scores were available in this group.

Study 4 -—Three hundred thirty-two community-dwelling participants (>60 years) were recruited from the Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO, for a longitudinal study related to health aging and dementia. These patients were followed up for a median of 5.4 years (2.5–7.6). Cognitive decline was assessed by the MMSE and Clinical Dementia Rating (CDR) scale. In this cohort, some patients with a CDR of 0.5 met the criteria for MCI and some were more mildly impaired and were considered "pre-MCI". To study the progression of MCI to dementia, patients with a baseline CDR of 0.5 and a follow up CDR greater than 0.5 were considered converters to AD. Non-progressors were defined as those patients with a CDR of 0.5 which remained stable, and those patients with a CDR of 0 which their cognitive status did not progress further than 0.5. Plasma and CSF collection protocols were previously reported elsewhere [26–29].

2.3 Biomarker measurements

In study 1, serum CCL23 levels were determined by quantitative enzyme-linked immunosorbent assay (ELISA) (Human MPIF-1 ELISA Kit RayBio, RayBiotech Inc, Norcross, GA). Plasma CCL23 levels in studies 2 and 3 were also analyzed by ELISA using a different kit (Human CCL23/MPIF-1 DuoSet ELISA Development System, R&D Systems Inc, Minneapolis, MN). The plasma and serum CCL23 levels measured by the two kits were correlated ($R=0.88$, $p<0.0001$). All assays were performed according to the manufacturer's instructions. Each sample was measured in duplicate and the mean of the two values was used to analyze the data (given in pg/ml). In each 96-well plate, a commercial sample pool was assayed in quadruplicate to be used as an interassay control (plasma from Innovative Research, Novi, MI, cat. #: IPLA-N; serum, cat. #: H16914). Interplate differences among the assays were assessed using the coefficient of variation (CV). If the CV was higher than 20%, the values were standardized to a control sample measured in all plates of the assay for statistical analyses.

In case of study 4, plasma and CSF CCL23 levels were measured as part of a quantitative immunoassay panel (Discovery MAP® v. 3.3, Myriad RBM, Austin, TX). Furthermore, in this cohort CSF Aβ42, total tau, and phospho-tau181 levels were analyzed by quantitative ELISA (β-amyloid (1–42), hTAUAg, and phosphotau (181P); Fujirebio, Ghent, Belgium). The specifications of the biomarker measurements of study 4 were reported previously [26,27].

2.4 Statistical analyses

Statistical analyses were performed with SPSS software (version 22; SPSS Inc.; Chicago, IL) and R software (version 3.6.1; R Foundation for Statistical Computing; Vienna, Austria), and figures were constructed with GraphPad Prism (version 6; GraphPad Software; La Jolla, CA). Each study was analyzed separately. In studies 1 and 2, comparisons were performed at baseline levels; in study 3, the analyses were performed at follow-up, and in study 4, the analyses were performed at baseline and follow-up. The normality of the variables was assessed with the Kolmogorov-Smirnov test. The values of normal variables are represented as the mean ± standard deviation (SD), and non-normal variables are represented as the median (IQR).

In all studies, *T* test and nonparametric Mann-Whitney and Kruskal-Wallis tests were used to evaluate the associations between categorical and continuous variables. Bonferroni's corrections were applied when appropriate. Correlations were performed using Spearman's Rho to compare two continuous variables, and the chi-squared test was used to assess intergroup differences for categorical variables. The best cut-off points for CCL23 levels were established as the highest Youden index (specificity + sensitivity −1) calculated in areas under the receiver operating characteristic (ROC) curve (AUC).

In studies 3 and 4, as progression data were available, survival analyses were performed using Kaplan-Meier estimation to study survival throughout the follow-up period. Cox proportional hazards regression analysis was carried out by the forward stepwise method to investigate the association between survival time and one or more predictors. A likelihood ratio test was used to compare the fitness of the models. A p-value lower than 0.05 was considered statistically significant for all tests.

3. RESULTS

3.1 Group comparisons in studies 1, 2 and 4

In the pilot study (study 1), the blood levels of CCL23 were compared between AD patients and healthy controls. We measured the serum levels of CCL23 in 47 subjects, 36 AD patients and 11 healthy controls. The demographic data are shown in Table S1 of the supplemental data. AD patients tended to have higher standardized serum CCL23 levels than those of healthy controls (2.35 ± 0.78 vs. 1.89 ± 0.71, p=0.09).

To compare cognitively normal patients with AD patients as well as aMCI patients and other dementia patients, we measured plasma CCL23 levels in study 2. The descriptive analysis of these patients is shown in Table S2 of the supplemental data. The plasma levels of CCL23 were inversely correlated with MMSE scores in the entire group (R=−0.2, p=0.008) (Figure

1A). These two variables were also inversely correlated when only AD patients were analyzed (R=−0.29, p=0.05). Individuals from the groups with cognitive impairment (AD, aMCI and other dementias) had higher levels of plasma CCL23 than those of controls, as shown in Table S2, even though these differences were not statistically significant.

In longitudinal study 4, we also studied the levels of CCL23 in CSF and plasma at baseline. The demographic data of this cohort are shown in Table S4 of the supplemental data. Differences were observed in CSF CCL23 levels among the groups (CDR=0, CDR=0.5 and CDR=1) (p=0.01) (Figure 1C). When performing post hoc pairwise comparisons, patients with a CDR of 0.5 had higher levels of CCL23 in the CSF than those of patients with a CDR of 0 (8280 pg/mL (7120–9645) vs. 7340 pg/mL (5970–9260), adjusted p=0.001). Such differences were not observed in the plasma.

3.2 Prediction of progression in studies 3 and 4

Next, we studied the possible role of CCL23 as a predictor of the progression of MCI to AD.

First, this was studied in a prospective case-control study. In study 3, 74 MCI patients were analyzed; 42 patients progressed to AD, and 36 did not progress.

Patients with progression tended to have higher plasma CCL23 levels at baseline (351.4 pg/mL (±21.9) vs. 303.93 pg/mL (±23.65), p=0.13). For the prediction of progression to AD, plasma CCL23 had an area under the curve (AUC) value of 0.6 (CI 95%: 0.472–0.732). A cut-off point of 265 pg/mL with a sensitivity of 76.3% and a specificity of 47.2% was selected for the prediction of progression. Patients who had plasma CCL23 levels >265 pg/mL were more likely to progress (progression was noted in 60.4% of patients with CCL23 levels >265 pg/mL vs. 34.6% of patients with CCL23 levels <265 pg/mL, p=0.03) (Figure 2A).

Kaplan-Meier curves showed that plasma CCL23 levels were associated with progression, discriminating the patients at the established cut-off (p=0.02) (Figure 2B). In the Cox regression analysis, plasma CCL23 levels >265 pg/mL (hazard Ratio (HR)=2.5 (1.2–5.3), p=0.02) and baseline MMSE scores (HR=0.86 (0.76–0.97), p=0.01) were independent predictors of AD conversion after adjusting for age and sex. According to the likelihood ratio test, the addition of the biomarker to the clinical model improved the fitness of the model (p=0.019).

Progression was also assessed in patients in study 4. In this case, patients with a baseline CDR of 0.5 that increased to greater than 0.5 in the follow-up were considered to have progressed to dementia and non-progressors were defined as those patients with a CDR of 0.5 which remained stable, and those patients with a CDR of 0 which their cognitive status did not progress further than 0.5. Forty-two subjects (13.8%) progressed to dementia, and 262 subjects (86.2%) did not.

There were no differences in plasma CCL23 levels (p=0.43) when comparing patients who progressed and patients who did not, but patients who progressed had higher CCL23 levels in the CSF (8500 pg/mL (7100–9300) vs. 7400 pg/mL (6000–9200), p=0.02). CSF CCL23 levels had an AUC value of 0.61 (0.53–0.69) for the progression of dementia. We defined a

cut-off point of 7080 pg/mL, which showed 81% sensitivity and 46.9% specificity. Patients with CSF CCL23 levels >7080 pg/mL were more likely to progress to dementia (6.1% of patients with CSF CCL23 levels <7080 pg/mL progressed, and 19.8% of patients with CSF CCL23 levels >7080 pg/mL progressed, p=0.001) (Figure 2C).

We also performed Kaplan-Meier analyses by using the same cut-off CSF CCL23 level (>7080 pg/mL). Individuals with lower CCL23 levels progressed more slowly than those with higher levels (p<0.0001) (Figure 2D). In the Cox regression analysis, CSF CCL23 levels >7080 pg/mL (HR=3.05 (1.02–5), p=0.04), MMSE scores (HR=0.626 (0.56–0.7), p<0.0001), age (1.072 (1.01–1.13), p=0.011), female sex (0.447 (0.223–0.89, p=0.023) and the presence of the *APOE* ε4 allele (4.05 (2.01–8.12, p<0.0001) were independently associated with progression to dementia. In this model, the addition of CSF CCL23 levels >7080 pg/mL improved the fitness of the model when compared with the model with the clinical variables (p=0.005).

3.3 *APOE* association in studies 2 and 4

The association of the *APOE* genotype with CCL23 expression was explored in studies which these data were available. In study 2, AD patients followed by aMCI patients showed a greater load of the *APOE ε4* allele (p=0.005) (Figure 3A). When AD patients were compared to patients with other dementias or control patients, the differences were statistically significant (p=0.02 in both cases). *APOE ε4* carriers showed higher plasma levels of CCL23 than those of noncarriers (470.33 pg/mL (303.33–597.76) vs. 377.94 pg/mL (267.16–529.19), p=0.01) (Figure 3B).

APOE genotype was also available in 302 patients of cohort 4. *APOE* ε4 carriers progressed to dementia more frequently (p<0.001) than noncarriers. There were no differences in CCL23 levels in CSF between *APOE* ε4 carriers and noncarriers, but *APOE* ε4 carriers had lower levels of CCL23 in the plasma (90 pg/mL (77.7–105) vs. 98 pg/mL (89.1–114), p=0.03).

3.4 Association of CCL23 with CSF biomarkers in study 4

To further understand the relationship between CCL23 and AD, we studied the association of this chemokine with other widely accepted CSF biomarkers ($A\beta_{42}$, Tau and P-Tau) in cohort 4. Correlations were performed between these biomarkers and plasma and CSF CCL23 levels. In the plasma, no significant correlations were found. In the CSF, CCL23 levels correlated with Tau (R=0.172, p=0.002) and P-tau (R=0.170, p=0.002), but not with $A\beta_{42}$, in the entire cohort (Figure 3C and D, respectively). However, when including tau and P-tau in the previous Cox regression analysis, CSF CCL23levels >7080 pg/mL were not further associated with the prediction of dementia.

4. DISCUSSION

Here, we studied the role of CCL23 in neurodegenerative diseases, especially at the early stages of the disease. Our study is the first to provide evidence of a relationship between CCL23 and AD.

The activation of the immune system in the central nervous system (CNS), called neuroinflammation, occurs in most CNS diseases. In AD, misfolded proteins and β-amyloid (as Aβ plaques) become damage-associated molecular patterns (DAMPs), driving an immune response by CNS resident cells (microglia, astrocytes and perivascular myeloid cells) [30]. Due to this activation, peripheral inflammatory markers in the plasma (IL-1β, IL-6 [31], BDNF [32] or CRP [33]) are of interest as possible biomarkers for AD. Nevertheless, these markers are not good enough on their own, and systematic reviews have not provided enough evidence supporting these and other candidates, in part because of the heterogeneity between studies [34]. Chemokines have also been studied as biomarkers for AD. CCL2, also known as MCP-1, IL-8 and CCL5 (also known as RANTES), are some examples of chemokines that have been studied in AD [35]. The CCL23 receptor, CCR1, has been found to be an early marker for AD, and a close relationship between CCR1 and Aβ42 has been demonstrated [36]. CCL23 was not previously related to AD; therefore in our study, we have assessed an association of this chemokine, both in plasma and CSF, with AD and the progression of MCI patients to dementia.

Some studies have already described an increase in cytokines and chemokines in MCI patients. Magaki et al. (2007) found increased production of inflammatory cytokines (IL-6, IL-8 and IL-10) in the peripheral blood mononuclear cells of MCI patients. Similarly, Galimberti et al. (2006) observed an increase in the CSF levels of IP-10, CCL2 and IL-8 in MCI patients compared with AD patients and age-matched controls. These studies suggest an upregulation of chemokines at the very beginning of AD pathogenesis. Thus, inflammation might represent an initiating factor of the disease. Our results show that CCL23 could play a role in the early stages of the disease. The early increase of this chemokine and other cytokines in the preclinical stages of AD may not only serve as a biomarker but also represent a new therapeutic strategy for preventing or slowing the progression of this disease.

The higher levels of CCL23 in *APOE ε4* carriers found in study 2 supports the role of this chemokine as a predictor of AD. The allele *ε4* of *APOE* was identified in 1993 as a major genetic risk factor for AD [39]. The protein product of the *APOE* gene, the Apolipoprotein E (Apo E), modulates inflammatory and immune responses in an isoform-dependent manner, with *APOE ε4* carriers having a higher concentration of inflammatory mediators in the plasma [40,41]. Ringman and Elashoff (2012) demonstrated that the levels of plasma inflammatory markers are related to the *APOE* genotype; thus it is plausible to hypothesize that the *APOE* genotype influences CCL23 plasma levels. Specifically, being carrier of the *APOE ε4* allele increases the risk of developing AD, but the precise mechanisms leading to this increased risk are not fully understood. An increase in the plasma levels of pro-inflammatory mediators, such as CCL23, might be related to the increased probability of progression from MCI to AD. The results obtained in study 4 does not support this hypothesis; however, the two studies are not comparable due to the differences in the types of patients studied and the CCL23 detection method.

It is important to highlight that we measured CCL23 in both the plasma and CSF. We found increased levels of CCL23 in the blood and CSF depending on the study. The predictive value of CCL23 was assessed in both fluids, although there was a disparity in plasma

CCL23 levels in study 4 compared with the rest of the findings of this study. We suggest that this disparity is the result from the different detection methods used in this study, as mentioned earlier.

Although $A\beta_{42}$, tau and P-tau in the CSF are widely accepted biomarkers, lumbar puncture required to obtain CSF is an invasive technique; therefore, blood would be a more ideal fluid to develop new biomarkers for AD. However, studying blood has several pitfalls; for example, molecules from the CNS have to cross the blood-brain barrier and, as a consequence, are less concentrated in the blood than in the CSF [15]. Due to this and for several other reasons, there are no good candidate blood biomarkers for AD and its progression so far, even though great efforts have been made to identify them. Our results suggest a possible role of CCL23 levels in plasma to predict MCI progression to AD in one of the studies, but more research and more powerful results are needed to confirm increases of CCL23 levels in the blood as predictors for this endpoint. In the future, a panel of several biomarkers would be required to be clinically useful and provide sufficient accuracy required for diagnosis.

Our study has some limitations. The method of CCL23 quantification was different in 3 of the studies, using 2 different ELISA detection kits and a quantitative immunoassay panel. Each of these methods has different sensitivities, detection limits and binding to CCL23. This disparity, as well as the different scales used to evaluate patients' cognitive condition in each study, may have influenced our results. For these reasons, it has not been possible to merge the data from the 4 studies, and the analyses of each study were performed separately. It is also important to note that in the 4 studies, we were not able to analyze vascular risk factors and previous cerebrovascular events due to the unavailability of these data. These variables could act as confounders, and this is especially relevant due to the relationship between stroke and CCL23 that our group described previously [9]. Moreover, different cut-off points were calculated for the different studies, which may have limited the reproducibility and broader application of the results.

Another limitation of our study is the disparity in our results, which may have weakened our final message. However, this is an exploratory study, and more research is needed to clarify the role of CCL23 in MCI and AD. Future research should focus on large prospective studies with standardized methods to evaluate whether our findings could be useful for the clinical management of MCI patients. This study should include patients in preclinical stages of AD (asymptomatic patients) and prodromal AD patients, and use neuroimaging, CSF biomarkers (Aβ, tau and P-tau) measurements and blood collection at baseline and at the end of the patient follow-up.

4.1 Conclusions

This study provides evidence of a relationship between CCL23 and AD. The association of CCL23 levels in both the blood and CSF with AD progression in MCI patients, and an increase in CCL23 levels in *APOE ε4* carriers in one of the studies was assessed. CCL23 seems to have a role in the early stages of the disease and could be a candidate blood biomarker for the prediction of AD progression in MCI patients. Further studies are needed to validate these results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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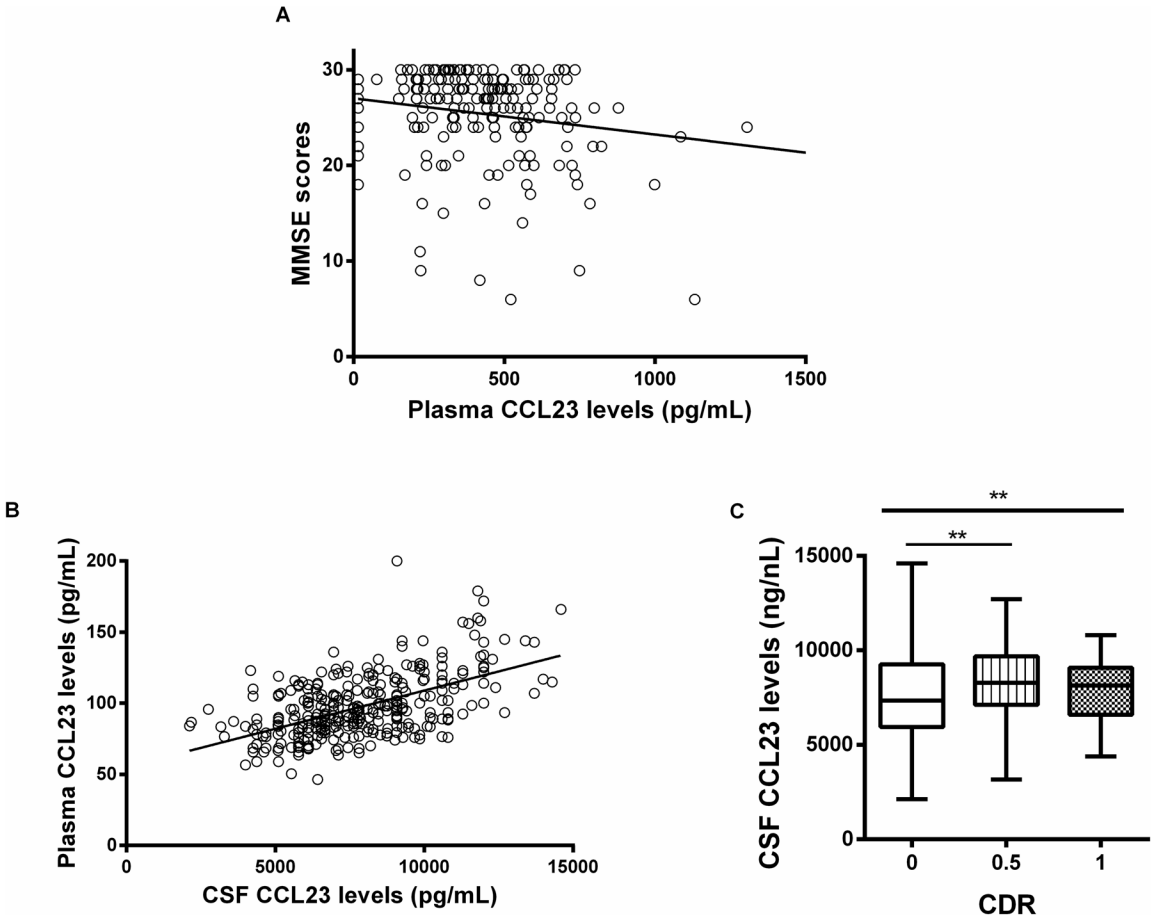

Figure 1. Comparisons of CCL23 levels at baseline in studies 2 and 4.

A. Correlation between plasma CCL23 levels and MMSE scores in study 2 (N=200) (R= −0.2, p=0.008). **B**. Correlation between plasma CCL23 levels and CSFCCL23 levels in patients in cohort 4 (N=332) (R=0.509, p<0.0001) **C**. Box plot comparing CSF CCL23 levels in patients with CDRs of 0, 0.5 and 1 at baseline. The thick line represents the p value of the Kruskal-Wallis test (p=0.005) and the thin line represents the p value of the post hoc comparison with Bonferroni's correction (p=0.01). In the box plots, *p<0.05, **p<0.01, ***p<0.001, and the values are presented as the median and IQR.

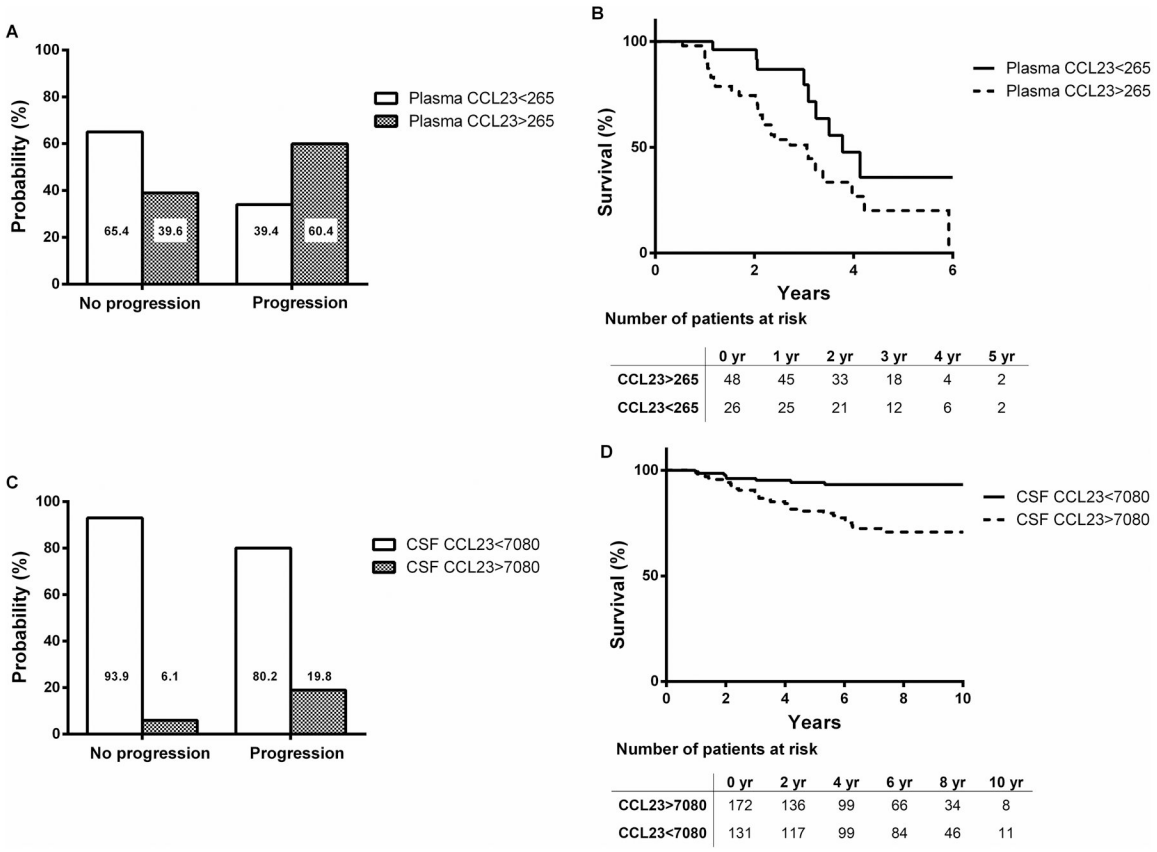

Figure 2. Follow-up studies of cohorts 3 and 4.

A. Bar plot representing the probabilities of MCI progression to AD. The light bars represent patients with CCL23 levels lower than 265 pg/mL, and the dark bars are those with levels higher than 265 pg/mL (p=0.03). **B**. Kaplan-Meier survival analysis of patients with CCL23 levels higher or lower than 265 pg/mL (p=0.02). **C**. Bar plot representing the probabilities of the progression of patients with a CDR of 0.5 to a CDR >0.5. The light bars represent patients with CSF CCL23 levels lower than 7080 pg/mL, and the dark bars represent those with levels higher than 7080 pg/mL (p=0.001). **D**. Kaplan-Meier survival analysis dividing patients into two groups depending on whether their CCL23 levels were higher or lower than 7080 pg/mL (p<0.0001).

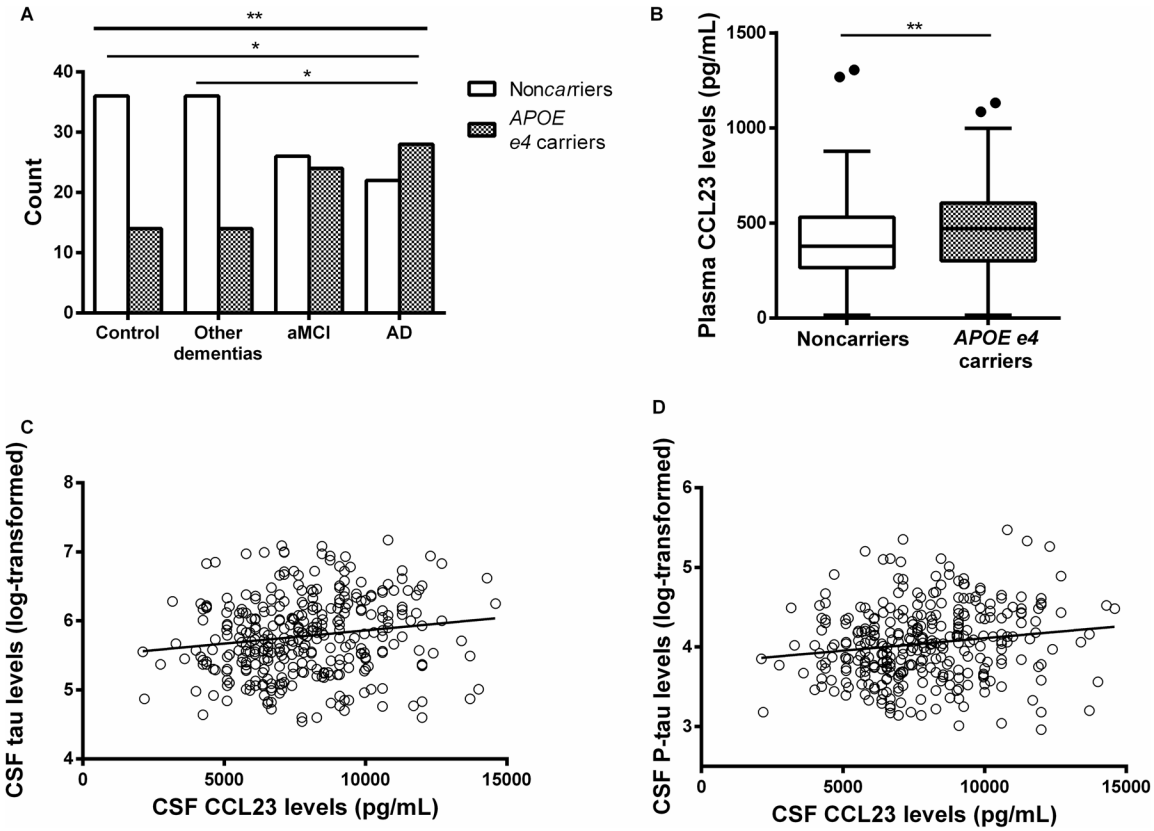

Figure 3. Association of CCL23 with the *APOE* genotype and other widely accepted AD biomarkers in studies 2 and 4.

A. Bar plot representing the different clinical groups according to *APOE* genotype in study 2 (p=0.005). This is represented by the thick line on the graph. The AD patient group had more *APOE* ε4 carriers than the other dementias and control groups (p=0.02 in both cases) and is represented by the thin line. **B**. Box plot of plasma CCL23 levels in study 2 comparing *APOE* ε4 carriers and noncarriers (p=0.017). **C**. Correlation of tau and CCL23 levels in the CSF (R=0.172, p=0.002) in study 4. **D**. Correlation of P-tau and CCL23 levels in the CSF (R=0.17, p=0.002) in study 4. In the box plots, *p<0.05, **p<0.01, ***p<0.001, and the values are presented as the median and IQR.

Table 1.

Demographic characteristics of each study.

Variables without a normal distribution (age and follow-up) are expressed as median (IQR), and categorical variables (sex) are expressed as the proportion of patients who presented the specified condition. Abbreviations: NINCDS-ADRDA: National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association; NIA-AA: National Institute on Aging-Alzheimer's Association.

	Study 1 (n=47)	**Study 2 (n=200)**	**Study 3 (n=74)**	**Study 4 (n=332)**
Center	Hospital Universitari Vall d'Hebron, Barcelona, Spain	Hospital de la Santa Creu i Sant Pau, Barcelona, Spain	Alzheimer Center, VU Medical Center, Amsterdam, Netherlands	Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MO
Type of study	Cross-sectional	Cross-sectional	Longitudinal case-control	Longitudinal
Follow-up (years)	N/A	N/A	2.6 (2–3.1)	5.4 (2.5–7.6)
Subjects	36 AD patients 11 cognitive normal patients	50 AD patients 50 MCI patients 50 patients with other dementias 50 cognitive normal patients	74 MCI patients	332 community-dwelling patients (>60 years)
Diagnostic criteria	NINCDS-ADRDA	NIA-AA	NIA-AA	NINCDS-ADRDA
Age (years)	77 (72.5–83)	67 ± 0.64	68 (62–72)	72 (66.5–78)
Sex (female)	66.7%	55.2%	32.1%	60.1%
CCL23 measurement	Human MPIF-1 ELISA Kit RayBio, RayBiotech Inc, Norcross, GA	Human CCL23/MPIF-1 DuoSet ELISA Development System, R&D Systems Inc, Minneapolis, MN	Human CCL23/MPIF-1 DuoSet ELISA Development System, R&D Systems Inc, Minneapolis, MN	Discovery MAP® v. 3.3, Myriad RBM, Austin, TX
Biospecimen	Serum	Plasma	Plasma	Plasma and CSF