Cerebrospinal fluid ceruloplasmin levels predict cognitive decline and brain atrophy in people with underlying β-amyloid pathology

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Abstract

Objectives: The mechanisms leading to neurodegeneration in Alzheimer’s disease (AD) may involve oxidative stress and neuroinflammation. Ceruloplasmin (Cp) is a circulating protein that intersects both these pathways, since its expression is increased during the acute phase response, and the protein acts to lower pro-oxidant iron in cells. Since the role of Cp in AD, and its potential for use as a biomarker is not established, we investigated CSF Cp and its association with longitudinal outcome measures related to AD.

Methods: This was an observational study of 268 people from the Alzheimer’s Disease Neuroimaging (ADNI) cohort. Subjects were classified clinically as having AD, mild cognitive impairment (MCI) or were cognitively normal (CN), and were also classified as being positive for β-amyloid using established thresholds in the CSF t-tau/β42 ratio. Subjects underwent cognitive tests and MRI studies every 6 months for 2 years, then yearly thereafter for up to 6 years.

Results: At baseline, CSF Cp was not associated with clinical or pathological diagnosis, but we found an unexpected association between CSF Cp and levels of CSF apolipoprotein E. In longitudinal analysis, high level of CSF Cp was associated with accelerated cognitive decline (as assessed by ADAS-Cog, CDR-SB, and MMSE) and ventricular volume enlargement in people classified as MCI and who had underlying β-amyloid pathology.

Conclusion: These results raise new questions into the role of Cp in neuroinflammation, oxidative stress, and APOE pathways involved in AD, and reveal the potential for this protein to be used as a biomarker in disease prognostication.

1. Introduction

Cognitive decline begins in the years prior to the diagnosis of Alzheimer’s disease (AD), as a clinical manifestation of insidious neurodegeneration. While β-amyloid (Aβ) and neurofibrillary tangle proteinopathy accumulate in this prodromal phase, the toxic mechanisms leading to neurodegeneration remain unknown. One third of cognitively normal people over the age of 65 have elevated plaque pathology as measured by Aβ PET or a ratio of total tau (t-tau) to Aβ42 in the CSF (Rowe et al., 2013; Schindler et al., 2018); these people are likely to undergo cognitively decline in the ensuing years, but with large degree of variability between people (Lim et al., 2014). The discovery of biomarkers that predict decline upon pathology has been prioritised for their clinical value in disease prognostication, and also for the potential insight into the disease mechanism that may be gleaned from the association of a biomarker with disease outcomes.

Furthermore, since lowering Aβ pathology has proven unsuccessful in halting disease progression in many large-scale clinical trials...
(Nikseresht et al., 2019), there is renewed impetus to investigate other components of the disease mechanism, such as neuroinflammation and oxidative stress. Ceruloplasmin (Cp) is a protein that intersects these pathways, given that it is an acute phase protein whose expression is induced during inflammation (Mackiewicz et al., 1988), and is a protein that acts to lower iron-induced oxidative damage. This function may be important since elevated brain iron in AD (measured using ferritin in CSF, MRI, and directly in post mortem tissue) predicts accelerated disease progression (Aytou et al., 2015; Aytou et al., 2017a; Aytou et al., 2017b; Aytou et al., 2019; Aytou et al., 2018; Diouf et al., 2019). But the role of Cp in AD pathophysiology, or its potential for use as a biomarker is not established.

Cp is the major copper binding protein of plasma, but it is not involved in copper metabolism, rather Cp functions to promote iron export from cells via the iron exporting protein, ferroportin (Harris et al., 1999). Ferroportin is a membrane channel that presents ferrous (Fe\(^{2+}\)) iron to the extracellular surface where it is oxidized by Cp so that the extracellular ferric (Fe\(^{3+}\)) transporting protein, transferrin, can bind and remove iron from the cell (Eid et al., 2014).

Two isoforms of Cp exist, a soluble form, and a glycosylphosphatidylinositol (GPI)-linked form that has alternative splice sites for exon 19 and 20. The GPI-linked Cp is expressed in astrocytes in the brain (Patel and David, 1997), and also the testis (Fornta et al., 1999). In the brain, Cp is additionally present in soluble form in CSF (Loeffer et al., 1994; Capo et al., 2008; Kallianpur et al., 2019). It is not known whether this soluble Cp is supplied by the blood, released by another cell type, or the result of GPI cleavage (Kondoh et al., 2005). The level of Cp in CSF is however (~1.6 ng/ml (Kallianpur et al., 2019)) much lower than that of plasma (0.2 mg/ml (Mak et al., 2008)).

Loss or impaired Cp activity is damaging to the brain, indeed aceruloplasminemia, a genetic disease where mutations in Cp lead to low levels of the protein, is one cause of Neurodegeneration with Brain Iron Accumulation (NBIA). This disease is characterized by iron elevation and progressive neurological symptoms that may include (but are not limited to) cognitive decline, parkinsonism, ataxia, and chorea (McNeill et al., 2008). In mice, loss of Cp protein causes iron-mediated neurodegeneration accompanyng motor and cognitive impairments (Zheng et al., 2018), and elevation of Cp by supplementation has been reported to improve animal models of iron overload (Aytou et al., 2013; Aytou et al., 2014; Tuo et al., 2017; Zanardi et al., 2017). In an animal model of AD, loss of Cp exacerbated the phenotype (Zhao et al., 2017).

Low activity of Cp may therefore promote iron retention and contribute to neurodegeneration, whereas elevated Cp may be a reporter of an inflammatory state. In AD patients, decreased Cp activity has been reported in serum (Sirot et al., 2016; Torsdottir et al., 2011), whereas the level of Cp protein in serum has been inconsistently reported as elevated (Giommetto et al., 1988), decreased (Kessler et al., 2006), or unchanged (Rembach et al., 2013). Cp was shown to be increased in CSF of 17 non-pathologically confirmed AD patients (Loeffler et al., 1994), whereas another paper reported unchanged levels but decreased specific activity of Cp in 10 non-pathologically confirmed AD patients (Capo et al., 2008). Here, we investigated the level of Cp in CSF of a well-characterized AD cohort, and investigated its association with longitudinal disease progression.

2. Methods

2.1. Study design and participants

Data used in the preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu) on 16/8/2018. The data files include ADNI-MERGE and “Biomarkers Consortium ADNI CSF Multiplex Raw”. The ADNI study has been previously described in detail (Weiner et al., 2012). The ADNI study was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see www.adni-info.org.

2.2. Recruitment inclusion and exclusion criteria

Consent was obtained according to the Declaration of Helsinki and the Ethical Committees of each Institution in which the work was performed approved the study. Inclusion criteria were as follows: 1) Hachinski Ischemic Score ≤ 4; 2) Permitted medications stable for 4 weeks prior to screening; 3) Geriatric Depression Scale score < 6; 4) visual and auditory acuity adequate for neuropsychological testing; good general health with no diseases precluding enrollment; 5) 6 grades of education or work history equivalent; 6) Ability to speak English or Spanish fluently; 7) A study partner with 10 h per week of contact either in person or on the telephone who could accompany the participant to the clinic visits. Cognitively normal (CN) subjects must have no significant cognitive impairment or impaired activities of daily living. Clinical diagnosed Alzheimer’s disease patients (AD) must have had mild AD and had to meet the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for probable AD (McKhan et al., 1984), whereas mild cognitive impairment subjects (MCI) should not meet these criteria, have largely intact general cognition as well as functional performance, but meet defined criteria for MCI.

2.3. CSF biomarkers

CSF was collected on a sample of the ADNI cohort at baseline. CSF samples were frozen within one hour of collection and stored at -80°C without prior centrifugation. Ferritin and apolipoprotein E levels were measured with the Rules Based Medicine (RBM) multiplex platform (Trojanowski et al., 2010), while levels of CSF A42 and total tau (t-tau) were measured with the Elecsys immunoassay platform, as previously described (Shaw et al., 2018).

Cp was measured using multi-reaction monitoring mass spectrometry (peptides IYHSHIDAPK and NNEGTYYSPYNYPQSR). Prior to MS analysis, high abundant serum proteins (excluding Cp) were depleted in the CSF using a MARS-14 immunoaffinity resin, run in batches of 12, 20, or 21 over 15 days, using two separate MARS-14 columns. Details of run order and column usage have been previously described (McKhan et al., 1984). Three in-run QC samples (HGS-CSF, human gold standard CSF) (Bioreclamation, lot BRH631340)) were included per depletion day and were not well aligned with the others, the sample was excluded. The ADNI study CSF biomarkers were run order and column usage have been previously described (McKhan et al., 1984).

Peptide quantification was then conducted using a LC/MRM-MS system (AB Sciex), as previously described (McKhan et al., 1984).

The raw mass spectrometer data files (WIFF) were converted to mzXML format and loaded into the Elucidator software (version 3.3.0.1 SP4.25, Rosetta Biosciences) and processed using the “PeakTeller” processing pipeline for chromatogram alignment, noise filtering, data smoothing, peak detection and quantitation. The peak alignment was then manually reviewed. If more than 20% of the peaks of a sample were not well aligned with the others, the sample was excluded. The
following set of 5 additional peptide verification criteria were implemented to using Perl scripts developed in-house and orchestrated using Elucidator’s “visual scripts” plug-in interface (for further details see (McKhan et al., 1984)). Peptides with one or more flags were manually reviewed and were either kept or discarded, depending on the overall peak shape, the quality of the alignment and the presence of a neighboring interference. Once the final set of transitions was validated, the peak area data were transformed on the natural log scale. The amount of Cp was calculated by the summation of the peptides and expressed in relative units, since heavy-isotope labelled internal standard peptides were not used to allow for absolute quantitation.

2.4. Cognitive assessments

Cognitive assessment protocol has been previously described in detail (See www.loni.ucla.edu/ADNI, and for detail (Aisen et al., 2010)). Assessments were undertaken by a trained rater in site visits every 6 months for 2 years, then yearly for up to 6 years. Only CN and MCI subjects were included in the cognitive analysis because of low follow up numbers for AD subjects. Subjects were only included in the analysis if they had more than 1 cognitive assessment. The cognitive tests utilized were the ADAS-Cog (Alzheimer’s disease assessment Scale-cognitive subscale), CDR-SB (clinical dementia rating scale, sum of boxes) and MMSE (mini mental state exam) obtained in the ADNIMERGE primary table as part of the ADNIMERGE R package, downloaded on the 16/8/2018.

2.5. Structural MRI acquisition and processing

Subjects with a 1.5-T MRI and a sagittal volumetric 3D MPRAGE with variable resolution around the target of 1.2 mm isotropically were included in the analysis. See (www.loni.ucla.edu/ADNI), and for detail (Jack Jr et al., 2008). The hippocampal and ventral volumes utilized were those in the ADNIMERGE primary table as part of the ADNIMERGE R package, downloaded on the 16/8/2018. Only CN and MCI subjects were included in the MRI analysis because of low follow up numbers for AD subjects. Subjects were only included in the analysis if they had more than one MRI scan. MRI scans were performed at baseline, six months, one year, and then yearly for six years.

2.6. Statistical analysis

Baseline demographics of participants included in this study were described in strata of APOE pathology, based on previously published CSF t-tau/Aβ_{42} ratio threshold: t-tau/Aβ_{42} ratio < 0.27 for low pathology and ≥ 0.27 for high pathology (Shaw et al., 2018). Factors associated with baseline CSF Cp were analysed in a linear regression model of Cp, with the following covariates: age, gender, APOE ε4 genotype, diagnosis (NC, MCI), CSF levels of apolipoprotein E, ferritin and t-tau/Aβ_{42} (included as a continuous variable in each of the high-low t-tau/Aβ_{42} groups) as independent variables. To assess associations between longitudinal changes in cognition and brain volume with baseline CSF Cp, participants were stratified by diagnosis and t-tau/Aβ_{42} ratio. Data were modelled with mixed effects linear models including age, sex, APOE ε4, apolipoprotein E levels, and t-tau/Aβ_{42} (included as a continuous variable in each of the high/low t-tau/Aβ_{42} groups) as covariates. Models were performed with R (version 3.5.0) and tested for multicollinearity and normal distribution of residuals. Bonferroni adjustment was used to correct for multiple comparisons of the cognitive tests (α = 0.05, m = 3: adjusted α = 0.017) and volumetric analysis (α = 0.05, m = 2: adjusted α = 0.025).

3. Results

We identified 268 ANDI subjects with complete data for biomarkers (CSF values of Cp, tau, Aβ_{42}, ferritin, and apolipoprotein E) to perform cross-sectional analysis (Table 1). To determine whether AD-related clinical variables were associated with CSF Cp levels, we stratified the cohort based on previously described threshold in the CSF t-tau/Aβ_{42} ratio (0.27), indicating underlying amyloid deposition (Shaw et al., 2018), and performed separate multiple regressions of CSF Cp (Table 2). This pathology stratification is important since AD is frequently misdiagnosed clinically, and MCI may be due to another pathological change (e.g. vascular dementia). Furthermore, there are many people with prodromal AD, who have the pathology of AD but are cognitive normal; these people are at high risk of future cognitive decline (Lim et al., 2014) and therefore shouldn’t be considered as normal controls. In people with high t-tau/Aβ_{42}, Cp levels were not associated with diagnosis, sex, t-tau/Aβ_{42} or CSF ferritin levels, or APOE ε4 genotype (Table 2), but was unexpectedly associated levels of CSF apolipoprotein E (β S.E. = 0.244 [0.551] 0.660 0.278 [0.613] 0.879). In participants with low t-tau/Aβ_{42}, there was no significant association between Cp levels and the different independent variables (Table 2). In this group, the association between CSF apoE levels and CSF Cp levels was similar to what we observed in the high t-tau/Aβ_{42} but did not reach statistical significance (β S.E. = 3.275 [1.662]; P = .054; Fig. 1B). The higher p value in this group might reflect the lower N compared to the high t-tau/Aβ_{42} group (72 vs 196).

The associations between baseline Cp and disease progression over 6 years (see Table 3 for N) were investigated in pathology strata (t-tau/Aβ_{42} < 0.27 and t-tau/Aβ_{42} ≥ 0.27) and disease category (CN and MCI). In CN subjects, Cp was not associated with longitudinal change in any of the cognitive tests regardless of t-tau/Aβ_{42} status. Similarly, Cp was not associated with change in cognition in MCI subjects with low t-tau/Aβ_{42} (Table 4). However, in MCI subjects with high t-tau/Aβ_{42}, elevated baseline CSF Cp levels were associated with declining cognitive performance measured by CDR-SB (β S.E. = 0.744 [0.879]; P = 3 x 10^{-5}; Fig. 2A), ADAS-Cog (β S.E. = 0.153 [0.069]; P = 3 x 10^{-5}; Fig. 2B), and MMSE (β S.E. = 0.149 [0.041]; P = 3 x 10^{-4} Fig. 2A).

### Table 1

Baseline clinical variables. S.E.: Standard error. Dx: Diagnosis.

| Covariate | Low t-tau/Aβ_{42} | High t-tau/Aβ_{42} |
|-----------|------------------|-------------------|
| N         |                  |                   |
| 72        |                  | 196               |
| Age (mean, S.E.) | 75.4 (6.69) | 75.0 (7.10) |
| Male sex (N, %) | 45 (62.5%) | 118 (60.2%) |
| Dx – CN (N, %) | 45 (62.5%) | 23 (11.7%) |
| Dx – MCI (N, %) | 23 (33.3%) | 109 (55.6%) |
| Dx – AD (N, %) | 3 (4.2%) | 64 (32.7%) |
| APOE ε4 + ve (N, %) | 7 (9.7%) | 130 (66.3%) |

### Table 2

Association between baseline clinical variables and CSF Cp levels in people with low and high t-tau/Aβ_{42}. Data are from a multiple regression model of CSF Cp levels including the variables indicated in the table (CSF t-tau/Aβ_{42} was additionally included as a continuous variable in the separate models stratified by this biomarker). β for the CSF values represent standard deviation shift of each analyte. P for age is in years.

| Covariate | Low t-tau/Aβ_{42} | High t-tau/Aβ_{42} |
|-----------|------------------|-------------------|
| β         | S.E. | P      |  β         | S.E. | P      |
| Age (years) | -0.003 | (0.024) | 0.915 | 0.036 | (0.011) | 0.002 |
| Male sex | -0.269 | (0.327) | 0.414 | 0.171 | (0.163) | 0.294 |
| Dx – MCI | 0.271 | (0.344) | 0.434 | 0.227 | (0.242) | 0.349 |
| Dx – AD | NA | NA | NA | 0.473 | (0.263) | 0.075 |
| APOE ε4 + ve | 0.244 | (0.551) | 0.660 | 0.278 | (0.170) | 0.103 |
| CSF apolipoprotein E | 3.275 | (1.662) | 0.054 | 2.51 | (0.650) | 0.002 |
| CSF ferritin | -1.57 | (1.30) | 0.377 | 0.094 | (0.613) | 0.879 |
| CSF t-tau/Aβ_{42} | -3.37 | (3.75) | 0.373 | -0.380 | (0.311) | 0.223 |

S.E.: Standard Error. Dx: diagnosis. *represents significant P values.
was set at 0.05, and a Bonferroni adjustment was used to correct for multiple comparisons for cognitive and volumetric studies. APOE, sex, biomarker, apolipoprotein E, and ferritin, and all of these interacted with time.

effects linear models of outcome (either CDR-SB, ADAS-Cog, MMSE, Hippocampal Volume or Lateral ventricular volume) and include the following covariates: age, sex, APOE ε4, and CSF levels of Cp, t-tau/Aβ

Table 3
Number of subjects who underwent cognitive and MRI assessments at each timepoint, for each disease category.

| Outcome | Clinical t-tau/ Aβ_{42} | Years | Dx | Dx | Dx |
|---------|------------------------|-------|----|----|----|
|         |                        |       | 0 | 0.5 | 1 |
| Cognition | CN Low                  | 45 | 45 | 45 | 0 | 42 | 43 | 31 | 25 | 28 |
|          | CN High                 | 23 | 21 | 21 | 0 | 22 | 17 | 13 | 11 | 12 |
|          | MCI Low                 | 108 | 107 | 108 | 102 | 93 | 74 | 39 | 30 | 29 |
|          | MCI High                | 24 | 24 | 24 | 24 | 22 | 18 | 12 | 10 | 7 |
| MRI      | CN Low                  | 40 | 40 | 35 | 0 | 31 | 28 | 22 | 14 | 17 |
|          | CN High                 | 21 | 21 | 21 | 0 | 19 | 12 | 11 | 7 | 10 |
|          | MCI Low                 | 20 | 18 | 17 | 19 | 15 | 13 | 7 | 4 |
|          | MCI High                | 83 | 78 | 76 | 70 | 61 | 47 | 27 | 18 | 13 |

4. Discussion

Neurodegeneration in AD may involve oxidative stress and neuroinflammatory mechanisms, and efforts to identify biomarkers of these processes may be clinically useful in disease prognostication, as well as to help shine light on therapeutic targets to slow down these processes. Cp is an acute phase protein whose expression is induced during inflammation (Mackiewicz et al., 1988), and it is a protein that functions to lower iron burden (Harris et al., 1999), which may promote neurodegeneration in AD (Aytón et al., 2015; Aytón et al., 2017a; Aytón et al., 2017b; Aytón et al., 2019; Aytón et al., 2018; Diouf et al., 2019). Cp expression is also dependent on copper availability, and since low brain copper is also a feature of AD (Deibel et al., 1996), it is possible that this may further affect the levels of Cp in CSF. While Cp is linked to these neurochemical changes occurring in AD, we could only find 2 prior papers that investigated CSF Cp levels in a total of 27 AD patients, studied cross sectionally (Loeffler et al., 1994; Cape et al., 2008). Here, in a cohort of 268 along the spectrum of AD clinical and pathological

Table 4
Association between baseline CSF Cp levels and t-tau/Aβ_{42} ratio with change in longitudinal cognitive and MRI-volumetric outcomes. Data are from separate mixed effects linear models of outcome (either CDR-SB, ADAS-Cog, MMSE, Hippocampal Volume or Lateral ventricular volume) and include the following covariates: age, sex, APOE ε4, and CSF levels of Cp, t-tau/Aβ_{42} (CSF t-tau/Aβ_{42} was additionally included as a continuous variable in the separate models stratified by this biomarker), apolipoprotein E, and ferritin, and all of these interacted with time. β represent interaction with time with either CSF Cp levels or t-tau/Aβ_{42} levels. α was set at 0.05, and a Bonferroni adjustment was used to correct for multiple comparisons for cognitive and volumetric studies.

| Outcome | Clinical t-tau/ Aβ_{42} | CSF Cp levels | CSF t-tau/ Aβ_{42} levels |
|---------|------------------------|---------------|--------------------------|
|         | Dx | Dx | β | S.E. | P | β | S.E. | P |
| CDR-SB  | CN Low                  | 0.004 (0.009) | 0.630 | 0.616 | (0.258) | 0.018* |
|          | CN High                 | 0.001 (0.042) | 0.987 | 0.121 | (0.210) | 0.564 |
|          | MCI Low                 | 0.047 (0.039) | 0.233 | 3.304 | (1.141) | 0.004* |
|          | MCI High                | 0.149 (0.041) | 3 × 10^{-4.} | 0.473 | (0.178) | 0.008* |
| ADAS-Cog | CN Low                  | −0.036 (0.085) | 0.676 | −4.01 | (2.56) | 0.118 |
|          | CN High                 | 0.287 (0.848) | 0.249 | 0.979 | (0.848) | 0.249 |
|          | MCI Low                 | −0.066 (0.162) | 0.685 | 10.05 | (4.71) | 0.033* |
|          | MCI High                | 0.744 (0.145) | 3 × 10^{-7.} | 1.40 | (0.647) | 0.301* |
| MMSE    | CN Low                  | 0.017 (0.028) | 0.537 | −0.966 | (0.825) | 0.242 |
|          | CN High                 | −0.061 (0.065) | 0.348 | 0.008 | (0.325) | 0.980 |
|          | MCI Low                 | −0.072 (0.074) | 0.336 | −5.410 | (2.147) | 0.012* |
|          | MCI High                | −0.153 (0.069) | 0.027* | −1.130 | (0.297) | 2 × 10^{-4.} |
| Hippocampal volume | CN Low                  | −8.29 (6.22) | 0.184 | −321.4 | (207.3) | 0.122 |
|          | CN High                 | −5.23 (8.11) | 0.519 | −281.5 | (44.4) | 9 × 10^{-3.} |
|          | MCI Low                 | 13.8 (12.0) | 0.253 | 552.3 | (433.9) | 0.203 |
|          | MCI High                | −9.02 (9.64) | 0.350 | −18.5 | (32.8) | 0.572 |
| Ventricle volume | CN Low                  | 106.9 (53.7) | 0.063 | −11.2 | (146.4) | 0.940 |
|          | CN High                 | 424.6 (133.8) | 0.002 | −231.7 | (627.6) | 0.712 |
|          | MCI Low                 | −3022 (1898) | 0.112 | −2562 | (4691) | 0.585 |
|          | MCI High                | 479.2 (1126) | 4 × 10^{-5.} | −479.1 | (477.0) | 0.316 |

#: indicates nominally significant P values. *indicates significant P values after Bonferroni correction. S.E.: Standard Error. Dx: diagnosis. The number of subjects who underwent cognitive and MRI assessments at each timepoint, and for each disease category is presented in Table 3.
diagnosis, we found that CSF Cp levels were not elevated in AD, however, relatively high levels of Cp were associated with accelerated disease progression in people with MCI and underlying Aβ-pathology (confirmed with CSF tau/Aβ42).

Cp functions to lower cellular iron burden, which has been reported to be elevated in AD cortex, with a degree of variability between areas and studies (Aytont et al., 2019; Kenkhusi et al., 2019; Tao et al., 2014), so it may seem surprising that higher level of this protein is associated with accelerated disease progression toward AD. A similar observation was reported in HIV patients, where elevated Cp levels (and other acute phase proteins) were associated with increased risk of cognitive impairment (Kallianpur et al., 2019). Since elevation of Cp levels have been shown to be protective against brain iron overload in animal models (Aytont et al., 2013; Aytont et al., 2014; Tuo et al., 2017; Zanardi et al., 2017), it is likely that higher Cp levels are not damaging to the brain, but rather might reflect neuroinflammation (since Cp is an acute phase protein that increases during inflammation). It is possible that Cp functions to limit the toxicity of iron in this pro-inflammatory state. However, another possibility is that Cp acts to exacerbate neuroinflammation, since Cp has been shown to promote an inflammatory response in microglial cells in vitro (Lee et al., 2007; Lazzaro et al., 2014).

This first report on the associations between CSF Cp levels and longitudinal AD outcomes highlights that CSF Cp was more informative for prognostication than for cross-sectional changes. In the three prior longitudinal studies that measured blood Cp in the context of AD, we identified one paper that showed no change in Cp levels in serum (Rembach et al., 2013), while another showed that plasma Cp levels (and other acute phase proteins) predicted MCI conversion to AD (Westwood et al., 2018), which was not replicated in another cohort (Squitti et al., 2014). It is unclear how Cp levels in the serum relate to Cp levels in the CSF (although experiments in mice demonstrate evidence of a communication of Cp between these biofluid pools (Aytont et al., 2013; Aytont et al., 2014; Tuo et al., 2017; Zanardi et al., 2017)), but it is possible that Cp in both the serum and CSF reflect an increased state of inflammation, which drives disease progression.

Finally, we report a novel association between CSF Cp and CSF apolipoprotein E, but CSF Cp was not associated with APOE genotype. It is not known whether Cp promotes apolipoprotein E secretion (or vice versa), or whether they have a common mechanism that affects their expression (and are therefore not causally related to each other). A prior GWAS on 570 subjects did not identify the Cp gene as a significant predictor of CSF apoE (Cruchaga et al., 2012); while this is evidence against a role for Cp in causing changes in apoE levels, a study with higher power may yet reveal a significant result. We previously showed that CSF ferritin was associated with apoE and also APOE genotype (Aytont et al., 2015), but in the current study we found no association between CSF Cp and CSF ferritin. So, both CSF Cp and CSF ferritin have shared variance with CSF apoE, but they do not correlate with each other. This is surprising since ferritin is also an acute phase protein, and we may also expect that CSF Cp would impact CSF ferritin levels by affecting cellular iron load (and in turn secretion of ferritin).

In contrast to ferritin, which is mainly produced by microglia in the brain (Kaneko et al., 1989), Cp and apoE are both produced by astrocytes (Patel and David, 1997; Harris et al., 2004). The correlation we observe between CSF Cp and apoE may therefore relate to the abundance and/or activation of astrocytes. However, CSF apoE (regardless of genotype) has been previously reported to be negatively associated with disease progression (i.e. low CSF apoE is associated with worse outcomes) (Aytont et al., 2015; Toledo et al., 2014), whereas we report that Cp is positively associated with disease progression (i.e. high CSF apoE is associated with worse outcomes). So, these proteins may reveal different aspects of astrocyte pathobiology in AD and/or different activation states of these cells, which warrants future experimental investigation.

The results from this study raise new questions into the role of Cp in AD, and associated pathways including oxidative stress and neuroinflammation. It is presently unclear whether Cp levels report inflammatory changes, copper metabolism, or impact on iron biochemistry. But regardless, our results show that CSF Cp could be useful in predicting near-term disease progression (cognitive decline and neurodegeneration) in MCI people who are positive for Aβ. Future work should focus on Cp specific activity (which can be affected by ATP7B pump dysfunction (Yamada et al., 1993) or oxidation of Cp (Olivieri et al., 2011)), and non-Cp bound copper in CSF, which has shown to be informative biomarker in serum (Squitti et al., 2014).

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