Original research

CSF ferritin in the clinicopathological progression of Alzheimer's disease and associations with APOE and inflammation biomarkers

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ABSTRACT

Background A putative role for iron in driving Alzheimer's disease (AD) progression is complicated by previously reported associations with neuroinflammation, apolipoprotein E and AD proteinopathy. To establish how iron interacts with clinicopathological features of AD and at what disease stage iron influences cognitive outcomes, we investigated the association of cerebrospinal fluid (CSF) biomarkers of iron (ferritin), inflammation (acute phase response proteins) and apolipoproteins with pathological biomarkers (CSF $A\beta_{42}$/t-tau, p-tau181), clinical staging and longitudinal cognitive deterioration in subjects from the BioFINDER cohort, with replication of key results in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort.

Methods Ferritin, acute phase response proteins (n=9) and apolipoproteins (n=6) were measured in CSF samples from BioFINDER (n=1239; 4 years cognitive follow-up) participants stratified by cognitive status (cognitively unimpaired, mild cognitive impairment, AD) and for the presence of amyloid and tangle pathology using CSF $A\beta_{42}$/t-tau (A+) and p-tau181 (T+). The ferritin and apolipoprotein E associations were replicated in the ADNI (n=264) cohort.

Results In both cohorts, ferritin and apoE were elevated in A-T+ and A+T+ subjects (16%–40%), but not clinical diagnosis. Other apolipoproteins and acute phase response proteins increased with clinical diagnosis, not pathology. CSF ferritin was positively associated with p-tau181, which was mediated by apolipoprotein E. An optimised threshold of ferritin predicted cognitive deterioration in mild cognitive impairment subjects in the BioFINDER cohort, especially those people classified as A-T- and A+T-.

Conclusions CSF markers of iron and neuroinflammation have distinct associations with disease stages, while iron may be more intimately associated with apolipoprotein E and tau pathology.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Brain iron is implicated in Alzheimer's disease, with uncertain pathogenic significance. Iron also associates with clinicopathological features such as proteinopathy, inflammation and APOE, so it is unclear if iron is an independent lesion of Alzheimer's disease or epiphenomenal.

WHAT THIS STUDY ADDS

⇒ By interrogating cerebrospinal fluid (CSF) biomarkers of iron (ferritin), inflammation (acute phase response proteins) and apolipoproteins with pathological biomarkers (CSF $A\beta_{42}$/t-tau and p-tau181), in a large clinical cohort (with replication of key results in a smaller cohort), we reveal that iron associates with tangle pathology, which is mediated by apolipoprotein E. Iron does not change with clinical severity or with inflammation. Iron, inflammation and tau biomarkers all predict disease progression, but in different disease stages.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We propose criteria for high iron pathology (I+), and in what disease stage this may have clinical utility.

INTRODUCTION

Brain iron burden is a copathology increasingly implicated in Alzheimer's disease (AD) pathogenesis; higher iron burden can promote inflammation or lipid peroxidation, which signals the regulated cell death pathway, ferroptosis.[1] Brain iron levels assayed both by ferritin (the major iron storage biomarker) in cerebrospinal fluid (CSF),[2–5] by quantitative susceptibility mapping-MRI,[6] or directly measured post mortem,[7 8] predict longitudinal cognitive deterioration and brain atrophy in people within the AD clinical spectrum.

Iron also has both physiological and pathological associations with the canonical AD proteins: Aβ and the amyloid precursor protein (APP), tau and apolipoprotein E (apoE—protein; *APOE*—gene). APP and tau have been reported to have physiological roles in neuronal iron homoeostasis.[9–14] In neuropathological and neuroimaging surveys, brain iron levels have been reported as associating with plaque and tangle pathology.[6 7 15–18] We previously identified an unexpected positive correlation between CSF apoE and ferritin levels.[2] We also found that CSF ferritin was elevated in carriers of the AD risk allele, *APOE* ε4. Higher MRI-determined brain iron has been reported in *APOE* ε4 carriers in some studies,[19] but not

others,[6 20] while *APOE* ε4 genotype has been shown to influence the impact of iron on synchronised default mode network activity.[21]

Iron neurochemistry is linked to neuroinflammation, which is implicated in the natural history of AD by uncertain mechanisms. Most,[22–29] but not all[30 31] studies of translocator protein (TSPO) PET ligand (which reports activated glia) describe increased uptake in AD and mild cognitive impairment (MCI) subjects. Yet, PET-determined Aβ plaque has been reported consistently as not positively associated with TSPO.[23 27 31] In agreement, we found that acute phase response proteins in CSF (including α1-antichymotrypsin, α1-antitrypsin, ceruloplasmin, C reactive protein, complement C3, ferritin, α-fibrinogens, β-fibrinogens, γ-fibrinogens, haptoglobin, haemopexin), as reporters of proinflammatory microglia, were not associated with amyloid burden, but were increased with clinical diagnosis.[32]

Several acute phase response proteins have functional roles in iron regulation. These include ceruloplasmin (required for cellular iron export), ferritin, haptoglobin and haemopexin (bind and clear iron-rich haemoglobin and heme, respectively), and lactoferrin (binds and transports extracellular iron). Iron sequestration from extracellular fluid into cells is an innate immune manoeuvre to deprive pathogens of iron nutrition,[33] thus, activated microglia retain iron.[34–36] Indeed a subset of microglia that are infiltrated by plaque were recently characterised as particularly enriched with ferritin and iron in AD cases.[37] Conversely, iron promotes the NALP3-inflammasome as well as Aβ-induced activation of IL-1β.[38] But the role of iron in AD neuroinflammation is uncertain.

Since iron is associated with other putative causative factors of AD such as neuroinflammation,[34–37] proteinopathy (amyloid plaque, neurofibrillary tangles are enriched with iron[6 7 15–18] or apolipoprotein E[2 19 39], iron might be epiphenomenal to other principal lesions. To test the hypothesis that iron is a copathology of AD that acts independently on disease progression we used biomarkers of iron (CSF ferritin), inflammation (CSF acute phase response proteins), proteopathy (CSF Aβ42, total-tau, p-tau181) and APOE (CSF protein levels and ε4 genotype) to explore: (1) changes in ferritin during the clinicopathology staging of AD, (2) associations of ferritin with other biomarker and clinical variables, and (3) the performance of ferritin against other biomarkers in predicting longitudinal disease outcomes according to disease stage in the BioFINDER cohort, with replication in the Alzheimer's disease neuroimaging initiative (ADNI) cohort where data availability allowed.

METHODS

BioFINDER cohort

We included Cognitively Unimpaired participants (CU; n=788) and patients with MCI (n=263) and AD dementia (n=188) from the BioFINDER study. Participants underwent a medical history, complete neurological examination, neuropsychological testing and lumbar puncture. The inclusion and exclusion criteria are described in online supplemental methods.

The characteristics of the study participants are given in table 1. Subjects were assessed with Mini–Mental State Examination (MMSE) and Clinical Dementia Rating Scale, sum of boxes (CDR-SB) at baseline, annually for 4 years (subjective cognitive decline and MCI subjects) or every second year (CU subjects without subjective cognitive decline). The N at each timepoint is displayed in online supplemental table 1. The measurement of CSF samples is described in online supplemental methods.

Table 1 Baseline demographics of BioFINDER subjects stratified by cognitive severity (CU, MCI, AD) and biomarker (A± and T+/-) criteria

Dx	CU						MCI						AD					
A	–		+		–	+	–		+		–	+	–		+		–	+
T	–		–		+	+	–		–		+	+	–		–		+	+
N	558		85		22	123	90		34		7	132	9		19		0	160
Age: mean (SD)	70.3	(5.8)	72.6	(4.8)	75.5	72.9	(5.2)	69.0	(5.7)	72.3	(5.2)	69.7	(4.5)	72.1	(5.0)	75.0	(7.1)	78.3	(4.6)	NA	74.5	(7.4)
Female sex: N (%)	344	(61.6)	56	(65.9)	12	65	(52.8)	28	(31.1)	11	(32.4)	2	(28.6)	68	(51.5)	6	(66.7)	9	(47.4)	NA	104	(65.0)
APOE ε4+ve: N (%)	135	(24.4)	44	(52.4)	7	(31.8)	81	(65.9)	17	(18.9)	21	(61.8)	3	(42.9)	92	(69.7)	4	(44.4)	10	(58.8)	NA	105	(66.9)
$A\beta_{42}$: mean (SD)	734	(233)	371	(137)	1002	(272)	374	(119)	689	(214)	327	(114)	961	(343)	342	(122)	526	(293)	255	(110)	NA	317	(138)
t-tau mean (SD)	275	(74)	352	(98)	456	(101)	551	(190)	265	(76)	358	(120)	430	(136)	571	(209)	282	(97)	386	(154)	NA	648	(206)
$A\beta_{42}$/t-tau: mean (SD)	0.13	(0.02)	0.08	(0.02)	0.11	(0.02)	0.06	(0.02)	0.13	(0.02)	0.08	(0.03)	0.11	(0.03)	0.06	(0.02)	0.13	(0.03)	0.09	(0.03)	NA	0.06	(0.02)
p-tau181: mean (SD)	35.1	(9.9)	44.7	(10.7)	71.4	(14.7)	100.2	(37.1)	34.5	(9.8)	43.2	(11.3)	77.8	(13.2)	115.1	(43.3)	37.5	(11.1)	37.2	(12.2)	NA	128.3	(42.6)
CDR-SB: mean (SD)	0.15	(0.41)	0.09	(0.29)	0.00	(0.00)	0.32	(0.54)	1.34	(0.95)	1.45	(0.87)	1.00	(0.58)	1.48	(0.97)	NA	(NA)	NA	(NA)	NA	NA	(NA)
MMSE: mean (SD)	29.0	(1.1)	29.0	(1.0)	28.9	(1.1)	28.5	(1.3)	27.6	(1.8)	27.2	(2.0)	26.7	(2.2)	26.6	(1.6)	19.6	(5.1)	22.0	(4.6)	NA	21.0	(3.9)

Values of $A\beta_{42}$, t-tau and p-tau181 are in pg/mL.
AD, Alzheimer's disease; CDR-SB, Clinical Dementia Rating Scale, sum of boxes; CU, cognitively unimpaired; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; NA, not available.

ADNI Cohort

Data were obtained from the ADNI database (adni.loni.usc.edu; 09/06/2019). ADNI was launched in 2003 as a public–private partnership, led by principal investigator Michael W Weiner. 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.

The ADNI study protocols and patient inclusion criteria have been reported previously.[2] Subject demographics are reported in online supplemental table 4. The measurement of CSF samples is described in online supplemental methods.

Statistics

Baseline demographics of participants included in this study were described in strata of CU, MCI and AD diagnosis (determined clinically, described above) as well as A+and T+criteria using previously published thresholds, respectively, for CSF $A\beta_{42}$/t-tau and p-tau181 for BioFINDER[39] and ADNI[40] cohorts. The acute phase proteins and apolipoproteins were log and Z transformed. Ferritin and apoE levels were used in the primary analysis; unadjusted p values are reported, but the Bonferroni-adjusted alpha is noted in the table legends and text. The other acute phase response proteins and lipoproteins were used in exploratory analysis, therefore, correction for multiple comparisons was not performed. Factors associated with each of the CSF proteins were analysed using separate multiple linear regressions with the following covariates: age, sex, *APOE* ε4 genotype, and either A&T stratification or cognitive status (CU, MCI, AD). Multiple regressions of each CSF protein included the following additional covariates: age, sex and *APOE* ε4. Mixed effects models of CDR-SB or MMSE were constructed for CU and MCI subjects in strata of A± and T±, and included the following variables: age, sex, *APOE* ε4 and the CSF protein of interest. Due to insufficient N we did not model A-T+subjects (of any clinical status) or AD dementia subjects in BioFINDER. For the same reason, we did not perform longitudinal modelling in ADNI subjects. Data were not imputed if missing. Models were performed with R (V.4.03).

RESULTS

CSF ferritin and apoE levels are markedly elevated in T+ subjects

Similar to our previous report[32] in the BioFINDER cohort, CSF ferritin varied little with cognitive status (CU, MCI, AD dementia) in a multiple regression model including cognitive status, age, sex and *APOE* ε4 (figure 1A, table 2—values, online supplemental table 2—statistics). Ferritin was elevated to 11.6 ng/mL in AD dementia subjects compared with 10.2 ng/mL in CU, which was at the threshold of significance (p=0.05). But, when the cohort was categorised by A/T grouping, ferritin was markedly increased in A-T+ (β(S.E.)=0.621 (0.183); p=0.001), and A+T+ (β(S.E.)=0.426 (0.068); $p=3.3\times10^{-11}$) subjects compared with A-T- (figure 1B, table 3—values, online supplemental table 3—statistics). The trend for ferritin to be elevated in T+subjects was observed in each diagnostic category, although with few subjects in some of the categories when grouped in this way (online supplemental table 4).

APOE ε4 status was not associated with ferritin (p>0.05; online supplemental table 5), and apoE levels did not differ according to diagnosis (figure 1C, table 2−values, online supplemental table 2−statistics). Compared with A-T- subjects, apoE levels were decreased in A+T subjects (β(S.E.)=−0.283 (0.095); p=0.003), but, like ferritin, were increased in A-T+subjects (β(S.E.)=0.709; (0.184); $p=1.2\times10^{-4}$) and A+T+ subjects (β(S.E.)=0.468 (0.070)); $p=8.8\times10^{-14}$; figure 1D, table 3—values, online supplemental table 3—statistics)

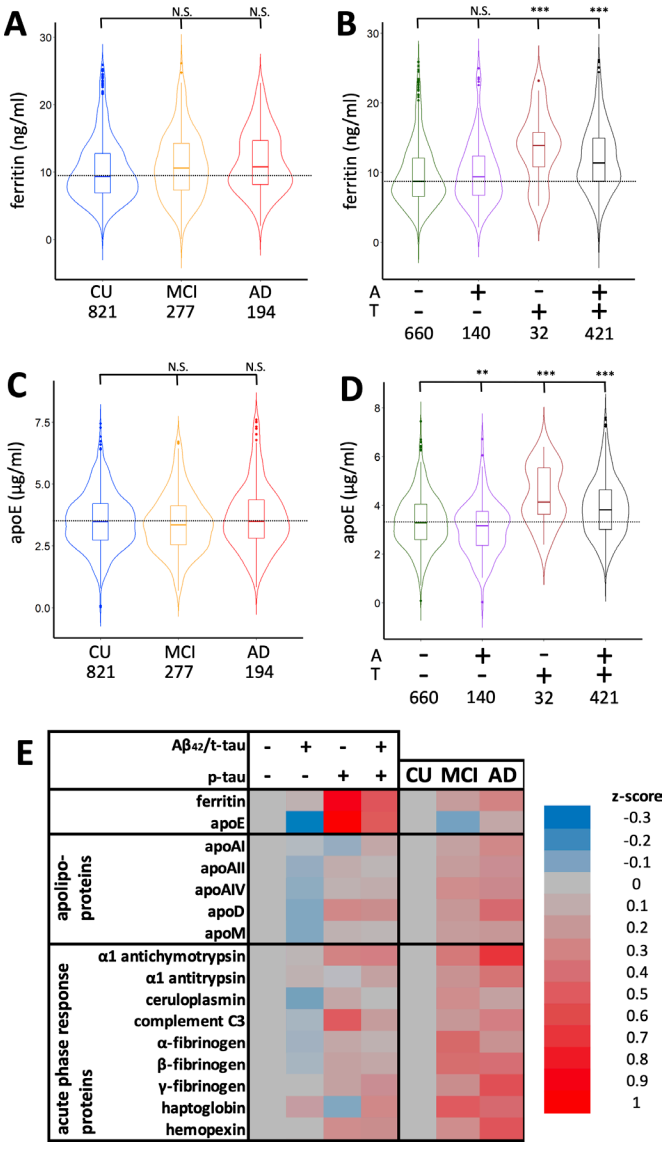

Figure 1 Ferritin and apoE levels in BioFINDER subjects. (A, B) Violin plots of CSF ferritin (A) stratified by cognitive status and (B) A&T criteria. (C, D) Violin plots of CSF apoE (C) stratified by cognitive status and (D) A&T criteria. Statistics are from multiple regression models including age, sex, *APOE* ε4, apoE, ferritin and either cognitive status or A&T criteria, where **p<0.01; ***p<0.001. (E) Heat map of changes in apoE, ferritin, and other apolipoproteins and acute phase response proteins in subjects stratified by either cognitive status or A&T criteria. AD, Alzheimer's disease; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment.

Changes in ferritin and apoE were recapitulated in the ADNI cohort (online supplemental table 6). As previously reported,[2] neither ferritin nor apoE differed according to diagnosis (assessed in multiple regression models of each of the analytes, including age, sex, *APOE* ε4 and cognitive status as covariates; online supplemental figure 1A,C; online supplemental table 7). But, when analysed according to A/T criteria, ferritin was increased in A-T+ (β(S.E.)=0.945 (0.375); p=0.012) and A+T+ subjects (β(S.E.) = 0.445 (0.163); p=0.007; online supplemental figure 1B; online supplemental table 8). ApoE was likewise increased

Table 2 Comparison of changes in CSF apoE and ferritin with other apolipoproteins and acute phase proteins across clinical diagnosis in the BioFINDER cohort

		CU			MCI			AD		
		Mean	SD	P value	Mean	SD	P value	Mean	SD	P value
Apolipoproteins	apoE	3.53	(1.13)	NA	3.41	(1.18)	0.092	3.66	(1.33)	0.625
	apoAI	2.56	(1.32)	NA	2.73	(1.42)	0.422	2.92	(1.48)	**0.007**
	apoAII	0.42	(0.23)	NA	0.46	(0.26)	0.263	0.47	(0.26)	**0.016**
	apoAIV	0.23	(0.12)	NA	0.26	(0.15)	**0.022**	0.26	(0.14)	**0.027**
	apoD	2.20	(0.78)	NA	2.35	(0.88)	0.197	2.53	(0.94)	**5.3×10^{-5}**
	apoM	10.8	(8.20)	NA	12.3	(9.70)	0.070	12.5	(9.71)	**0.022**
Acute phase proteins	ferritin	10.2	(4.60)	NA	11.0	(4.90)	0.264	11.6	(4.59)	**0.050**
	α1 antichymotrypsin	0.87	(0.39)	NA	1.01	(0.48)	**7.8×10^{-5}**	1.13	(0.52)	**2.2×10^{-9}**
	α1 antitrypsin	3.14	(1.58)	NA	3.48	(1.79)	0.054	3.75	(1.79)	**3.1×10^{-4}**
	ceruloplasmin	0.49	(0.48)	NA	0.62	(0.67)	**0.017**	0.56	(0.38)	0.058
	complement C3	3.48	(1.35)	NA	3.75	(1.52)	0.070	3.91	(1.53)	**0.001**
	α-fibrinogen	0.43	(0.53)	NA	0.67	(0.93)	**5.2×10^{-5}**	0.55	(0.43)	**0.001**
	β-fibrinogen	0.17	(0.19)	NA	0.24	(0.30)	**0.001**	0.24	(0.21)	**2.5×10^{-5}**
	γ-fibrinogen	0.14	(0.09)	NA	0.16	(0.10)	**0.008**	0.19	(0.12)	**2.0×10^{-6}**
	haptoglobin	1.47	(1.43)	NA	2.18	(2.13)	**1.1×10^{-5}**	2.08	(2.04)	**0.001**
	haemopexin	3.06	(1.31)	NA	3.38	(1.55)	**0.033**	3.75	(1.65)	**1.2×10^{-6}**

The apolipoprotein composite was formed by averaging the Z(log()) transformed variables: apoAI, apoAII, apoAIV, apoD, apoM. The APR composite was formed by averaging the Z(log()) transformed variables: α1 antichymotrypsin, α1 antitrypsin, ceruloplasmin, complement C3, α-fibrinogen, β-fibrinogen and γ-fibrinogen, haptoglobin and haemopexin. Statistics are from multiple regression models of each of the analytes including age, sex, *APOE* ε4 gene and diagnosis as covariates. Protein concentrations are in µg/mL CSF except for ferritin and apoM which are ng/mL. Ferritin and apoE were highlighted as the primary analysis, the other analytes are included as exploratory comparative results. Bold indicates p<0.05.
*Indicates correction after multiple comparison between the two primary analysis of ferritin and apoE (α/2=0.025).
AD, Alzheimer's disease; CSF, cerebrospinal fluid; CU, Cognitively Unimpaired; MCI, mild cognitive impairment; NA, not available.

in A-T+ (β(S.E.) = 0.913 (0.347); p=0.009) and A+T+ subjects (β(S.E.)=0.545 (0.151); p=$0.30.6\times10^{-4}$; online supplemental figure 1D).

Cross-sectional CSF ferritin and apoE discriminate A/T status while lipoproteins and acute phase proteins discriminate diagnosis

Since the pattern in changes according to the A/T biomarkers was similar for both ferritin and apoE, we explored whether other lipoproteins (ApoA-I, apoA-II, apoA-IV, apoM and apoD) might change similarly. Unlike apoE or ferritin, these additional lipoproteins were modestly elevated in AD compared with CU subjects ($0.187<\beta<0.339$; $5.3\times10^{-5}<p<0.027$; figure 1E, table 2—values, online supplemental table 2—statistics). ApoA-IV was also modestly elevated in MCI subjects (β(S.E.)=0.160 (0.070); p=0.022). Levels of these lipoproteins were generally not associated with A or T status (figure 1E, table 3—values, online supplemental table 3—statistics), except

Table 3 Comparison of changes in CSF apoE and ferritin with other apolipoproteins and acute phase proteins in strata of A and T criteria in the BioFINDER cohort

		A-T-			A+T-			A-T+			A+T+		
		Mean	SD	P value	Mean	SD	P value	Mean	SD	P value	Mean	SD	P value
Apolipoprotein	apoE	3.34	(1.08)	NA	3.07	(1.09)	**0.003***	4.42	(1.19)	**1.2×10^{-4}***	3.9	(1.20)	**8.8×10^{-14}***
	apoA-I	2.61	(1.35)	NA	2.59	(1.40)	0.280	2.52	(1.26)	0.473	2.75	(1.39)	0.279
	apoA-II	0.43	(0.24)	NA	0.42	(0.23)	0.140	0.45	(0.26)	0.746	0.44	(0.24)	0.846
	apoA-IV	0.23	(0.13)	NA	0.22	(0.14)	**0.012**	0.24	(0.16)	0.852	0.25	(0.13)	0.506
	apoD	2.22	(0.81)	NA	2.14	(0.84)	**0.027**	2.44	(0.72)	0.361	2.42	(0.86)	**0.001**
	apoM	11.4	(9.27)	NA	10.5	(7.13)	0.672	11.9	(9.95)	0.931	11.8	(8.65)	0.275
Acute phase proteins	ferritin	9.66	(4.45)	NA	9.99	(4.47)	0.701	13.59	(4.95)	**0.001***	12.06	(4.67)	**3.3×10^{-11}***
	α1 antichymotrypsin	0.89	(0.42)	NA	0.91	(0.42)	0.308	1.02	(0.39)	0.252	1.02	(0.48)	0.257
	α1 antitrypsin	3.21	(1.65)	NA	3.32	(1.75)	0.250	3.21	(1.55)	0.665	3.45	(1.68)	0.606
	ceruloplasmin	0.53	(0.60)	NA	0.46	(0.39)	0.108	0.60	(0.49)	0.601	0.55	(0.42)	0.370
	complement C3	3.51	(1.41)	NA	3.46	(1.46)	0.123	4.22	(1.18)	**0.019**	3.75	(1.41)	**0.001**
	α-fibrinogen	0.48	(0.71)	NA	0.45	(0.50)	0.391	0.57	(0.55)	0.095	0.53	(0.55)	0.077
	β-fibrinogen	0.19	(0.23)	NA	0.18	(0.21)	0.141	0.22	(0.22)	0.529	0.21	(0.22)	**5.3×10^{-10}**
	γ-fibrinogen	0.14	(0.09)	NA	0.14	(0.10)	0.169	0.16	(0.10)	0.620	0.16	(0.11)	**0.001**
	haptoglobin	1.55	(1.54)	NA	1.78	(1.61)	0.158	1.41	(1.04)	0.868	1.96	(2.02)	0.205
	haemopexin	3.10	(1.37)	NA	3.13	(1.48)	0.151	3.44	(1.42)	0.395	3.43	(1.51)	0.362

Statistics are from multiple regression models of each of analyte including age, sex, *APOE* ε4 and A/T criteria as covariates. Protein concentrations are in µg/ml CSF except for ferritin and apoM which are ng/mL. Ferritin and apoE were highlighted as the primary analysis, the other analytes are included as exploratory comparative results. Bold indicates p<0.05.
*Indicates correction after multiple comparison between the two primary analysis of ferritin and apoE (α/2=0.025).
CSF, cerebrospinal fluid; NA, not available.

for a decrease in apoA-IV (β(S.E.)=−0.241 (0.095); p=0.012) and apoD β(S.E.)=−0.208 (0.094); p=0.027) levels in subjects who were A+T-, and an elevation in apoD in A+T+ subjects (β(S.E.)=0.220 (0.068); p=0.001). Thus, the association of ferritin and apoE with A/T biomarkers cannot be explained by generalised changes in CSF lipoproteins.

Ferritin is an acute phase response protein, so it is possible that changes in ferritin reflect inflammatory changes and not brain iron burden. We; therefore, investigated a panel of other CSF acute phase response proteins in the BioFINDER cohort: α1-antichymotrypsin, α1-antitrypsin, ceruloplasmin, complement C3, α-fibrinogen, β-fibrinogen, γ-fibrinogen, haptoglobin and haemopexin. In contrast to ferritin levels, all acute phase response proteins except for α1 antitrypsin and complement C3 were significantly elevated in MCI ($0.149<\beta<0.316$; $1.1\times10^{-5}<p<0.033$) and, except for ceruloplasmin, in AD dementia ($0.271<\beta<0.499$; $2.2\times10^{-9}<p<0.001$; figure 1E; table 2—values, online supplemental table 2—statistics), similar to what we have previously reported.[32]

Several of the acute phase proteins also varied according to A&T criteria, although these changes were more modest compared with ferritin and apoE (figure 1E; table 3—values, online supplemental table 3—statistics). In multiple regression models of each analyte (including age, sex, *APOE* ε4 and A/T criteria covariates), no protein was changed in A+T subjects, and only complement C3 was changed (increased) in A-T+subjects (β(S.E.)=0.434 (0.185); p=0.019). Complement C3 was also elevated in A+T+ subjects, along with γ-fibrinogen and β-fibrinogen ($0.173<\beta<0.186$; $5.3\times10^{-10}<p<0.001$). Therefore, changes in ferritin and apoE were unlikely to be explained by a generalised response of acute phase proteins.

Associations between CSF ferritin, apoE, Aβ and tau

We explored associations between ferritin, apoE, $A\beta_{42}$/t-tau and p-tau in regressions of all BioFINDER subjects, then separately according to cognitive status. In the BioFINDER cohort, ferritin and apoE were correlated in the overall cohort (figure 2A,B; partial $r^2=0.056$; $p=3.8\times10^{-15}$) and within each diagnostic category (online supplemental figure 2A–C), consistent with our previous report of ADNI samples.[2] Both ferritin (figure 2C; partial $r^2=0.038$ $p=2.0\times10^{-11}$) and apoE (figure 2D; partial $r^2=0.069$; $p=5.3\times10^{-19}$) levels were correlated with p-tau levels in the overall cohort, and in each clinical category (online supplemental figure 2D–F; J–L). Ferritin was not associated with $A\beta_{42}$/t-tau when all subjects were included in the modelling (figure 2E), or when the diagnostic categories were separately explored (online supplemental figure 2G–I). ApoE was negatively associated with $A\beta_{42}$/t-tau in all subjects (figure 2F; partial $r^2=0.010$; $p=5.4\times10^{-4}$), but not in any group when stratified by cognitive status (online supplemental figure 2M–O). When we replicated this analysis in the ADNI cohort, finding that ferritin was again correlated with apoE (partial $r^2=0.236$; $p=4.3\times10^{-15}$), but not in this cohort with p-tau or $A\beta_{42}$/t-tau (online supplemental figure 3). ApoE was again correlated with p-tau (partial $r^2=0.098$; $p=1.9\times10^{-8}$) and, to a lesser extent, $A\beta_{42}$/t-tau (partial $r^2=0.045$; p=0.027).

In prior work measuring iron by in vivo QSM MRI,[18] or by direct assay in post mortem brain tissue,[7] we have found evidence that iron promotes neurodegeneration downstream of tangle pathology in the natural history of AD. Since ferritin was associated with both p-tau (β (95% CI)=0.33 (0.27 to 0.38)) and apoE (β (95% CI)=0.24, (0.19 to 0.30)), we tested whether apoE mediated the association between p-tau and ferritin in the BioFINDER cohort. Indeed, we found that the indirect effect between p-tau and ferritin was significant (β (95% CI)=0.074 (0.055 to 0.098)), indicating partial mediation of the relationship between p-tau and ferritin by apoE (figure 2G). This underscores a neurochemical relationship between brain iron burden, reflected by CSF ferritin, and the canonical AD proteins, apoE and p-tau.

Predicting cognitive decline using ferritin and related biomarkers in different disease stages

We next investigated whether baseline ferritin or apoE predicted clinical deterioration on the CDR-SB scale over 4 years of annual follow-up of the BioFINDER subjects (online supplemental table 1 for N). Change in MMSE was additionally used as an outcome variable in secondary analysis. Baseline ferritin predicted increased CDR-SB score (deterioration) when tested in mixed-effects models in MCI (β(S.E.)=0.179 (0.043); $p=2.8\times10^{-5}$) but not CU subjects (figure 3A,B; online supplemental table 9). Within the MCI cohort, baseline ferritin predicted CDR-SB deterioration in A-T- (figure 3D; β(S.E.)=0.228 (0.089); p=0.011) and, most prominently, A+T- (figure 3F; β(S.E.)=0.633 (0.141); $p=2.0\times10^{-5}$), but not A+T+. Among CU subjects, the only

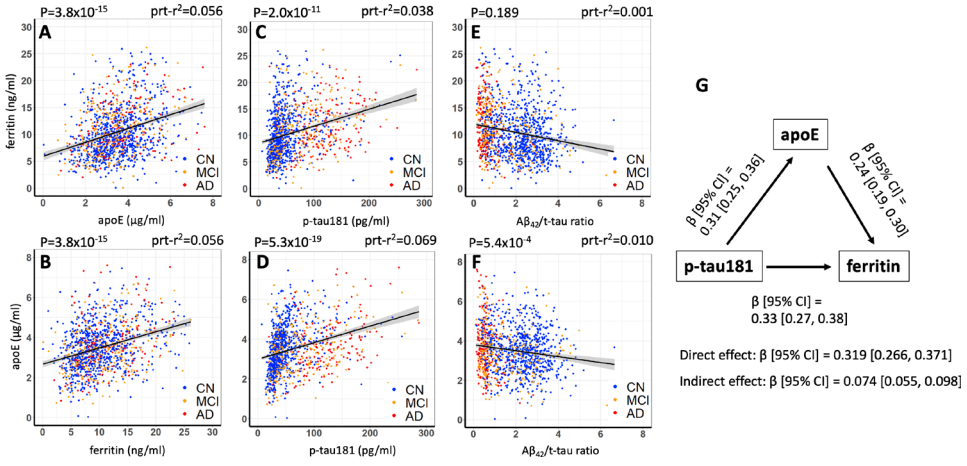

Figure 2 Correlations between ferritin, apoE, p-tau and $A\beta_{42}$/t-tau in subjects from the BioFINDER cohort and mediation analysis. (A, B) are the same data that are alternatively presented for ease of comparison. Statistics are from multiple regression model of either ferritin or apoE, including age, sex, *APOE* ε4, p-tau181, $A\beta_{42}$/t-tau and either ferritin or apoE. prt-r^2 represents the partial r^2. AD, Alzheimer's disease; MCI, mild cognitive impairment.

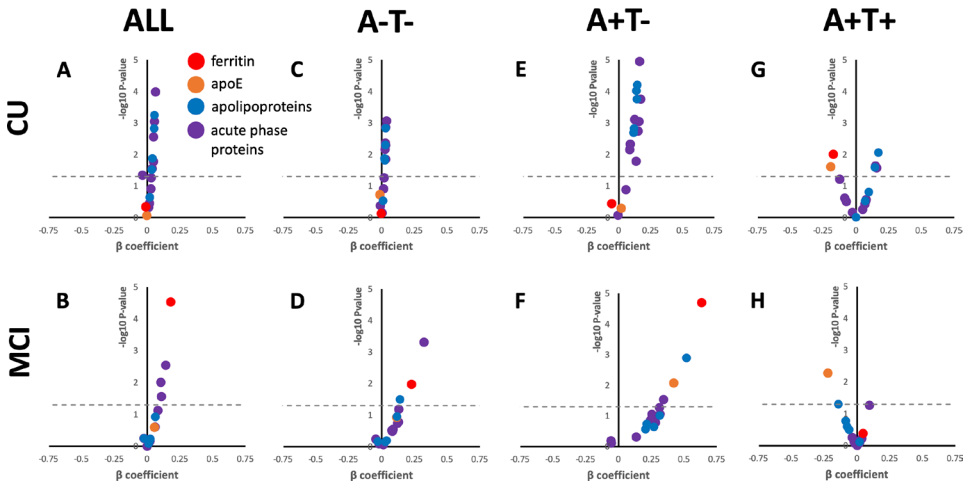

Figure 3 Baseline CSF analytes predict longitudinal deterioration on CDR-SB in BioFINDER subjects. Volcano plots for the β coefficient and p values (-log10 for visual display) of the interaction between each analyte and time in separate mixed effects linear models including the following additional variables: age, sex, *APOE* ε4. Positive β signifies that higher values of the analyte are associated with worsening performance (faster accumulated score on CDR-SB), negative β signifies that lower values of the analyte are associated with worsening performance. Acute phase response proteins and apolipoproteins are grouped for visual display, actual statistics for each variable can be found in online supplemental table 7. Above the dotted line represents p<0.05. CDR-SB, Clinical Dementia Rating Scale, sum of boxes; CSF, cerebrospinal fluid; MCI, mild cognitive impairment.

category where ferritin was significantly predictive was in A+T+ subjects, where it predicted improvement in CDR-SB (β(SE)=−0.176 (0.068); p=0.010).

When MMSE was used as the cognitive outcome variable our results were similar to that of the CDR-SB models; baseline ferritin predicted decline in all MCI subjects, and in A+T subjects, but predicted improvement (as did apoE, see below) in A+T+ CU subjects (online supplemental table 9).

Baseline apoE levels did not predict change in CDR-SB in either CU or MCI unless additionally stratified by A/T criteria (figure 3A,B; online supplemental table 9). But on stratifying by A/T criteria, apoE predicted CDR-SB deterioration in A+T MCI subjects (figure 3F; β(S.E.)=0.420 (0.156); p=0.008), like ferritin. In both CU and MCI subjects that were designated A+T+, apoE predicted improvement in CDR-SB (figure 3G,H; CU: β(S.E.)=−0.195 (0.086); p=0.025; MCI β(S.E.)=−0.222 (0.079); p=0.005). When using MMSE as the outcome variable, apoE was also associated with improvement on this scale in CU A+T+ subjects (β(S.E.)=0.268 (0.127); p=0.036), but not MCI A+T+ subjects. Rather, apoE predicted deterioration on MMSE when all MCI subjects were combined into one group (β(S.E.)=−0.139 (0.065); p=0.034).

The results relating to ferritin and apoE were unlikely due to a biochemical class effects since the other acute phase proteins and lipoproteins were generally more strongly associated with decline on CDR-SB of CU subjects, and in particular those who were A+T- (figure 3; online supplemental table 9) where all proteins were significant predictors of CDR-SB except for haptoglobin and γ-fibrinogen.

To determine whether CSF ferritin could be a useful diagnostic test in tandem with the A/T pathology biomarkers (which already have established pathological thresholds), we defined a pathological threshold of ferritin value (high/low). We stratified the subjects into a series of high/low ferritin dichotomies, which were based on incremental increases in the ferritin threshold beginning at one SD below the mean ferritin of the CU group, to one SD above the CU mean. We included these groupings, individually, as predictive variables (interacted with time) in a series of mixed effects linear models of CDR-SB in MCI subjects (including age, sex, *APOE* ε4, apoE). Ferritin was significantly associated with decline with almost every threshold used, but the best performing (determined by highest β and lowest P; figure 4A) threshold was 12.47 ng/mL ferritin (β(S.E.)=0.492 (0.094); $p=9.9\times10^{-7}$; figure 4B), which was almost exactly ½

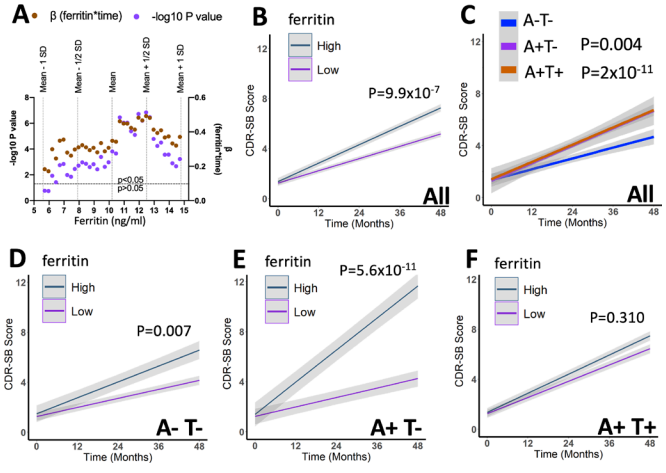

Figure 4 Defining a high ferritin/iron threshold (I+) to predict cognitive decline in BioFINDER MCI cases. (A) Statistical readouts from time*ferritin (high/low) when included as a variable with increasing ferritin threshold (over ±1 SD from the referent mean of CU subjects) value in a series of mixed effects models of longitudinal CDR-SB scores from all MCI subjects. A 12.47 ng/mL was the best performing threshold (determined by lowest p value and highest β), which was almost exactly 0.5 SD above the mean of the CU reference group. Above this threshold was considered high ferritin, or Iron+ (I+) in future analysis. (B, C) Association of A+, T+ and I+ with change in CDR-SB in all MCI subjects. Statistics are from mixed effects models including the additional covariates: age, sex, *APOE* ε4 gene and apoE levels. (D–G) Association between I+and change in CDR-SB in MCI subjects stratified by A&T criteria. Statistics are from mixed effects models including the additional covariates: age, sex, *APOE* ε4 and apoE levels. Data are means±SE. CDR-SB, Clinical Dementia Rating Scale, sum of boxes; CU, cognitively unimpaired; uMCI, mild cognitive impairment.

an SD increase from the mean ferritin values in the CU reference group. So, we designated this category of elevated ferritin 'I+', for high iron (demographics: online supplemental table 10).

The association with I+and decline on CDR-SB in MCI subjects (figure 4B) was similar in magnitude to the effect of A+/T- (β(S.E.)=0.362 (0.190); p=0.004) and A+/T+ (β(S.E.) = 0.659 (0.097); $p=2\times10^{-11}$) categorisations when they were compared with A-T- MCI subjects (figure 4C). The I+designation also proved valuable for predicting decline on CDR-SB after stratifying by A/T criteria. I+predicted accelerated decline in CDR-SB in A-T- subjects (β(S.E.)=0.533 (0.198); p=0.007; figure 4D), most prominently in A+T subjects (β(S.E.)=1.813 (0.277); $p=5.6\times10^{-11}$; figure 4E) but was not predictive in A+T+ subjects (β(S.E.)=0.119 (0.117); p=0.310; figure 4F).

DISCUSSION

Here, we report an elevation of CSF ferritin associated with p-tau181 that was significantly mediated by CSF apoE levels, which were likewise elevated in T+cases. Ferritin and apoE levels did not change according to clinical diagnosis in the BioFINDER cohort, which is similar to our prior findings in the ADNI cohort.[2] The changes in ferritin and apoE according to A/T criteria that we now report in both ADNI and BioFINDER cohorts differed to all other apolipoproteins and acute phase response proteins that we investigated in the BioFINDER study. We found CSF ferritin predicts accelerated clinical deterioration in MCI subjects, particularly T-subjects, whereas other inflammatory acute phase response proteins associate with cognitive decline in subjects classified as CU at baseline. As will be discussed, these findings provide insight into the pathophysiological mechanisms of AD, the timing of their influence in disease progression, and a potentially clinically useful ferritin threshold for prognostication.

Associations between ferritin and apoE levels and genotype

We previously observed a positive association between CSF apoE and ferritin in the ADNI cohort,[2] which was replicated in this study. It has been shown that apoE inhibits the degradation of ferritin by ferritinophagy independent of genotype,[41] which may mechanistically explain these findings. But in ADNI we also observed that *APOE* ε4 carriers express elevated CSF ferritin, which was not replicated here. In other studies, *APOE* ε4 carriers were reported to have higher MRI-reported brain iron levels, which adversely influences the impact of iron on functional MRI[21 42] and cognitive decline.[3] Therefore, the association between apoE and ferritin levels appears to be robust, but an association with *APOE* ε4 genotype warrants additional validation.

Ferritin and apoE associations with plaque pathology

We have not observed positive associations between cross-sectional tau/$A\beta_{42}$ and apoE or ferritin in either the current or prior studies.[5] One factor that may confound the interpretation of ferritin and apoE in CSF is the potential for these proteins to be sequestered into plaque. As plaque increases, $A\beta_{42}$ levels decrease because the peptide is sequestered into plaque; it is possible that ferritin and apoE may also be sequestered, since they are both resident plaque proteins.[17 43 44] This would result in a lower level of ferritin and apoE than we would otherwise expect. However, it is impossible to tell from these datasets whether sequestration could account for our findings, or whether these results simply reflect ferritin and apoE status at different disease states/stages. We conclude that ferritin and apoE rise in concert with T+status, but the lack of elevation of apoE and ferritin in A+ subjects may reflect either the underlying pathophysiology, or sequestration of these proteins in accumulating plaque pathology.

Ferritin and apoE associations with p-tau181 and cognitive decline

Ayton *et al*[7 18 15 16] have reported tau-related iron elevation, which is supported by the current study. However, the association of ferritin and p-tau181 with cognitive decline was complex. Ferritin, but not p-tau181, was predictive of decline in MCI T-subjects despite neither being elevated compared with any other clinicopathology group. Conversely, p-tau181, but not ferritin, was associated with decline in MCI A+T+ subjects even though both ferritin and p-tau181 were elevated in this group. So, while ferritin and p-tau181 are associated in the clinicopathology staging, they represent different facets of disease progression.

Neurofibrillary tangles differ to iron since tangles are an abnormality, whereas iron is endogenously present within the brain. Prior studies have shown that iron acts as a risk factor for cognitive decline in AD, since it associates with disease progression even when not elevated during disease staging.[2 3 6 8] We observe similar associations in the current study—when MCI subjects are considered as a whole, ferritin predicted decline despite no elevation to ferritin in MCI subjects. The same is true in MCI subjects classified as T—where ferritin levels are likewise unchanged. But in MCI T+subjects, where ferritin levels are markedly elevated, the degree of variation in ferritin levels within this group may have been insufficient to observe a statistical association with decline. Indeed, in defining a threshold for I+, we used 12.47 ng/mL as the optimum threshold to predict decline—thresholds beyond this value did not provide improved prognostic performance. Yet, this threshold approximated the mean ferritin value of A+T+ =12.06 ng/mL. Thus, the ferritin values in the MCI T+group may have lacked the variance needed to demonstrate an association with cognitive outcome.

The opposite is likely true for p-tau181. Values of p-tau181 below the defined threshold are unlikely to be associated with tangle pathology since the threshold is used to classify patients with tangle pathology. So it is not surprising that p-tau181 levels are only associated with cognitive decline in people with tangle pathology (above threshold p-tau181 levels). Therefore, while the clinical value of I+ in MCI subjects that are also T+ is not apparent from these data, we show the prognostic value of I+ in T- subjects (which represent 48% of MCI subjects in BioFINDER), where p-tau181 is not associated with decline when used as a continuous variable.

Associations between ferritin, apoE and p-tau181 warrant further mechanistic investigations. ApoE inhibits the degradation of intracellular ferritin via ferritinophagy[41]—which may be a mechanism that could explain our current findings. The elevation to ferritin downstream of tau agrees with prior findings that loss of soluble tau—which occurs during the aggregation of the protein in neurofibrillary tangles—limits APP mediated iron export from neurons.[13] But since neurofibrillary tangles also accumulate iron, it remains to be determined whether the elevation of ferritin downstream of p-tau181 reports iron accumulating with tangle pathology directly, or loss of functional tau that occurs with tau phosphorylation and aggregation that then disables iron export.

It is also possible that the effect of apoE and ferritin on disease outcomes varies according to disease stage. Higher levels of apoE has previously been reported to predict better cognitive outcomes,[2 45] and we observe this in the current study in both

A+T+ CU and MCI subjects. Conversely, higher apoE levels were associated with worse outcomes in MCI A+T subjects. We do not believe apoE has been investigated in this specific patient group previously, and this result raises the possibility that apoE has a complex role on disease outcomes, which would require further validation. Similarly, we observed in CU A+T+ subjects that higher ferritin was associated with better cognitive outcomes. Again, we are not aware that ferritin has been tested in this particular patient group previously, but may suggest a complex role for ferritin that varies by disease stage.

Ferritin is distinct to neuroinflammatory changes of the acute phase response

Ferritin, along with several acute phase response proteins, have functional roles in iron regulation. Activated microglia retain iron[34–36] and a subset of microglia that are infiltrated by plaque are particularly enriched with ferritin.[37] Prior to this study it was plausible to interpret CSF ferritin as a part of the acute phase response that reports neuroinflammation rather than iron levels. Yet we reveal that ferritin differs to other acute phase response proteins by elevating according to p-tau181 status, whereas other acute phase proteins elevate with symptomatic progression. Ferritin predicted decline in MCI subjects, whereas acute phase response proteins were more associated with decline in CU subjects. Our findings agree with research into other neuroinflammation biomarkers, such as PET TSPO, where no or an inverse relationship with amyloid PET is reported.[23 27 31] Most prior findings regarding TSPO report elevation with clinical diagnosis,[22–29] which we also observe with acute phase proteins.

Limitations and therapeutic implications

Small samples sizes in the A-T+group limit our interpretation. Insufficient longitudinal data for AD subjects (regardless of A/T designation) restricts our interpretations to the pre-dementia phase.

We have used CSF ferritin as a biomarker of iron, but acknowledge that this has not been validated against iron in the brain measured postmortem. The rationale supporting CSF ferritin as a biomarker of brain iron burden is as follows: plasma ferritin is an established biomarker of body iron burden, CSF ferritin is decreased in restless legs syndrome, a disease of low brain iron[46] and ferritin expression and secretion from cultured glia is dependent on iron.[47]

Do these associations have implications for iron or inflammation as therapeutic targets? Associations between acute phase proteins and cognitive deterioration was observed only in CU subjects, suggesting that therapeutically targeting neuroinflammation might be most effective in the early stages of disease. Preventing brain iron elevation that rises in concert with tau pathology might be effective in slowing disease progression in people who have MCI. Whether there is benefit in targeting iron and inflammation after they have already been induced remains difficult to forecast, since the biomarkers of iron and inflammation we used were not associated with longitudinal decline at the disease stages where they were significantly elevated.

Collaborators Alzheimer's Disease Neuroimaging Initiative; Swedish BioFINDER study.

Contributors Primary data collection: SJ, SP, ES and OH. Sample analysis: SA, SJ, SP, ES, OH, AAB, AR and BR. Statistical analysis: SA, PK and AIB. Manuscript drafting: SA, SJ, PK, SP, AAB, ES, AR, BR, OH and AIB.

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Competing interests AIB is a shareholder in Alterity Cogstate and Mesoblast. He is a paid consultant for, and has a profit share interest in, Collaborative Medicinal Development. AIB and SA hold a patent related to CSF ferritin as a diagnostic for dementia (US patent app: 15/562,801). OH has acquired research support (for the institution) from Roche, Pfizer, GE Healthcare, Biogen, Eli Lilly and AVID Radiopharmaceuticals. In the past 2 years, he has received consultancy/speaker fees (paid to the institution) from Biogen and Roche. BR is an inventor on patent application AU2014/00849 for diagnosis of neurological disorders and receives research support from Agilent Technologies, eMSion and Neurovision.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Regional Ethics Committee in Lund, Sweden:2016-120-32. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Anonymised data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

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Supplementary Methods

BioFINDER Inclusion criteria

In the BioFINDER-1 cohort, the inclusion criteria for cognitively unimpaired (CU) elderly were 1) absence of cognitive symptoms as assessed by a physician with special interest in cognitive disorders, 2) age ≥60 years, 3) MMSE 28-30 points at screening visit, 4) did not fulfill the criteria for mild cognitive impairment (MCI) or any dementia disorder, and 5) fluency in Swedish. The exclusion criteria were 1) significant unstable systemic illness, such as terminal cancer, or organ failure that made it difficult to participate in the study, 2) current significant alcohol or substance misuse and 3) significant neurological or psychiatric illness. In accordance with the research framework by the National Institute on Aging-Alzheimer's Association study patients with SCD and cognitively healthy controls were considered as the CU group[1].

Following neuropsychological assessment including a test battery evaluating verbal ability, episodic memory function, visuospatial construction ability, and attention and executive functions, patients were classified as MCI as previously described[2]. The inclusion criteria for patients with MCI were (1) referred to a participating memory clinic because of cognitive complaints, (2) age 60 to 80 years, (3) did not fulfil the criteria for any dementia disorder and (4) fluency in Swedish. The exclusion criteria were 1) significant unstable systemic illness or organ failure, such as terminal cancer, that made it difficult to participate in the study, 2) current significant alcohol or substance misuse and 3) cognitive impairment that without doubt could be explained by other specific non-neurodegenerative disorders, such as brain tumor or subdural hematoma. Patients with AD dementia were required to meet the criteria for probable AD dementia defined by NINCDS-ADRDA[3].

BioFINDER CSF collection and processing

CSF samples were collected before noon with participants non-fasting. Lumbar puncture and CSF handling followed a structured protocol[4]. CSF was assayed for $A\beta_{42}$, t-tau and p-tau181 using ELISA (Euroimmun AG, Lübeck, Germany). The laboratory technicians performing the biochemical analyses were blinded to the clinical data. Subjects were classified positive for Aβ (A+) if their CSF $A\beta_{42}$/t-tau was <1.44, and were classified positive for tau pathology (T+) if their p-tau181 was >60.2, as previously described for this manufacturer[5].

CSF was stored in 0.5ml tubes and thawed on ice for 1 hr, briefly mixed and centrifuged at 4,000 x g for 5 minutes. All samples were processed on the sample day. Then 20 µL of CSF was transferred to low-bind 96-well plates (Eppendorf, Germany) and mixed with 42 µL of 9 M urea, 20 mM dithiolthreitol (DTT) and 300 mM Tris (pH 8.0). The samples were mixed and incubated at 37˚C in an incubator. After incubation, 23 µL of 0.2 M iodoacetamide was added (50 mM final concentration), mixed and incubated at room temperature in the dark for 30 minutes. The sample was then diluted with 326 µL of 100 mM Tris (pH 8.0) to yield a final urea concentration of 0.9 M. To this 25 µL of a 2 $mg*mL^{-1}$ solution of trypsin was added, and the sample was mixed and then incubated overnight at 37˚C. The following day, 55 µL of 9% formic acid was added to yield a final volume of 500 µL. Following acidification, a mixture of the stable isotope labelled peptides was added to each sample (10 µL of 25 pmol/µL stock solution). The samples were then desalted and concentrated using Oasis µelution HLB 96-well plates (Waters) and a positive pressure-96 Processor (Waters), peptides were eluted from the µelution plates with 75 µL of 50% acetonitrile 0.1% formic acid into a skirted PCR plate (Eppendorf, Germany). The samples were then dried using a vacuum concentrator (Labconco). Plates were sealed with sealing film (Sigma) and stored at -20˚C until analysis. For LC-MS/MS analysis the sealing film was removed, and peptides

were resuspended with 40 µL of 2.5% acetonitrile 0.1% formic acid, with bath sonication for 5 min at room temperature. Samples were then centrifuged for 2 min at 4,000 x g to collect the sample and the sealing film was replaced with X-pierce™ film (Thomas Scientific). The samples were then loaded into an autosampler (1290 Agilent Technologies) maintained at 6˚C. For analysis 20µL of each sample was loaded on to a 2.1 x 150 mm AdvancedBio C_{18} column (Agilent Technologies) maintained at 50˚C. The peptides were monitored using a triple quadrupole mass spectrometer (6495 QQQ, Agilent Technologies). Data analysis was conducted using Skyline[6-8].

MRM mass spectrometry

For liquid chromatography-MS/MS analysis the sealing film was removed, and peptides were resuspended with 40 µL of 2.5% acetonitrile 0.1% formic acid, with bath sonication for 5 min at room temperature. Samples were then centrifuged for 2 min at 4,000 x g to collect the sample. The sealing film was replaced with X-pierce™ film (Thomas Scientific). The samples were then loaded into an autosampler (1290 Agilent Technologies) maintained at 6˚C.

For analysis, 20 µL of each sample was loaded on to a 2.1 x 150 mm AdvancedBio C_{18} column (Agilent Technologies) maintained at 50˚C. The peptides were monitored using a triple quadrupole mass spectrometer (6495 QQQ, Agilent Technologies).

Data analysis was conducted using Skyline[6-8]. The quantitative selective reaction monitoring (SRM) assay for the acute phase response protein (α1 antichymotrypsin, α1 antitrypsin, ceruloplasmin, complement C3, α-fibrinogen, β-fibrinogen, γ-fibrinogen, haptoglobin and hemopexin; **Supplementary Methods Table 1** for peptide sequences) targets was developed by MRM proteomics (Montreal, Canada)[9], as previously described[10]. This included the synthesis and quantitation of the heavy isotopically labelled (^{15}N, ^{13}C enriched K and R residues) peptides used as standards. The concentration of the heavy standards was determined by amino acid analysis. Purity was checked by capillary electrophoresis. The development of the assay by MRM proteomics was comprehensive and included the generation of a standard curve, quality controls and artificial CSF. Qualification of the mass spectrometry setting used to monitor the peptides included the liquid chromatography (LC) gradient. Due to the large (>1000) number of samples we needed to measure we reduced the run time for the LC from 60 minutes sample to sample, down to 10 minutes.

Selectivity and specificity were addressed using heavy isotope labels of each of the peptide analytes measured. The heavy isotopically labelled peptides were added to every sample as stable isotope internal standard (SIS). The development of the assay monitored 4 transitions for each of the heavy peptides to ensure positive identification. Further blanks of CSF material without the SIS were used to assess any matrix interferences with the 4 transitions. Each peptide transition was evaluated for signal intensity and potential matrix interference signal and reduced to two well defined transitions per peptide. The number of transitions was reduced so that we could maintain a longer dwell time per transition (e.g. 20ms). Parallelism of the external standard curve was assessed by comparing the slope of the artificial CSF matrix with SIS spiked into a pooled CSF matrix. The artificial CSF was used because we needed a matrix that did not contain the endogenous peptide so we could determine the forward and reverse standard curves to ensure the ratio of the endogenous/heavy internal standard was in the linear range. The standard curve consisted of 12 points and covered nearly four orders of magnitude (dilution factor of the highest concentration standard 1, 2.5, 6.25, 25, 50, 200, 400, 1000, 2000, 4000, 8000). Standards and quality controls were run in replicate to determine assay precision and accuracy.

Limits of quantitation were 3 times the limit of detection with a coefficient of variation (CV) limit of 30%. Outliers were excluded if they were outside 3 SD from the mean. All sampling and initial quantitative data analysis was conducted blinded. Samples were measured with a single injection and in a randomized order. The MS based assay was developed using the Food and Drug Administration (FDA) guidance on biomarker LC-MS assays[11]. This included external standard curves and four quality controls which spanned the concentration range used to determine accuracy. The quality controls ranged from 78.8-120.7%. In addition, a pooled quality control was included and injected every 24 samples to monitor performance over the 6-week data collection phase (CV range 11-32%, median 17%).

Supplementary Methods table 1: Details of SRM assay for protein quantification

Protein Name	**UniProt KB Accession #**	**Average Protein MW [Da]**	**Peptide Sequence**	Ion	Ion Charge	Fragment Ion charge
Alpha-1-antichymotrypsin	P01011	45265.82	EIGELYLPK	y5	2	1
Alpha-1-antichymotrypsin	P01012	45266.82	EIGELYLPK	y3	2	1
Alpha-1-antitrypsin	P01009	44324.55	LQHLENELTHDIITK	y2	3	1
Alpha-1-antitrypsin	P01010	44325.55	LQHLENELTHDIITK	y13	2	2
Apolipoprotein A-I	P02647	28078.62	ATEHLSTLSEK	y10	3	2
Apolipoprotein A-I	P02648	28079.62	ATEHLSTLSEK	y8	3	2
Apolipoprotein A-II	P02652	8707.91	SPELQAEAK	y6	2	1
Apolipoprotein A-II	P02653	8708.91	SPELQAEAK	y2	2	1
Apolipoprotein A-IV	P06727	43402.53	LGEVNTYAGDLQK	y9	2	1
Apolipoprotein A-IV	P06728	43403.53	LGEVNTYAGDLQK	y6	2	1
Apolipoprotein D	P05090	19303.08	NILTSNNIDVK	y9	2	1
Apolipoprotein D	P05091	19304.08	NILTSNNIDVK	b3	2	1
Apolipoprotein E	P02649	34236.68	SELEEQLTPVAEETR	y9	2	1
Apolipoprotein E	P02650	34237.68	SELEEQLTPVAEETR	y7	2	1
Apolipoprotein M	O95445	21253.29	AFLLTPR	y5	2	1
Apolipoprotein M	O95446	21254.29	AFLLTPR	b2	2	1
Ceruloplasmin	P00450	120085.49	EYTDASFTNR	y6	2	1
Ceruloplasmin	P00451	120086.49	EYTDASFTNR	y5	2	1
Complement C3	P01024	184951.34	TGLQEVEVK	y6	2	1
Complement C3	P01025	184952.34	TGLQEVEVK	y5	2	1
Fibrinogen alpha chain	P02671	91358.87	NSLFEYQK	y5	2	1
Fibrinogen alpha chain	P02672	91359.87	NSLFEYQK	b2	2	1
Fibrinogen beta chain	P02675	50762.93	AHYGGFTVQNEANK	y6	3	1
Fibrinogen beta chain	P02676	50763.93	AHYGGFTVQNEANK	y5	3	1
Fibrinogen gamma chain	P02679	48483.03	YEASILTHDSSIR	y11	3	2
Fibrinogen gamma chain	P02680	48484.03	YEASILTHDSSIR	y10	3	2
Haptoglobin	P00738	43349.01	DYAEVGR	y5	2	1
Haptoglobin	P00739	43350.01	DYAEVGR	y4	2	1
Hemopexin	P02790	49295.43	NFPSPVDAAFR	y9	2	2
Hemopexin	P02791	49296.43	NFPSPVDAAFR	y7	2	2

Multiplex ELISA

CSF ferritin in the BioFINDER cohort was measured using the MILLIPLEX MAP kit (EMD Millipore) as previously described[10]. All samples were measured using the same kit batch number following a blinded and randomization protocol. The assay was conducted in a 96 well plate and all working solutions were prepared according to the manufacturer's instructions. Briefly, 25 µl of assay buffer was added to each well of a 96 well plate. Then, CSF was centrifuged at 15,000 g and a 25 µl aliquot was added to sample wells followed by 25 µl of the magnetic bead suspension. Blank, standards, kit and pooled CSF controls were included in each plate. The plate was sealed, wrapped with foil and incubated on a plate shaker overnight (16-18 h) at 4 °C. Following day, the plate was washed three times with wash buffer using a Bio-Plex Pro II wash station (Bio-Rad) and 25 µl of the detection antibody was added to each well and the plate was sealed, covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature. The plate was washed three times with wash buffer and 25 µl of the streptavidin-Phycoerythrin solution was added to each well and the plate was sealed, covered with foil and incubated on a plate shaker for 30 min at room temperature. After a final wash step, 100 µl of sheath fluid was added to each well and incubated on a plate shaker for 5 min to resuspend the beads. Finally, fluorescence was measured with a Bioplex 200 instrument (Bio-Rad) and median fluorescent intensity data using a 5-parameter logistic curve-fitting method was used to determine sample concentration. For all plates, the internal standard control duplicate analyses were within the accepted percentage of coefficient of variance and all samples' concentrations were above the limit of quantification, which we determined experimentally (LoQ: 0.52 ng/ml). Internal calibration curves were constructed from 8 ferritin concentrations included in each plate. A duplicate quality control was used for each plate consisting of 2 quality control samples (lyophilized ferritin standard) QC1 (expected concentration of 0.15-0.32 ng/ml), and QC2 (expected concentration of 3.2-6.7 ng/ml). QC2 was within the expected range for all measured plates and ranged from 3.21to 4.58 ng/ml, while QC1, was below the limit of quantification determined experimentally (LoQ: 0.52 ng/ml). QC1 showed slightly more variation and ranged from 0.22 to 0.63 ng/ml. The CV_{mean} between all plates over different days was determined experimentally using the QC2 standard (Inter-plate CV_{mean}: 2.4 %).

ADNI inclusion criteria

Inclusion criteria for ADNI are previously described[12] and were as follows: (1) Hachinski Ischaemic Score <4; (2) permitted medications stable for 4 weeks before screening; (3) Geriatric Depression Scale score <6; (4) visual and auditory acuity adequate for neuropsychological testing; good general health with no diseases precluding enrolment; (5) six 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 AD patients 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, whereas mild cognitive impairment subjects (MCI) could not meet these criteria, have largely intact general cognition as well as functional performance, but meet defined criteria for MCI.

ADNI CSF analysis

CSF levels of apoE, and ferritin were measured with the RBM multiplex platform[12], and CSF $A\beta_{42}$, t-tau, and p-tau181 with the multiplex xMAP Luminex platform with Innogenetics immunoassay kit-based reagents (INNO-BIA AlzBio 3; Ghent, Belgium)[12-15]. Subjects were

classified positive for Aβ pathology (A+) if CSF $A\beta_{42}$/t-tau levels were <1.27, and positive for tau pathology (T+) if CSF p-tau181 was >26.6 pg/ml, as previously described for this manufacturer[15].

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Supplementary Tables

Supplementary Table 1. N table of BioFINDER subjects with cognitive performance data over time.

	Year→	0	1	2	3	4
CU	**A-T-**	558	160	353	146	329
	A+T-	85	18	61	16	56
	A-T+	25	2	15	1	10
	A+T+	125	59	94	52	84
MCI	**A-T-**	89	81	78	65	61
	A+T-	34	30	31	26	24
	A-T+	7	7	7	7	7
	A+T+	133	124	118	106	96

Supplementary Table 2. Comparison of changes in CSF apoE and ferritin with other apolipoproteins and acute phase proteins across diagnostic categories in the BioFINDER cohort. Statistics are from multiple regression models of each of the analytes including age, sex, *APOE* ε4, and diagnosis as covariates. β represents one standard deviation change associated with diagnosis category (reference category is CU). Ferritin and apoE were highlighted as the primary analysis, the other analytes are included as exploratory comparative results. Bold indicates P<0.05; *Indicates correction after multiple comparison between the two primary analysis of ferritin and apoE (α/2 = 0.025).

		CU			MCI			AD		
		β	**S.E.**	**P**	**β**	**S.E.**	**P**	**β**	**S.E.**	**P**
apolipoprotein	**apoE**	NA	NA	NA	-0.121	0.072	0.092	0.043	0.087	0.625
	apoAI	NA	NA	NA	0.057	0.071	0.422	0.232	0.086	**0.007**
	apoAII	NA	NA	NA	0.080	0.071	0.263	0.207	0.086	**0.016**
	apoAIV	NA	NA	NA	0.160	0.070	**0.022**	0.187	0.084	**0.027**
	apoD	NA	NA	NA	0.089	0.069	0.197	0.339	0.084	**$5.3x10^{-5}$**
	apoM	NA	NA	NA	0.132	0.073	0.070	0.202	0.088	**0.022**
acute phase proteins	**ferritin**	NA	NA	NA	0.077	0.069	0.264	0.163	0.083	**0.050**
	α1 antichymotrypsin	NA	NA	NA	0.272	0.069	**$7.8x10^{-5}$**	0.499	0.083	**$2.2x10^{-9}$**
	α1 antitrypsin	NA	NA	NA	0.136	0.071	0.054	0.309	0.086	**$3.1x10^{-4}$**
	ceruloplasmin	NA	NA	NA	0.186	0.078	**0.017**	0.179	0.094	0.058
	complement C3	NA	NA	NA	0.128	0.071	0.070	0.271	0.085	**0.001**
	α-fibrinogen	NA	NA	NA	0.309	0.076	**$5.2x10^{-5}$**	0.313	0.093	**0.001**
	β-fibrinogen	NA	NA	NA	0.245	0.075	**0.001**	0.389	0.092	**$2.5x10^{-5}$**
	γ-fibrinogen	NA	NA	NA	0.188	0.070	**0.008**	0.405	0.085	**$2.0x10^{-6}$**
	haptoglobin	NA	NA	NA	0.316	0.072	**$1.1x10^{-5}$**	0.281	0.087	**0.001**
	hemopexin	NA	NA	NA	0.149	0.070	**0.033**	0.410	0.084	**$1.2x10^{-6}$**

Supplementary Table 3. Comparison of changes in CSF apoE and ferritin with other apolipoproteins and acute phase proteins in strata of A and T criteria in the BioFINDER cohort. Statistics are from mixed effects models of each of analyte including age sex *APOE* ε4 A/T criteria as covariates. β represents one standard deviation change associated with A/T category (reference category is A-T-). Ferritin and apoE were highlighted as the primary analysis, the other analytes are included as exploratory comparative results. Bold indicates P<0.05. ; *Indicates correction after multiple comparison between the two primary analysis of ferritin and apoE (α/2 = 0.025).

		A-T-			A+T-			A-T+			A+T+		
		β	S.E.	P	β	S.E.	P	β	S.E.	P	β	S.E.	P
apolipoprotein	**apoE**	NA	NA	NA	-0.283	0.095	**0.003***	0.709	0.184	**$1.2x10^{-4}$***	0.468	0.070	**$8.8x10^{-14}$***
	apoA-I	NA	NA	NA	-0.105	0.097	0.280	-0.134	0.187	0.473	0.080	0.071	0.279
	apoA-II	NA	NA	NA	-0.143	0.097	0.140	0.059	0.183	0.746	0.037	0.071	0.846
	apoA-IV	NA	NA	NA	-0.241	0.095	**0.012**	-0.034	0.181	0.852	0.062	0.070	0.506
	apoD	NA	NA	NA	-0.208	0.094	**0.027**	0.163	0.178	0.361	0.220	0.068	**0.001**
	apoM	NA	NA	NA	-0.042	0.100	0.672	-0.016	0.187	0.931	0.129	0.073	0.275
acute phase proteins	**ferritin**	NA	NA	NA	0.036	0.094	0.701	0.621	0.183	**0.001***	0.426	0.068	**$3.3x10^{-11}$***
	α1 antichymotrypsin	NA	NA	NA	-0.096	0.094	0.308	0.205	0.179	0.252	0.236	0.069	0.257
	α1 antitrypsin	NA	NA	NA	-0.111	0.096	0.250	-0.081	0.186	0.665	0.090	0.071	0.606
	ceruloplasmin	NA	NA	NA	-0.176	0.109	0.108	0.105	0.201	0.601	0.072	0.079	0.370
	complement C3	NA	NA	NA	-0.148	0.096	0.123	0.434	0.185	**0.019**	0.173	0.070	**0.001**
	α-fibrinogen	NA	NA	NA	-0.092	0.108	0.391	0.333	0.199	0.095	0.185	0.077	0.077
	β-fibrinogen	NA	NA	NA	-0.155	0.105	0.141	0.122	0.194	0.529	0.186	0.076	**$5.3x10^{-10}$**
	γ-fibrinogen	NA	NA	NA	-0.132	0.096	0.169	0.090	0.182	0.620	0.175	0.070	**0.001**
	haptoglobin	NA	NA	NA	0.140	0.099	0.158	0.032	0.190	0.868	0.189	0.072	0.205
	hemopexin	NA	NA	NA	-0.137	0.095	0.151	0.154	0.180	0.395	0.180	0.070	0.362

Supplementary Table 4. Comparison of changes in CSF apoE and ferritin in strata of A and T criteria in the BioFINDER cohort. Subjects were limited to those that had ferritin values measured.

Dx	**CU**				**MCI**				**AD**				**All**
A	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**All**
T	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**All**
N	531	83	21	113	89	33	6	124	8	19	0	149	1211
Ferritin: ng/ml (SD)	9.63 (4.38)	9.74 (4.39)	13.49 (4.38)	11.98 (4.70)	9.49 (4.90)	10.44 (4.52)	13.03 (6.18)	11.76 (4.67)	7.93 (4.22)	9.9 (4.88)	NA NA	11.95 (4.51)	10.47 (4.65)
apoE: µg/ml (SD)	3.38 (1.06)	3.22 (1.09)	4.51 (1.05)	4.16 (1.11)	3.12 (1.12)	2.8 (0.96)	3.81 (1.48)	3.74 (1.15)	2.35 (1.03)	2.78 (1.21)	NA NA	3.82 (1.27)	3.51 (1.16)

Supplementary Table 5. Comparison of changes in CSF ferritin in strata of APOE and diagnosis in the BioFINDER cohort. Subjects were limited to those that had ferritin values measured. Statistics are from a multiple regression model containing the following covariates: age, sex, APOE ε4, apoE level.

	APOE ε4 -ve			APOE ε4 +ve			
	N	**Mean**	**SD**	**N**	**Mean**	**SD**	**P**
All	692	10.35	(4.72)	509	10.63	(4.69)	0.994
CU	502	10.14	(4.52)	265	9.97	(4.63)	0.670
MCI	132	10.66	(4.97)	131	11.11	(4.74)	0.724
AD	58	11.54	(4.61)	113	11.61	(4.69)	0.214

Supplementary Table 6. Demographics Table of ADNI subjects stratified by clinical (CU MCI dementia) and biomarker (A+/- and T+/-) criteria. Values of $A\beta_{42}$, t-tau and p-tau181 are in pg/ml.

Dx	**CU**				**MCI**				**AD**			
A	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
T	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**-**	**-**	**+**	**+**
N	41	8	4	15	22	16	2	83	2	8	1	52
Age: Mean (SD)	75.0 (5.6)	74.1 (4.2)	77.9 (4.7)	78.1 (5.4)	74.6 (7.9)	76.7 (3.4)	72.3 (15.6)	74.2 (7.6)	82.4 (3.0)	78.8 (4.6)	87.7 (NA)	74.2 (7.8)
Male Sex: N (%)	22 (53.7)	4 (50.0)	1 (25.0)	10 (66.7)	19 (86.4)	12 (75.0)	1 (50.0)	50 (60.2)	1 (50.0)	6 (75.0)	1 (100)	27 (51.9)
APOE ε4: N (%)	4 (9.8)	6 (75.0)	1 (25.0)	7 (46.7)	1 (4.5)	10 (62.5)	1 (50.0)	57 (68.7)	0 (0.0)	6 (75.0)	0 (0.0)	39 (75.0)
$A\beta_{42}$: mean (SD)	1299 (263)	599 (103)	1333 (294)	693 (194)	1153 (353)	502 (128)	1422 (134)	627 (173)	1189 (166)	570 (140)	1539 (NA)	580 (207)
t-tau: mean (SD)	188 (37)	218 (25)	298 (53)	327 (60)	185 (42)	202 (32)	287 (15)	379 (102)	154 (0.1)	204 (28)	294 (NA)	396 (117)
$A\beta_{42}$/t-tau: mean (SD)	7 (1.5)	2.8 (0.5)	4.5 (0.6)	2.2 (0.7)	6.3 (1.7)	2.5 (0.7)	5 (0.2)	1.7 (0.6)	7.7 (1.1)	2.8 (0.6)	5.2 (NA)	1.5 (0.6)
p-tau181: mean (SD)	16.7 (3.2)	20.1 (2.2)	27.2 (4.7)	33.3 (7.2)	16 (3.8)	19.1 (3.3)	26.4 (1.4)	38.9 (11.6)	12.5 (0.9)	18.9 (3.0)	24.5 (NA)	40.4 (13.9)
CDR-SB: mean (SD)	0.04 (0.13)	0 (0.0)	0 (0.0)	0 (0.0)	1.46 (0.82)	1.56 (1.0)	2.50 (0.71)	1.60 (0.86)	4.75 (1.77)	5.06 (2.16)	3.5 (NA)	4.26 (1.46)
MMSE: mean (SD)	29 (1.0)	28.9 (1.0)	29.5 (0.6)	29.3 (1.0)	27.5 (1.7)	26.4 (1.6)	29 (0.0)	26.7 (1.7)	25.5 (0.7)	22 (1.8)	24 (NA)	23.6 (1.8)

Supplementary Table 7. Comparison of changes in CSF apoE and ferritin across diagnostic categories in the ADNI cohort. Statistics are from multiple regression models of each of the analytes including age, sex, *APOE* ε4, and diagnosis as covariates. β represents one standard deviation change associated with diagnosis category (reference category is CU).

	CU			MCI			AD		
apoE (µg/ml)									
-mean; (S.D.)	7.34	(1.99)		7.06	(2.08)		6.76	(2.33)	
-β; (S.E.); P	NA	NA	NA	-0.042	(0.139)	0.760	-0.296	(0.167)	0.078
ferritin (ng/ml)									
-mean; (S.D.)	6.47	(2.00)		7.03	(2.75)		7.28	(3.04)	
-β; (S.E.); P	NA	NA	NA	0.093	(0.141)	0.513	0.061	(0.170)	0.720

Supplementary Table 8. Comparison of changes in CSF apoE and ferritin in strata of A and T criteria in the ADNI cohort. Statistics are from multiple regression models of each of analyte including age, sex, *APOE* ε4, and A/T criteria as covariates. β represents one standard deviation change associated with A/T criteria category (reference category is A-T-). Bold indicates P<0.05.

	A-T-			A+T-			A-T+			A+T+		
apoE (µg/ml)												
-mean; (S.D.)	6.55	(1.66)		5.58	(1.83)		8.13	(1.82)		7.24	(2.16)	
-β; (S.E.); P	NA	NA	NA	-0.354	(0.204)	0.084	0.913	(0.347)	**0.009**	0.545	(0.151)	**$3.6x10^{-4}$**
ferritin (ng/ml)												
-mean; (S.D.)	5.93	(2.01)		5.98	(1.71)		7.92	(1.13)		7.42	(2.93)	
-β; (S.E.); P	NA	NA	NA	-0.160	(0.220)	0.468	0.945	(0.375)	**0.012**	0.445	(0.163)	**0.007**

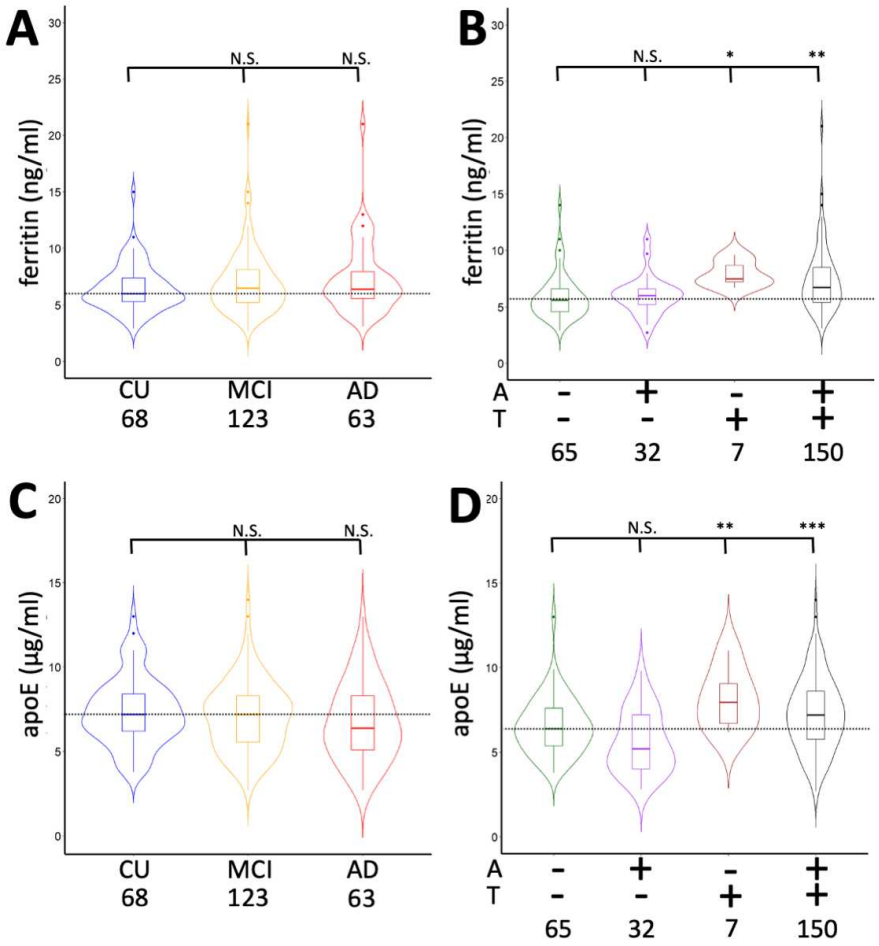
Supplementary Table 9. Association between ferritin and apoE levels with longitudinal CDR-SB and MMSE in CU and MCI BioFINDER subjects. Data are from mixed effects models of CDR-SB/MMSE including the indicated variables and their interaction with time. β represents a unit change in cognitive score per year per standard deviation change in each analyte. Ferritin and apoE were highlighted as the primary analysis of CDR-SB, the other analytes are included as exploratory comparative results. MMSE was included as supportive analysis. Bold indicates P<0.05. *Indicates correction after multiple comparison between the two primary analysis of ferritin and apoE, and the duel analysis of the group as a whole, and when stratified by A/T criteria (α/4 = 0.0125).

Diagnosis	**CU**												**MCI**											
A	**All**			**-**			**+**			**+**			**All**			**-**			**+**			**+**		
T	**All**			**-**			**-**			**+**			**All**			**-**			**-**			**+**		
CDR-SB	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**
apoE	-0.003	0.016	0.860	-0.014	0.010	0.185	0.020	0.029	0.502	-0.195	0.086	**0.025**	0.054	0.047	0.252	0.116	0.074	0.118	0.420	0.156	**0.008***	-0.222	0.079	**0.005***
apoAI	0.055	0.016	**0.001**	0.032	0.010	**0.001**	0.144	0.035	**$6.2x10^{-5}$**	0.142	0.063	**0.025**	0.024	0.043	0.585	0.037	0.079	0.642	0.214	0.159	0.184	-0.059	0.059	0.311
apoAII	0.040	0.016	**0.013**	0.028	0.010	**0.005**	0.139	0.035	**$9.2x10^{-5}$**	0.096	0.067	0.153	0.022	0.045	0.627	-0.030	0.075	0.692	0.314	0.188	0.097	0.021	0.066	0.753
apoAIV	0.053	0.017	**0.001**	0.011	0.010	0.293	0.119	0.037	**0.001**	0.170	0.064	**0.009**	-0.025	0.044	0.564	0.029	0.072	0.693	0.206	0.187	0.274	-0.075	0.064	0.248
apoD	0.037	0.017	**0.030**	0.025	0.011	**0.015**	0.117	0.037	**0.002**	0.070	0.067	0.300	0.063	0.041	0.120	0.141	0.065	**0.032**	0.520	0.157	**0.001**	-0.085	0.062	0.168
apoM	0.019	0.016	0.226	0.023	0.009	**0.013**	0.142	0.037	**$1.7x10^{-4}$**	-0.003	0.068	0.967	0.010	0.047	0.824	0.117	0.073	0.111	0.270	0.224	0.230	-0.141	0.072	**0.049**
ferritin	-0.012	0.016	0.458	-0.003	0.009	0.741	-0.055	0.059	0.358	-0.176	0.068	**0.010***	0.179	0.043	**$2.8x10^{-5}$***	0.228	0.089	**0.011***	0.633	0.141	**$2.0x10^{-5}$***	0.045	0.055	0.409
α1 antichymotrypsin	0.055	0.016	**0.001**	0.029	0.010	**0.004**	0.124	0.036	**0.001**	0.075	0.068	0.266	0.080	0.045	0.074	0.132	0.071	0.065	0.317	0.184	0.088	0.015	0.068	0.831
α1 antitrypsin	0.030	0.016	0.054	0.019	0.010	0.054	0.085	0.031	**0.007**	0.066	0.073	0.366	0.109	0.049	**0.028**	0.121	0.090	0.180	0.313	0.160	0.054	0.038	0.070	0.581
ceruloplasmin	-0.036	0.018	**0.045**	-0.008	0.010	0.422	0.136	0.055	**0.016**	-0.126	0.067	0.061	0.020	0.048	0.672	0.012	0.074	0.867	0.134	0.193	0.489	0.019	0.075	0.801
complement C3	0.036	0.016	**0.028**	0.016	0.010	0.123	0.091	0.032	**0.005**	0.073	0.070	0.297	0.000	0.048	0.995	0.088	0.082	0.283	-0.057	0.204	0.779	-0.023	0.068	0.732
α-fibrinogen	0.017	0.019	0.353	0.030	0.011	**0.005**	0.158	0.046	**0.001**	-0.088	0.075	0.243	-0.025	0.043	0.566	-0.044	0.078	0.576	0.244	0.164	0.140	-0.036	0.061	0.554
β-fibrinogen	0.029	0.019	0.121	0.037	0.011	**0.001**	0.153	0.047	**0.002**	-0.076	0.075	0.312	0.011	0.044	0.803	-0.021	0.083	0.804	0.255	0.148	0.089	0.002	0.061	0.977
γ-fibrinogen	0.048	0.016	**0.003**	0.031	0.010	**0.001**	0.056	0.037	0.131	0.156	0.070	**0.027**	0.142	0.048	**0.003**	0.324	0.092	**$4.9x10^{-4}$**	0.342	0.155	**0.030**	0.019	0.065	0.767
haptoglobin	0.011	0.016	0.480	0.004	0.009	0.706	-0.005	0.023	0.835	-0.029	0.069	0.679	0.104	0.040	**0.010**	0.081	0.083	0.330	0.258	0.172	0.137	0.095	0.049	0.053
hemopexin	0.064	0.016	**$1.0x10^{-4}$**	0.027	0.010	**0.007**	0.162	0.035	**$1.1x10^{-5}$**	0.147	0.064	**0.023**	0.018	0.042	0.659	0.077	0.075	0.306	-0.058	0.131	0.657	0.027	0.062	0.668
MMSE	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**	**β**	**SE**	**P**
apoE	0.011	0.025	0.656	0.027	0.023	0.235	-0.011	0.052	0.834	0.268	0.127	**0.036**	-0.139	0.065	**0.034**	0.072	0.088	0.410	-0.266	0.165	0.109	0.012	0.122	0.922
apoAI	-0.042	0.025	0.089	-0.025	0.021	0.237	-0.112	0.068	0.102	-0.159	0.097	0.100	-0.011	0.062	0.865	-0.005	0.097	0.956	-0.141	0.176	0.427	0.124	0.093	0.185
apoAII	-0.022	0.025	0.377	-0.015	0.021	0.492	-0.108	0.067	0.107	-0.121	0.101	0.233	0.033	0.064	0.605	0.070	0.088	0.430	-0.383	0.204	0.063	0.110	0.102	0.280
apoAIV	-0.026	0.026	0.322	-0.004	0.023	0.863	-0.093	0.066	0.159	-0.078	0.101	0.440	0.006	0.064	0.924	-0.028	0.089	0.754	-0.156	0.206	0.451	0.016	0.104	0.881
apoD	0.009	0.026	0.747	0.021	0.023	0.353	-0.145	0.068	**0.035**	-0.006	0.102	0.952	-0.074	0.058	0.203	-0.053	0.079	0.505	-0.402	0.179	**0.027**	0.073	0.098	0.453
apoM	-0.018	0.023	0.449	-0.024	0.019	0.211	-0.173	0.068	**0.012**	0.106	0.107	0.321	-0.002	0.066	0.971	-0.065	0.089	0.468	-0.627	0.234	**0.009**	0.212	0.112	0.059
ferritin	0.043	0.025	0.088	0.045	0.020	0.026	0.098	0.098	0.316	0.250	0.101	**0.014**	-0.185	0.060	**0.002**	-0.128	0.107	0.231	-0.322	0.162	**0.050**	-0.061	0.083	0.464
α1 antichymotrypsin	-0.050	0.027	0.063	-0.024	0.023	0.284	-0.176	0.066	**0.009**	-0.041	0.108	0.703	0.000	0.066	0.998	-0.004	0.088	0.960	-0.500	0.203	**0.016**	0.084	0.109	0.444
α1 antitrypsin	-0.021	0.025	0.399	-0.019	0.021	0.379	-0.082	0.057	0.154	-0.061	0.112	0.587	-0.064	0.069	0.353	0.008	0.109	0.940	-0.288	0.170	0.094	0.026	0.105	0.806
ceruloplasmin	0.034	0.027	0.218	0.003	0.023	0.904	-0.237	0.095	**0.015**	0.208	0.101	**0.040**	0.095	0.068	0.161	0.132	0.087	0.132	-0.413	0.213	0.055	0.131	0.115	0.254
complement C3	-0.019	0.026	0.456	-0.001	0.022	0.959	-0.082	0.059	0.165	-0.070	0.105	0.508	0.091	0.067	0.176	0.031	0.098	0.751	0.081	0.223	0.719	0.097	0.103	0.350
α-fibrinogen	0.023	0.028	0.416	-0.005	0.023	0.822	-0.156	0.085	0.070	0.229	0.109	**0.036**	-0.010	0.061	0.871	0.128	0.093	0.168	-0.494	0.183	**0.008**	-0.016	0.094	0.866
β-fibrinogen	0.022	0.029	0.446	-0.019	0.024	0.434	-0.095	0.082	0.247	0.277	0.108	**0.011**	0.009	0.064	0.882	0.096	0.098	0.329	-0.418	0.173	**0.017**	0.045	0.100	0.651
γ-fibrinogen	-0.036	0.026	0.166	-0.039	0.022	0.068	-0.024	0.071	0.731	-0.136	0.112	0.226	-0.081	0.069	0.245	-0.174	0.114	0.130	-0.287	0.173	0.101	0.053	0.102	0.603
haptoglobin	-0.004	0.025	0.881	-0.001	0.021	0.954	-0.006	0.055	0.911	0.036	0.101	0.719	-0.063	0.057	0.268	-0.026	0.099	0.790	-0.139	0.188	0.462	-0.043	0.076	0.573
hemopexin	-0.051	0.026	0.052	-0.018	0.022	0.420	-0.166	0.067	**0.015**	-0.137	0.100	0.171	0.034	0.060	0.567	0.033	0.091	0.722	0.070	0.144	0.630	0.031	0.098	0.753

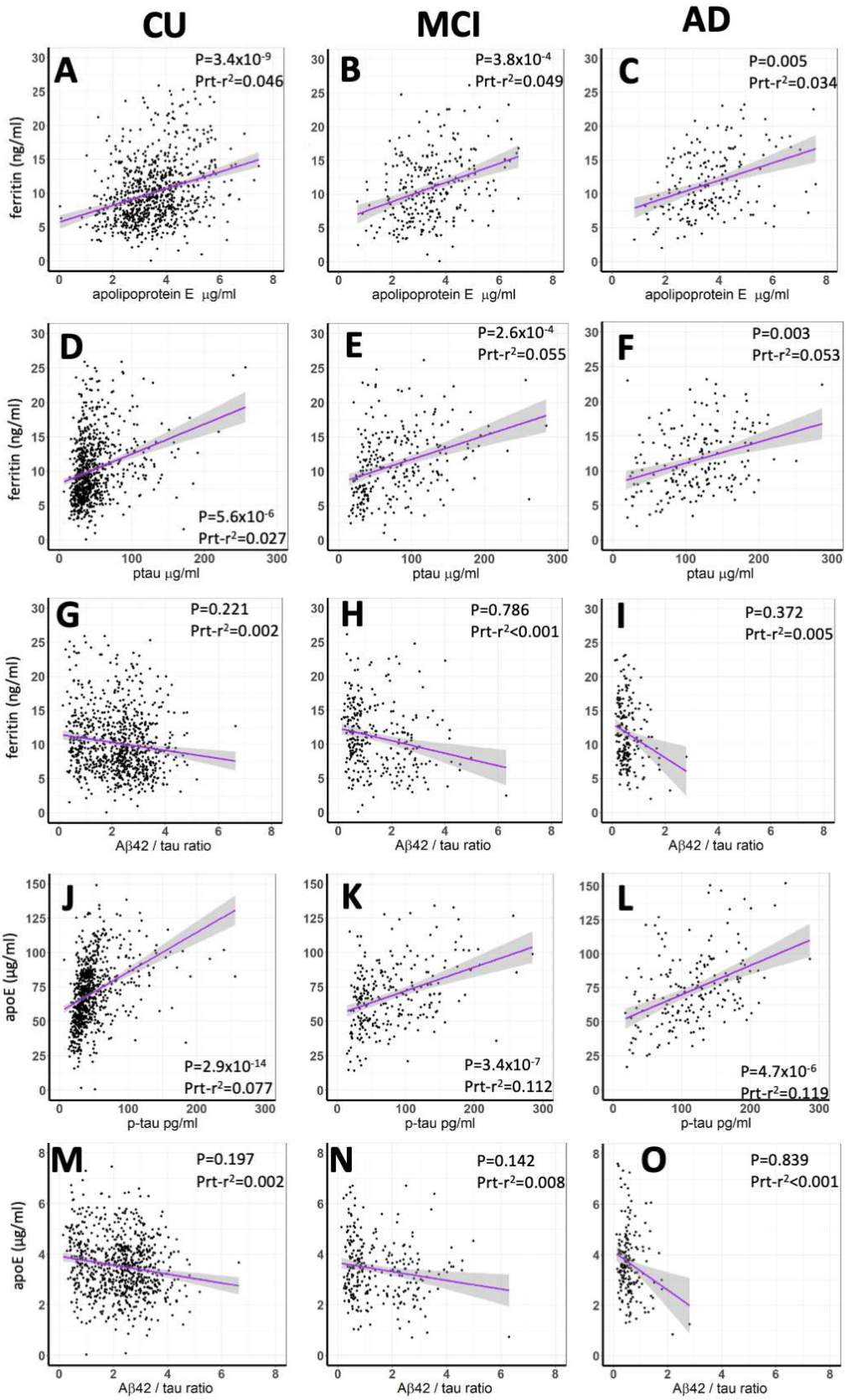
Supplementary Table 10. Demographics table of BioFINDER subjects stratified by clinical diagnosis and I+ criteria. Values of $A\beta_{42}$, t-tau and p-tau181 are in pg/ml.

Dx	**CN**								**MCI**							
A	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
T	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
I	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
N	425	133	67	18	7	15	69	54	72	18	23	11	3	4	71	61
Age: Mean (SD)	70.0 (6.0)	71.4 (5.3)	71.7 (4.4)	75.9 (4.9)	76.3 (4.2)	75.1 (8.2)	73.2 (5.2)	72.6 (5.2)	68.5 (5.9)	71.2 (4.7)	71.8 (5.3)	73.5 (5.1)	69.7 (3.2)	69.8 (5.7)	71.1 (5.0)	73.2 (4.6)
Female Sex: N (%)	148 (35)	66 (50)	21 (31)	8 (44)	2 (29)	8 (53)	27 (39)	31 (57)	45 (63)	17 (94)	15 (65)	8 (73)	1 (33)	4 (100)	31 (44)	33 (54)
APOE ε4+ve: N (%)	108 (26)	27 (21)	36 (55)	8 (44)	3 (43)	4 (27)	43 (62)	38 (70)	15 (21)	2 (11)	16 (70)	5 (46)	1 (33)	2 (50)	49 (69)	43 (71)
$A\beta_{42}$: mean (SD)	711 (227)	809 (239)	370 (142)	377 (118)	962 (313)	1,020 (260)	351 (97)	404 (138)	664 (206)	790 (219)	336 (115)	310 (117)	901 (356)	1,006 (380)	317 (106)	372 (135)
t-tau: mean (SD)	266 (72)	305 (72)	346 (97)	372 (98)	396 (110)	484 (86)	523 (148)	588 (231)	254 (76)	310 (60)	338 (99)	400 (152)	466 (193)	402 (99)	503 (155)	650 (235)
$A\beta_{42}$/t-tau: mean (SD)	0.13 (0.02)	0.13 (0.02)	0.08 (0.02)	0.08 (0.02)	0.12 (0.02)	0.11 (0.02)	0.06 (0.02)	0.06 (0.01)	0.14 (0.02)	0.13 (0.01)	0.09 (0.03)	0.08 (0.03)	0.11 (0.03)	0.10 (0.04)	0.06 (0.02)	0.06 (0.02)
p-tau181: mean (SD)	33.9 (9.5)	39.0 (10.2)	44.7 (11.0)	44.4 (9.9)	75.9 (24.4)	69.4 (7.3)	94.6 (29.1)	107.5 (44.6)	33.1 (9.6)	40.3 (8.8)	40.8 (11.1)	48.1 (10.5)	78.5 (20.8)	77.3 (7.7)	102.8 (36.0)	129.6 (46.8)
CDR-SB: mean (SD)	0.2 (0.4)	0.1 (0.4)	0.1 (0.3)	0.1 (0.3)	0.0 (0.0)	0.0 (0.0)	0.3 (0.6)	0.3 (0.5)	1.3 (0.9)	1.6 (1.1)	1.3 (0.9)	1.8 (0.7)	0.5 (0.0)	1.4 (0.5)	1.3 (0.9)	1.7 (1.0)
MMSE: mean (SD)	29.0 (1.0)	28.9 (1.1)	29.0 (0.9)	28.9 (1.0)	28.9 (1.1)	28.9 (1.1)	28.6 (1.3)	28.4 (1.4)	27.5 (1.9)	27.8 (1.5)	27.7 (2.0)	26.2 (1.8)	28.0 (2.0)	25.8 (2.1)	26.6 (1.7)	26.6 (1.6)

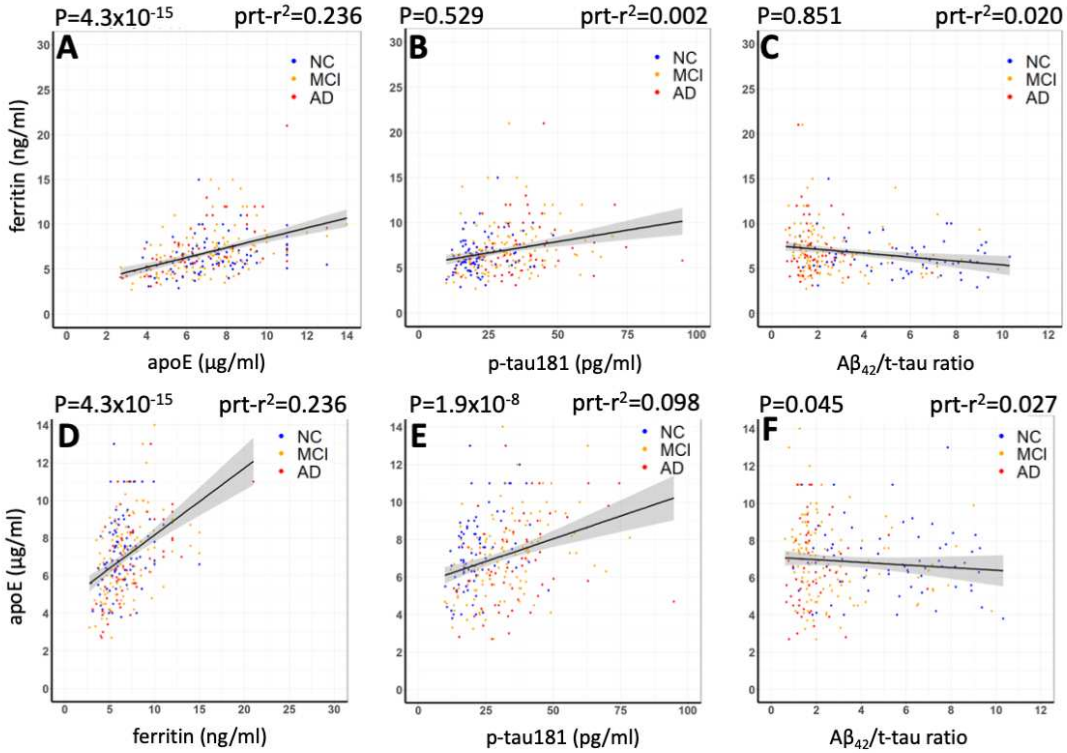
Supplementary Figures



Supplementary Figure 1. Ferritin and apoE levels in ADNI subjects. (A&B) Violin plots of CSF ferritin (**A**) stratified by cognitive status and (**B**) A&T criteria. **(C&D)** Violin plots of CSF apoE (**C**) stratified by cognitive status and (**D**) A&T criteria. Statistics are from multiple regression models including age, sex, *APOE* ε4, apoE, ferritin, and either cognitive status or A&T criteria, where *p<0.05; **P<0.01; ***P<0.001.



Supplementary Figure 2. Correlations between ferritin, apoE, p-tau and $A\beta_{42}$/t-tau in subjects from the BioFINDER cohort stratified by cognitive severity. Statistics are from multiple regression model of either ferritin or apoE, including age, sex, *APOE* ε4, p-tau, $A\beta_{42}$/t-tau and either ferritin or apoE. Prt-r^2 represents the partial r^2.



Supplementary Figure 3. Correlations between ferritin, apoE, p-tau and Aβ$_{42}$/t-tau in subjects from the ADNI cohort. Statistics are from multiple regression model of either ferritin or apoE, including age, sex, *APOE* ε4, p-tau, Aβ$_{42}$/t-tau and either ferritin or apoE. prt-r^2 represents the partial r^2.