Letters

RESEARCH LETTER

Association of Cerebrospinal Fluid Ferritin Level With Preclinical Cognitive Decline in *APOE*- ϵ 4 Carriers

The ϵ 4 allele of *APOE* confers the greatest genetic risk for Alzheimer disease (AD), and recent data implicate brain-iron load as a pathogenic mechanism because ϵ 4 carriage elevates the level of cerebrospinal fluid (CSF) ferritin.¹ Controlled for potential confounders like inflammation and bleeding, CSF ferritin level, although not elevated in AD, was associated with longitudinal cognitive performance and the risk of developing AD.¹ Herein, we investigate whether CSF ferritin level combines with established AD risk variables in predicting cognitive decline over 7 years in the preclinical phase.

Methods | This study used data obtained from the Alzheimer Disease Neuroimaging Initiative (ADNI) database.² The ADNI study and patient inclusion criteria have been reported.³ 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 magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. The ADNI study was approved by institutional review boards of all participating institutions. Informed written consent was obtained from all participants at each site. There was no compensation.

Baseline CSF levels of $A\beta$ 1-42, tau, *APOE*, ferritin, factor H (an inflammation marker), and hemoglobin (a blood leakage marker) were analyzed as previously described.¹ The scores for cognition using the longitudinal Rey Auditory-Visual Learning Task (RAVLT) (sensitive to early changes⁴) and the AD Assessment Scale-cognitive subset (ADAS-Cog13) were analyzed using linear mixed-effects models with R software (version 3.2.1). Normality and the absence of multicollinearity were confirmed. Data from individuals who left the study prematurely were included to the point of leaving. Statistical significance was set at $P = .05$ for all results.

Results | In participants who, at baseline, were cognitively normal or were categorized as having MCI, CSF ferritin level predicted cognition in a 4-way interaction with time, *APOE*- ϵ 4, and diagnosis (RAVLT: β [SE] = -1.45 [0.60], $P = .02$; ADAS-Cog13: β [SE] = 0.14 [0.07], $P = .03$) (Table). In contrast, the tau/ $A\beta$ 1-42 ratio interacted with time and not *APOE*- ϵ 4, diagnosis, CSF ferritin levels, or *APOE* levels, to predict cognitive performance (RAVLT: β [SE] = -0.39 [0.15]; $P = .01$; ADAS-Cog13: β [SE] = 0.06 [0.02]; $P = .001$).

In separate modeling of cognitively normal individuals and those with MCI, the tau/ $A\beta$ 1-42 ratio predicted cognitive dete-

rioration for those with MCI (RAVLT: β [SE] = -0.30 [0.17], $P = .07$; ADAS-Cog13: β [SE] = 0.05 [0.02], $P = .03$) and those who are cognitively normal (RAVLT: β [SE] = -0.68 [0.33], $P = .04$; ADAS-Cog13: β [SE] = 0.07 [0.03], $P = .06$) (Figure, A and B), and this index did not interact with the other included variables. Ferritin level was associated with cognitive performance in those with MCI (RAVLT: β [SE] = -2.24 [0.95], $P = .02$; ADAS-Cog13: 0.17 [0.08], $P = .04$), but it did not interact with time or the other included variables. For cognitively normal individuals, however, ferritin level was associated with cognitive deterioration in a 3-way interaction with time and ϵ 4 (RAVLT: β [SE] = -1.58 [0.54], $P = .004$; ADAS-Cog13: β [SE] = 0.11 [0.04], $P = .01$) (Figure, C and D). Categorization of cognitively normal individuals according to ϵ 4 revealed that ferritin level was strongly associated with cognitive decline in ϵ 4 carriers (RAVLT: β [SE] = -1.4 [0.4], $P < .001$; ADAS-Cog13: β [SE] = 0.09 [0.04], $P = .02$). For ϵ 4-negative individuals, lower ferritin levels predicted a modest deterioration in cognition (ADAS-Cog13: β [S.E.] = -0.04 [0.016], $P = .02$ but not RAVLT: β [SE] = 0.16 [0.22], $P = .48$), which might signify that abnormally low brain-iron could impair performance.

Finally, baseline CSF ferritin level was used to discriminate stable from declining (≥ 1 point/year worsening on RAVLT) cognitively normal ϵ 4-positive individuals. The area under the receiver operating characteristic (ROC) curve was 0.96, at a threshold predictive value of 6.6 ng/mL of ferritin per milliliter (Figure, E).

Discussion | These findings demonstrate the potential for CSF ferritin as a biomarker, especially for ϵ 4 carriers, and also provide new insight into the pathophysiologic mechanisms of AD. Cerebrospinal ferritin level might potentially be affected by abnormal vascular permeability, which occurs early in AD.⁵ However, in our model of the relationship between paired CSF and plasma samples from this cohort, plasma ferritin levels accounted for only 4% of the variance of CSF ferritin levels, regardless of diagnosis.¹ Thus, plasma ferritin permeating into CSF is unlikely to explain the adverse prognosis associated with higher CSF ferritin levels. Rather, CSF ferritin level probably reflects brain-iron burden (analogous to the periphery). Our observation that ferritin had a markedly divergent impact on ϵ 4 carriers and noncarriers agrees with findings of prior genetic studies demonstrating an epistatic interaction between the *APOE*- ϵ 4 allele and the iron-accumulating H63D variant of the hemochromatosis protein, HFE, leading to earlier onset of AD (by 5.5 years) (Ali-Rahmani et al⁶). Therefore, while CSF ferritin level is an indirect measure of ferritin level in the brain, our findings are consistent with a role for iron in the pathogenesis of AD.

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Table. Patient Demographics and Statistical Models^a

Characteristic	Only		Those Who Are CN Only		CN $\epsilon 4$	
	All Patients				Negative	Positive
Patients, No. (%)	234 (NA)		144 (NA)		69 (NA)	21 (NA)
APOE $\epsilon 4$ -positive	96 (41)		75 (52)		0 (0)	21 (100)
Female sex	93 (40)		47 (33)		38 (55)	8 (35)
Patient characteristic, mean (SD)						
Age, y	75.2 (6.6)		74.9 (7.2)		75.6 (5.2)	76 (6.4)
Education, y	15.8 (3)		15.9 (3)		15.7 (2.8)	15.5 (3.4)
CSF ferritin level, ng/mL	6.72 (2.5)		6.95 (2.72)		6.1 (1.83)	7.19 (2.48)
Average annual decline in RAVLT	-1.22 (1.04)		-1.74 (0.78)		-0.12 (0.19)	-1.26 (1.39)
Average annual decline in ADAS-cog13	1.74 (1.22)		2.47 (0.94)		0.43 (0.23)	1.04 (0.7)
RAVLT	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value
Controlling variables						
Diagnosis	-10.96 (1.45)	<.001	NA	NA	NA	NA
Sex	3.89 (1.12)	<.001	2.45 (1.45)	.10	6.89 (1.71)	<.001
Education, y	0.48 (0.18)	.01	0.11 (0.23)	.62	1.20 (0.29)	<.001
Testing variable/interaction						
Tau/A β 1-42	-0.67 (0.64)	.30	-0.84 (0.70)	.23	-0.34 (1.58)	.83
Tau/A β 1-42 $\times$ time	-0.39 (0.15)	.01	-0.30 (0.17)	.07	-0.68 (0.33)	.04
Ferritin ^b	-0.27 (1.05)	.80	-2.24 (0.95)	.02	-0.14 (1.01)	.89
Ferritin ^{a,b,c} $\times$ time $\times$ $\epsilon 4$ $\times$ diagnosis ^c	1.45 (0.60)	.02	-0.21 (0.31)	.49	-1.58 (0.54)	.004
ADAS-Cog13 ^d	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value
Controlling variables						
Diagnosis	1.27 (0.12)	<.001	NA	NA	NA	NA
Sex	-0.19 (0.09)	.045	-0.07 (0.13)	.60	-0.41 (0.13)	.002
Education, y	-0.04 (0.02)	.02	-0.02 (0.02)	.31	-0.07 (0.02)	.003
Testing variable/interaction						
Tau/A β 1-42	0.09 (0.05)	.11	0.10 (0.06)	.10	0.03 (0.12)	.81
Tau/A β 1-42 $\times$ time	0.06 (0.02)	.001	0.05 (0.02)	.03	0.07 (0.03)	.006
Ferritin ^b	0.12 (0.09)	.22	0.17 (0.08)	.04	0.10 (0.08)	.20
Ferritin ^b $\times$ time $\times$ $\epsilon 4$ $\times$ diagnosis ^c	-0.14 (0.07)	.03	-0.02 (0.04)	.63	0.11 (0.04)	.01

Abbreviations: ADAS-Cog13, Alzheimer's Disease Assessment Scale-cognitive subset; CN, cognitively normal;

CSF, cerebrospinal fluid; MCI, mild cognitive impairment; NA, not applicable; RAVLT, Rey Auditory-Visual Learning

Task; SE, standard error.

^a Separate covariate-adjusted mixed effects linear models of longitudinal (7-year) cognitive performance (RAVLT, ADAS-Cog13) in CN individuals and patients with MCI. Variables initially included in modeling were age, sex, body-mass index, years of education, APOE $\epsilon 4$ allele, baseline diagnosis, CSF tau/A β , CSF ApoE, CSF ferritin, CSF factor H (to control for inflammation), CSF hemoglobin level (to control for blood contamination), before minimal models were obtained using Akaike information criterion and Bayesian information criterion. CSF ApoE,

CSF factor H, and CSF hemoglobin level were not predictive in any of the models.

^b CSF ferritin was natural log-transformed.

^c This interaction variable was simplified to lower-order terms when the cohort was restricted according to the column titles (ie, the diagnosis interaction variable was removed when MCI and CN were modeled separately. The $\epsilon 4$ interaction variable was removed when performing separate modeling for $\epsilon 4$ -positive and $\epsilon 4$ -negative patients).

^d ADAS-Cog13 variable was square-root transformed.

Figure. Cognitive Decline in 90 Cognitively Normal Individuals at Baseline as Predicted by Baseline Cerebrospinal Fluid (CSF) Factors Stratified by *APOE*- ϵ 4 Allelic Status

A and B, The association between the baseline CSF tau/A β 1-42 ratio with annual change in (A) the Rey Auditory Visual Learning Test (RAVLT) score and (B) Alzheimer's Disease Assessment Scale-cognition (ADAS-Cog13) score in 21 *APOE*- ϵ 4 carriers and 69 noncarriers over 7 years. C and D, The association between baseline ferritin level with annual change in (C) RAVLT and (D) ADAS-Cog13 in *APOE*- ϵ 4 carriers and noncarriers over 7 years. Means $\pm$ 95% CIs are shown. E, The receiver under the operating curve of baseline CSF ferritin level (indicated by the colors) for predicting stable or deteriorating (≥ 1 RAVLT unit decrease per year) cognition in cognitively normal ϵ 4 carriers over 7 years. Area under the curve = 0.96.

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Author Contributions: Drs Ayton and Faux had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Faux and Ayton contributed equally to the manuscript. *Concept and design:* All authors.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: All authors.

Critical revision of the manuscript for important intellectual content: All authors. *Statistical analysis:* All authors.

Administrative, technical, or material support: Bush.

Study supervision: Faux, Bush.

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Use of Genetic Testing in Amyotrophic Lateral Sclerosis by Neurologists

There have been a number of publications describing the important role of genetic counseling in amyotrophic lateral sclerosis (ALS).^{1,2} While such attempts at guiding who should undergo genetic testing are welcome, they are put forth in a vacuum because there are no data on where ALS neurologists stand in terms of genetic testing and counseling for the disease, and in terms of what is considered to be familial and sporadic ALS.³ We attempted to fill this gap by surveying members of the Northeast Amyotrophic Lateral Sclerosis Consortium (NEALS, <http://www.alsconsortium.org>), one of the largest clinical research organizations for ALS.

We sought to understand in which situations genetic testing is used, which genes are tested for, and the attitudes of respondents toward genetic testing and counseling.

Methods | The survey was sent via email on June 20, 2016, to 134 principal investigators who are members of NEALS. The deadline for survey completion was July 29, 2016. Data were collated and analyzed using Microsoft Excel (Table). The NIH Office of Human Subjects Research Protection has determined that this type of research falls under exemption for institutional review board approval.

Table. Survey Questions and Responses of Neurologists Who Study Amyotrophic Lateral Sclerosis (ALS) (N = 43)

Question	Yes	No	No Response
Do you screen familial ALS cases for genetic mutations for clinical trials?	31	11	1
Do you screen sporadic ALS cases for genetic mutations for clinical trials?	8	34	1
Do you screen familial ALS cases for genetic mutations in everyday clinical practice?	40	3	0
Do you screen sporadic ALS cases for genetic mutations in everyday clinical practice?	13	30	0
Do you use next-generation sequencing techniques such as exome sequencing?	21	17	5
Do you use genetic panel testing?	24	17	2
Do you believe panel testing is cost-effective?	18	22	3
Do you provide genetic counseling to patients?	42	1	0
Would your attitude toward genetic testing change if an effective gene therapy became available?	39	4	0

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