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Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE

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Brain iron elevation is implicated in Alzheimer's disease (AD) pathogenesis, but the impact of iron on disease outcomes has not been previously explored in a longitudinal study. Ferritin is the major iron storage protein of the body; by using cerebrospinal fluid (CSF) levels of ferritin as an index, we explored whether brain iron status impacts longitudinal outcomes in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. We show that baseline CSF ferritin levels were negatively associated with cognitive performance over 7 years in 91 cognitively normal, 144 mild cognitive impairment (MCI) and 67 AD subjects, and predicted MCI conversion to AD. Ferritin was strongly associated with CSF apolipoprotein E levels and was elevated by the Alzheimer's risk allele, *APOE-ε4*. These findings reveal that elevated brain iron adversely impacts on AD progression, and introduce brain iron elevation as a possible mechanism for *APOE-ε4* being the major genetic risk factor for AD.

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Cortical iron elevation is increasingly reported as a feature of Alzheimer's disease (AD)[1], and might contribute to the oxidative damage observed in AD brains[2]. A single-blind, 2-year trial of 48 AD patients with the iron chelator, deferoxamine, reported decreased cognitive decline[3], but this has not been followed up. While evidence in animal models argue in favour of brain iron accumulation propelling atrophy and dementia[4], prospective evidence about the link between brain iron status and clinical outcomes in AD is lacking.

CSF ferritin could be an index of brain iron load. Ferritin is the iron storage protein of the body and is elevated in AD brain tissue[5–8]. In cultured systems, ferritin expression[9,10] and secretion[11] by glia is dependent on cellular iron levels. Ferritin levels in CSF likely reflect iron levels in the brain and can have clinical utility. For example, in Restless Legs Syndrome, a disorder of low brain iron that is treated with iron supplementation, CSF ferritin levels are decreased[12]. CSF ferritin was reported to be elevated in AD in one study[13], but this was not repeated in subsequent studies using larger clinical cohorts[14,15].

Here, we examined the association of baseline CSF-ferritin data with biomarker, cognitive, anatomical and diagnostic outcomes over 7 years in the Alzheimer's disease Neuroimaging Initiative (ADNI) prospective clinical cohort. We show that CSF ferritin levels have similar utility compared with more established AD CSF biomarkers, the tau/Aβ_{1-42} ratio and apolipoprotein E (ApoE) levels, in predicting various outcomes of AD. However, the nature of the relationship between CSF ferritin levels and cognitive performance was different from the other biomarkers, and, in contrast, CSF ferritin appears as a trait variable, and not a marker of disease.

Results

The relationship between CSF ferritin and biomarkers of AD. In agreement with other reports[14,15], CSF ferritin levels were not different between cognitively normal (CN; $=91$), mild cognitive impairment (MCI; $=144$) and AD ($=67$) subjects (ANCOVA: $P=0.591$; Table 1) in the ADNI cohort. Neither were there changes in ferritin levels when we separated the cohort according to CSF Aβ_{1-42} levels (192 ng l^{-1} cutoff; as proposed previously[16]) to reflect likely cerebral amyloid burden (ANCOVA: $P=0.946$; Supplementary Fig. 1). But in multiple regression modelling of ferritin including the established CSF biomarkers of AD[17] (tau, p-tau, Aβ_{1-42}), CSF ferritin levels were predicted by Aβ_{1-42} (partial $R^2=0.013$, $P=0.029$) and tau (partial $R^2=0.086$, $P<0.001$; model 1, Supplementary Table 1), although not by p-tau. Since the apolipoprotein E gene (*APOE*) alleles are the major genetic risk for AD[18] and CSF apolipoprotein E protein (ApoE) levels are associated with Aβ_{1-42} (refs 19,20) and tau[20,21], we re-built the model to include CSF ApoE levels. This abolished the relationship between ferritin and the other biomarkers (Aβ_{1-42}: $R^2<0.001$, $P=0.904$; tau: $R^2=0.003$, $P=0.219$; model 2, Supplementary Table 1). This led us to detect a surprisingly strong relationship between ApoE and ferritin (linear term partial $R^2=0.243$, $P=7.69\times10^{-22}$), which was improved when Aβ_{1-42} and tau

Table 1 | Baseline characteristics of subjects from the ADNI cohort used in this study, stratified by diagnosis.

	Units	CN	MCI	AD	P-value
n	—	91	144	67	NA
Age	Years (s.d.)	75.74 (5.43)	74.85 (7.2)	74.57 (7.61)	0.502
Female	*n* (%)	46 (50.55)	47 (32.64)	29 (43.28)	*0.021*
Education	Years (s.d.)	15.67 (2.94)	15.91 (2.95)	15.01 (2.96)	0.123
APOE-ε4 +ve	*n* (%)	22 (24.18)	75 (52.08)	46 (68.66)	6.50×10^{-8}
ADAS-Cog13	Units (s.d.)	9.51 (4.16)	19.19 (5.94)	29.22 (8.21)	2.75×10^{-56}
CSF Ferritin	ng ml^{-1} (s.d.)	6.4 (2.07)	6.95 (2.72)	6.94 (2.99)	0.591
CSF ApoE	μg ml^{-1} (s.d.)	7.3 (2.21)	7.1 (2.22)	6.35 (2.27)	*0.012*
CSF tau	pg ml^{-1} (s.d.)	69.78 (28.01)	104.3 (52.41)	122.63 (57.47)	4.57×10^{-7}
CSF ptau	pg ml^{-1} (s.d.)	24.77 (13.34)	36.39 (16.09)	41.39 (20.76)	1.13×10^{-6}

(non-significant terms) were removed from the model (linear term partial $R^2 = 0.341$, $P = 1.52 \times 10^{-29}$; model 3, Supplementary Table 1, Fig. 1a).

In model 3, *APOE* genotype strongly influenced CSF ferritin ($P = 1.10 \times 10^{-8}$), with the major AD risk allele, *ε4*, inducing 22% higher levels than non-*ε4* carriers (Fig. 1b). Reciprocally, in multiple regression modelling of CSF ApoE, *APOE ε4*-positive subjects had lower ApoE levels (-16%; $P = 2.50 \times 10^{-9}$) compared with non-*ε4* carriers (Fig. 1c). Plasma ferritin levels were not associated with plasma ApoE levels or *APOE ε4* allele status (Supplementary Fig. 2), but there was a modest association between plasma ferritin and CSF ferritin levels ($\beta = 0.075$, $P = 0.0002$; Supplementary Fig. 3).

Association of ferritin with neuropsychiatric assessments. Next, we explored whether CSF ferritin was related to cognitive performance in AD. Baseline ADAS-Cog13 (The Alzheimer's Disease Assessment Scale) score was associated with CSF ferritin ($P = 0.006$; Table 2), ApoE levels ($P = 0.0003$) and tau/Aβ_{1-42} ratio ($P = 0.025$), independently, in a multiple regression model containing the AD biomarkers and other clinical variables. In tertile analysis, high ($>7.2\,ng\,ml^{-1}$), compared with low ($<5.4\,ng\,ml^{-1}$), levels of ferritin were associated with an $\approx$3 point poorer ADAS-cog13 score (Fig. 2a). Similarly, in tertiles, lower levels of ApoE (Fig. 2b) were associated with a $\approx$4 point worse ADAS-Cog13, and higher tau/Aβ_{1-42} ratio was associated with a $\approx$2 point worse ADAS-Cog13 (Fig. 2c), as previously reported[21,22].

To determine whether baseline values of CSF ferritin predict longitudinal cognitive outcome, we constructed a mixed effects model of annual ADAS-Cog13 scores over 7 years (Table 2 for statistics, Supplementary Table 2 for patient numbers) and observed that both ApoE ($P = 0.006$) and tau/Aβ_{1-42} ratio

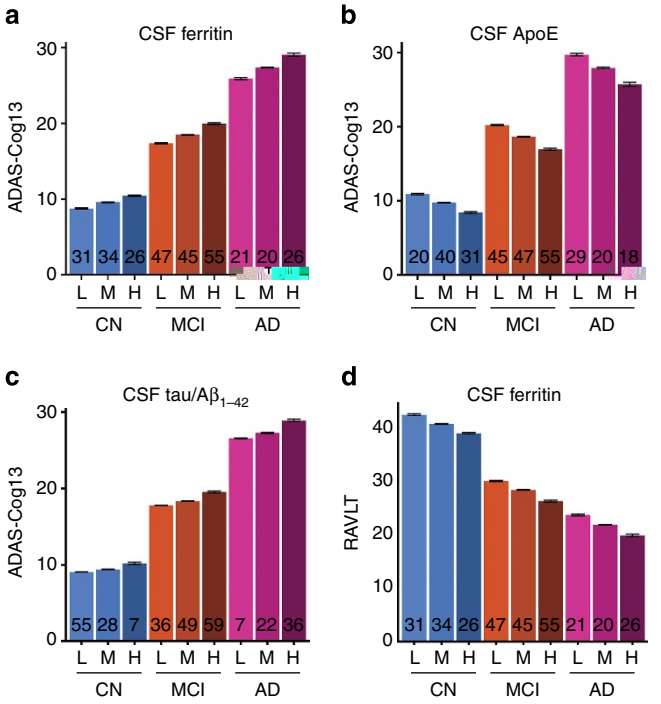

Figure 2 | CSF ferritin levels independently predict cognitive status. (**a**–**c**) Multiple regression of baseline ADAS-Cog13 score expressed as tertiles of CSF (**a**) ferritin (L<5.5; H>7.3 ng ml^{-1}), (**b**) ApoE (L< 5.8; H>7.8 μg ml^{-1}) and (**c**) tau/Aβ_{1-42} (L<0.35; H>0.76). (**d**) Multiple regression of baseline RAVLT score expressed as CSF ferritin tertiles. Data are adjusted for baseline diagnosis, gender, years of education and the AD CSF biomarkers in the minimal models. Data are means + s.e. '*n*' is shown in graph columns. CN, cognitively normal; MCI, mild cognitive impairment.

Table 2 | Modelling the association of CSF biomarkers on AD outcomes.

Model	Ferritin*		tau/Aβ_{1-42}		ApoE	
Cross-sectional cognition (MR)	β (s.e.)	*P* value	β (s.e.)	*P* value	β (s.e.)	*P* value
ADAS-Cog13†	0.139 (0.050)	*0.006*	0.104 (0.046)	*0.025*	−0.178 (0.049)	*0.0003*
RAVLT	−1.77 (0.559)	*0.0017*	NS	NS	1.033 (0.564)	0.0677
Longitudinal cognition (MELM)	β (s.e.)	*P* value	β (s.e.)	*P* value	β (s.e.)	*P* value
ADAS-Cog13†						
Main effect	0.178 (0.051)	*0.0005*	0.129 (0.049)	*0.009*	−0.180 (0.051)	*0.0004*
Interaction time	0.0005 (0.016)	0.977	0.081 (0.016)	2.70×10^{-7}	−0.044 (0.016)	*0.006*
RAVLT						
Main effect	−1.60 (0.63)	*0.012*	−0.847 (0.608)	0.165	1.03 (0.63)	0.104
Interaction time	−0.035 (0.152)	0.817	−0.610 (0.150)	4.85×10^{-5}	0.279 (0.152)	0.066
MCI conversion to AD	Statistic‡	*P* value	Statistic‡	*P* value	Statistic‡	*P* value
Cox (Hazard ratio)	1.10 (1.01-1.19)	*0.030*	1.53 (1.03-2.28)	*0.037*	0.83 (0.73-0.95)	*0.008*
LR (Odds ratio)	2.32 (1.86-2.90)	8.001×10^{-15}	1.45 (1.16-1.80)	*0.0001*	0.38 (0.30-0.48)	1.88×10^{-17}
Rate of MRI atrophy (MELM)	β (s.e.)	*P* value	β (s.e.)	*P* value	β (s.e.)	*P* value
Hippocampus	−18.33 (7.86)	*0.019*	−35.31 (7.79)	6.81×10^{-6}	21.38 (8.02)	*0.008*
Lateral ventricles§	0.007 (0.003)	*0.008*	0.013 (0.002)	4.19×10^{-8}	−0.009 (0.003)	*0.0002*

Cox, Cox proportional hazard model; LR: logistic regression; MELM, mixed effects linear model; MR, multiple regression; NS, not significant. All models initially contained the variables: age, gender, BMI, *APOE* genotype, baseline diagnosis; the MRI models additionally included intracranial volume. Minimal models for the cognition models included baseline diagnosis, gender, years of education and the AD CSF biomarkers. Minimal model for the Cox proportional hazard model (Cox) contained only the AD CSF biomarkers. Minimal models for the MRI models contained age, gender, baseline diagnosis, years of education, *APOE ε4* status and intracranial volume. All subjects with available data were included in the cross-sectional cognition models. Only CN and MCI subjects were included in modelling of longitudinal cognition because short follow up of AD subjects (Supplementary Table 3). Only subjects who were classed as MCI at baseline were included in the MCI conversion models. The MRI models contained subjects who were classed as cognitively normal or MCI at baseline. AD subjects at baseline were not included because of low numbers and lack of follow-up (Supplementary Tables 3).
*Ferritin values were log-transformed, excluding non-parametric Cox and LR models.
†The β-coefficient is for the square root of ADAS-Cog13.
‡The statistics for the conversion models were based on one interquartile range change for each analyte (ferritin: 3.3 ng ml^{-1}, tau/Aβ_{1-42}: 0.67 units; ApoE: 3.1 μg ml^{-1}).
§For Lateral ventricles, the β-coefficient is for natural log of the ventricle volume.

($P = 2.70 \times 10^{-7}$) were still associated with rate of cognitive change (interacted with time), as previously reported[21,22].

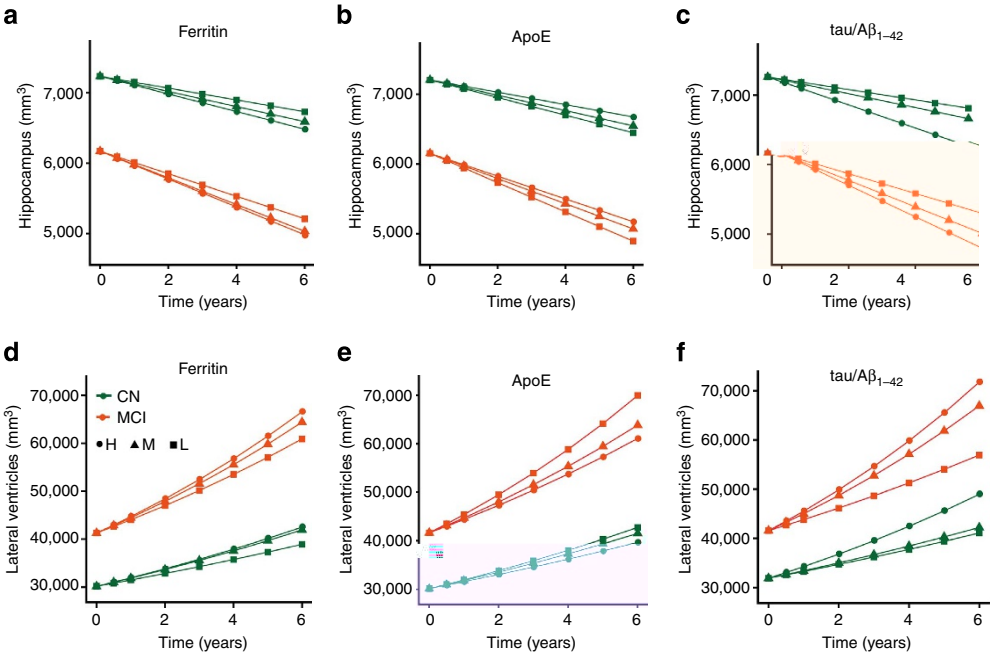

Figure 4 | CSF ferritin levels independently predict brain structural changes. (**a**–**c**) Longitudinal hippocampal changes based on tertiles of CSF (**a**) ferritin (L < 5.5; H > 7.3 ng ml^{-1}) (**b**) ApoE (L < 5.8; H > 7.8 μg ml^{-1}) and (**c**) tau/Aβ$_{1-42}$ (L < 0.35; H > 0.76) tertiles (refer to Table 2). (**d**–**f**) Longitudinal lateral ventricular changes based on CSF (**d**) ferritin (**e**) ApoE and (**f**) tau/Aβ$_{1-42}$ tertiles (refer to Table 2). These mixed effects models were adjusted for age, gender, baseline diagnosis, years of education, APOE *ε4* status and intracranial volume. Tertiles at baseline were not significantly different for all models, therefore for visual display the baseline values were held at the adjusted means for each diagnostic group. CN, cognitively normal; H, highest tertile; M, middle tertile; MCI, mild cognitive impairment; L, lowest tertile.

shown to predict atrophy of various brain structures when considered in isolation[24]. Baseline ApoE, ferritin and tau/Aβ$_{1-42}$ values each independently predicted hippocampal volume in an adjusted longitudinal model (Table 2). The rate of atrophy of the hippocampus was greater in individuals with high CSF ferritin ($P = 0.02$; Fig. 4a). Low CSF ApoE ($P = 0.008$; Fig. 4b) or high tau/Aβ$_{1-42}$ ($P = 6.80 \times 10^{-6}$; Fig. 4c) also predicted atrophy, as previous reported[21,25]. Lateral ventricular enlargement over time was similarly associated independently with high-CSF ferritin ($P = 0.008$; Fig. 4d), low-CSF ApoE ($P = 0.0002$; Fig. 4e), or high tau/Aβ$_{1-42}$ ($P = 4.19 \times 10^{-8}$; Fig. 4f).

Discussion

Our analyses show that CSF ferritin levels were independently related to cognitive performance in the ADNI cohort and predicted MCI conversion to AD. The magnitude impact of ferritin on these outcomes was comparable to the established biomarkers, ApoE and tau/Aβ$_{1-42}$; however, the nature of the effect of ferritin was not the same. Ferritin was associated with constant shift in cognitive performance over the study period (Fig. 5a), whereas the decrements associated with the other biomarkers were exaggerated over time (Fig. 5b). A downward shift (poorer cognitive presentation) in response to high ferritin levels (1.77 RAVLT points per 1 ng ml^{-1} ferritin; Table 2) results in an earlier age of diagnosis (3 months per 1 ngng ml^{-1} ferritin; Fig. 3b). This would be consistent with findings that patients with an early age of AD onset have greater neocortical iron burden than late-onset patients[1,7]. Collectively these data support consideration of therapeutic strategies that lower brain iron, which have reported beneficial outcomes in Phase II trials of Alzheimer's[3] and Parkinson's[26] diseases. Lowering CSF ferritin, as might be expected from a drug like deferiprone[26], could conceivably delay MCI conversion to AD by as much as 3 years.

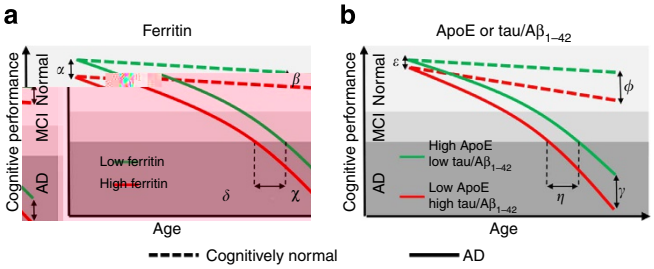

Figure 5 | Schematic: the impact of ferritin and other biomarkers on AD presentation. (**a**) CSF ferritin has a qualitatively different impact to (**b**) CSF tau/Aβ$_{1-42}$ and ApoE on cognitive performance over time in cognitively normal (dotted lines) and in subjects who develop AD (solid lines). Higher CSF ferritin levels are associated with poorer baseline cognitive status (for example, RAVLT) by [α] points, where [α] = Ln[ferritin (ng ml^{-1})]*1·77 (refer to Table 2). This effect is constant over time, such that [α] = [β,χ]. Consequently, ferritin causes a shift to the left in age of conversion to AD by [δ] months, where [δ] = ferritin (ng ml^{-1})*3 (refer to Fig. 3b). Levels of tau/Aβ$_{1-42}$ or ApoE are associated with both baseline cognitive status [ε] and the rate of cognitive deterioration, such that [ε] < [ϕ,γ]. The effect causes a shift in age of diagnosis by [η] months where [η] = ApoE (μg ml^{-1})*8 or tau/Aβ$_{1-42}$ (units)*17 (refer to Fig. 3b).

An unresolved question arising from this study is why are CSF ferritin levels not elevated in AD, where brain iron levels are reported as elevated[2]? We hypothesize that ferritin levels in the CSF reflect global brain iron burden, whereas iron elevation in AD has only been reported in affected regions (for example, frontal cortical tissue[27]). Possibly, iron elevation in brain regions affected by AD is too confined regionally to be reflected in CSF. An altered relationship between tissue and CSF ferritin in AD, however, cannot yet be excluded.

Our data also provide exploratory insights into iron in AD aetiopathogenesis, identifying an unexpected interaction of ApoE with ferritin. That ferritin levels are increased by the *APOE-ε4* allele argues that ApoE influences ferritin levels, rather than the reverse. Our current findings indicate that *APOE* genotype should influence constitutive brain iron burden. However, to our knowledge, a post mortem study of iron or ferritin in brain tissue, stratified according to *APOE* genotype, has not been reported. Focal changes to iron and ferritin have been observed in AD brains post mortem[1,2,5–8], and on the basis of our findings we propose that the *ε4* genotype raises the baseline iron load of the brain, thus lowering the threshold for iron-mediated neuronal loss in disease. This proposal awaits experimental confirmation, but it is possible

Associations between the baseline Alzheimer's Disease Assessment Scale Cognition (ADAS-cog13) and Rey Auditory Verbal Learning Test (RAVLT) scores with CSF ferritin, the CSF tau/Aβ_{1-42} ratio and CSF ApoE were tested with a covariate-adjusted multiple regression for each cogntive scale. For these analyses, age, gender, BMI, years of education, *APOE-ε4* allele and baseline diagnosis were initially included as covariates. To assess the association of baseline CSF ferritin levels with the longitudinal clinical outcomes (ADAS-cog13 and RAVLT scores over 7 years), linear mixed effects models were used. These models were adjusted for the same variables as the baseline models of cognition, and additionally included time as interacting variable with each of the CSF biomarkers. AD subjects were excluded from the longitudinal analysis because of low rate of follow up (Supplementary Table 2). A significant value for any of these interaction terms would indicate that the variable affected the rate of cognitive change. For the ADAS-cog13, longitudinal analysis, the minimal model included years of education, gender and *APOE-ε4* allele. For the longitudinal analysis with RAVLT, the minimal model included years of education and gender.

Cox proportional hazards model was used to assess the impact of CSF analytes on the time to AD conversion. The initial model contained age at baseline, gender, years of education and *APOE-ε4* genotype as confounding variables together with CSF ApoE, tau/Aβ_{1-42} and ferritin. A minimal model containing only the CSF biomarkers was identified via BIC step down procedure and log likelihood test.

Logistic regression analysis was used to assess the impact of CSF analytes on risk of conversion to AD. Combinations of CSF ferritin, ApoE and tau/Aβ_{1-42} analytes were included in logistic regression models of MCI conversion to AD that were adjusted for age at baseline, gender, years of education, *APOE* genotype and BMI. These models determined the predictive performance of these analytes in identifying stable MCI participants from MCI participants who, up to 102 months, had a diagnosis change to AD. The receiver-operator curves and the area under the curve were derived from the predictive probabilities of the logistic regression models.

The relationships between CSF ferritin, ApoE, tau/Aβ_{1-42} with longitudinal structural (MRI) changes to hippocampus and lateral ventricle were analysed using linear mixed models adjusted for age, years of education, BMI, gender and *APOE* genotype and intracranial volume. For all models, CSF ferritin, ApoE, tau/Aβ_{1-42} and baseline diagnosis were included as fixed effects and were not removed from a minimal model. Two random effects were also included, intercepts and slope (time). An interaction between time and diagnosis, time and CSF ferritin, time and CSF ApoE, as well as time and CSF tau/Aβ_{1-42} were also included for all models. All the AD subjects were excluded from MRI analyses due to low numbers and short follow-up. PET imaging data from ADNI were not included in the analysis because there were too few patients who had CSF ferritin measured and who also underwent PET imaging at baseline.

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Author contributions

S.A. provided-scientific concept, modelling of data and wrote manuscript. N.G.F. helped in modelling of data and wrote manuscript. A.I.B. supervised modelling, wrote manuscript and funded analysis project.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: A.I.B. is a shareholder in Prana Biotechnology, Cogstate, Eucalyptus, Mesoblast, Brighton Biotech, LLC, and a payed consultant for Collaborative Medicinal Developments, LLC and Brighton Biotech, LLC. S.A. and N.G.F. declare no competing financial interests.

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