

1 **Biological Brain Age Prediction Using Machine Learning on**
2 **Structural Neuroimaging Data: Multi-Cohort Validation Against**
3 **Biomarkers of Alzheimer's Disease and Neurodegeneration stratified**
4 **by sex**
5
6

7 Irene Cumplido-Mayoral^{1,2}, Marina García-Prat¹, Grégory Operto^{1,3,4}, Carles Falcon^{1,3,5},
8 Mahnaz Shekari^{1,2,3}, Raffaele Cacciaglia^{1,3,4}, Marta Milà-Alomà^{1,2,3,4}, Luigi Lorenzini⁶,
9 Silvia Ingala⁶, Alle Meije Wink⁶, Henk JMM Mutsaerts⁶, Carolina Minguillón^{1,3,4},
10 Karine Fauria^{1,4}, José Luis Molinuevo^{1,*}, Sven Haller⁷, Gael Chetelat⁸, Adam
11 Waldman⁹, Adam Schwarz¹⁰, Frederik Barkhof^{6,11}, Ivonne Suridjan¹², Gwendlyn
12 Kollmorgen¹³, Anna Bayfield¹³, Henrik Zetterberg^{14,15,16,17,18}, Kaj Blennow^{14,15}, Marc
13 Suárez-Calvet^{1,3,4,19}, Verónica Vilaplana^{#20}, Juan Domingo Gispert^{#1,3,5}
14 ALFA study, EPAD study, ADNI study, OASIS study

15
16
17
18 1) Barcelonaβeta Brain Research Center (BBRC), Pasqual Maragall Foundation,
19 Barcelona, Spain

20 2) Universitat Pompeu Fabra, Barcelona, Spain

21 3) IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain

22 4) CIBER Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain

23 5) Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y
24 Nanomedicina (CIBER-BBN), Madrid, Spain

25 6) Department of Radiology and Nuclear Medicine, Amsterdam Neuroscience, Vrije
26 Universiteit Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands

27 7) CIRD Centre d'Imagerie Rive Droite, Geneva, Switzerland

28 8) Normandie Univ, UNICAEN, INSERM, U1237, PhIND "Physiopathology and
29 Imaging of Neurological Disorders", Institut Blood and Brain @ Caen-Normandie,
30 Cyceron, Caen, France

31 9) Centre for Dementia Prevention, Edinburgh Imaging, and UK Dementia Research
32 Institute at The University of Edinburgh, Edinburgh, UK

33 10) Takeda Pharmaceutical Company Ltd, Cambridge, MA, USA

34 11) Institutes of Neurology and Healthcare Engineering, University College London,
35 London, UK

36 12) Roche Diagnostics International Ltd, Rotkreuz, Switzerland

37 13) Roche Diagnostics GmbH, Penzberg, Germany

38 14) Institute of Neuroscience and Physiology, University of Gothenburg, Mölndal,
39 Sweden

40 15) Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal,
41 Sweden

42 16) Department of Neurodegenerative Disease, UCL Queen Square Institute of
43 Neurology, London, United Kingdom

44 17) UK Dementia Research Institute at UCL, London, United Kingdom

45 **NOTE:** This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
18) Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

46 19) Servei de Neurologia, Hospital del Mar, Barcelona, Spain
47 20) Department of Signal Theory and Communications, Universitat Politècnica de
48 Catalunya, Barcelona, Spain
49 * Current affiliation: H.Lundbeck A/S, Copenhagen, Denmark

50

51

52 # Corresponding authors

53

54

55

56

57 **ABSTRACT (150 words)**

58

59 Brain-age can be inferred from structural neuroimaging and compared to chronological
60 age (brain-age delta) as a marker of biological brain aging. Accelerated aging has been
61 found in neurodegenerative disorders like Alzheimer's disease (AD), but its validation
62 against markers of neurodegeneration and AD is lacking. Here, imaging-derived
63 measures from the UK Biobank dataset (N=22,661) were used to predict brain-age in
64 2,314 cognitively unimpaired (CU) individuals at higher risk of AD and mild cognitive
65 impaired (MCI) patients from four independent cohorts with available biomarker data:
66 ALFA+, ADNI, EPAD and OASIS. Brain-age delta was associated with abnormal
67 amyloid- β , more advanced stages (AT) of AD pathology and *APOE*- ϵ 4 status. Brain-age
68 delta was positively associated with plasma neurofilament light, a marker of
69 neurodegeneration, and sex differences in the brain effects of this marker were found.
70 These results validate brain-age delta as a non-invasive marker of biological brain aging
71 related to markers of AD and neurodegeneration.

72

73

74 **INTRODUCTION**

75

76 Age is the main risk factor for Alzheimer's Disease (AD) and most neurodegenerative
77 diseases. However, the mechanisms underlying this association are still poorly
78 understood (Fjell et al., 2014). Both normal aging and AD are associated with region-
79 specific cerebral morphological changes characterized by the occurrence of atrophy
80 (Bakkour et al., 2013; Fjell et al., 2014). Both aging and AD have differential and partially
81 overlapping effects on specific regions of the cerebral cortex like, for instance, the
82 dorsolateral prefrontal cortex (Bakkour et al., 2013; Fjell et al., 2014; Pichet Binette et
83 al., 2020). Conversely, some regions are predominantly affected by age (e.g., calcarine
84 cortex) and some others are predominantly affected by AD (e.g., medial temporal cortex)
85 (Bakkour et al., 2013). A better understanding of the mechanistic links between the brain
86 aging process and neurodegenerative diseases is an urgent priority to develop effective
87 strategies to deal with their rising burden amid an ageing population (Franke & Gaser,
88 2019). Therefore, a growing amount of research is focusing on using neuroimaging
89 techniques to develop a biomarker of biological brain aging. In this framework, the
90 concept of brain-age has emerged as an appealing comprehensive marker that enables
91 determining on an individual basis, the risk for age-associated brain diseases (James H.
92 Cole et al., 2017; James H. Cole & Franke, 2017; Franke et al., 2010; Franke & Gaser,
93 2019). However, this is a challenging task because, even though the cerebral structural
94 changes related to aging are well established, the older population is characterized by

95 substantial variation in neurobiological aging trajectories (J. H. Cole et al., 2018; Fjell et
96 al., 2014).

97

98 Recently, machine learning techniques have gained popularity as brain-age prediction
99 models (James H. Cole et al., 2017; Dafflon et al., 2020; de Lange et al., 2019; Franke &
100 Gaser, 2019), due to their ability in identifying relevant data-driven patterns within
101 complex data (Zhavoronkov et al., 2019). These models learn the association between
102 chronological age and cerebral morphological features derived from structural magnetic
103 resonance imaging (MRI) in healthy individuals, yielding a predicted brain-age for each
104 individual. Individuals with a predicted brain-age higher than their chronological age may
105 have an “older” brain than expected, whereas an individual with an estimated brain-age
106 lower than their chronological age has a “younger” brain. Subtracting chronological age
107 from estimated brain-age hence provides an estimate of accelerated brain aging, namely
108 the brain-age delta. Recent literature has shown the adequacy of using a brain-age
109 predicted measurement in the assessment of the clinical severity of AD, by finding higher
110 brain-age deltas in AD and individuals with mild cognitive impairment (MCI) with
111 respect to cognitively unimpaired (CU) individuals (Beheshti et al., 2018; Kaufmann et
112 al., 2019). A higher brain-age delta has also been reported in other diseases, such
113 as multiple sclerosis, epilepsy and psychiatric disorders, with respect to healthy controls
114 (Beheshti et al., 2018; Kaufmann et al., 2019). In addition, brain-age delta has also been
115 associated with other biological measures such as: lifestyle factors (James H. Cole, 2020),
116 cognition (Beheshti et al., 2018; James H. Cole, 2020) hypertension (de Lange, et al.,
117 2020) and prediction of mortality (J. H. Cole et al., 2018).

118

119 Even though these studies support the association of brain-age delta as a biomarker of
120 biological aging with relevance to various brain diseases, there are no comprehensive
121 studies validating this measurement in association with specific biological markers of AD
122 pathology (*i.e.* Amyloid- β [$A\beta$] and tau pathology), neurodegeneration and
123 cerebrovascular disease. This is a very relevant aspect since the recent AD research
124 framework criteria defines AD as a biological construct, namely the presence of both
125 abnormal $A\beta$ (A+) and tau (T+) biomarkers, regardless of clinical manifestations (Jack et
126 al., 2018). The term “Alzheimer’s pathological change” is proposed whenever there is
127 evidence of $A\beta$ but not tau pathology (A+T-). The umbrella term “Alzheimer’s
128 *continuum*” includes both “Alzheimer’s pathological change” (A+T-) and “Alzheimer’s
129 Disease” (A+T+). Under this definition, A-T+ individuals would not fall into the AD
130 *continuum*. Then, under this framework, neurodegeneration biomarkers (N) and cognitive
131 status (*i.e.* CU, MCI and dementia syndromes) are used to stage disease progression.

132

133 A recent study used brain-age measurements to identify amnesic MCI (aMCI), the
134 typical clinical presentation of prodromal AD, from other individuals with MCI, by
135 studying the association with AD risk factors such as apolipoprotein E (*APOE*) and
136 $A\beta$ (Huang et al., 2021). Another study focusing on the impact of training the brain-age
137 prediction model in individuals with $A\beta$ pathology ($A\beta+$) showed that CU $A\beta+$
138 individuals had a higher brain-age delta than CU $A\beta-$ individuals (Ly et al., 2020).
139 Nonetheless, there remains a need to study the associations between brain-age prediction
140 and AD as well as neurodegeneration biomarkers in preclinical stages in different and
141 independent cohorts and in a larger sample size. Moreover, given that female individuals
142 have a higher AD prevalence compared to males (Nebel et al., 2018) and display different
143 lifetime trajectories in the brain morphological features (Gennatas et al., 2017), it is of
144 interest to determine the effect of sex on brain age delta and its interaction with AD

145 biomarkers. Literature describes sex differences in AD biomarkers, such as that females
146 with abnormal $A\beta$ who are *APOE-ε4* carriers show greater subsequent increase in
147 cerebrospinal fluid (CSF) tau than their male counterparts (Buckley et al., 2019), or that
148 females with higher $A\beta$ burden show higher entorhinal cortical tau than their male
149 counterparts (Buckley et al., 2019). Conversely, levels of the neurodegeneration
150 biomarker CSF neurofilament light (NfL) have been widely reported to be higher in males
151 than in females (Mielke, 2020; Milà-Alomà et al., 2020). In line with this, AD risk factors
152 are associated with greater brain aging in women than men (Subramaniapillai et al.,
153 2021).

154
155 Therefore, in the present study, we aim to validate brain-age delta as a clinically relevant
156 marker related to markers of AD and neurodegeneration. For this purpose, we determine
157 the association between the predicted structural brain-age delta with biomarkers and risk
158 factors for AD and neurodegeneration in non-demented individuals, as well as to study
159 the effect of sex on these associations. We trained a model to predict the brain-age
160 separately for females and males, using machine learning on imaging-derived measures
161 of cortical thickness, cortical volume, and subcortical volume from the UK BioBank
162 cohort (N=22,661). Using this model, we then estimated brain-age in four independent
163 cohorts: ALFA+ (N=380), ADNI (N=719), EPAD (N=808) and OASIS (N=407). In each
164 cohort, we studied the associations of brain-age delta with biomarkers of AD pathology
165 (CSF $A\beta$ and p-tau as continuous values, as well as categorized in AT stages), the *APOE-*
166 $\epsilon 4$ genotype which is the main genetic risk factor for AD, neurodegeneration (CSF and
167 plasma NfL), and small vessel disease (White Matter Hyperintensities [WMH]). Finally,
168 we studied the sex differences in brain age prediction and the sex effects with these
169 biomarkers on brain-age delta.

170

171

172 RESULTS

173

174 Participants' Characteristics

175

176 Table 1 summarizes the demographic characteristics of the cohorts included in the study.
177 ADNI and EPAD cohorts included both CU and MCI individuals, while the UK BioBank,
178 ALFA+ and OASIS cohorts only included CU individuals. Table 2 summarizes the
179 variables used to study the associations with brain-age delta, which included biomarkers
180 for AD ($A\beta$ positron emission tomography [PET] and CSF $A\beta$ and p-tau),
181 neurodegeneration (CSF and plasma NfL), and cerebrovascular pathology (WMH on
182 MRI), as well as the aging signature composite (Bakkour et al., 2013), both cross-sectional
183 and longitudinally. The aging signature composite is a map of specific brain regions that
184 undergo cortical thinning in normal aging, which has been used as a proxy measurement
185 for brain aging. These validation variables were correlated with chronological age for all
186 cohorts (see Supplementary Table 1). Some of the participants for ALFA+ (N=25), ADNI
187 (N=116) and EPAD (N=71) fell into the A-T+ group, corresponding to non-AD
188 pathologic change. Since our aim was to specifically validate the brain-age delta
189 measurements in the AD *continuum*, we excluded these participants from subsequent
190 analyses; and they are reported within Table 1 and Table 2 solely for descriptive purposes.
191 In addition, the number of MCI individuals with available data of CSF NfL and of aging
192 signature change was relatively low and, therefore, these variables were excluded from
193 the analysis in MCI individuals.

194

Table 1. Sample demographics and characteristics separated by cohort and by diagnosis.

Characteristics	CU					MCI	
	UK BioBank (n=22,661)	ALFA+ (n=380)	ADNI (n=284)	EPAD (n=653)	OASIS (n=407)	ADNI (n=435)	EPAD (n=155)
Age, years	64.54 (7.55)	60.61 (4.72)	71.42 (6.36)	64.96 (7.01)	69.07 (9.42)	71.09 (7.31)	69.08 (6.97)
Age range, years	[44, 81]	[48, 73]	[55, 89]	[50, 88]	[42, 89]	[55, 91]	[52, 88]
Female, n (%)	11,767 (51.92)	254 (60.76)	126 (50.00)	386 (59.11)	244 (59.95)	249 (50.00)	81 (47.74)
Education, years	17.75 (5.42)	13.43 (3.71)	16.54 (2.49)	14.83 (3.56)	15.93 (2.59)	16.23 (2.71)	14.17 (3.77)
<i>APOE</i> - ϵ 4 carriers, n (%)	6,334 (27.95)	221 (52.87)	72 (28.57)	217 (33.23)	118 (28.99)	218 (43.78)	60 (38.71)
MMSE	-	29.15 (0.95)	28.985 (1.24)	28.82 (1.40)	29.03 (1.31)	27.57 (2.19)	27.86 (1.97)

Notes: Data are expressed as mean (M) and standard deviation (SD) or percentage (%), as appropriate. Abbreviations: *APOE*, apolipoprotein E; MMSE, Mini-Mental State Examination.

Table 2. Biomarkers separated by cohort and by diagnosis

BIOMARKERS	CU						MCI					
	ALFA+		ADNI		EPAD		OASIS		ADNI		EPAD	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Centiloids	0	-	0	-	0	-	407	13.468 (28.138)	0	-	0	-
CSF Aβ42 (pg/mL) ^a	380	1318.059 (599.223)	284	1223.890 (556.648)	653	1403.617 (681.736)	0	-	435	986.248 (446.402)	155	1245.181 (741.756)
CSF p-tau (pg/mL)	380	16.289 (7.813)	283	22.234 (9.692)	627	18.326 (8.380)	0	-	434	26.490 (14.402)	151	24.715 (14.897)
CSF NfL (pg/mL) ^b	380	82.717 (29.124)	26	1052.444 (376.095)	0	-	0	-	48	1383.638 (918.231)	0	-
Plasma NfL (pg/mL)	368	10.519 (3.739)	184	35.843 (17.988)	0	-	0	-	404	38.157 (18.908)	0	-
WMH volume	360	0.045 (0.845)	240	-0.0085 (1.267)	456	0.038 (1.072)	0	-	458	-0.005 (1.229)	108	0.048 (1.076)
Ageing signature ^b	360	2.387 (0.071)	240	2.284 (0.105)	456		0	-	458	2.251 (0.109)	0	-
Ageing signature V2 ^b	187	2.376 (0.072)	45	2.299 (0.118)	0	-	0	-	46	2.257 (0.119)	0	-
Ageing signature change ($V2 - V1 / \Delta t$) ^b	187	-0.003 (0.011)	45	-0.0007 (0.037)	0	-	0	-	46	-0.003 (0.050)	0	-

Notes: Data are expressed as mean (M) and standard deviation (SD) or percentage (%), as appropriate. Amyloid- β status was defined by CSF (ALFA+, ADNI and EPAD) or amyloid PET (OASIS). For ALFA+ and ADNI, we calculated the ageing signature from MRI scans acquired 3 years later than the original MRI scan, called ageing signature V2. Ageing signature change was calculated as the difference in ageing signature over these two MRI scans.

^a Individuals that fell into the A-T+ group: 25 from ALFA+, 116 from ADNI and 71 from EPAD.

^b As the number of MCI individuals with CSF NfL and ageing signature change was relatively low, we excluded them from the following results.

Abbreviations: CSF, cerebrospinal fluid; NfL, neurofilament light; WMH, White Matter Hyperintensities.

196 Brain-Age Prediction and Chronological Age

197

198 We trained the prediction model using the UK BioBank cohort and tested the model using
 199 four independent cohorts (ALFA+, ADNI, EPAD and OASIS), as shown
 200 in Supplementary Fig. 1. Table 3 shows the prediction accuracy before and after age-bias
 201 correction for the combined female and male predictions. The average prediction
 202 accuracy of the model run on UK BioBank using ten-fold cross-validation as measured
 203 by the mean absolute error (MAE) and by Pearson's correlation were, originally,
 204 MAE=4.19 and R=0.71 and, after correction, MAE=2.95 and R=0.89 (Table 3
 205 and Supplementary Fig. 2).

206

207 We then investigated the association of predicted brain-age with chronological age on
 208 each of the independent cohorts. All the cohorts showed a similar positive correlation and
 209 fitting performance metrics as measured by the mean absolute error (MAE), R and root
 210 mean squared error (RMSE) between chronological age and predicted brain-age.
 211 Correlation coefficients were not different between cohorts ($P>0.05$, for all comparisons,
 212 see Supplementary Table 2). As an example, after bias correction, the highest numerical
 213 difference was between ALFA+ and OASIS, with quite similar MAE=3.25 and R=0.729
 214 and MAE=3.81 and R=0.910, respectively.

215

216 In order to study the effect of sex on brain age prediction, we also computed the
 217 performance metrics stratified by females and males (Supplementary Table 3 and 4).
 218 Correlations and fitting performance metrics were not significantly different between
 219 females and males (Pearson's r (William's test), $P>0.05$; RMSE (F-test) $P>0.05$), see
 220 Supplementary Table 5. Plots of the correlations between predicted brain-age and
 221 chronological age for females and males in each of the cohorts can be seen in
 222 the Supplementary Fig. 3.

223

Table 3. Prediction metrics for all independent cohorts.

Cohorts	Correlation with age		MAE (y)	R ²	RMSE
	R	P-value			
Before bias correction					
UK BioBank	0.712 (0.007)	<0.001	4.19 (0.07)	0.51 (0.03)	5.25 (0.08)
ALFA+	0.448	<0.001	4.31	0.20	4.18
ADNI	0.587	<0.001	7.21	0.34	5.47
EPAD	0.629	<0.001	4.63	0.40	5.62
OASIS	0.733	<0.001	6.99	0.54	6.42
After bias correction					
UK BioBank	0.898 (0.004)	<0.001	2.95 (0.10)	0.89 (0.01)	3.29 (0.10)
ALFA+	0.729	<0.001	3.25	0.53	3.99
ADNI	0.807	<0.001	4.47	0.65	5.47
EPAD	0.847	<0.001	3.29	0.72	4.07
OASIS	0.910	<0.001	3.81	0.82	4.83

The Pearson's correlation coefficient (R) between predicted brain-age and chronological age, R², root mean square error (RMSE), and mean absolute error (MAE) for UKBioBank and for each of the independent cohorts before and after bias correction. Age bias (Pearson's correlation between brain-age delta and chronological age) is also computed. For UKBioBank, the metrics, given as mean (standard deviation) are computed from 10-fold cross validation with 10 repetitions per fold.

224 **Brain Regions Associated with Aging**

225

226 We computed the SHapley Additive exPlanation (SHAP) values, which reflect the
227 marginal contribution of each brain region to the brain-age prediction, using the
228 UKBioBank dataset. SHAP values interpret the impact in the prediction of the values of
229 volume or cortical thickness for a given brain region. In other words, they reflect the most
230 important features that consistently influenced the prediction of brain-age and whether
231 the decrease or increase of each region impacted into predicting a higher or lower brain-
232 age. The SHAP values were computed separately for females and males. We compared
233 the regions with higher SHAP values for females and males, and vice-versa, by averaging
234 the SHAP values within each sex separately and then subtracting the mean SHAP of
235 males to the mean SHAP of females.

236

237 There were regions whose SHAP values were high in both females and males, including
238 the volumes of the amygdala, nucleus accumbens, cerebellar white matter, lateral
239 ventricles and the insula, as well as the cortical thickness of the superior-temporal cortex.
240 All the brain regions with consistent highest SHAP values for females and males are
241 shown in Fig. 1a-b, as well as the effect of each region (larger or lower value) on
242 predicting a higher brain-age. Conversely, the thickness of regions such as the transverse
243 temporal cortex, the pars triangularis, the inferior parietal cortex and the left frontal pole
244 thickness, as well as the volume of the left entorhinal cortex had higher SHAP values in
245 females than in males, while the opposite occurred with the thickness of the left isthmus
246 cingulate and the right cuneus and the cortical volume of the superior frontal and right
247 rostral middle regions (Fig. 1c).

248

249 In Fig. 1d we can see the aging trajectories of three regions whose SHAP values were
250 different for females and males. For example, the bi-lateral superior frontal volumes
251 decreased more over the years within males than females. This result was seen as the
252 interaction of sex with age ($P_{interatcion}<0.001$). We also found an interaction effect of sex
253 and age for the isthmus cingulate thickness ($P_{interatcion}<0.001$), by which the thickness of
254 males decreased more over the years than from the females. On the contrary, we also
255 found regions, such as the middle temporal thickness, which followed the same trajectory
256 over time for both sexes ($P_{interaction}=0.671$), but which was lower for females than for
257 males ($P<0.001$).

258

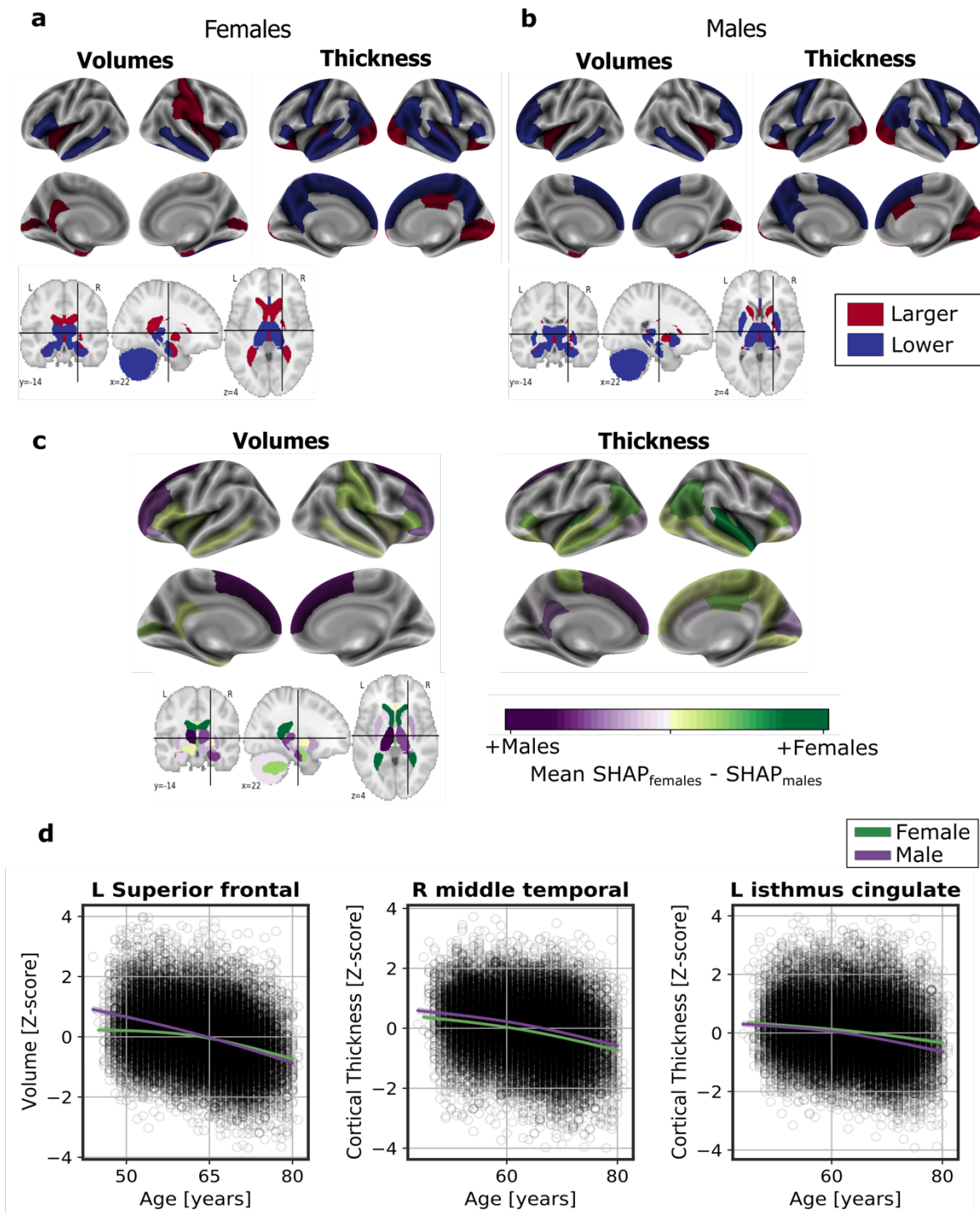


Figure 1. Significant SHAP-selected brain regions most important in prediction for **a** females and **b** males separately. Significance was studied by assessing the stability of the region's importance by performing subsampling of data over 1,000 permutations. Colored regions had a p -value < 0.05 corrected for multiple comparisons using Bonferroni correction approach. Regions in red show larger volume or cortical thickness, while regions in blue show lower volume or cortical thickness. In **c**, the difference between the SHAP values of the significant SHAP-selected regions for females and males. In green, higher values for females and in red, higher for males. In **d**, examples of different aging trajectories for females and males of different significant SHAP-selected regions. For visualization purposes, nonparametric smoothing spline functions were used to fit the data.

259 **Associations with AD biomarkers and risk factors**

260

261 We studied the association between brain-age delta and AD biomarker classifications (A β
262 status, AT stages) and *APOE*- ϵ 4 status in all the independent cohorts pooled together,
263 with a linear model adjusting for the effect of age and sex (Figure 2 and Table 4). A β
264 status was defined by CSF (ALFA+, ADNI and EPAD) or amyloid PET (OASIS) using
265 pre-established cut-off values (Hansson et al., 2018; Milà-Alomà et al., 2020; Salvadó et
266 al., 2019; Schindler et al., 2018). Brain-age delta was higher in MCI with respect to CU
267 individuals ($P < 0.001$). In both CU and MCI, a higher brain-age delta was significantly
268 associated with abnormal A β status (CU: $P < 0.001$ and MCI: $P < 0.001$) and with
269 progressive AT stages (CU: $P < 0.001$ and MCI: $P < 0.001$) (see Table 4 and Supplementary
270 Table 6 for more details). The mean brain-age delta values for the different A β status and
271 AT stages can be found in Supplementary Table 7. The brain-age effect on AT stages was
272 progressive, as that of the A+T- group was larger than that of A-T-, while the brain-age
273 delta of A+T+ was larger than those of the other two previous stages (Table 4 and Fig
274 2a). Brain-age delta was also significantly associated with *APOE* status (CU: $P < 0.001$
275 and MCI: $P = 0.029$). In particular, *APOE*- ϵ 4 carriers had larger brain-age deltas (i.e.,
276 older-appearing brains than expected for their chronological age) compared to *APOE*- ϵ 33
277 individuals for both CU ($\beta = 0.105$, $P = 0.032$) and MCI ($\beta = 0.266$, $P = 0.005$) (see Table 4
278 and Figure 2). The mean brain-age delta values for the different *APOE* status can be found
279 in Supplementary Table 7. These results were consistent with the results from the within-
280 cohort analyses (see Supplementary Table 8).

281

282 We next studied the association between brain-age delta and AD biomarkers and risk
283 factors stratified by sex (Table 5). In general, the same associations found with the whole
284 sample was seen for females and males separately. However, although a higher brain-age
285 delta was significantly associated with progressive AT stages both for females (CU:
286 $P < 0.001$ and MCI: $P < 0.001$) and males (CU: $P = 0.009$ and MCI: $P < 0.001$), brain-age
287 delta of A+T+ was significantly larger than those of the other two previous stages (A-T-
288 and A+T-) in CU females ($\beta = 0.431$, $P = 0.001$) but not in CU males ($\beta = 0.139$, $P = 0.364$).
289 We conducted regression analyses to test the interaction effect of sex and AT stages on
290 CU brain-age delta. Although we found a trend by which the proportion of A+T+ with
291 larger brain-age delta was larger in females than in males, the interaction effect was not
292 significant ($P_{\text{interaction}} = 0.071$).

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

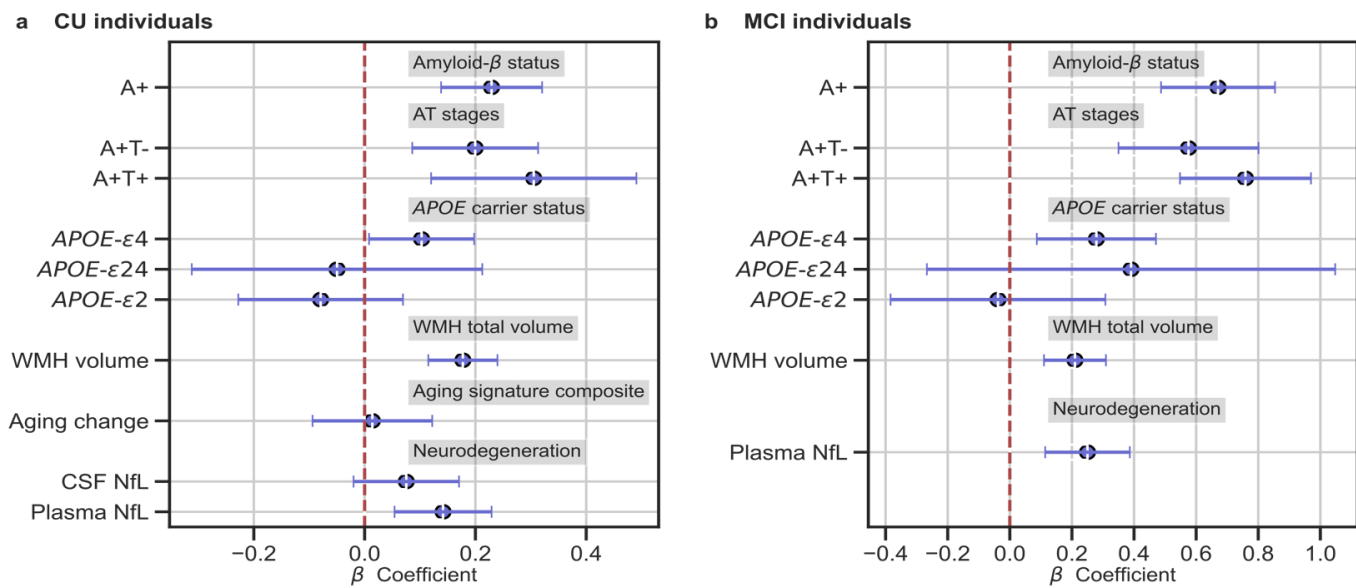


Figure 2. In **a** and **b**, the standardized associations ($\beta \pm 95\%$ CI) between measures of brain-age delta validation variables for a) CU individuals and b) MCI individuals. Variables include AD biomarkers and risk factors: amyloid- β status, AT stages and APOE status; and neurodegeneration markers (available in ALFA+ and ADNI): CSF NfL, plasma NfL and aging signature change. The analyses included age and sex as covariates.

Table 4. Relationships between validation variables and brain-age delta for all CU and MCI individuals.

Model	β	SE	P-Value	[0.025	0.975]	N	Effect size
CU Individuals							
Amyloid-β pathology (ref: A-)	0.233	0.047	<0.001	0.140	0.325	1,634	0.222
Amyloid-β / Tau pathology (ref: A-T-)	A+T-	0.2023	0.001	0.087	0.318	1,162	0.205
	A+T+	0.310	0.001	0.122	0.498		0.311
APOE status (ref: APOE-ϵ33)	APOE- ϵ 2	-0.081	0.295	-0.232	0.070	1,634	0.079
	APOE- ϵ 4	0.105	0.032	0.008	0.201		0.100
	APOE- ϵ 24	-0.051	0.795	-0.317	0.216		0.033
WMH volume †	0.171	0.030	<0.001	0.111	0.231	972	0.033
CSF NfL ‡	0.077	0.049	0.122	-0.021	0.173	378	0.006
Plasma NfL ‡	0.142	0.045	0.002	0.054	0.229	508	0.020
Brain Atrophy ‡	0.014	0.056	0.799	-0.096	0.124	152	0.000
Aging signature V1 ‡	-0.366	0.053	<0.001	-0.471	-0.261	152	0.175
Aging signature V2 ‡	-0.302	0.053	<0.001	-0.407	-0.198	152	0.120
MCI Individuals							
Amyloid-β pathology	0.640	0.089	<0.001	0.465	0.816	218	0.665
Amyloid-β / Tau pathology (ref: A-T-)	A+T-	0.550	<0.001	0.334	0.765	218	0.581
	A+T+	0.7245	<0.001	0.523	0.926		0.722
APOE status (ref: APOE-ϵ33)	APOE- ϵ 2	-0.036	0.829	-0.367	0.294	218	0.036
	APOE- ϵ 4	0.266	0.005	0.083	0.450		0.272
	APOE- ϵ 24	0.372	0.319	0.244	-0.255		1.000
WMH volume	0.222	0.054	<0.001	0.117	0.327	191	0.040
Plasma NfL †	0.242	0.067	<0.001	0.110	0.374	134	0.043

Notes: Relationships between validation variables and Brain-Age delta from all CU pooled subjects (including ALFA+, ADNI, EPAD and OASIS) and all MCI pooled subjects (including ADNI and EPAD). Results given by the linear model: brain-age delta ~ each variable + chronological age + sex. The regression coefficients (β), standard errors (SE), P-value, 95% Confidence Interval, number of individuals (N) and effect size are depicted for each variable.

Significant values ($P < 0.05$) are marked in bold.

Effect size in categorical variables was calculated as Cohen's D, while Cohens f^2 was calculated for continuous measurements. Amyloid- β status was defined by CSF (ALFA+, ADNI and EPAD) or amyloid PET (OASIS). MCI individuals only contained individuals from ADNI and EPAD.

† Contains data from ALFA+, ADNI and EPAD.

‡ Contains data from ALFA+ and ADNI.

‡ Contains data from ADNI.

Abbreviations: APOE, apolipoprotein E; WMH, White Matter Hyperintensities; CSF, cerebrospinal fluid; NfL, neurofilament light; ref, reference.

310 **Associations with neurodegeneration biomarkers**

311

312 We next tested the associations between brain-age delta and neurodegeneration
313 biomarkers (Fig. 2 and Table 4). CSF NfL, plasma NfL and longitudinal change of the
314 aging signature were available in ALFA+ and ADNI. The positive associations between
315 brain-age deltas and plasma NfL were significant within the CU ($\beta=0.142$, $P=0.002$) and
316 MCI individuals ($\beta=0.242$, $P<0.001$). CSF NfL was not significantly associated with brain-
317 age delta ($\beta=0.077$, $P=0.122$). The aging signature composite at both visits was
318 negatively associated with brain-age delta (Visit 1: $\beta=-0.366$, $P<0.001$ and Visit 2: $\beta=-$
319 0.302 , $P<0.001$). That is, larger brain-age delta was associated with reduced cortical
320 thickness in aging-vulnerable regions. However, the association between the longitudinal
321 change in the aging signature and brain-age delta was not statistically significant
322 ($\beta=0.014$, $P=0.799$).

323

324 We next studied the association between brain-age delta and neurodegeneration
325 biomarkers stratified by sex (Table 5). The associations between brain-age delta and CSF
326 NfL were significant within the CU females ($\beta=0.131$, $P=0.042$), but not within the CU
327 males ($\beta=-0.004$, $P=0.959$). However, the interaction effect of sex and CSF NfL on CU
328 brain-age delta (Fig. 3a) did not reach significance ($P_{\text{interaction}}=0.170$). In the same line,
329 the associations between brain-age delta and plasma NfL were significant within the CU
330 and MCI females (CU: $\beta=0.193$, $P=0.001$ and MCI: $\beta=0.342$, $P=0.001$), but not within
331 the males (CU: $\beta=0.079$, $P=0.254$ and MCI: $\beta=0.157$, $P=0.086$). The interaction effect of
332 sex and plasma NfL on brain-age delta (Fig. 3a) revealed a trend within CU individuals,
333 by which, although not significant, plasma NfL was larger on females when the brain-age
334 delta showed larger values (older-appearing brain) than in males (CU: $P_{\text{interaction}}=0.092$
335 and MCI: $P_{\text{interaction}}=0.194$).

336

337 In addition, we tested for the interaction effect of age and these biomarkers on brain-age
338 delta. We found a significant interaction effect of age and CSF NfL on CU brain-age delta
339 ($P_{\text{interaction}}<0.001$) within the CU individuals (Fig 4a), by which the measures of CSF NfL
340 were higher with age and with larger brain-age deltas (older-appearing brain). When
341 stratifying by sex, this interaction effect of age was seen in females ($P_{\text{interaction}}<0.001$),
342 but not in males ($P_{\text{interaction}}=0.241$). Regarding plasma NfL (Fig. 4b), although we found
343 a similar trend by which the measures of plasma NfL were higher with age and with larger
344 brain-age deltas for CU and MCI individuals, the interaction effects were not significant
345 (CU: $P_{\text{interaction}}=0.136$ and MCI: $P_{\text{interaction}}=0.145$). When stratifying by sex, this
346 interaction effect of age was seen in CU females ($P_{\text{interaction}}<0.016$) and not in CU males
347 ($P_{\text{interaction}}=0.656$). On the contrary, this interaction effect of age and plasma NfL on brain-
348 age delta was seen in MCI males ($P_{\text{interaction}}<0.017$) and not in MCI females
349 ($P_{\text{interaction}}=0.621$).

350

351

352 **Associations with markers of cerebrovascular disease**

353

354 We lastly tested the associations between brain-age delta and markers of cerebrovascular
355 disease WMH; WMH data were available in ALFA+, ADNI and EPAD. In both CU and
356 MCI, brain-age delta was significantly associated with WMH (CU: $\beta=0.171$, $P<0.001$
357 and MCI: $\beta=0.222$, $P<0.001$) (see Table 4). These results were consistent with the results
358 from the within-cohort analyses (see Supplementary Table 7).

359

360 When studying the association between brain-age delta and WMH stratified by sex (Table
361 5) we found that the brain-age delta was positively associated with WMH both in CU
362 females ($\beta=0.202$, $P<0.001$) and CU males ($\beta=0.132$, $P=0.007$). The interaction effect of
363 sex and WMH on CU brain-age delta (Fig. 3a) was not significant ($P_{\text{interaction}}=0.182$).
364 Conversely, we found that the brain-age delta was positively associated with WMH MCI
365 males ($\beta=0.154$, $P<0.001$), but not in females ($\beta=0.140$, $P=0.100$). The interaction effect
366 of sex and WMH on MCI brain-age delta (Fig. 3b) was also not significant
367 ($P_{\text{interaction}}=0.112$).

368

Table 5. Relationships between validation variables and brain-age delta stratified by sex for all CU and MCI individuals.

Model	Females					Males					
	β	SE	P-Value	N	Effect size	β	SE	P-Value	N	Effect size	
CU Individuals											
Amyloid- β pathology (ref: A-)	0.224	0.063	<0.001	966	0.226	0.2434	0.072	0.001	668	0.246	
Amyloid- β / Tau pathology (ref: A-T-)	A+T-	0.197	0.078	688	0.191	0.206	0.091	0.024	474	0.208	
	A+T+	0.431	0.123		0.001	0.425	0.139	0.154		0.367	0.142
APOE status (ref: APOE- ϵ 33)	APOE- ϵ 2	-0.1301	0.104	966	0.128	-0.019	0.115	0.869	668	0.018	
	APOE- ϵ 4	0.081	0.064		0.203	0.080	0.130	0.079		0.098	0.122
	APOE- ϵ 24	-0.001	0.187		0.998	0.001	-0.126	0.200		0.532	0.117
WMH volume †	0.202	0.039	<0.001	580	0.046	0.132	0.049	0.007	392	0.019	
CSF NfL ‡	0.131	0.064	0.042	228	0.019	-0.004	0.078	0.959	150	0.000	
Plasma NfL ‡	0.1923	0.059	0.001	298	0.037	0.079	0.069	0.254	210	0.006	
Brain Atrophy ‡	0.073	0.074	0.328	171	0.005	-0.074	0.087	0.400	102	0.007	
Aging signature V1 ‡	-0.410	0.067	<0.001	171	0.223	-0.301	0.092	0.001	102	0.109	
Aging signature V2 ‡	-0.356	0.069	<0.001	171	0.159	-0.229	0.085	0.009	102	0.072	
MCI Individuals											
Amyloid- β pathology	0.714	0.130	<0.001	217	0.752	0.5612	0.124	<0.001	286	0.578	
Amyloid- β / Tau pathology (ref: A-T-)	A+T-	0.558	0.175	214	0.576	0.509	0.175	<0.001	284	0.529	
	A+T+	0.8156	0.146		<0.001	0.818	0.626	0.145		<0.001	0.626
APOE status (ref: APOE- ϵ 33)	APOE- ϵ 2	0.006	0.295	217	0.006	-0.044	0.209	0.834	286	0.041	
	APOE- ϵ 4	0.270	0.142		0.06	0.283	0.259	0.126		0.041	0.259
	APOE- ϵ 24	0.3278	0.418		0.435	0.348	0.415	0.812		0.417	0.406
WMH volume	0.140	0.085	0.100	181	0.154	0.291	0.070	<0.001	252	0.069	
Plasma NfL †	0.342	0.098	0.001	128	0.097	0.157	0.091	0.086	173	0.017	

Notes: Relationships between validation variables and Brain-Age delta from all CU pooled subjects (including ALFA+, ADNI, EPAD and OASIS) and all MCI pooled subjects (including ADNI and EPAD). Results given by the linear model: brain-age delta ~ each variable + chronological age + sex. The standardized regression coefficients (β), standard errors (SE), P-value, 95% Confidence Interval, number of individuals (N) and effect size are depicted for each variable. Significant values ($P < 0.05$) are marked in bold. Effect size in categorical variables was calculated as Cohen's D, while Cohens f^2 was calculated for continuous measurements. Amyloid- β status was defined by CSF (ALFA+, ADNI and EPAD) or amyloid PET (OASIS).

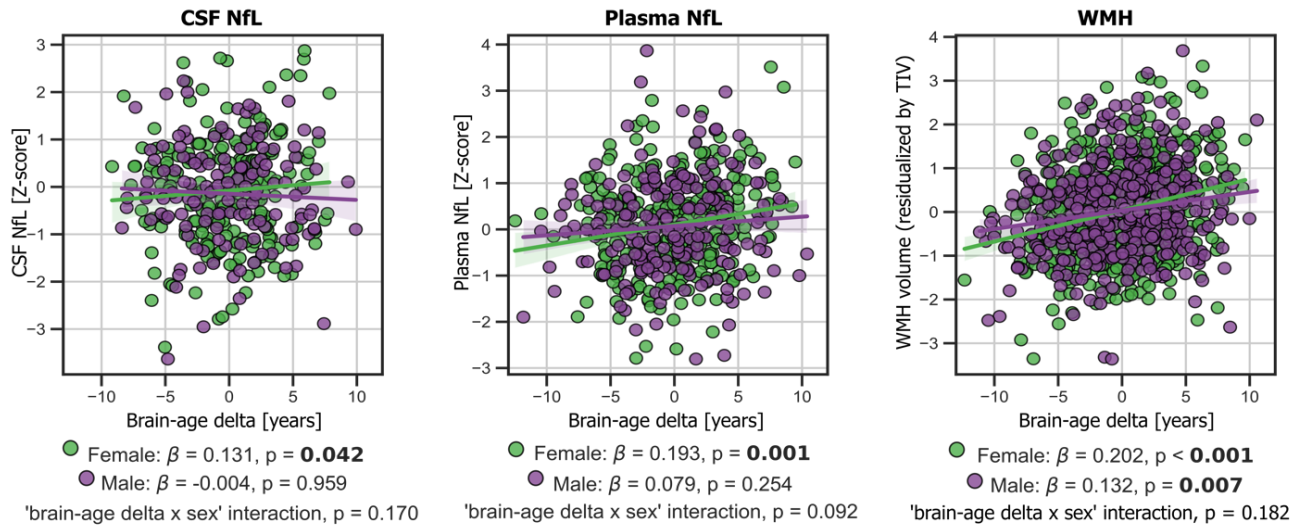
† Contains data from ALFA+, ADNI and EPAD.

‡ Contains data from ALFA+ and ADNI.

† Contains data from ADNI.

Abbreviations: APOE, apolipoprotein E; WMH, White Matter Hyperintensities; CSF, cerebrospinal fluid; NfL, neurofilament light; ref, reference.

a CU individuals



b MCI individuals

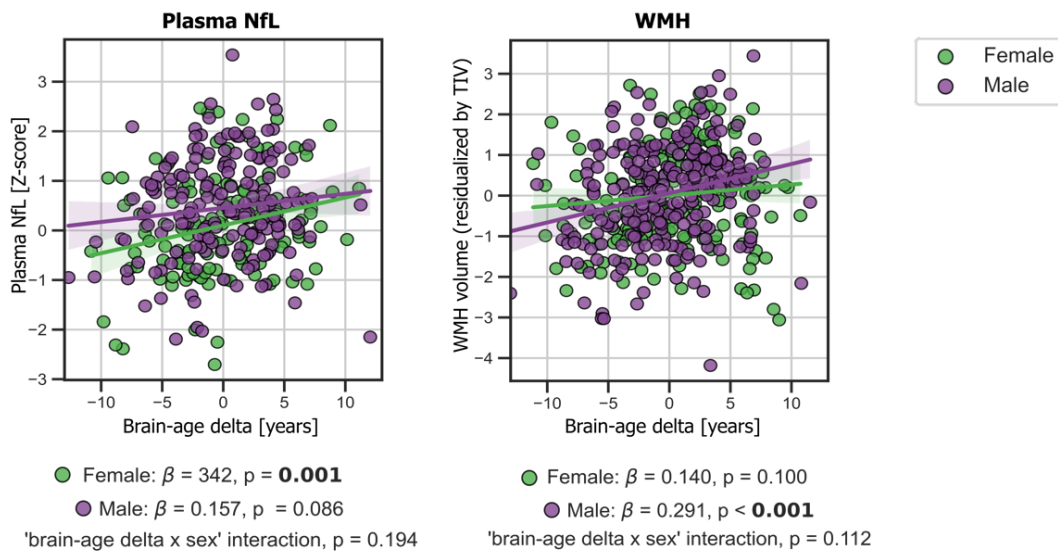


Figure 3. In **a** and **b**, the associations of brain-age delta and validation variables stratified by sex for a) CU individuals and b) MCI individuals. Scatter plots representing the associations of CSF NfL, plasma NfL and WMH with brain-age delta in females (green) and males (red). Each point depicts the value of the validation biomarkers of an individual and the solid lines indicate the regression line for each of the groups. The standardized regression coefficients (β) and the P-values are shown and were computed using a linear model adjusting for age and sex. Additionally, we also computed the “brain-age delta x sex” interaction term.

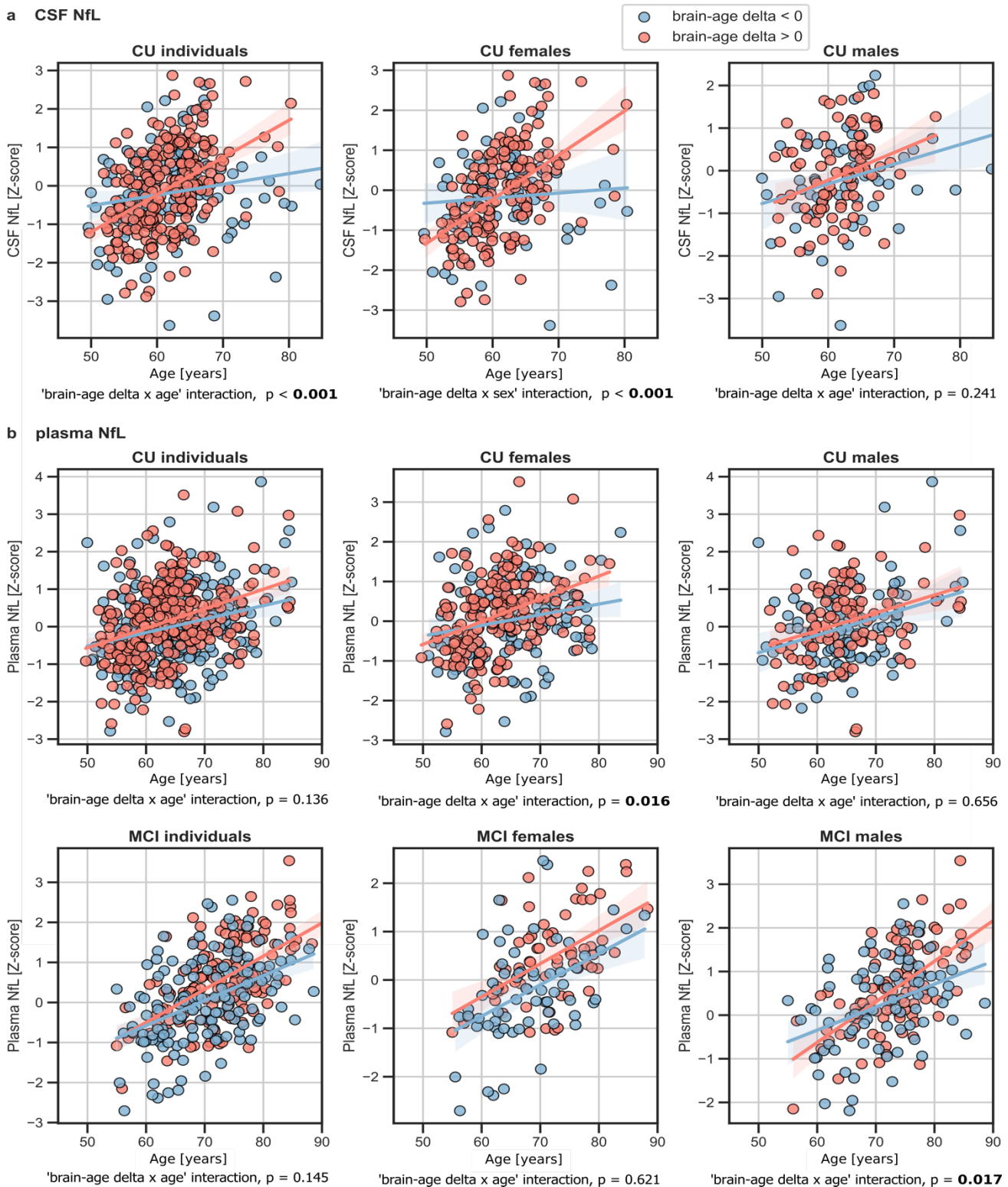


Figure 4. The associations of brain-age delta and **a** CSF NfL and **b** plasma NfL for all CU and, when available, MCI individuals. Scatter plots representing the associations of CSF NfL, plasma NfL and WMH with age in accelerated brain aging (purple) and decelerated brain aging (green). Each point depicts the value of the validation biomarkers of an individual and the solid lines indicate the regression line for each of the groups. The regression coefficients (β) and the P-values are shown and were computed using a linear model adjusting for age and sex. Additionally, we also computed the “brain-age delta x sex” interaction term.

1 DISCUSSION

2
3 In this study, we show that, in non-demented individuals, the predicted brain-age delta is
4 associated with specific AD biomarkers (amyloid- β PET, CSF A β 42 and CSF pTau) and
5 risk factors (*APOE* genotype), as well as with unspecific neurodegeneration biomarkers
6 (plasma NfL), and markers of cerebrovascular disease (WMH volume). Our results also
7 indicate that there are sex differences in the development of brain aging trajectories.
8 Taken together, our findings validate the use of machine learning predicted brain-age
9 deltas as biomarkers of brain aging and AD pathology.

10
11 We have studied, to our knowledge for the first time, the associations between brain-age
12 delta and different biomarkers of AD pathology and neurodegeneration in CU
13 individuals. We are aware of the complexity of disentangling the effects of aging and
14 pathology in brain aging. In this work, we do not aim to disentangle to what extent the
15 brain structural differences are caused by AD pathology (as measured by the biomarkers
16 that we study) or aging. Here, we show that an unspecific estimation of biological brain
17 aging, agnostic of the underlying mechanisms is associated with the specific biological
18 process of AD.

19
20 Regarding the associations with AD biomarkers and risk factors, regression analyses
21 revealed significant positive associations of brain-age delta with increased A β pathology
22 and with AT stages for CU and MCI individuals. We also found significant associations
23 with *APOE* status in the CU and MCI individuals, in which larger brain-age deltas were
24 associated with the presence of *APOE*- ϵ 4 allele. This result is in line with previous
25 literature that has shown that *APOE*- ϵ 4 carriership may accelerate AD-related brain
26 atrophy (Evans et al., 2014; Filippini et al., 2011), as accelerated brain aging has also
27 been found in MCI and AD patients (Beheshti et al., 2018; Kaufmann et al., 2019). The
28 association of brain-age delta with *APOE*- ϵ 4 was also previously studied, for which
29 significant associations were found in MCI individuals (J. H. Cole et al., 2018; Löwe et
30 al., 2016). Taken together, our results advocate for an effect of *APOE*- ϵ 4 in physiological
31 brain aging, albeit of a lesser magnitude than when AD pathology is present. These results
32 with AD biomarkers and risk factors were highly reproducible in within-cohort analyses.

33
34 With the aim of studying the associations between brain-age delta and neurodegeneration,
35 we computed the associations with NfL, a marker of neuro-axonal damage (Khalil et al.,
36 2018) which can be measured both in CSF and in plasma, and with longitudinal changes
37 in the aging signature composite as marker of age-related brain atrophy. The particular
38 use of NfL in this context is supported by its correlation with age throughout the lifespan,
39 as well as its strong association with all-cause mortality in the elderly (Kaeser et al.,
40 2021). We found significant positive associations between brain-age delta and plasma
41 NfL both in CU and MCI individuals, but we did not find significant associations between
42 CSF NfL and CU brain-age delta. Even though we found NfL to be positively associated
43 with chronological age in CU individuals, in line with previous studies (Beheshti et al.,
44 2018; Kaufmann et al., 2019; Khalil et al., 2020; Milà-Alomà et al., 2020), the expected
45 annual change of NfL in CU individuals whose mean age range was 65 years old is around
46 3.5% (Khalil et al., 2020). Therefore, we expected to find weak associations with brain-
47 age delta. Still, we found a significant interaction effect of age and CSF NfL on CU brain-
48 age delta, for which the individuals with larger brain-age delta had increased CSF NfL
49 over the years, whereas the decelerated ones remained more stable. This trend was also
50 seen in plasma NfL for CU and MCI individuals, although the interaction did not reach

51 significance. Taken together, a strong association between brain-age delta and plasma
52 NfL was observed across all individuals whereas the association on CU brain-age delta
53 and CSF NfL was milder and could only be detected as an interaction with age. Overall,
54 these mild associations between brain-age delta and NfL suggest that the morphological
55 effects of aging in the brain are not fully driven by neurodegeneration. In this regard, it is
56 worth noting that cortical thinning with age has also been linked to loss of volume of the
57 neuropil and other non-neuronal processes which are not necessarily implicated in
58 neurodegeneration (Vidal-Pineiro et al., 2020).

59

60 We also studied the associations between brain-age delta and cerebrovascular disease.
61 Regression analyses revealed significant associations of brain-age delta with increased
62 WMH for both CU and MCI individuals. These results were expected, as the increase in
63 WMH with age has been previously studied (Maniega et al., 2015) and it has been shown
64 that individuals with high WMH burden display spatial patterns of atrophy that partially
65 overlap with those of brain aging (Brugulat-Serrat, Salvadó, et al., 2020; Habes et al.,
66 2016). In addition, WMH have been linked to cognitive dysfunction and dementia
67 (Brugulat-Serrat, Salvadó, et al., 2020; Brugulat-Serrat, Salvadó, et al., 2020; Maniega et
68 al., 2015) and a potential pathway has been proposed, in which small vessel
69 cerebrovascular disease affects cognition by promoting neurodegenerative changes
70 (Rizvi et al., 2018). In summary, our results support an effect of cerebrovascular disease
71 in physiological brain aging.

72

73 Brain structure aging-associated changes have been widely studied (Bakkour et al., 2013;
74 Fjell et al., 2014). In our study, the brain regions that had highest impact on the machine
75 learning prediction were similar to regions previously mentioned in literature (Arenaza-
76 Urquijo et al., 2019; Bakkour et al., 2013). We found an overlap between some of our
77 selected regions and regions included in the aging signature for both females and males,
78 such as the precentral sulcus, insula, superior frontal and rostral middle frontal regions.
79 In addition, the effect of sex on age-related changes in brain structure has also been
80 studied in the recent years, with some studies reporting age–sex interactions in volumes
81 of certain brain structures (Coffey et al., 1998; DeCarli et al., 2005), and others not finding
82 such interactions (Greenberg et al., 2008). In our study, we found that, even though most
83 of the regions with highest impact were the same for males and females, there were some
84 regions that were sex specific. In particular, we found reduction in the superior-frontal,
85 isthmus-cingulate and pars orbitalis regions within males and regions such as inferior-
86 parietal, pars triangularis and paracentral within females. Most of these sex-specific
87 regions were in concordance with previous studies (Armstrong et al., 2019; Podgórski et
88 al., 2021). The mechanisms underlying these sex-specific brain aging differences are not
89 well-known. Sexual hormones such as estrogen, progesterone and androgen could play a
90 role in brain atrophy (Armstrong et al., 2019); in the WHIMS-MRI study (Resnick et al.,
91 2009), women under menopausal hormone therapy were associated with greater brain
92 atrophy. Others, however, have proposed that estrogen and progesterone may play a
93 protective effect in women (Armstrong et al., 2019; Green & Simpkins, 2000). Other
94 possible biological mechanisms influencing these results could be developmental (Baron-
95 Cohen et al., 2005) or the influence of a greater presence of adverse lifestyle-related
96 factors in men (DeCarli et al., 2005).

97

98 In line with the effect of sex on age-related changes in brain structure, we studied the
99 effect of sex on the associations between brain-age delta and the above-mentioned
100 variables. Regarding the AD biomarkers and risk factors, we found that the association

101 between brain-age delta and a larger proportion of A+T+ was only seen in females for the
102 CU individuals, but the interaction effect of sex and AT stages on brain-age delta was not
103 significant. Regarding the neurodegeneration variables, we found a mild positive
104 association between brain-age delta and CSF NfL in CU females, as well as a stronger
105 positive association between brain-age delta and plasma NfL in CU and in MCI females.
106 These associations were not seen in their male counterparts. However, the interaction
107 effect of sex and CSF and plasma NfL on CU and MCI brain-age delta was not significant.
108 In addition, we found a significant interaction effect of age and CSF and plasma NfL on
109 brain-age delta, for which the CU females with larger brain-age delta had increased CSF
110 and plasma NfL over the years, whereas the decelerated ones remained more stable. On
111 the contrary, we found an effect of age interaction with plasma NfL on brain-age delta in
112 MCI males, but not in MCI females. These results were expected, as females have higher
113 chances of developing neurodegeneration and have showed to undergo faster cognitive
114 decline than males (Ferretti et al., 2018). Although the role of sex hormones still needs to
115 be clarified, it has been suggested that the menopausal drop of estrogen increases
116 vulnerability to neurological events (Green & Simpkins, 2000; Maioli et al., 2021). On
117 the contrary, results suggest that morphological effects of aging in the CU males' brain
118 are not fully driven by neurodegeneration, although these effects might increase with
119 older age in MCI males. Lastly, regarding the cerebrovascular disease biomarkers, we
120 found a positive association between brain-age delta and WMH for both CU females and
121 males, while no interaction effect of sex was found. Conversely, in MCI individuals, we
122 only found positive associations between brain-age delta and WMH on males, but no
123 interaction effect of sex was found in MCI individuals. Overall, we found sex differences
124 in the associations between brain-age delta and markers of neurodegeneration and
125 cerebrovascular disease. NfL was only positively associated with brain-age delta in
126 females and, although WMH were positively associated with both CU females and males,
127 only MCI males showed this positive association. Positive associations between NfL and
128 WMH have been previously demonstrated, for both CU and MCI (Osborn et al., 2018),
129 and the different AD stages (Walsh et al., 2021). Moreover, it has been proposed that
130 WMH may reflect two different pathological pathways, one including amyloid
131 aggregation and another including axonal injury (Osborn et al., 2018). Our results may
132 suggest that brain aging in males might be driven more strongly by the former pathway,
133 while brain aging in females might be driven more by the latter one.

134
135 Our purpose was to study the clinical validity of using the brain-age delta as a proxy
136 biomarker of brain aging associated to AD and neurodegeneration. Therefore, our main
137 aim was studying the characteristics of the individuals whose brain age is more
138 accelerated or decelerated. One of the strengths of this study was the robustness of the
139 brain-age delta measurement using a widely used segmentation atlas such as the Desikan-
140 Killiany. Notably, we demonstrated the robustness of our method by training our model
141 with one cohort and testing independently on four independent cohorts. The similar
142 results obtained in all cohorts allowed us to seek associations in a large sample of
143 participants with biomarker data and to further stratify the data by sex. This aspect is
144 critical for this type of analyses as the effects of biological aging are necessarily very
145 small, particularly in CU individuals of limited age range. This may explain why we could
146 not detect significant effects versus longitudinal brain atrophy, as the available sample
147 size for these analyses were smaller since this variable was not available in all cohorts.
148 Another strength of our work was that we were able to include a wide range of different
149 biomarkers of AD pathology allowing us to perform an in-depth analysis of the effect of
150 these measurements with the brain-age delta. Conversely, our model used a smaller

151 number of features and a training set with a more limited age range than other models
152 seen in literature recently (Liem et al., 2017; Peng et al., 2021), leading to a performance
153 which cannot be compared against these state-of-the-art models. However, the
154 performance of age prediction was similar to other publications that used similar
155 methodologies (Beheshti et al., 2018; Dafflon et al., 2020; de Lange, et al., 2020) and,
156 most importantly, was successful in studying the utility of brain-age delta as biomarker
157 for AD and neurodegeneration. Future work should focus on developing a model with
158 larger number of features or a 3D model and should study the effect of these validation
159 measurements for AD and neurodegeneration with the brain-age delta more in depth.

160

161 In conclusion, we validated that machine-learning based brain age prediction obtained
162 from a widely used segmentation atlas can be used as a biomarker of biological brain
163 aging associated with AD pathology, risk factors and neurodegeneration. Moreover, our
164 results confirm the presence of sex-related brain aging structural changes and suggest the
165 prevalence of different neuropathological pathways involved in brain aging within
166 females and males. Therefore, these results show the necessity to consider different
167 approaches for assessing aging and neurodegeneration differently for each sex.

168

169

170 MATERIALS AND METHODS

171

172 Participants

173

174 We used a collection of T1-weighted brain MRI scans included in the UKBiobank
175 (www.ukbiobank.ac.uk) dataset for training the proposed model and for calculating cross-
176 validated brain age predictions. The dataset consisted of CU individuals ($N = 22,661$),
177 after excluding subjects with ICD-9 and ICD-10 diagnosis, covering individuals of ages
178 44 to 81.

179

180 We also used four different cohorts to investigate the association between brain-age deltas
181 with different sets of biomarker and AD risk factor measurements. Inclusion criteria for
182 the independent cohorts consisted of: (i) availability of T1-weighted MRI brain scans; (ii)
183 and availability of apolipoprotein E (*APOE*) categories and of CSF or PET measures for
184 amyloid- β pathology acquired in less than a year from the MRI acquisition. These
185 datasets included CU and MCI subjects from ADNI 1,2 and 3 ($N = 751$, CU = 253, MCI
186 = 498), CU and MCI (as specified by a Clinical Dementia Rating = 0.5) subjects from the
187 EPAD cohort ($N = 808$, CU = 653, MCI = 155), CU subjects from the ALFA+ cohort
188 ($N = 380$) and CU subjects from the OASIS cohort ($N = 407$).

189

190 All the individuals had available data for the following clinical variables: chronological
191 age, sex, MMSE and years of education, which will be referred as clinical variables from
192 now on. A more detailed description of the clinical variables of these datasets is given in
193 Table 1. Regarding AD-related variables, ALFA+, ADNI and EPAD cohorts included
194 CSF $A\beta_{42}$ measurements for categorizing $A\beta$ pathology status, AT status determined by
195 CSF $A\beta_{42}$ and CSF p-tau, *APOE* categories and WMH. OASIS, meanwhile, only had
196 data available for $A\beta$ PET and *APOE* categories. In addition, ALFA+ and ADNI included
197 biomarkers of neurodegeneration such as CSF NfL, plasma NfL and cortical atrophy
198 measured by longitudinal changes in the so-called aging signature (Bakkour et al., 2013).
199 The combination of available AD-related variables and neurodegeneration biomarkers

200 will be referred as validation variables from so on. A more detailed description of the
201 validation variables can be seen in Table 2.

202

203 **Image Acquisition and Preprocessing**

204

205 The UKBioBank, ADNI and OASIS datasets had available T1-weighted magnetic
206 resonance (MR) images that had already been segmented with Freesurfer and had been
207 parcellated using the FreeSurfer's cortical Desikan-Killiany(Desikan et al., 2006) and
208 subcortical aseg(Fischl et al., 2002) labeling pipelines, which had undergone a quality
209 control procedure. Taking advantage of this available data, we decided to use the same
210 segmentation pipeline with the ALFA+ and EPAD cohorts. All the image acquisition and
211 preprocessing done is as follows.

212

213 The UKBiobank dataset consisted of T1-weighted magnetic resonance (MR) images, all
214 collected using a 3T Siemens Skyra scanner and preprocessed as previously explained in
215 more detail (https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf). Images
216 were previously segmented with Freesurfer 6.0 and underwent a quality control
217 procedure.

218

219 For ADNI participants (Petersen et al., 2010), MRI acquisition methods are described in
220 more detail elsewhere (<http://adni.loni.usc.edu/methods/documents/>). In brief, most of
221 the T1-weighted MR were MP-RAGE, acquired with 1.5T or 3T scanners. Images were
222 segmented with Freesurfer 5.1 and 6.0 and subjected to a quality control procedure. When
223 possible, we also included a second T1-weighted MRI image sequence for the participants
224 that underwent another MRI visit 3 years later. These scans were also segmented
225 following the previously explained procedure.

226

227 For the OASIS subjects (Marcus et al., 2007), the MRI scans were acquired on 1.5 T or
228 on 3.0 T scanners. T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE)
229 scans were obtained according to previously explained protocol
230 ([https://theunitedconsortium.com/wp-content/uploads/2021/07/OASIS-](https://theunitedconsortium.com/wp-content/uploads/2021/07/OASIS-3_Imaging_Data_Dictionary_v1.8.pdf)
231 [3_Imaging_Data_Dictionary_v1.8.pdf](https://theunitedconsortium.com/wp-content/uploads/2021/07/OASIS-3_Imaging_Data_Dictionary_v1.8.pdf)). All MRI sessions were segmented using
232 FreeSurfer 5.1 or 5.3 and followed quality control measures. The PET images were
233 acquired with [¹¹C]PIB Pittsburgh's compound 60-minute dynamic PET scan in 3D
234 mode and the corresponding analysis analyses were performed using the PET unified
235 pipeline (PUP, <https://github.com/ysu001/PUP>). Mean standardized uptake value
236 (SUVR) values were converted to Centiloid scale as previously explained.

237

238 For the ALFA+ participants, a high-resolution 3D T1-weighted MRI sequence was
239 acquired in a 3T Philips Ingenia CX scanner (TE/TR=4.6/9.9 ms, Flip Angle = 8°; voxel
240 size= 0.75x0.75x0.75 mm). Images were segmented with Freesurfer 6.0 and subjected to
241 a quality control procedure to identify and remove incidental findings (Brugulat-Serrat et
242 al., 2017) and segmentation errors(Huguet et al., 2021). Some of these ALFA+ subjects
243 (N=187) underwent a second MRI visit 3 years after the initial visit, where another T1-
244 weighted MRI sequence was acquired and segmented following the same procedure as in
245 the first visit.

246

247 For the EPAD cohort(Solomon et al., 2018), which is a multisite study, T1-weighted
248 MRIs were inversion-recovery prepare 3D gradient-echo sequences, acquired with 3T

249 scanners. Images were segmented with Freesurfer 6.0 and subjected to a quality control
250 procedure (Lorenzini et al., 2021).

251

252 For all the cohorts, subsequent to the FreeSurfer segmentation, tissue regions were
253 parcellated into 183 different anatomical regions of interest (ROI)s using the widely-used
254 FreeSurfer's cortical Desikan-Killiany(Desikan et al., 2006) and subcortical aseg(Fischl
255 et al., 2002) labeling pipelines. As mentioned before, we used the available FreeSurfer
256 segmentations from UKBioBank, ADNI and OASIS cohorts. All volumes were
257 residualized with respect to total intracranial volume (TIV) and to scanning site, while all
258 cortical thicknesses were residualized with respect to scanning site, using linear models.
259 Lastly, we performed a standardization procedure by computing z-score measurements
260 feature-wise within each cohort, as previously performed (Casamitjana et al., 2018;
261 Subramaniapillai et al., 2021; Ten Kate et al., 2018). We then assessed that there were
262 not statistical differences in mean cortical thickness and volumes between the cohorts
263 (see Supplementary Fig. 4).

264

265

266 **Biomarkers**

267

268 **CSF and plasma collection, processing and biomarkers measurements**

269

270 CSF and blood collection, processing and storage in the ALFA+ study have been
271 described previously (Milà-Alomà et al., 2020a; Suárez-Calvet et al., 2020). CSF p-
272 tau181 was measured using the Elecsys[®] Phospho-Tau (181P) CSF
273 electrochemiluminescence immunoassay on a fully automated cobas e 601 instrument
274 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). CSF A β 42 and NfL were
275 measured with the NeuroToolKit on a cobas e 411 or cobas e 601 instrument (Roche
276 Diagnostics International Ltd, Rotkreuz, Switzerland). Plasma NfL was measured using
277 the commercial Quanterix[®] assay (Simoa[®] NF-light Kit cat. no. 103186) on a HD-X
278 analyzer following the manufacturer's instructions (Quanterix, Billerica, MA, USA). All
279 these measurements were previously reported (Milà-Alomà et al., 2020; Suárez-Calvet et
280 al., 2020). All measurements were performed at the Clinical Neurochemistry Laboratory,
281 University of Gothenburg, Mölndal, Sweden, by laboratory technicians and scientists
282 blinded to participants' clinical information.

283

284 In the ADNI study, CSF samples were measured according to the kit manufacturer's
285 instructions and as described in previous studies (Bittner et al., 2016), using the Elecsys
286 β -amyloid(1–42) CSF (Bittner et al., 2016) , and the Elecsys Phospho-Tau (181P) and
287 Elecsys Total-Tau CSF immunoassays on a cobas e 601 analyzer at the Biomarker
288 Research Laboratory, University of Pennsylvania, USA. Plasma NfL was measured on
289 an in-house immunoassay on the single-molecule array (Simoa) platform, using the same
290 methodology as described previously, at the Clinical Neurochemistry Laboratory,
291 University of Gothenburg, Mölndal, Sweden.

292

293 In the EPAD study, CSF was measured using the Elecsys β -amyloid (1–42) and the
294 Elecsys Phospho-Tau (181P) CSF electrochemiluminescence immunoassay on a fully
295 automated cobas e 601 instrument (Roche Diagnostics International Ltd.). All
296 measurements were performed at the Clinical Neurochemistry Laboratory, University of
297 Gothenburg, Mölndal, Sweden, by laboratory technicians and scientists blinded to

298 participants' clinical information. Concentrations of CSF A β 42 and p-tau181 were
299 determined according to the manufacturer's instructions(Solomon et al., 2018).

300

301

302 **Amyloid- β positivity cutoffs**

303

304 For ALFA+, ADNI and EPAD, AT stages were defined by CSF A β 42 and CSF p-tau,
305 respectively. Previously used cut-offs were applied to each cohort, consisting of 1098
306 pg/mL for CSF A β 42 for ALFA+ and EPAD (Schindler et al., 2018) and of 880 pg/mL
307 for CSF A β 42 for ADNI (Hansson et al., 2018) and of 24 pg/mL for p-tau for the three
308 cohorts (Milà-Alomà et al., 2020). For OASIS, we used the cut-off value of 17 Centiloids
309 from literature(Salvadó et al., 2019).

310

311

312 **WMH Volumes**

313

314 WMH volumes were generated for ALFA+ and EPAD cohorts using Bayesian Model
315 Selection (BaMoS) procedure (Sudre et al., 2015), which has been provided previously.
316 We also obtained the already available WMH volumes for ADNI cohort, in which the
317 method of WMH volumetric quantification was performed using probabilistic models in
318 a Markov Random Field framework, as previously provided (Schwarz et al., 2009). For
319 each cohort, total WMH volumes were derived by summing and multiplying the number
320 of labeled voxels by voxel dimensions. Total WMH volumes were natural log
321 transformed and residualized with respect to TIV using linear models.

322

323

324 **Aging signature measurements**

325

326 For ALFA+ and ADNI, we computed the weighted Dickerson's aging signature (Bakkour
327 et al., 2013), which has been used as a proxy measurement for brain aging. The aging
328 signature is a map of specific brain regions that undergo cortical atrophy in normal aging.
329 This meta-ROI is composed of the surface-area weighted average of the mean cortical
330 thickness in the following individual ROIs: calcarine, caudal fusiform, caudal insula,
331 cuneus, inferior frontal gyrus, medial superior frontal and precentral cortices. A Z-score
332 of this aging-specific measure was calculated based on the mean and standard deviation
333 of the CU individuals, as done previously (Bakkour et al., 2013). This is referred as Aging
334 Signature V1.

335

336 In addition, we also computed this measurement on the scanners from the second MRI
337 visit, referred as Aging Signature V2. We then computed a longitudinal brain atrophy
338 measurement by computing the aging signature change over the years between the MRI
339 acquisitions. Therefore, longitudinal aging signature change was computed as:

$$340 \quad \text{Aging signature change} = \frac{\text{aging signature Visit 2} - \text{aging signature Visit 1}}{\text{Time between visits}}$$

341

342 For another secondary analysis shown in Supplementary Table 9, we also computed the
343 aging signature (Aging Signature V1) for the remaining independent cohorts: EPAD and
344 OASIS.

345

346

347 **Brain-Age Prediction**

348

349 **Regression Model**

350

351 **Model Workflow**

352

353 For the current study, we used a gradient boosting framework: the XGBoost regressor
354 model from the XGBoost python package ([https://xgboost.readthedocs.io/en/](https://xgboost.readthedocs.io/en/latest/python)
355 [latest/python](https://xgboost.readthedocs.io/en/latest/python)) to run the brain age prediction. This regressor, which is based on a decision-
356 tree based ensemble algorithm, was selected due to its speed and performance and its
357 advanced regularization to reduce overfitting (Chen & Guestrin, 2016). In addition, large-
358 scale brain age studies have demonstrated its adequacy (Bashyam et al., 2020; de Lange
359 et al., 2019; de Lange, Barth, et al., 2020; Kaufmann et al., 2019). As it has been shown
360 that there are sex-related trajectories in normal aging (Podgórski et al., 2021), we trained
361 separate models for females and males. For each model, we first performed Bayesian
362 parameter optimization based on a cross-validation scheme with ten folds and ten repeats
363 per fold using the FreeSurfer volumes and thickness of the UK BioBank as input. For the
364 optimization we used HYPEROPT (Bergstra et al., 2013), with which we scanned for
365 maximum depth, number of estimators, learning rate, alpha regularization, lambda
366 regularization, subsample, gamma and colsample by tree. The optimized parameters were
367 maximum depth = 4, number of estimators = 800, learning rate = 0.03, alpha
368 regularization = 4, lambda regularization = 1, subsample = 0.36, gamma = 3 and colsample
369 by tree = 0.89 for the males model; and maximum depth = 4, number of estimators = 850,
370 learning rate = 0.03, alpha regularization = 8.5, lambda regularization = 14.5, subsample =
371 0.449, gamma = 3.5 and colsample by tree = 0.72 for the females model. We trained these
372 two models and performed the brain-age prediction on the independent cohorts. We
373 decided to compute a ROI based model using these 183 FreeSurfer regions because they
374 are widely used and available in most of the neuroimaging datasets. Therefore, our aim
375 was not to compare our performance to the one achieved by a model trained with larger
376 number of ROIs or with the full 3D images, but to study the generalizability and the
377 relevance of our model in the AD field.

378

379 **Contribution of Brain Regions in prediction**

380

381 We computed SHAP (SHapley Additive exPlanation) values
382 (<https://github.com/slundberg/shap>) to measure the contribution of each brain region in
383 the prediction of age for each subject. SHAP assigns an importance value within the
384 prediction to each feature (in this case, brain region), which is based on its unique
385 consistent and locally accurate attribution (Lundberg et al., 2020). We calculated the
386 average SHAP value for each region for all females and males of the UK BioBank cohort.

387

388 In addition, to assess that the regions with highest SHAP values were stable, we
389 performed a permutation approach to study the significance of each region, separately for
390 females and males. With this aim we compared the averaged SHAP value (region-
391 specific) obtained when using the entire train set on the model to a null distribution
392 calculated from 1,000 permutations performing subsample of the subjects, in which we
393 trained and tested the model using 80% and 20% of the individuals, respectively.

394

395 **Brain-Age Delta Estimation**

396

397 We predicted brain-age on the independent cohorts separately: ALFA+, EPAD, ADNI
398 and OASIS, using the previously trained model. To investigate the prediction
399 performance, correlation analyses were run for predicted brain-age versus chronological
400 age, R^2 , root mean square error (RMSE), and mean absolute error (MAE) were calculated
401 for each independent cohort separately, as well as for females and males separate pooled
402 from all independent cohorts. We also investigated the prediction performance on the
403 UKBioBank cohort by computing the average latter metrics from a cross validation with
404 ten splits and ten repetitions.

405

406 As recent research has shown that brain-age estimation involves a proportional bias (de
407 Lange & Cole, 2020; Le et al., 2018; Liang et al., 2019; Smith et al., 2019), we applied a
408 well-established age-bias correction procedure to our data (de Lange & Cole, 2020; Le et
409 al., 2018). This correction, as originally proposed (de Lange & Cole, 2020; Le et al.,
410 2018), consists of a linear regression between age (Ω) and brain-predicted age (Y) on each
411 of the independent cohorts, $Y = \alpha \times \Omega + \beta$. The derived values of slope (α) and
412 intercept (β) from the training set were used to correct the predicted brain-age in each test
413 set by applying: Corrected Predicted Brain Age = Predicted Brain Age + [$\Omega - (\alpha \times$
414 $\Omega + \beta)$]. By subtracting the chronological age from the Corrected Predicted Brain Age,
415 we obtained the brain-age delta which was used to test the associations with the validation
416 measurements. The result of the correction is shown in Supplementary Fig.3.

417

418 **Statistical Analyses**

419

420 All statistical analyses were conducted using Python 3.7.0. We tested for normality of the
421 distribution for each biomarker using the Kolmogorov-Smirnov test and visual inspection
422 of histograms. CSF NfL and plasma NfL did not follow a normal distribution and were
423 thus natural log transformed. In addition, to compare the measurements for CSF NfL and
424 for plasma NfL coming from different cohorts (ALFA+ and ADNI), CSF and plasma NfL
425 was converted to z-scores.

426

427 To study the performance and accuracy of the brain-age prediction for each cohort,
428 correlation analyses were run for predicted brain-age versus chronological age. We also
429 computed R^2 , RMSE and MAE for each cohort, as well as the age bias of the prediction
430 after bias correction. We assessed statistically whether the accuracy of the predicted
431 brain-age was different between cohorts by using Fisher's z-transformation for
432 correlation coefficients. In addition, we computed these performance metrics to assess
433 the differences in the model for females and males in the pooled cohorts. Results from
434 another secondary analysis are also shown in Supplementary Table 2, in which we
435 assessed the performance and accuracy of the aging signature for all cohorts, by
436 performing correlation analyses between the aging signature versus chronological age
437 and computing the R^2 and RMSE. In this secondary analyses, we also studied whether
438 the performance obtained for the predicted brain-age was better than the aging signature
439 by performing the William's test (Williams, 1959) for the Pearson's correlation
440 coefficient and a F-test to assess which model was statistically better.

441

442 We used the brain-age delta as a measure of brain aging to study the associations between
443 this measurement and the different AD and neurodegeneration biomarkers and risk
444 factors. With this aim, we pooled all the subjects from all cohorts together and computed
445 linear regression models for each validation variable, in which chronological age and sex
446 were included as covariates. Local effect size of each of the continuous validation

447 variables was calculated using Cohen's f^2 (Cohen, 2013). The mean brain age delta
448 among A β pathology, AT stages and *APOE* status, were assessed by a one-way analysis
449 of covariance (ANCOVA) adjusting for age and sex. Effect size of the different levels
450 was calculated by dividing the estimated difference in the brain-age delta between the
451 different categories by the estimated standard deviation. We also stratified the individuals
452 by sex and studied the associations between brain-age delta and the different validation
453 variables by computing linear regression models in which chronological age was included
454 as covariate. We next tested for interactions between sex and the validation variables on
455 brain-age delta using linear regression models and including chronological age as
456 covariate. Lastly, we tested for interactions between age and the validation variables on
457 brain-age delta for CU and MCI individuals.

458
459 We also studied the differences in volumes and cortical thickness between females and
460 males in the UKBioBank for the brain regions that contributed the most to the prediction
461 according to the SHAP values. With this aim we performed regression models for each
462 ROI with sex as predictor variable, in which linear and quadratic expansions of age, site
463 and TIV (only included for volume ROIs), were included as covariates.

464
465 As a secondary analysis we wanted to identify the individuals whose predicted brain-age
466 deviate the most from chronological aging, i.e., individuals with the highest positive or
467 lowest negative brain-age deltas, to study the above-mentioned associations. With this
468 aim, we selected the individuals whose brain-age delta was included within the 10th and
469 90th percentile of the distribution for each independent cohort and studied the differences
470 between these groups. The methodology and the results of this analysis can be found in
471 Supplementary Appendix A.

472
473
474

475 **ACKNOWLEDGEMENTS & FUNDING**

476
477 This publication is part of the ALFA study (ALzheimer and FAMilies). The authors would
478 like to express their most sincere gratitude to the ALFA project participants, without
479 whom this research would have not been possible. Authors would like to thank Roche
480 Diagnostics International Ltd. for kindly providing the kits for the CSF analysis of
481 ALFA+ participants and GE Healthcare for kindly providing [18 F]flutemetamol doses of
482 ALFA+ participants. Collaborators of the ALFA Study are: Müge Akinci, Annabella
483 Beteta, Alba Cañas, Irene Cumplido, Carme Deulofeu, Ruth Dominguez, Maria Emilio,
484 Karine Fauria, Sherezade Fuentes, Oriol Grau-Rivera, Laura Hernandez, Gema Huesa,
485 Jordi Huguet, Eider M Arenaza-Urquijo, Eva M Palacios, Paula Marne, Tania Menchón,
486 Carolina Minguillon, Eleni Palpatzis, Cleofé Peña-Gómez, Albina Polo, Sandra Pradas,
487 Blanca Rodríguez-Fernández, Aleix Sala-Vila, Gemma Salvadó, Mahnaz Shekari, Anna
488 Soteras, Laura Stankeviciute, Marc Vilanova and Natalia Vilor-Tejedor.

489
490 The project leading to these results has received funding from “la Caixa” Foundation (ID
491 100010434), under agreement LCF/PR/GN17/50300004 and the Alzheimer's
492 Association and an international anonymous charity foundation through the TriBEKa
493 Imaging Platform project (TriBEKa-17-519007). Additional support has been received
494 from the Universities and Research Secretariat, Ministry of Business and Knowledge of
495 the Catalan Government under the grant no. 2017-SGR-892 and the Spanish Research
496 Agency (AEI) under project PID2020-116907RB-I00 of the call MCIN/ AEI

497 /10.13039/501100011033. FB is supported by the NIHR biomedical research center at
498 UCLH. MSC receives funding from the European Research Council (ERC) under the
499 European Union's Horizon 2020 research and innovation programme (Grant agreement
500 No. 948677), the Instituto de Salud Carlos III (PI19/00155), and from a fellowship from
501 "la Caixa" Foundation (ID 100010434) and from the European Union's Horizon 2020
502 research and innovation programme under the Marie Skłodowska-Curie grant agreement
503 No 847648 (LCF/BQ/PR21/11840004).

504

505 COBAS, COBAS E and ELECSYS are trademarks of Roche. All other product names
506 and trademarks are the property of their respective owners. The Roche NeuroToolKit is
507 a panel of exploratory prototype assays designed to robustly evaluate biomarkers
508 associated with key pathologic events characteristic of AD and other neurological
509 disorders, used for research purposes only and not approved for clinical use.

510

511

512

513 **CONFLICTS OF INTEREST**

514

515 Ivonne Suridjan is a full-time employee and shareholder of Roche Diagnostics
516 International Ltd; Gwendlyn Kollmorgen is a full-time employee of Roche Diagnostics
517 GmbH; Anna Bayfield is a full-time employee and shareholder of Roche Diagnostics
518 GmbH.

519

520 MSC has served as a consultant and at advisory boards for Roche Diagnostics
521 International Ltd and has given lectures in symposia sponsored by Roche Diagnostics,
522 S.L.U, Roche Farma, S.A and Roche Sistemas de Diagnósticos, Sociedade Unipessoal,
523 Lda.

524

525 J.L.M is currently a full-time employee of H. Lundbeck A/S and previously has served as
526 a consultant or on advisory boards for the following for-profit companies or has given
527 lectures in symposia sponsored by the following for-profit companies: Roche
528 Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green
529 Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, and ProMIS Neurosciences.

530

531

532

533 **REFERENCES**

534

535 Arenaza-Urquijo, E. M., Przybelski, S. A., Lesnick, T. L., Graff-Radford, J., Machulda, M. M.,
536 Knopman, D. S., Schwarz, C. G., Lowe, V. J., Mielke, M. M., Petersen, R. C., Jack, C. R.,
537 Jr., & Vemuri, P. (2019). The metabolic brain signature of cognitive resilience in the 80+:
538 beyond Alzheimer pathologies. *Brain*, *142*(4), 1134.
539 <https://doi.org/10.1093/BRAIN/AWZ037>

540 Armstrong, N. M., An, Y., Beason-Held, L., Doshi, J., Erus, G., Ferrucci, L., Davatzikos, C., &
541 Resnick, S. M. (2019). Sex differences in brain aging and predictors of neurodegeneration
542 in cognitively healthy older adults. *Neurobiology of Aging*, *81*, 146–156.
543 <https://doi.org/10.1016/J.NEUROBIOLAGING.2019.05.020>

544 Bakkour, A., Morris, J. C., Wolk, D. A., & Dickerson, B. C. (2013). The effects of aging and

- 545 Alzheimer's disease on cerebral cortical anatomy: Specificity and differential relationships
546 with cognition. *NeuroImage*, 76, 332–344.
547 <https://doi.org/10.1016/j.neuroimage.2013.02.059>
- 548 Baron-Cohen, S., Knickmeyer, R. C., & Belmonte, M. K. (2005). Sex differences in the brain:
549 Implications for explaining autism. *Science*, 310(5749), 819–823.
550 <https://doi.org/10.1126/SCIENCE.1115455>
- 551 Bashyam, V. M., Erus, G., Doshi, J., Habes, M., Nasrallah, I., Truelove-Hill, M., Srinivasan, D.,
552 Mamourian, L., Pomponio, R., Fan, Y., Launer, L. J., Masters, C. L., Maruff, P., Zhuo, C.,
553 Völzke, H., Johnson, S. C., Fripp, J., Koutsouleris, N., Satterthwaite, T. D., ... Davatzikos,
554 C. (2020). MRI signatures of brain age and disease over the lifespan based on a deep brain
555 network and 14 468 individuals worldwide. *Brain : A Journal of Neurology*, 143(7), 2312–
556 2324. <https://doi.org/10.1093/brain/awaa160>
- 557 Beheshti, I., Maikusa, N., & Matsuda, H. (2018). The association between “Brain-Age Score”
558 (BAS) and traditional neuropsychological screening tools in Alzheimer's disease. *Brain
559 and Behavior*, 8(8), e01020. <https://doi.org/10.1002/brb3.1020>
- 560 Bergstra, J., Yamins, D., & Cox, D. D. (2013). Making a Science of Model Search:
561 Hyperparameter Optimization in Hundreds of Dimensions for Vision Architectures. *Proc.
562 of the 30th International Conference on Machine Learning (ICML 2013)*, 28, I-115 to I–
563 23.
- 564 Bittner, T., Zetterberg, H., Teunissen, C. E., Ostlund, R. E., Militello, M., Andreasson, U.,
565 Hubeek, I., Gibson, D., Chu, D. C., Eichenlaub, U., Heiss, P., Kobold, U., Leinenbach, A.,
566 Madin, K., Manuilova, E., Rabe, C., & Blennow, K. (2016). Technical performance of a
567 novel, fully automated electrochemiluminescence immunoassay for the quantitation of β -
568 amyloid (1-42) in human cerebrospinal fluid. *Alzheimer's and Dementia*, 12(5), 517–526.
569 <https://doi.org/10.1016/j.jalz.2015.09.009>
- 570 Brugulat-Serrat, A., Rojas, S., Bargalló, N., Conesa, G., Minguillón, C., Fauria, K., Gramunt,
571 N., Molinuevo, J. L., & Gispert, J. D. (2017). Incidental findings on brain MRI of
572 cognitively normal first-degree descendants of patients with Alzheimer's disease: a cross-
573 sectional analysis from the ALFA (Alzheimer and Families) project. *BMJ Open*, 7(3).
574 <https://doi.org/10.1136/BMJOPEN-2016-013215>
- 575 Brugulat-Serrat, A., Salvadó, G., Operto, G., Cacciaglia, R., Sudre, C. H., Grau-Rivera, O.,
576 Suárez-Calvet, M., Falcon, C., Sánchez-Benavides, G., Gramunt, N., Minguillon, C.,
577 Fauria, K., Barkhof, F., Molinuevo, J. L., & Gispert, J. D. (2020). White matter
578 hyperintensities mediate gray matter volume and processing speed relationship in
579 cognitively unimpaired participants. *Human Brain Mapping*, 41(5), 1309–1322.
580 <https://doi.org/10.1002/hbm.24877>
- 581 Brugulat-Serrat, A., Salvadó, G., Sudre, C. H., Grau-Rivera, O., Suárez-Calvet, M., Falcon, C.,
582 Sánchez-Benavides, G., Gramunt, N., Fauria, K., Cardoso, M. J., Barkhof, F., Molinuevo,
583 J. L., Gispert, J. D., Camí, J., Cacciaglia, R., Operto, G., Skouras, S., Minguillón, C., Polo,
584 A., ... Huguet, J. (2020). Patterns of white matter hyperintensities associated with
585 cognition in middle-aged cognitively healthy individuals. *Brain Imaging and Behavior*,
586 14(5), 2012–2023. <https://doi.org/10.1007/s11682-019-00151-2>

- 587 Buckley, R. F., Mormino, E. C., Rabin, J. S., Hohman, T. J., Landau, S., Hanseeuw, B. J.,
588 Jacobs, H. I. L., Papp, K. V., Amariglio, R. E., Properzi, M. J., Schultz, A. P., Kirn, D.,
589 Scott, M. R., Hedden, T., Farrell, M., Price, J., Chhatwal, J., Rentz, D. M., Villemagne, V.
590 L., ... Sperling, R. A. (2019). Sex Differences in the Association of Global Amyloid and
591 Regional Tau Deposition Measured by Positron Emission Tomography in Clinically
592 Normal Older Adults. *JAMA Neurology*, *76*(5), 542–551.
593 <https://doi.org/10.1001/jamaneurol.2018.4693>
- 594 Casamitjana, A., Petrone, P., Tucholka, A., Falcon, C., Skouras, S., Molinuevo, J. L., Vilaplana,
595 V., & Gispert, J. D. (2018). MRI-Based Screening of Preclinical Alzheimer’s Disease for
596 Prevention Clinical Trials. *Journal of Alzheimer’s Disease : JAD*, *64*(4), 1099–1112.
597 <https://doi.org/10.3233/JAD-180299>
- 598 Chen, T., & Guestrin, C. (2016). XGBoost: A scalable tree boosting system. *Proceedings of the*
599 *22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*,
600 785–794. <https://doi.org/10.1145/2939672.2939785>
- 601 Coffey, C. E., Lucke, J. F., Saxton, J. A., Ratcliff, G., Unitas, L. J., Billig, B., & Bryan, R. N.
602 (1998). Sex differences in brain aging: A quantitative magnetic resonance imaging study.
603 *Archives of Neurology*, *55*(2), 169–179. <https://doi.org/10.1001/archneur.55.2.169>
- 604 Cohen, J. (2013). Statistical Power Analysis for the Behavioral Sciences. In Lawrence Erlbaum
605 Associates Inc (Ed.), *Statistical Power Analysis for the Behavioral Sciences*. Routledge.
606 <https://doi.org/10.4324/9780203771587>
- 607 Cole, J. H., Ritchie, S. J., Bastin, M. E., Valdés Hernández, M. C., Muñoz Maniega, S., Royle,
608 N., Corley, J., Pattie, A., Harris, S. E., Zhang, Q., Wray, N. R., Redmond, P., Marioni, R.
609 E., Starr, J. M., Cox, S. R., Wardlaw, J. M., Sharp, D. J., & Deary, I. J. (2018). Brain age
610 predicts mortality. *Molecular Psychiatry*, *23*(5), 1385–1392.
611 <https://doi.org/10.1038/mp.2017.62>
- 612 Cole, James H. (2020). Multimodality neuroimaging brain-age in UK biobank: relationship to
613 biomedical, lifestyle, and cognitive factors. *Neurobiology of Aging*, *92*, 34–42.
614 <https://doi.org/10.1016/j.neurobiolaging.2020.03.014>
- 615 Cole, James H., & Franke, K. (2017). Predicting Age Using Neuroimaging: Innovative Brain
616 Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690.
617 <https://doi.org/10.1016/J.TINS.2017.10.001>
- 618 Cole, James H., Poudel, R. P. K., Tsagkrasoulis, D., Caan, M. W. A., Steves, C., Spector, T. D.,
619 & Montana, G. (2017). Predicting brain age with deep learning from raw imaging data
620 results in a reliable and heritable biomarker. *NeuroImage*, *163*, 115–124.
621 <https://doi.org/10.1016/J.NEUROIMAGE.2017.07.059>
- 622 Dafflon, J., Pinaya, W. H. L., Turkheimer, F., Cole, J. H., Leech, R., Harris, M. A., Cox, S. R.,
623 Whalley, H. C., McIntosh, A. M., & Hellyer, P. J. (2020). An automated machine learning
624 approach to predict brain age from cortical anatomical measures. *Human Brain Mapping*,
625 *41*(13), 3555–3566. <https://doi.org/10.1002/hbm.25028>
- 626 de Lange, A. M. G., Anatórk, M., Suri, S., Kaufmann, T., Cole, J. H., Griffanti, L., Zsoldos, E.,
627 Jensen, D. E. A., Filippini, N., Singh-Manoux, A., Kivimäki, M., Westlye, L. T., &

- 628 Ebmeier, K. P. (2020). Multimodal brain-age prediction and cardiovascular risk: The
629 Whitehall II MRI sub-study. *NeuroImage*, 222, 117292.
630 <https://doi.org/10.1016/j.neuroimage.2020.117292>
- 631 de Lange, A. M. G., Barth, C., Kaufmann, T., Maximov, I. I., van der Meer, D., Agartz, I., &
632 Westlye, L. T. (2020). Women’s brain aging: Effects of sex-hormone exposure,
633 pregnancies, and genetic risk for Alzheimer’s disease. *Human Brain Mapping*, 41(18),
634 5141–5150. <https://doi.org/10.1002/hbm.25180>
- 635 de Lange, A. M. G., & Cole, J. H. (2020). Commentary: Correction procedures in brain-age
636 prediction. In *NeuroImage: Clinical* (Vol. 26). Elsevier Inc.
637 <https://doi.org/10.1016/j.nicl.2020.102229>
- 638 de Lange, A. M. G., Kaufmann, T., Van Der Meer, D., Maglanoc, L. A., Alnæs, D., Moberget,
639 T., Douaud, G., Andreassen, O. A., & Westlye, L. T. (2019). Population-based
640 neuroimaging reveals traces of childbirth in the maternal brain. *Proceedings of the*
641 *National Academy of Sciences of the United States of America*, 116(44), 22341–22346.
642 <https://doi.org/10.1073/pnas.1910666116>
- 643 DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., Beiser, A., D’Agostino, R.,
644 & Wolf, P. A. (2005). Measures of brain morphology and infarction in the framingham
645 heart study: establishing what is normal. *Neurobiology of Aging*, 26(4), 491–510.
646 <https://doi.org/10.1016/J.NEUROBIOLAGING.2004.05.004>
- 647 Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R.
648 L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An
649 automated labeling system for subdividing the human cerebral cortex on MRI scans into
650 gyral based regions of interest. *NeuroImage*, 31(3), 968–980.
651 <https://doi.org/10.1016/j.neuroimage.2006.01.021>
- 652 Evans, S., Dowell, N. G., Tabet, N., Tofts, P. S., King, S. L., & Rusted, J. M. (2014). Cognitive
653 and neural signatures of the APOE E4 allele in mid-aged adults. *Neurobiology of Aging*,
654 35(7), 1615–1623. <https://doi.org/10.1016/J.NEUROBIOLAGING.2014.01.145>
- 655 Ferretti, M. T., Iulita, M. F., Cavado, E., Chiesa, P. A., Dimech, A. S., Chadha, A. S., Baracchi,
656 F., Girouard, H., Misoch, S., Giacobini, E., Depypere, H., & Hampel, H. (2018). Sex
657 differences in Alzheimer disease — The gateway to precision medicine. In *Nature*
658 *Reviews Neurology* (Vol. 14, Issue 8, pp. 457–469). Nature Publishing Group.
659 <https://doi.org/10.1038/s41582-018-0032-9>
- 660 Filippini, N., Ebmeier, K. P., MacIntosh, B. J., Trachtenberg, A. J., Frisoni, G. B., Wilcock, G.
661 K., Beckmann, C. F., Smith, S. M., Matthews, P. M., & Mackay, C. E. (2011). Differential
662 effects of the APOE genotype on brain function across the lifespan. *NeuroImage*, 54(1),
663 602–610. <https://doi.org/10.1016/J.NEUROIMAGE.2010.08.009>
- 664 Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., Van Der Kouwe,
665 A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., & Dale,
666 A. M. (2002). Whole brain segmentation: Automated labeling of neuroanatomical
667 structures in the human brain. *Neuron*, 33(3), 341–355. <https://doi.org/10.1016/S0896->
668 [6273\(02\)00569-X](https://doi.org/10.1016/S0896-6273(02)00569-X)

- 669 Fjell, A. M., McEvoy, L., Holland, D., Dale, A. M., & Walhovd, K. B. (2014). What is normal
670 in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex
671 and the hippocampus. In *Progress in Neurobiology* (Vol. 117, pp. 20–40). Elsevier Ltd.
672 <https://doi.org/10.1016/j.pneurobio.2014.02.004>
- 673 Franke, K., & Gaser, C. (2019). Ten years of brainage as a neuroimaging biomarker of brain
674 aging: What insights have we gained? *Frontiers in Neurology*, *10*, 789.
675 <https://doi.org/10.3389/fneur.2019.00789>
- 676 Franke, K., Ziegler, G., Klöppel, S., & Gaser, C. (2010). Estimating the age of healthy subjects
677 from T1-weighted MRI scans using kernel methods: Exploring the influence of various
678 parameters. *NeuroImage*, *50*(3), 883–892.
679 <https://doi.org/10.1016/J.NEUROIMAGE.2010.01.005>
- 680 Gennatas, E. D., Avants, B. B., Wolf, D. H., Satterthwaite, T. D., Ruparel, K., Ciric, R.,
681 Hakonarson, H., Gur, R. E., & Gur, R. C. (2017). Age-Related Effects and Sex
682 Differences in Gray Matter Density, Volume, Mass, and Cortical Thickness from
683 Childhood to Young Adulthood. *The Journal of Neuroscience*, *37*(20), 5065.
684 <https://doi.org/10.1523/JNEUROSCI.3550-16.2017>
- 685 Green, P. S., & Simpkins, J. W. (2000). Neuroprotective effects of estrogens: Potential
686 mechanisms of action. In *International Journal of Developmental Neuroscience* (Vol. 18,
687 Issues 4–5, pp. 347–358). John Wiley & Sons, Ltd. [https://doi.org/10.1016/S0736-](https://doi.org/10.1016/S0736-5748(00)00017-4)
688 [5748\(00\)00017-4](https://doi.org/10.1016/S0736-5748(00)00017-4)
- 689 Greenberg, D. L., Messer, D. F., Payne, M. E., MacFall, J. R., Provenzale, J. M., Steffens, D.
690 C., & Krishnan, R. R. (2008). Aging, gender, and the elderly adult brain: An examination
691 of analytical strategies. *Neurobiology of Aging*, *29*(2), 290–302.
692 <https://doi.org/10.1016/j.neurobiolaging.2006.09.016>
- 693 Habes, M., Erus, G., Toledo, J. B., Zhang, T., Bryan, N., Launer, L. J., Rosseel, Y., Janowitz,
694 D., Doshi, J., Van Der Auwera, S., Von Sarnowski, B., Hegenscheid, K., Hosten, N.,
695 Homuth, G., Völzke, H., Schminke, U., Hoffmann, W., Grabe, H. J., & Davatzikos, C.
696 (2016). White matter hyperintensities and imaging patterns of brain ageing in the general
697 population. *Brain*, *139*(4), 1164. <https://doi.org/10.1093/BRAIN/AWW008>
- 698 Hansson, O., Seibyl, J., Stomrud, E., Zetterberg, H., Trojanowski, J. Q., Bittner, T., Lifke, V.,
699 Corradini, V., Eichenlaub, U., Batrla, R., Buck, K., Zink, K., Rabe, C., Blennow, K., &
700 Shaw, L. M. (2018). CSF biomarkers of Alzheimer's disease concord with amyloid- β PET
701 and predict clinical progression: A study of fully automated immunoassays in BioFINDER
702 and ADNI cohorts. *Alzheimer's and Dementia*, *14*(11), 1470–1481.
703 <https://doi.org/10.1016/j.jalz.2018.01.010>
- 704 Huang, W., Li, X., Li, H., Wang, W., Chen, K., Xu, K., Zhang, J., Chen, Y., Wei, D., Shu, N.,
705 & Zhang, Z. (2021). Accelerated Brain Aging in Amnesic Mild Cognitive Impairment:
706 Relationships with Individual Cognitive Decline, Risk Factors for Alzheimer Disease and
707 Clinical Progression. *Radiology: Artificial Intelligence*, e200171.
708 <https://doi.org/10.1148/ryai.2021200171>
- 709 Huguet, J., Falcon, C., Fusté, D., Girona, S., Vicente, D., Molinuevo, J. L., Gispert, J. D.,
710 Operto, G., & Study, for the A. (2021). Management and Quality Control of Large

- 711 Neuroimaging Datasets: Developments From the Barcelonaβeta Brain Research Center.
712 *Frontiers in Neuroscience*, 15. <https://doi.org/10.3389/FNINS.2021.633438>
- 713 Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B.,
714 Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine,
715 T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., ...
716 Silverberg, N. (2018). NIA-AA Research Framework: Toward a biological definition of
717 Alzheimer's disease. *Alzheimer's and Dementia*, 14(4), 535–562.
718 <https://doi.org/10.1016/j.jalz.2018.02.018>
- 719 Kaeser, S. A., Lehallier, B., Thinggaard, M., Häsler, L. M., Apel, A., Bergmann, C., Berdnik,
720 D., Jeune, B., Christensen, K., Grönke, S., Partridge, L., Wyss-Coray, T., Mengel-From,
721 J., & Jucker, M. (2021). A neuronal blood marker is associated with mortality in old age.
722 *Nature Aging*, 1(2), 218–225. <https://doi.org/10.1038/s43587-021-00028-4>
- 723 Kaufmann, T., van der Meer, D., Doan, N. T., Schwarz, E., Lund, M. J., Agartz, I., Alnæs, D.,
724 Barch, D. M., Baur-Streubel, R., Bertolino, A., Bettella, F., Beyer, M. K., Bøen, E.,
725 Borgwardt, S., Brandt, C. L., Buitelaar, J., Celius, E. G., Cervenka, S., Conzelmann, A., ...
726 Westlye, L. T. (2019). Common brain disorders are associated with heritable patterns of
727 apparent aging of the brain. *Nature Neuroscience*, 22(10), 1617–1623.
728 <https://doi.org/10.1038/s41593-019-0471-7>
- 729 Khalil, M., Pirpamer, L., Hofer, E., Voortman, M. M., Barro, C., Leppert, D., Benkert, P.,
730 Ropele, S., Enzinger, C., Fazekas, F., Schmidt, R., & Kuhle, J. (2020). Serum
731 neurofilament light levels in normal aging and their association with morphologic brain
732 changes. *Nature Communications*, 11(1), 1–9. [https://doi.org/10.1038/s41467-020-14612-](https://doi.org/10.1038/s41467-020-14612-6)
733 6
- 734 Khalil, M., Teunissen, C. E., Otto, M., Piehl, F., Sormani, M. P., Gatringer, T., Barro, C.,
735 Kappos, L., Comabella, M., Fazekas, F., Petzold, A., Blennow, K., Zetterberg, H., &
736 Kuhle, J. (2018). Neurofilaments as biomarkers in neurological disorders. *Nature Reviews*
737 *Neurology*, 14(10), 577–589. <https://doi.org/10.1038/s41582-018-0058-z>
- 738 Le, T. T., Kuplicki, R. T., McKinney, B. A., Yeh, H. W., Thompson, W. K., Paulus, M. P.,
739 Aupperle, R. L., Bodurka, J., Cha, Y. H., Feinstein, J. S., Khalsa, S. S., Savitz, J.,
740 Simmons, W. K., & Victor, T. A. (2018). A Nonlinear Simulation Framework Supports
741 Adjusting for Age When Analyzing BrainAGE. *Frontiers in Aging Neuroscience*, 10, 317.
742 <https://doi.org/10.3389/fnagi.2018.00317>
- 743 Liang, H., Zhang, F., & Niu, X. (2019). Investigating systematic bias in brain age estimation
744 with application to post-traumatic stress disorders. *Human Brain Mapping*, 40(11), 3143–
745 3152. <https://doi.org/10.1002/hbm.24588>
- 746 Liem, F., Varoquaux, G., Kynast, J., Beyer, F., Kharabian Masouleh, S., Huntenburg, J. M.,
747 Lampe, L., Rahim, M., Abraham, A., Craddock, R. C., Riedel-Heller, S., Luck, T.,
748 Loeffler, M., Schroeter, M. L., Witte, A. V., Villringer, A., & Margulies, D. S. (2017).
749 Predicting brain-age from multimodal imaging data captures cognitive impairment.
750 *NeuroImage*, 148, 179–188. <https://doi.org/10.1016/J.NEUROIMAGE.2016.11.005>
- 751 Lorenzini, L., Ingala, S., Wink, A. M., Kuijser, J. P., Wottschel, V., Dijsselhof, M., Sudre, C. H.,
752 Haller, S., Molinuevo, J. L., Gispert, J. D., Cash, D. M., Thomas, D. L., Vos, S. B.,

- 753 Prados, F., Petr, J., Wolz, R., Palombit, A., Schwarz, A. J., Gael, C., ... for the EPAD
754 Consortium. (2021). The European Prevention of Alzheimer's Dementia (EPAD) MRI
755 Dataset and Processing Workflow. *BioRxiv*, 2021.09.29.462349.
756 <https://doi.org/10.1101/2021.09.29.462349>
- 757 Löwe, L. C., Gaser, C., Franke, K., & for the Alzheimer's Disease Neuroimaging Initiative.
758 (2016). The Effect of the APOE Genotype on Individual BrainAGE in Normal Aging,
759 Mild Cognitive Impairment, and Alzheimer's Disease. *PLoS ONE*, 11(7).
760 <https://doi.org/10.1371/JOURNAL.PONE.0157514>
- 761 Lundberg, S. M., Erion, G., Chen, H., DeGrave, A., Prutkin, J. M., Nair, B., Katz, R.,
762 Himmelfarb, J., Bansal, N., & Lee, S.-I. (2020). From local explanations to global
763 understanding with explainable AI for trees. *Nature Machine Intelligence*, 2(1), 56–67.
764 <https://doi.org/10.1038/s42256-019-0138-9>
- 765 Ly, M., Yu, G. Z., Karim, H. T., Muppidi, N. R., Mizuno, A., Klunk, W. E., & Aizenstein, H. J.
766 (2020). Improving brain age prediction models: incorporation of amyloid status in
767 Alzheimer's disease. *Neurobiology of Aging*, 87, 44–48.
768 <https://doi.org/10.1016/j.neurobiolaging.2019.11.005>
- 769 Maioli, S., Leander, K., Nilsson, P., & Nalvarte, I. (2021). Estrogen receptors and the aging
770 brain. In *Essays in Biochemistry* (Vol. 65, Issue 6, pp. 913–925). Portland Press Ltd.
771 <https://doi.org/10.1042/EBC20200162>
- 772 Maniega, S. M., Valdés Hernández, M. C., Clayden, J. D., Royle, N. A., Murray, C., Morris, Z.,
773 Aribisala, B. S., Gow, A. J., Starr, J. M., Bastin, M. E., Deary, I. J., & Wardlaw, J. M.
774 (2015). White matter hyperintensities and normal-appearing white matter integrity in the
775 aging brain. *Neurobiology of Aging*, 36(2), 909–918.
776 <https://doi.org/10.1016/j.neurobiolaging.2014.07.048>
- 777 Marcus, D. S., Wang, T. H., Parker, J., Csernansky, J. G., Morris, J. C., & Buckner, R. L.
778 (2007). Open Access Series of Imaging Studies (OASIS): Cross-sectional MRI data in
779 young, middle aged, nondemented, and demented older adults. *Journal of Cognitive
780 Neuroscience*, 19(9), 1498–1507. <https://doi.org/10.1162/jocn.2007.19.9.1498>
- 781 Mielke, M. M. (2020). Consideration of Sex Differences in the Measurement and Interpretation
782 of Alzheimer Disease-Related Biofluid-Based Biomarkers. In *The journal of applied
783 laboratory medicine* (Vol. 5, Issue 1, pp. 158–169). NIH Public Access.
784 <https://doi.org/10.1373/jalm.2019.030023>
- 785 Milà-Alomà, M., Salvadó, G., Gispert, J. D., Vilor-Tejedor, N., Grau-Rivera, O., Sala-Vila, A.,
786 Sánchez-Benavides, G., Arenaza-Urquijo, E. M., Crous-Bou, M., González-de-Echávarri,
787 J. M., Minguillon, C., Fauria, K., Simon, M., Kollmorgen, G., Zetterberg, H., Blennow,
788 K., Suárez-Calvet, M., & Molinuevo, J. L. (2020). Amyloid beta, tau, synaptic,
789 neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's
790 continuum. *Alzheimer's and Dementia*, 16(10), 1358–1371.
791 <https://doi.org/10.1002/alz.12131>
- 792 Nebel, R. A., Aggarwal, N. T., Barnes, L. L., Gallagher, A., Goldstein, J. M., Kantarci, K.,
793 Mallampalli, M. P., Mormino, E. C., Scott, L., Yu, W. H., Maki, P. M., & Mielke, M. M.
794 (2018). Understanding the impact of sex and gender in Alzheimer's disease: A call to

- 795 action. *Alzheimer's & Dementia*, 14(9), 1171–1183.
796 <https://doi.org/10.1016/J.JALZ.2018.04.008>
- 797 Osborn, K. E., Liu, D., Samuels, L. R., Moore, E. E., Cambronero, F. E., Acosta, L. M. Y., Bell,
798 S. P., Babicz, M. A., Gordon, E. A., Pechman, K. R., Davis, L. T., Gifford, K. A.,
799 Hohman, T. J., Blennow, K., Zetterberg, H., & Jefferson, A. L. (2018). Cerebrospinal fluid
800 β -amyloid42 and neurofilament light relate to white matter hyperintensities. *Neurobiology*
801 *of Aging*, 68, 18–25. <https://doi.org/10.1016/j.neurobiolaging.2018.03.028>
- 802 Peng, H., Gong, W., Beckmann, C. F., Vedaldi, A., & Smith, S. M. (2021). Accurate brain age
803 prediction with lightweight deep neural networks. *Medical Image Analysis*, 68, 101871.
804 <https://doi.org/10.1016/J.MEDIA.2020.101871>
- 805 Petersen, R. C., Aisen, P. S., Beckett, L. A., Donohue, M. C., Gamst, A. C., Harvey, D. J., Jack,
806 C. R., Jr, Jagust, W. J., Shaw, L. M., Toga, A. W., Trojanowski, J. Q., & Weiner, M. W.
807 (2010). Alzheimer's Disease Neuroimaging Initiative (ADNI): Clinical characterization.
808 *Neurology*, 74(3), 201. <https://doi.org/10.1212/WNL.0B013E3181CB3E25>
- 809 Pichet Binette, A., Gonneaud, J., Vogel, J. W., La Joie, R., Rosa-Neto, P., Collins, D. L.,
810 Poirier, J., Breitner, J. C. S., Villeneuve, S., Vachon-Preseau, E., for the Alzheimer's
811 Disease Neuroimaging, & the PREVENT-AD Research Group. (2020). Morphometric
812 network differences in ageing versus Alzheimer's disease dementia. *Brain*, 143(2), 635–
813 649. <https://doi.org/10.1093/BRAIN/AWZ414>
- 814 Podgórski, P., Bładowska, J., Sasiadek, M., & Zimny, A. (2021). Novel Volumetric and
815 Surface-Based Magnetic Resonance Indices of the Aging Brain – Does Male and Female
816 Brain Age in the Same Way? *Frontiers in Neurology*, 12.
817 <https://doi.org/10.3389/FNEUR.2021.645729>
- 818 Resnick, S. M., Espeland, M. A., Jaramillo, S. A., Hirsch, C., Stefanick, M. L., Murray, A. M.,
819 Ockene, J., Davatzikos, M. C., & Resnick, S. M. (2009). Postmenopausal hormone therapy
820 and regional brain volumes: the WHIMS-MRI Study. *Neurology*, 72(2), 135–142.
821 <https://doi.org/https://doi.org/10.1212/01.wnl.0000339037.76336.cf>
- 822 Rizvi, B., Narkhede, A., Last, B. S., Budge, M., Tosto, G., Manly, J. J., Schupf, N., Mayeux, R.,
823 & Brickman, A. M. (2018). The effect of white matter hyperintensities on cognition is
824 mediated by cortical atrophy. *Neurobiology of Aging*, 64, 25–32.
825 <https://doi.org/10.1016/j.neurobiolaging.2017.12.006>
- 826 Salvadó, G., Molinuevo, J. L., Brugulat-Serrat, A., Falcon, C., Grau-Rivera, O., Suárez-Calvet,
827 M., Pavia, J., Niñerola-Baizán, A., Perissinotti, A., Lomeña, F., Minguillon, C., Fauria, K.,
828 Zetterberg, H., Blennow, K., & Gispert, J. D. (2019). Centiloid cut-off values for optimal
829 agreement between PET and CSF core AD biomarkers. *Alzheimer's Research and*
830 *Therapy*, 11(1), 1–12. <https://doi.org/10.1186/s13195-019-0478-z>
- 831 Schindler, S. E., Gray, J. D., Gordon, B. A., Xiong, C., Batrla-Utermann, R., Quan, M., Wahl,
832 S., Benzinger, T. L. S., Holtzman, D. M., Morris, J. C., & Fagan, A. M. (2018).
833 Cerebrospinal fluid biomarkers measured by Elecsys assays compared to amyloid
834 imaging. *Alzheimer's and Dementia*, 14(11), 1460–1469.
835 <https://doi.org/10.1016/j.jalz.2018.01.013>

- 836 Schwarz, C., Fletcher, E., DeCarli, C., & Carmichael, O. (2009). Fully-Automated White Matter
837 Hyperintensity Detection with Anatomical Prior Knowledge and without FLAIR. *Lecture*
838 *Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence*
839 *and Lecture Notes in Bioinformatics)*, 5636 LNCS, 239–251. [https://doi.org/10.1007/978-](https://doi.org/10.1007/978-3-642-02498-6_20)
840 [3-642-02498-6_20](https://doi.org/10.1007/978-3-642-02498-6_20)
- 841 Smith, S. M., Vidaurre, D., Alfaro-Almagro, F., Nichols, T. E., & Miller, K. L. (2019).
842 Estimation of brain age delta from brain imaging. *NeuroImage*, 200, 528–539.
843 <https://doi.org/10.1016/j.neuroimage.2019.06.017>
- 844 Solomon, A., Kivipelto, M., Molinuevo, J. L., Tom, B., & Ritchie, C. W. (2018). European
845 Prevention of Alzheimer’s Dementia Longitudinal Cohort Study (EPAD LCS): Study
846 protocol. *BMJ Open*, 8(12), e021017. <https://doi.org/10.1136/bmjopen-2017-021017>
- 847 Suárez-Calvet, M., Karikari, T. K., Ashton, N. J., Lantero Rodríguez, J., Milà-Alomà, M.,
848 Gispert, J. D., Salvadó, G., Minguillon, C., Fauria, K., Shekari, M., Grau-Rivera, O.,
849 Arenaza-Urquijo, E. M., Sala-Vila, A., Sánchez-Benavides, G., González-de-Echávarri, J.
850 M., Kollmorgen, G., Stoops, E., Vanmechelen, E., Zetterberg, H., ... Vilor-Tejedor, N.
851 (2020). Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial
852 stages of the preclinical Alzheimer’s continuum when only subtle changes in A β
853 pathology are detected. *EMBO Molecular Medicine*, 12(12), 1–19.
854 <https://doi.org/10.15252/emmm.202012921>
- 855 Subramaniapillai, S., Rajagopal, S., Snytte, J., Otto, A. R., Einstein, G., & Rajah, M. N. (2021).
856 Sex differences in brain aging among adults with family history of Alzheimer’s disease
857 and APOE4 genetic risk. *NeuroImage: Clinical*, 30, 102620.
858 <https://doi.org/10.1016/J.NICL.2021.102620>
- 859 Sudre, C. H., Cardoso, M. J., Bouvy, W. H., Biessels, G. J., Barnes, J., & Ourselin, S. (2015).
860 Bayesian Model Selection for Pathological Neuroimaging Data Applied to White Matter
861 Lesion Segmentation. *IEEE Transactions on Medical Imaging*, 34(10), 2079–2102.
862 <https://doi.org/10.1109/TMI.2015.2419072>
- 863 Ten Kate, M., Redolfi, A., Peira, E., Bos, I., Vos, S. J., Vandenberghe, R., Gabel, S.,
864 Schaeferbeke, J., Scheltens, P., Blin, O., Richardson, J. C., Bordet, R., Wallin, A.,
865 Eckerstrom, C., Molinuevo, J. L., Engelborghs, S., Van Broeckhoven, C., Martinez-Lage,
866 P., Popp, J., ... Barkhof, F. (2018). MRI predictors of amyloid pathology: Results from the
867 EMIF-AD Multimodal Biomarker Discovery study. *Alzheimer’s Research and Therapy*,
868 10(1), 100. <https://doi.org/10.1186/s13195-018-0428-1>
- 869 Vidal-Pineiro, D., Parker, N., Shin, J., French, L., Grydeland, H., Jackowski, A. P., Mowinckel,
870 A. M., Patel, Y., Pausova, Z., Salum, G., Sørensen, Ø., Walhovd, K. B., Paus, T., & Fjell,
871 A. M. (2020). Cellular correlates of cortical thinning throughout the lifespan. *Scientific*
872 *Reports*, 10(1). <https://doi.org/10.1038/s41598-020-78471-3>
- 873 Walsh, P., Sudre, C. H., Fiford, C. M., Ryan, N. S., Lashley, T., Frost, C., & Barnes, J. (2021).
874 The age-dependent associations of white matter hyperintensities and neurofilament light in
875 early- and late-stage Alzheimer’s disease. *Neurobiology of Aging*, 97, 10–17.
876 <https://doi.org/10.1016/J.NEUROBIOLAGING.2020.09.008>
- 877 Williams, E. J. (1959). The Comparison of Regression Variables. *Journal of the Royal*

- 878 *Statistical Society: Series B (Methodological)*, 21(2), 396–399.
879 <https://doi.org/10.1111/J.2517-6161.1959.TB00346.X>
- 880 Zhavoronkov, A., Mamoshina, P., Vanhaelen, Q., Scheibye-Knudsen, M., Moskalev, A., &
881 Aliper, A. (2019). Artificial intelligence for aging and longevity research: Recent advances
882 and perspectives. *Ageing Research Reviews*, 49, 49–66.
883 <https://doi.org/10.1016/J.ARR.2018.11.003>