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1 **Microglial activation, tau and amyloid deposition in**
2 **TREM2 p.R47H carriers and mild cognitive impairment**
3 **patients: A multi-modal/multi-tracer PET/MRI imaging**
4 **study with influenza vaccine immune challenge.**

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30 **Running title:**

31 PHAGO-PET

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46 **Abstract**

47 **Background**

48 Microglia are increasingly understood to play an important role in the pathogenesis of
49 Alzheimer's disease. The rs75932628 (p.R47H) *TREM2* variant is a well-established risk factor
50 for Alzheimer's disease. *TREM2* is a microglial cell surface receptor. In this multi-
51 modal/multi-tracer PET/MRI study we investigated the effect of *TREM2* p.R47H carrier status
52 on microglial activation, tau and amyloid deposition, brain structure and cognitive profile.

53 **Methods**

54 We compared *TREM2* p.R47H carriers ($n = 8$; median age = 62.3) and participants with mild
55 cognitive impairment ($n = 8$; median age = 70.7). Participants underwent two [^{18}F]DPA-714
56 PET/MRI scans to assess TSPO signal, indicative of microglial activation, before and after
57 receiving the seasonal influenza vaccination, which was used as an immune stimulant.
58 Participants also underwent [^{18}F]florbetapir and [^{18}F]AV1451 PET scans to assess amyloid and
59 tau burden respectively. Regional tau and TSPO signal were calculated for regions of interest
60 linked to Braak stage. An additional comparison imaging healthy control group ($n = 8$; median
61 age = 45.5) had a single [^{18}F]DPA-714 PET/MRI. An expanded group of participants
62 underwent neuropsychological testing, to determine if *TREM2* status influenced clinical
63 phenotype.

64 **Results**

65 Compared to participants with mild cognitive impairment, *TREM2* carriers had lower TSPO
66 signal in Braak II ($P = 0.04$) and Braak III ($P = 0.046$) regions, despite having a similar burden
67 of tau and amyloid. There were trends to suggest reduced microglial activation following
68 influenza vaccine in *TREM2* carriers. Tau deposition in the Braak VI region was higher in

69 *TREM2* carriers ($P = 0.04$). Furthermore, compared to healthy controls *TREM2* carriers had
70 smaller caudate ($P = 0.02$), total brain ($P = 0.049$) and white matter volumes ($P = 0.02$); and
71 neuropsychological assessment revealed worse ADAS-Cog13 ($P = 0.03$) and Delayed
72 Matching to Sample ($P = 0.007$) scores.

73 **Conclusions**

74 *TREM2* p.R47H carriers had reduced levels of microglial activation in brain regions affected
75 early in the Alzheimer's disease course and differences in brain structure and cognition.
76 Changes in microglial response may underlie the increased Alzheimer's disease risk in *TREM2*
77 p.R47H carriers. Future therapeutic agents in Alzheimer's disease should aim to enhance
78 protective microglial actions.

79

80 **Keywords:**

81 *TREM2*; Neuroinflammation; TSPO; Florbetapir; AV1451; DPA714; Alzheimer's Disease;
82 Microglia; PET

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90 **Introduction**

91 The classical neuropathological hallmarks of Alzheimer's disease are abnormal amyloid-beta
92 (A β) and tau protein aggregates, and neurodegeneration. Microglia are the resident immune
93 cell of the brain and their importance in the pathogenesis of Alzheimer's disease is increasingly
94 recognised [1]. Advances in genetic research have revealed that microglia associated genes
95 account for approximately 25% of Alzheimer's disease risk genes [2, 3]. In Alzheimer's
96 disease, activated microglia cluster around amyloid plaques where they potentially act as a
97 barrier mitigating the neurotoxic effects of A β [4]. They may also clear A β via phagocytosis
98 [5]. Tau pathology initially accumulates in transentorhinal regions before propagation to limbic
99 then wider neocortical regions and is highly correlated with neuronal cell loss and the
100 emergence and progression of symptoms in patients [6]. This progressive spread has been
101 divided into six Braak stages, based on histopathological studies [7]. Recently microglia were
102 shown to internalise tau aggregates [8] and in so doing, appear to contribute to the spread of
103 tau pathology [9-11].

104

105 One of the highest impact risk gene variants for Alzheimer's disease is in the gene for *TREM2*
106 (triggering receptor expressed on myeloid cells 2). The rare *TREM2* rs75932628 non-
107 synonymous coding variant (p.R47H) has a similar effect size to APOE ϵ 4, with a two-to-three-
108 fold increased risk of Alzheimer's disease in heterozygous carriers [12, 13]. *TREM2* is a
109 microglia cell surface receptor, which promotes a change in microglial phenotype and
110 phagocytosis following the binding of ligands, including lipid species, APOE and A β [14-17].
111 Evidence suggests the *TREM2* p.R47H variant leads to impaired ligand binding [18]. *TREM2*
112 p.R47H may therefore act to increase the risk of Alzheimer's disease via a partial loss of a
113 protective function of microglia [19].

114

115 There have been a limited number of studies examining the clinical and pathological
116 characteristics of *TREM2* p.R47H carriers. One study reported a higher proportion of
117 psychiatric and parkinsonian symptoms in *TREM2* p.R47H carriers who received an
118 Alzheimer's disease diagnosis [20], while others failed to find any distinguishing clinical
119 symptoms [21, 22]. Smaller hippocampal volumes in older, but cognitively normal, carriers
120 have also been reported [23]. Amyloid burden detected via PET scan in people with
121 Alzheimer's disease was not found to differ between carriers and non-carriers of the *TREM2*
122 p.R47H variant [24]. However, recent preclinical research has shown that *TREM2* acts to
123 reduce tau seeding in the presence of significant A β pathology [25].

124

125 TSPO (translocator protein) is a mitochondrial membrane protein with an uncertain
126 physiological role. It is usually expressed at low levels in the brain [26]. However, TSPO
127 protein expression is upregulated in response to a variety of insults, including immune
128 challenges, and is a marker of microglial activation when examined at *post-mortem* [27]. We
129 interpret the increase in TSPO signal broadly as 'microglial activation' in this paper. However,
130 it is important to recognise that microglia are now understood to have a diverse array of
131 phenotypes beyond the traditionally recognised 'resting' and 'activated' states [28], which are
132 not measurable using *in vivo* TSPO-PET. We measured TSPO signal using the second-
133 generation TSPO PET tracer [¹⁸F]DPA-714, which has a good signal to noise ratio [29]. We
134 used the seasonal influenza vaccine as an immune challenge, which in mice has been shown to
135 increase microglial activation [30, 31]. Abnormal protein aggregation was also measured using
136 [¹⁸F]florbetapir for amyloid and [¹⁸F]AV1451 (Flortaucipir) for tau.

137

138 The primary aim of this study was to investigate if *TREM2* p.R47H risk variant carriers have
139 reduced *in vivo* microglial activation, measured using TSPO signal, compared to non-carriers

140 also at increased risk of Alzheimer's disease. We assessed microglia activation at baseline and
141 following an immune stimulant. Additional aims were to establish if the deposition of amyloid
142 or tau differs between *TREM2* p.R47H carriers and non-carriers, and simultaneously explore
143 the relationship between amyloid burden, tau burden and microglial activation in these cases.
144 We also investigated whether there were differences in brain structure and cognitive profiles
145 that distinguished *TREM2* p.R47H carriers from non-carriers.

146

147 **Materials and methods**

148 **Participants**

149 Participants were recruited from existing research cohorts established at King's College
150 London, including the Alzheimer's disease research cohorts AddNeuroMed and KHP-DCR
151 (King's Health Partners - Dementia Case Register) [32], and PROTECT (Platform for Research
152 Online to investigate Genetics and Cognition in Aging - REC reference 13/LO/1578), a cohort
153 of healthy older adults. All studies had consent for re-contact for future research studies [33].
154 AddNeuroMed, KHP-DCR and PROTECT are longitudinal studies involving annual cognitive
155 assessments. Imputed whole genome data (Human610-Quad genotyping platform,
156 AddNeuroMed and KHP-DCR; Illumina Global Screening Array with custom content,
157 PROTECT) was used by the cohort managers to invite a subset of cases heterozygous for the
158 rare *TREM2* p.R47H risk variant or homozygous for the common non-risk variant, to
159 participate in PHAGO. Genotypes linked to individuals were unknown to the PHAGO study
160 team at recruitment but were later established by sequencing exon 2 of *TREM2* using DNA
161 extracted from blood and/or saliva. Additionally, participants with mild cognitive impairment

162 were recruited from memory clinics within the South London and the Maudsley Hospital Trust
163 and the Join Dementia Research online platform.

164

165 General inclusion criteria for assessment were i) 50-80 years old and ii) able to give informed
166 consent. Exclusion criteria were i) history of significant neurological or psychiatric disorders
167 and ii) current or recent history of drug or alcohol abuse. Only participants found to be high-
168 affinity (HAB) or mixed-affinity (MAB) binding for the TSPO polymorphism rs6971
169 underwent imaging, as low-affinity binders show negligible TSPO PET signal [26]. Additional
170 exclusion criteria for imaging assessments were i) contraindications to the seasonal flu vaccine,
171 ii) pregnancy or breastfeeding, iii) contraindication to MRI, iv) history of cancer within the last
172 5 years, v) systemic steroid therapy. Participants with mild cognitive impairment had i) a
173 subjective memory complaint, ii) objective cognitive impairment measured on
174 neuropsychological testing (1.5 standard deviations below control mean), iii) Clinical
175 Dementia Rating (CDR) of 0.5 [34] and, iv) preserved activities of daily living. Participants
176 not eligible for imaging assessments following clinical and genetic screening were included
177 only for the clinical and neuropsychological assessments.

178

179 Additionally, healthy control data for TSPO PET and MRI were obtained from prior studies,
180 using the same PET scanner and protocol, to enable normative comparisons of baseline (pre-
181 vaccine challenge) TSPO levels and brain structure. These control participants met the general
182 inclusion and exclusion criteria described above, except participants aged under 50 were also
183 included. They were only genotyped for their TSPO binding status.

184

185 **Study activities**

186 Participants underwent an initial screening visit involving assessment of medical history and
187 physical examination. Detailed clinical assessments included the Geriatric Depression Scale
188 (GDS) [35], Hamilton Anxiety Rating Scale (HAM-A) [36], Apathy Evaluation Score (AES)
189 [37], Quality of life in Alzheimer's disease (QoL-AD) [38] and fatigue severity score [39].
190 Neuropsychological assessments included the Montreal Cognitive Assessment (MoCA) [40],
191 FAS and animal naming fluency tasks [41], Trail-Making Task (TMT) [42], ADAS-Cog 13
192 [43] and a CANTAB computerised battery (Reaction Time [RTI], Paired Associates Learning
193 [PAL], Spatial Working Memory [SWM], Delayed Matching to Sample [DMS], Rapid Visual
194 Information Processing [RVP], Spatial Span [SSP], Pattern Recognition Memory [PRM] and
195 One Touch Stockings of Cambridge [OTS]) [44]. Eligible participants underwent imaging
196 assessments.

197

198 **Genotyping**

199 Blood was collected in a 3ml EDTA Vacuette or alternatively, saliva was provided by
200 participants in a Genefix Saliva DNA/RNA collection and stabilisation tube (GFX-02,
201 Isohelix), where blood collection was not practical. DNA was isolated using standard protocols
202 followed by PCR and Sanger sequencing to establish the genotypes of the following variants:
203 *TREM2* rs75932628 (p.R47H), *TSPO* rs6971 and *APOE* rs429358 and rs7412 (to derive *APOE*
204 haplotypes ϵ 2, 3 or 4).

205

206 **PET and MRI Imaging**

207 Participants underwent MRI on the 3T SIEMENS Biograph mMR, a combined PET-MR
208 machine. Scans took place at the King's College London & Guy's and St Thomas' PET Centre,

209 London. A T1 weighted MPRAGE (magnetisation prepared rapid gradient echo) sequence with
210 1mm³ voxel size was obtained (repetition time = 2300 ms, echo time = 2.96 ms, flip angle of
211 9). Images from the baseline scan were processed using FreeSurfer version 6.0 [45]. The DKT
212 (Desikan-Killiany-Tourville) and ASEG (automated subcortical segmentation) atlases [46, 47]
213 were used to obtain volumes of the following regions of interest (ROI): total brain (sum of grey
214 matter and white matter), white matter, hippocampus, putamen and caudate, and frontal,
215 temporal, and parietal grey matter. Intracranial volume measurements were also obtained as
216 this can influence regional volumes [48]. The volume of T1 hypointensities was also obtained
217 from FreeSurfer as a measure of leukoaraiosis [49]. Manual quality control of FreeSurfer
218 output was undertaken as per the software manual –
219 <https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/TroubleshootingData>.

220

221 TSPO and tau uptake within grey matter was compared within FreeSurfer derived ROIs
222 mapped to the six Braak stages of tau deposition in Alzheimer's disease, further details of the
223 regions used are available elsewhere [50]. It should be noted that the tau signal in the Braak II
224 region may be affected by off-target binding to the choroid plexus, so results in this area should
225 be interpreted with caution [51]. The tau Braak regions have also used for TSPO uptake in a
226 prior study [9].

227

228 [¹⁸F]DPA-714 scans were performed before and seven days after the influenza vaccine. Three
229 *TREM2* p.R47H carriers and two participants with mild cognitive impairment did not undergo
230 repeat imaging due to tracer supply issues. A mean dose of 184.3 (±14.8) MBq was injected.
231 Dynamic data were collected over 60 minutes and binned in 26 frames (1x60, 8x15, 3x60,
232 5x120, 9x300). Scans took place on a SIEMENS Biograph mMR PET/MRI in the afternoon at
233 the King's College London & Guy's and St Thomas' PET Centre, London. Participants also

234 underwent a CT head scan, which was used for attenuation correction [52]. Distribution volume
235 ratio (DVR) values for ROIs were calculated using a simplified reference tissue model
236 accounting for vascular tracer activity (Tomasi et al. 2008; Yaqub et al. 2012; Rizzo et al 2019)
237 and a supervised reference region approach, which has previously been validated for use with
238 [¹⁸F]DPA-714 [53]. The method employed to derive the image-derived input function used to
239 account for vascular binding was adapted from a previous study (Tomasi et al. 2008). The
240 blood pool was defined by selecting the 50 voxels with the highest activity during the initial
241 1.5 minutes of the dynamic PET scan, before the signal peak. The supervised reference region
242 was determined using a set of pre-defined kinetic classes to identify cerebellar grey matter
243 voxels with kinetic behaviour most similar to healthy grey matter. Partial volume effects were
244 investigated by rerunning the analysis with partial volume correction (PVC) applied to each
245 dynamic PET frame using the PETPVC toolbox (Thomas et al. 2016).

246

247 The mean injected dose for the [¹⁸F]AV1451 scan was 180.3 (\pm 1.7) MBq. Participants had an
248 80-minute uptake time followed by a 30-minute dynamic scan. Scans took place on a Siemens
249 Biograph™ TruePoint™ PET/CT at the Invicro centre for imaging sciences, London.
250 Standardised uptake value ratio (SUVR) values were created by dividing the activity averaged
251 over ROI voxels by the activity averaged over cerebellar grey matter voxels [54].

252

253 The mean injected dose for the [¹⁸F]florbetapir scan was 192.2 (\pm 45.4) MBq. Participants had
254 a 40-minute uptake time followed by a 20-minute static scan. Scans took place on a GE
255 Discovery PET/CT 710 at the Department of Nuclear Medicine, King's College Hospital,
256 London. One participant had a delayed scan start, 84 minutes following injection, due to
257 scanner malfunction. At this time point the activity of [¹⁸F]florbetapir is expected to be
258 sufficiently stable to allow for the SUVR analysis [55], so the data were included. To determine

259 amyloid positivity a cortical summary region was created, comprised of the FreeSurfer grey
260 matter frontal, cingulate, lateral parietal, and lateral temporal regions. This value was divided
261 by the signal within the whole cerebellum and a cut of 1.11 was applied, as per prior studies
262 [56].

263

264 PET and MRI images were pre-processed using MIAKAT™ software, which allows for step-
265 by-step quality control checks [57]. MPRAGE MRI underwent brain extraction and
266 segmentation. Dynamic [¹⁸F]DPA-714 and [¹⁸F]AV1451 PET were corrected for motion and all
267 PET images were co-registered with baseline MPRAGE MRI. ROI maps were defined based
268 on the individual participant FreeSurfer template. The CIC (Clinical Imaging Centre) v2.0
269 neuroanatomical atlas [58] was non-linearly transformed to baseline MPRAGE MRI. The
270 reference regions used for TSPO and tau analyses were defined using a combination of grey
271 matter segmentation output and the transformed CIC atlas. Time activity curves were then
272 extracted from the pre-processed PET images.

273

274 **Influenza vaccine challenge**

275 Following the first TSPO PET scan participants were given the cell-based quadrivalent
276 influenza vaccine, Flucelvax Tetra™, based on the 2019/20, 2020/21 and 2021/22 composition.
277 This was consistent with established clinical practice in older eligible participants as part of
278 seasonal health protection measures. Scans were scheduled to coincide with participants'
279 planned vaccination or were delayed until after the winter flu season, if already vaccinated.
280 Blood samples were collected before and 4-10 weeks after influenza vaccination to establish
281 seropositivity. Serum was isolated and stored at -80°C, prior to being sent to Public Health
282 England for the evaluation of pre- and post-vaccination antibody levels against the 2020/21

283 influenza strains, using a haemagglutination inhibition assay (HAI). A HAI titre of 40 or more
284 was indicative of seroconversion to a protective antibody response [59].

285

286 **Statistical analysis**

287 The *TREM2* p.R47H carrier group was compared to the mild cognitive impairment group as
288 both were at higher risk of Alzheimer's pathology. *TREM2* p.R47H carriers were also
289 compared against a healthy control group (imaging control group) for the TSPO and MRI
290 imaging assessments, and against a separate healthy control group (clinical control group) for
291 clinical measures. Demographic variables were compared between comparison groups using
292 the Mann-Whitney U test for continuous variables (as distribution not normal) and Chi-squared
293 or Fisher's exact test for categorical variables depending on participant number.

294

295 A general linear model was also used to assess differences in levels of tau and baseline TSPO
296 across Braak defined regions of interest, with respect to study group, with TSPO status (for
297 TSPO results) and age as covariates, as increased TSPO signal is observed in HABs vs MABs
298 [26] and with increasing age [60]. For the response to influenza vaccination, a linear mixed
299 model was used to assess for an interaction between study group and change in TSPO signal
300 pre- and post-vaccination across the Braak regions. Age and TSPO genetic status were used as
301 covariates. Participant ID was used as a random factor, and random intercept and slope were
302 included to account for between participant variation. Linear regression was used to assess the
303 association between TSPO regional activity and tau deposition (Braak I), amyloid positivity,
304 age and TSPO status. Significant results for TSPO related outcomes (our primary aims)
305 underwent Bonferroni multiple comparison correction to account for 6 tests. Brain structure
306 volumes of interest extracted from structural MRI were compared using a general linear model
307 with age, sex and intracranial volume as covariates.

308

309 Clinical assessment scores were compared using the Mann-Whitney U test (non-normal
310 distribution) or t-test (normal distribution). Neuropsychological assessment scores were
311 compared using a general linear model with age and years of education as covariates. Positive
312 skew was corrected for by Log10 transformation for TMT-A, TMT-B, ADAS-Cog 13, OTS
313 mean choice to correct, OTS mean latency to correct, PAL (total errors adjusted), PAL (total
314 errors 6 shapes), RTI 5 choice reaction and RVP mean latency. Normality of residuals for the
315 general linear model and linear regression were established by inspection of the histograms and
316 Q-Q plots. All statistics were carried out in SPSS version 27.

317

318 **Results**

319 **Demographics**

320 Eight *TREM2* p.R47H carriers underwent PET and MRI assessments. Demographic
321 characteristics were compared against eight participants with mild cognitive impairment and
322 eight imaging controls (Table 1). The mild cognitive impairment group was older than the
323 *TREM2* p.R47H group (70.7 vs 62.3; $P = 0.01$). Imaging controls were younger (45.5 vs 62.3;
324 $P < 0.001$) and contained a higher proportion of men than the *TREM2* p.R47H group (100% vs
325 62.5%; $P = 0.03$). For the subgroup of participants undergoing repeat TSPO PET scans, the
326 mild cognitive impairment group was older than the *TREM2* p.R47H group (70.7 vs 61.7; $P =$
327 0.03; Supplementary Table 1).

328 **Table I** Demographic characteristics of participants undergoing MRI and PET

	Imaging Controls (n = 8)	MCI (n = 8)	<i>TREM2</i> p.R47H (n = 8)
Age (years) (Median + IQR)	45.5 (43.3 - 48.8)	70.7 (64.0 - 75.8)	62.3 (60.7 - 67.7) ^{a,b}

Sex (Male/Female)	8 / 0	6 / 2	5 / 3 ^a
TSPO genotype (MAB/HAB)	2 / 6	5 / 3	4 / 4
APOEε4 (carrier/non-carrier)	-	4 / 4	2 / 6
Amyloid status (positive/negative)	-	2/6	1/7
WM	-		
Hypointensity volume (cm ³) (Median + IQR)	1.0 (0.9 - 1.6)	1.9 (1.4 - 4.6)	1.5 (0.9 - 2.0)

329

330 HAB = high-affinity binder; IQR = interquartile range; MAB = mixed-affinity binder; MCI =
331 mild cognitive impairment; TSPO = translocator protein; WM = white matter.

332 APOEε4 carrier refers to the number with ≥1 ε4 allele.

333 Positive amyloid status refers to having a summary cortical SUVR of > 1.11 on Amyloid PET.

334 ^aTREM2 p.R47H carrier significant versus controls; P < 0.05.

335 ^bTREM2 p.R47H carrier significant versus MCI group; P < 0.05.

336

337 For the clinical and neuropsychological assessments, *TREM2* p.R47H carriers were compared
338 to a mild cognitive impairment and a healthy control group (clinical controls) (Table 2). The
339 clinical control group were a different group of people from the imaging control group and had
340 a similar age to the mild cognitive impairment group. *TREM2* p.R47H carriers were slightly
341 younger than the mild cognitive impairment group (64.9 vs 71.6; *P* = 0.02) and clinical control
342 group (64.9 vs 73.1; *P* = 0.005). The mild cognitive impairment group had worse MoCA scores
343 (25 vs 29; *P* = 0.003) and fewer years of education (14 vs 18; *P* = 0.01) compared to *TREM2*
344 p.R47H carriers. As would be anticipated, given their diagnosis, the mild cognitive impairment
345 group had a worse MoCA score than the clinical control group (25 vs 28; *P* < 0.001).

346 **Table 2** Demographic characteristics of participants undergoing clinical and neuropsychological assessments

	Clinical Controls (n=29)	MCI (n=11)	TREM2 p.R47H (n=12)
Age (Median + IQR)	73.1 (66.5-76.4)	71.6 (65.8-77.5)	64.9 (60.0 - 69.4) ^{a,b}
Sex (Male/Female)	10 / 19	8 / 3	6 / 6

Education (Median + IQR)	18 (16.5 - 20)	14 (12-18)	18 (17.3 - 20) ^b
APOEε4 (carrier/non-carrier)	8 / 21	4 / 7	2 / 10
MoCA (Median + IQR)	28.0 (27.0-30.0)	25.0 (23.0-26.0)	29.0 (26.5 - 30.0) ^b

347

348 IQR = interquartile range; MCI = mild cognitive impairment; MoCA = Montreal Cognitive
349 Assessment.

350 APOEε4 carrier refers to the number with ≥1 ε4 allele.

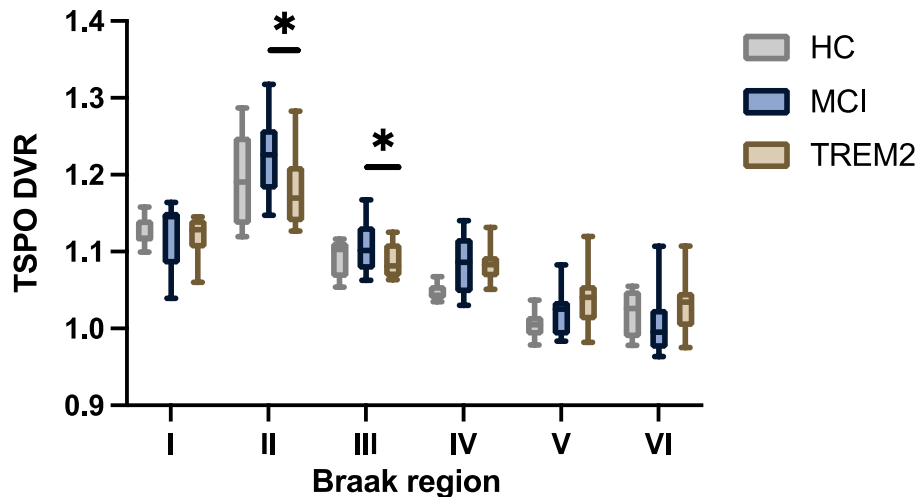
351 ^aTREM2 p.R47H carrier significant versus controls; $P < 0.05$.

352 ^bTREM2 p.R47H carrier significant versus MCI group; $P < 0.05$.

353

354 **Microglial activation**

355 We compared baseline differences in microglial activation between *TREM2* p.R47H carriers
356 and both participants with mild cognitive impairment and an imaging healthy control group
357 (Fig. 1 and Supplementary Table 2). Reduced TSPO signal was found in Braak II ($\eta^2 = 0.31$;
358 $F = 5.41$; $P = 0.04$) and III ($\eta^2 = 0.29$; $F = 4.95$; $P = 0.046$) regions of interest between *TREM2*
359 p.R47H carriers and mild cognitive impairment participants, which remained significant after
360 PVC application (Braak II: $P = 0.03$; Braak III: $P = 0.03$). These significant results do not
361 withstand multiple comparison correction. While TSPO signal in these regions was also lower
362 in *TREM2* p.R47H carriers compared to imaging controls, the difference was not statistically
363 significant. There were no differences in reference region SUV values between the groups.



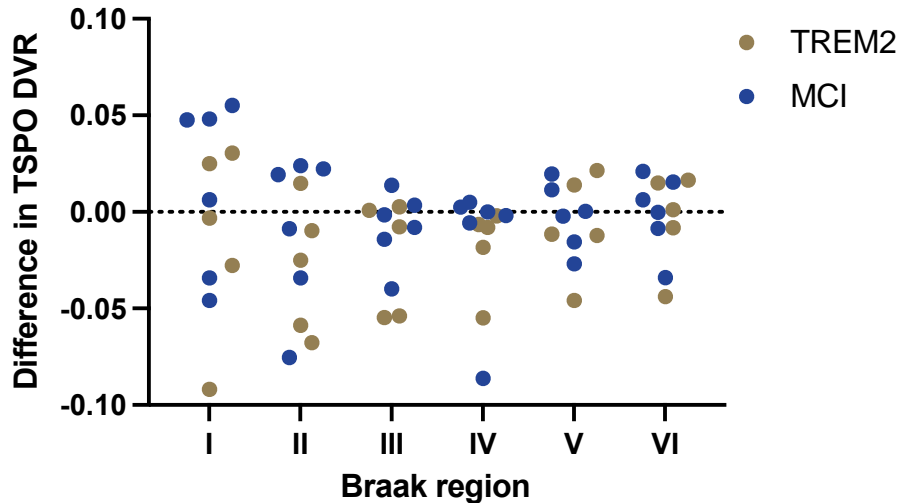
364

365 **Figure 1: Baseline comparison of regional microglial activation.** TSPO DVR values across
 366 Braak stages, comparing HC, MCI and *TREM2* p.R47H carriers, showing lower DVR in Braak
 367 II and Braak III regions in *TREM2* p.R47H carriers. Box plots show median values,
 368 interquartile range and range. Statistical comparisons between the *TREM2* p.R47H group and
 369 each of the control groups separately, with age and TSPO status as covariates. * $P < 0.05$. DVR
 370 = distribution volume ratio; HC = health control; MCI = mild cognitive impairment; TSPO =
 371 translocator protein.

372

373 **Microglial activation - influenza vaccine challenge**

374 The influenza vaccine did not result in a significant change in TPSO signal in any of the Braak
 375 regions (Fig. 2 and Supplementary Table 3). However, within Braak II ($\beta = -0.03 \pm 0.02$; $P =$
 376 0.08), Braak III ($\beta = -0.02 \pm 0.01$; $P = 0.08$), Braak IV ($\beta = -0.02 \pm 0.01$; $P = 0.06$) there were
 377 trends to suggest that influenza vaccine lowered the TSPO signal in *TREM2* p.R47H carriers
 378 compared to mild cognitive impairment participants.



379

380 **Figure 2: Microglia response to influenza vaccine.** Change in TSPO DVR values in response
 381 to influenza vaccine are presented based on Braak regions and participant group. DVR =
 382 distribution volume ratio; MCI = mild cognitive impairment; TSPO = translocator protein.

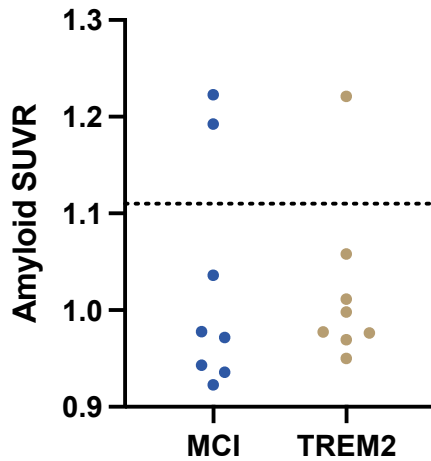
383

384 Where pre- and post-vaccine serum was available, serological conversion was assessed for
 385 participants, undergoing repeat TSPO scans, who were given the 2020/21 influenza vaccine (n
 386 = 7 out of 11). Four of these participants had evidence of seroconversion, whereas three
 387 participants had protective levels of antibodies both pre- and post-vaccination. Three additional
 388 participants, who only had baseline TSPO scans, also had evidence of serological conversion.

389

390 **Amyloid and tau pathology**

391 One *TREM2* p.R47H carrier and two mild cognitive impairment participants reached the
 392 threshold for abnormal amyloid pathology (Fig. 3) [56]. Given low participant numbers,
 393 amyloid positive and negative participants were therefore pooled together for subsequent
 394 analyses.



395

396 **Figure 3: Amyloid burden.** SUVR values for [¹⁸F]florbetapir between MCI and *TREM2*

397 p.R47H carrier groups. Dotted line represents the threshold of 1.11 for amyloid positivity. MCI

398 = mild cognitive impairment; SUVR = Standardised uptake value ratio.

399

400 There was no difference in regional tau PET signal between *TREM2* p.R47H and mild cognitive

401 impairment participants in Braak regions I-V regions of interest (Fig. 4, Supplementary Table

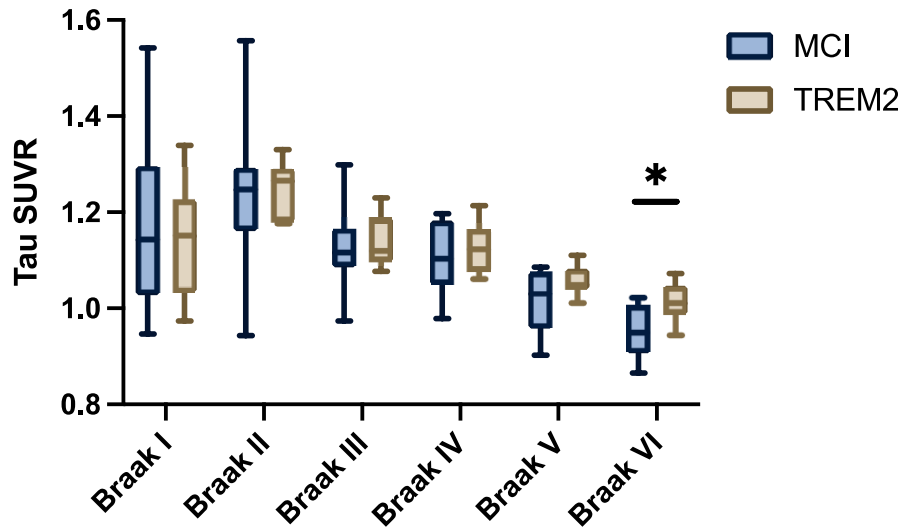
402 4). *TREM2* p.R47H carriers did however have higher tau PET signal in Braak VI than mild

403 cognitive impairment participants ($\eta^2 = 0.28$; $F = 5.0$; $P = 0.04$), although it should be noted

404 that uptake values overall were low in this region in all participants, which is to be expected in

405 the early-stage disease period we were focused on.

406



407

408 **Figure 4: Tau burden.** SUVR values for [¹⁸F]AV1451 across Braak stages, comparing MCI
 409 to *TREM2* p.R47H carrier groups. Box plots show median values, interquartile range and range.
 410 Statistical comparisons were made with age as a covariate. * $P < 0.05$. MCI = mild cognitive
 411 impairment; SUVR = Standardised uptake value ratio.

412

413 In order to confirm an anticipated association between amyloid positivity and early tau burden,
 414 a pooled analysis of all subjects was performed, demonstrating that amyloid positivity was
 415 associated with higher Braak I tau region of interest deposition ($r_{pb} = 0.60$; $P = 0.01$;
 416 Supplementary Fig. 1).

417

418 **Relationship between microglial activation, tau and amyloid pathology.**

419 We constructed linear regression models to assess if TSPO signal across the Braak stage
 420 regions of interest could be predicted by early tau burden (Braak I), amyloid positivity, age and
 421 TSPO binding status. There was no significant association in either the mild cognitive
 422 impairment group or *TREM2* p.R47H carriers.

423

424 **MRI**

425 ROI volumes were compared between *TREM2* p.R47H carriers and, both control and mild
 426 cognitive impairment groups (Table 3). Total brain volume ($\eta^2 = 0.31$; $F = 4.86$; $P = 0.049$),
 427 white matter volume ($\eta^2 = 0.42$; $F = 8.04$; $P = 0.02$) and caudate volume ($\eta^2 = 0.41$; $F = 7.67$;
 428 $P = 0.02$) were smaller in the *TREM2* p.R47H group compared to controls, with age, sex and
 429 intracranial volume included as covariates.

Table 3 FreeSurfer derived regions of interest volumes (cm³) were compared based on study group.

Region	Imaging controls (n = 8)	MCI (n = 8)	TREM2 p.R47H (n = 8)
TBV (mean \pm SD)	1,244 \pm 94.2	999.6 \pm 107.6	1,029 \pm 47.0 ^a
Frontal (mean \pm SD)	175.0 \pm 18.2	151.2 \pm 14.2	156.5 \pm 11.5
Temporal (mean \pm SD)	118.1 \pm 12.1	102.8 \pm 12.2	99.56 \pm 7.20
Parietal (mean \pm SD)	114.3 \pm 15.7	98.88 \pm 10.3	104.1 \pm 4.32
Hippocampus (mean \pm SD)	9.191 \pm 0.724	7.258 \pm 0.740	7.826 \pm 0.570
Putamen (mean \pm SD)	10.77 \pm 0.563	8.871 \pm 1.33	9.467 \pm 0.993
Caudate (mean \pm SD)	7.526 \pm 0.619	6.531 \pm 0.944	6.438 \pm 0.519 ^a
White matter (mean \pm SD)	559.5 \pm 47.7	410.3 \pm 63.4	424.8 \pm 23.5 ^a

430

431 MCI = mild cognitive impairment; SD = standard deviation TBV = total brain volume.

432 Age, sex and intracranial volume included as covariates for statistical comparison.

433 ^a*TREM2* p.R47H carrier significant versus controls; $P < 0.05$.

434 **Clinical and neuropsychological assessments**

435 Medical history revealed that none of the *TREM2* p.R47H carriers were experiencing features
 436 of parkinsonism, hallucinations or delusions. A series of clinical assessment scales were used
 437 to determine if *TREM2* p.R47H carriers exhibited differences in depression, anxiety, apathy,
 438 fatigue and quality of life compared to the clinical control and mild cognitive impairment
 439 group, who did not have the risk variant (Supplementary Table 5). The mild cognitive

440 impairment group had worse assessment scores than the *TREM2* p.R47H group, across all
441 domains. There was no difference between the *TREM2* p.R47H and clinical control group.

442

443 Neuropsychological battery revealed that the *TREM2* p.R47H group had a worse performance
444 on ADAS-Cog 13 ($F=5.3$; $\eta^2 = 0.13$; $P = 0.03$) and DMS ($F=8.3$; $\eta^2 = 0.19$; $P = 0.007$) when
445 compared to the clinical control group, with age and years of education as covariates. These
446 detailed neuropsychological assessments did not reveal a difference between the MCI and
447 *TREM2* p.R47H groups except a possible trend for participants with mild cognitive impairment
448 to have worse ADAS-Cog 13 scores than *TREM2* p.R47H carriers ($P = 0.09$).

449 **Discussion**

450 Within this multimodal PET/MRI imaging study we have shown that carriers of the rare
451 *TREM2* p.R47H Alzheimer's disease risk variant have lower levels of TSPO tracer uptake in
452 brain regions known to be affected in early Alzheimer's disease. This is consistent with our
453 hypothesis that *TREM2* p.R47H carriers have reduced microglial activation and shows this for
454 the first time *in vivo* in people. The influenza vaccine was not shown to be an effective
455 stimulator of brain microglial activation, measured by TSPO PET in any study group. We have
456 additionally shown that older *TREM2* p.R47H carriers have subclinical impaired cognitive
457 performance and areas of reduced brain volume compared to controls.

458

459 Lower relative TSPO uptake was specifically found in the hippocampus (included in Braak II
460 staging) and medial/inferior temporal lobe regions (Braak III) in *TREM2* p.R47H carriers,
461 although these results did not remain after stringently adjusting for multiple comparison testing.
462 This is in keeping with preclinical studies which have shown lower levels of hippocampal
463 microglial activation in mice carrying the human *TREM2* p.R47H variant, with or without

464 Alzheimer's disease pathology [61, 62]. The comparison group for this aspect of the study was
465 a mild cognitive impairment group. Therefore, the comparison was between two groups at
466 higher risk of Alzheimer's disease, although the ultimate diagnostic outcome of participants
467 was unknown. Additionally, no difference in tau deposition in these regions was found between
468 the two groups and there was a similar number of amyloid positive cases, suggesting reduced
469 microglial activation in *TREM2* p.R47H despite having a similar tau and amyloid burden to
470 MCI cases. Abnormal amyloid and tau protein accumulation can pre-empt Alzheimer's disease
471 clinical symptoms by many years [63], and it has recently been hypothesised that tau, rather
472 than amyloid, could be the initiating pathology [64]. The role of microglia in these initial stages
473 of Alzheimer's disease pathogenesis is thought to be protective, with microglia acting to reduce
474 the spread of amyloid and possibly tau [19, 65]. It is possible that the expected loss of function
475 in *TREM2* conferred by p.R47H may underlie the lower levels of TSPO signal seen in the
476 *TREM2* p.R47H carriers and that this reduced microglial activation may be a factor in the
477 increased risk of Alzheimer's disease in these carriers . This is consistent with *in vitro* work in
478 iPSC derived microglia that shows *TREM2* impairment leads to a locked immunometabolic
479 block which prevents microglial activation in the presence of damage stimuli [66].

480

481 The influenza vaccine was utilised as an immune challenge based on animal models which
482 demonstrated a brain microglial activation response [30, 31]. It was also chosen due to high
483 levels of participant acceptability and familiarity, with millions of people receiving the vaccine
484 each year in the UK [67]. Other immune challenges could have been considered including
485 lipopolysaccharide, which has been shown to cause raised brain TSPO PET signal [68].
486 However, the side effect profile makes this unacceptable for use in people [69]. The influenza
487 vaccine was not shown to cause microglial activation, measured by TSPO signal, in this study.
488 This was despite evidence of a peripheral antibody response in many of the participants.

489 Another potential stimulant of brain microglia, interferon-alpha, has also recently failed to
490 demonstrate TSPO signal change following administration [70]. The reason for this lack of
491 microglial activation could potentially relate to a reduction in blood-brain-barrier permeability,
492 and thus tracer transfer into the brain, in response to modest peripheral immune activation
493 typically obtained with interferon-alpha and flu vaccines, while lipopolysaccharide- like
494 stimulation is far more potent and may induce blood-brain-barrier leakage leading to higher
495 tracer binding [71].

496

497 There was no difference in tau deposition between regions with different Braak stages
498 comparing *TREM2* p.R47H carriers and non-carriers with mild cognitive impairment, except
499 for higher deposition in the Braak stage VI region in *TREM2* p.R47H carriers. It is unclear
500 whether this has biological relevance given that binding in this area was minimal across groups.
501 However, it is notable that a higher early tau burden in this region is seen in the posterior
502 cortical atrophy variant of Alzheimer's disease [72]. This would be in keeping with a report
503 that *TREM2* variant carriers are more likely to develop an atypical variant of Alzheimer's
504 disease [73]. There was an insufficient number of participants who were amyloid positive to
505 meaningfully investigate differences in amyloid deposition in this study. Furthermore, there
506 was no association between amyloid deposition, tau deposition, and TSPO signal in either
507 group. In prior PET imaging studies, TSPO signal has been shown to positively correlate with
508 both increasing amyloid and also with tau burden, especially in amyloid positive individuals
509 who are expected to have more advanced disease than those in the present study [74].
510 Preclinical research suggests that normally functioning *TREM2* acts to stop tau propagation,
511 but this only occurs in the presence of significant amyloid pathology [25]. TSPO and tau PET
512 signal have been shown to increase in tandem across the Braak stages, particularly in the
513 presence of a significant amyloid load [9]. We anticipate that when *TREM2* p.R47H carriers

514 develop greater levels of amyloid and tau, the association between these proteins and microglial
515 activation would be disrupted compared to non-carriers. Future research in *TREM2* p.R47H
516 carriers with Alzheimer's disease could address this but would be challenging due to the rarity
517 of the variant.

518

519 Little is known about how the behavioural phenotype differs between *TREM2* p.R47H carriers
520 and non-carriers. In our study, none of the *TREM2* p.R47H carriers exhibited parkinsonism or
521 reported psychotic symptoms, unlike previous reports from *TREM2* p.R47H carriers with
522 cognitive impairment [20]. Additionally, there was no difference in scores for depression,
523 anxiety, apathy, fatigue or quality of life between *TREM2* p.R47H carriers and non-carriers,
524 without cognitive impairment diagnosis. However, *TREM2* p.R47H carriers had worse
525 cognitive performance when measured by the ADAS-Cog 13 and DMS, despite none of these
526 participants meeting the criteria for mild cognitive impairment or Alzheimer's disease. ADAS-
527 Cog 13 is a broad cognitive assessment, that is sensitive for Alzheimer's disease-related
528 cognitive changes even in early stages of the disease [43]. Word recall and delayed recall are
529 major components of the assessment and assess episodic memory. DMS is a marker for visual
530 episodic memory [75]. These results hint at impaired temporal lobe functions relating to
531 episodic memory in *TREM2* p.R47H carriers, even without overt cognitive impairment.
532 Moreover, the worse visual episodic memory in *TREM2* p.R47H carriers could potentially
533 relate to the higher tau deposition in posterior brain regions (included within the Braak VI
534 region), seen in this study. Further work to evaluate pathology and symptoms linked to this
535 brain region are warranted in *TREM2* p.R47H carriers.

536

537 We also demonstrated smaller total brain, white matter and caudate volumes in *TREM2* p.R47H
538 carriers compared to controls. The white matter volume differences are of interest as other

539 *TREM2* variants are associated with leukoencephalopathy [76]. However, no evidence of
540 increased volume of white matter lesions was found in *TREM2* p.R47H carriers, suggesting no
541 marked leukoencephalopathy in this group. Progressive caudate atrophy in mild cognitive
542 impairment and Alzheimer's disease has previously been described [77]. Patients with
543 Alzheimer's disease and the *TREM2* p.R47H variant had smaller caudate as well as other
544 frontobasal brain areas [20]. Additionally, in young carriers of the rs143332484 (p.R62H)
545 *TREM2* variant the putamen was found to be smaller. This could suggest that subcortical areas
546 are particularly prone to neurodegeneration in *TREM2* variant carriers or that *TREM2* variants
547 lead to early neurodevelopmental effects in these regions that lead to later life vulnerability to
548 pathology.

549

550 There are several limitations of this study which need to be considered. Given the rarity of the
551 *TREM2* p.R47H risk variant, recruitment of only a relatively small number of participants was
552 possible. However, the higher signal to noise ratio of second-generation TSPO tracers can
553 provide sufficient statistical power using relatively modest numbers of participants [29]. None
554 of the *TREM2* p.R47H carriers had a diagnosis of mild cognitive impairment or Alzheimer's
555 disease. However, considering Alzheimer's disease pathogenesis initiates years prior to the
556 development of clinical symptoms [63] and that Alzheimer's disease treatments are
557 increasingly being trialled early in the disease course [78], healthy older high risk adults are an
558 important group of interest in Alzheimer's disease. The *TREM2* p.R47H carrier and mild
559 cognitive impairment groups had similar levels of Alzheimer's disease pathology detected on
560 PET. Both groups are at increased risk of Alzheimer's disease, although the low levels of
561 amyloid and tau pathology detected on PET indicates that conversion to Alzheimer's disease
562 was not likely imminent in most of the participants. A comparison between these two groups
563 is therefore an important prospective cohort from which future disease outcomes could

564 subsequently be re-evaluated. Other pragmatic decisions included the inclusion of both MAB
565 and HAB TSPO binders, and accepting an age imbalance between groups, which were
566 statistically controlled for. It should be noted that for the key comparison of TSPO signal
567 between the *TREM2* p.R47H carrier group and mild cognitive impairment group, the effect of
568 the 8 years age difference, while needing to be acknowledged, is likely to be marginal [60].
569 Women have been shown to have higher TSPO signal than men with an alternative TSPO tracer
570 [79]. However, despite the significant sex difference in the *TREM2* p.R47H carrier group with
571 more women compared to the imaging control group, and the older age, the *TREM2* p.R47H
572 carrier group still exhibited lower TSPO signal in early Braak regions, although this was not
573 significant. The microglial activation response is not homogeneous, with microglial phenotype
574 differing in response to the provoking factor, such as amyloid versus tau [80]. TSPO PET is
575 unable to differentiate between these different types of microglial response, highlighting the
576 need for ongoing preclinical research in this area. It should also be recognised that TSPO is
577 also expressed by astrocytes and endothelial, which may contribute to the signal [81].

578

579 Future research should aim to address the role of *TREM2* variants upon microglial activation
580 longitudinally, including later in the disease course after significant accumulation of amyloid
581 and tau, and if feasible with greater numbers of participants. This is especially important as the
582 role of microglia may change across the Alzheimer's disease course from protective to
583 antagonistic [65]. Moreover, research in younger age groups is important to establish when the
584 brain structural and clinical phenotype changes exhibited in *TREM2* p.R47H carriers first
585 emerge. Altered microglial activation, potentially via *TREM2* modulation, is an exciting future
586 target for novel therapeutics in Alzheimer's disease that is currently undergoing preclinical
587 trials [82, 83].

588 **Conclusions**

589 We have explored the *in vivo* impact of the *TREM2* p.R47H mutation in older high disease risk
590 carriers. Carriers of this variant had a suggestion of lower levels of microglial activation in
591 areas of the brain affected by tau pathology early in Alzheimer's disease pathogenesis. Minor
592 changes in brain structure and the cognitive profile of carriers suggest a different phenotypic
593 profile in *TREM2* p.R47H carriers than non-carriers. Future treatment avenues in Alzheimer's
594 disease should focus on enhancing the early protective effect of microglia with the aim of
595 stopping the progression of this devastating disease.

596

597 **Abbreviations**

598 A β : Amyloid-beta; AES: Apathy Evaluation Score; APOE: Apolipoprotein E; CDR: Clinical
599 Dementia Rating; DKT: Desikan-Killiany-Tourville; DMS: Delayed Matching to Sample;
600 DVR: Distribution Volume Ratio; GDS: Geriatric Depression Scale; HAB: High-Affinity
601 Binder; HAI: Haemagglutination Inhibition Assay; HAM-A: Hamilton Anxiety Rating Scale;
602 KHP-DCR: King's Health Partners - Dementia Case Register; MAB: Mixed-Affinity Binder;
603 MCC: Mean Choices to Correct; MCI: Mild Cognitive Impairment; MLC: Mean Latency to
604 Correct; MoCA: Montreal Cognitive Assessment; MPRAGE: Magnetisation Prepared Rapid
605 Gradient Echo; OTS: One Touch Stockings of Cambridge; PAL: Paired Associates Learning;
606 PRM: Pattern Recognition Memory; PROTECT: Platform for Research Online to investigate
607 Genetics and Cognition in Aging; QoL-AD: Quality of life in Alzheimer's disease; ROI:
608 Region of Interest; RVP: Rapid Visual Information Processing; RTI: Reaction Time; SD:
609 Standard Deviation; SUVR: Standardised Uptake Value Ratio; SSP: Spatial Span; SWM:
610 Spatial Working Memory; TBV: Total Brain Volume; TE: Total Errors; TMT: Trail Making

611 Task; *TREM2*: Triggering Receptor Expressed on Myeloid cells 2; TSPO: Translocator
612 Protein; WM: White Matter.

613

614 **Supplementary material**

615 The online version contains supplementary material. [Supplementarymaterial.pdf](#)

616

617 **Declarations**

618 **Ethics approval and consent to participate**

619 Written informed consent for all research participants recruited to PHAGO was obtained
620 according to the Declaration of Helsinki 1975, as revised in 2008. Protocols and procedures
621 contributing to this work complied with the ethical standards of the relevant national and
622 institutional committees on human experimentation and were approved by the London-
623 Bloomsbury ethics committee (reference number 17/LO/1266).

624

625 **Consent for publication**

626 Not applicable

627

628 **Availability of data and materials**

629 The datasets used and analysed during the current study are available from the corresponding
630 author on reasonable request.

631

632 **Competing interests**

633 The authors report no competing interests

634

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659

660 **Authors' contributions**

661 AH and FT: study concept and design. OC, AC, PV, ZK, RA, AH, IV, CB, AC, DA, LV and
662 OH: participant recruitment. OC, AC and PV: clinical data collection. AH, BC and OC: genetic
663 sequencing and analysis. OC, AC, IR, MO, DC and OH: imaging data collection. EA, RA and
664 KS: manufacturing of [¹⁸F]DPA-714. OC, JS, MV, AH, OH and FT: data analysis. OC:
665 manuscript first draft. All authors read and approved the final manuscript.

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673

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