

1           **Cortical and subcortical changes in Alzheimer’s**  
2           **disease: a longitudinal and quantitative MRI study**

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26   Short title: Longitudinal and quantitative MRI in AD

28

29 **Abstract**

30 Quantitative MRI provides important information about tissue properties in  
31 brain both in normal ageing and in degenerative disorders. Although it is well  
32 known that those with Alzheimer's disease (AD) show a specific pattern and  
33 faster rate of atrophy than controls, the precise spatial and temporal patterns  
34 of quantitative MRI in AD are unknown. We aimed to investigate  
35 neuroimaging correlates of AD using serial quantitative MRI. In our study,  
36 twenty-one subjects with AD and thirty-two similar-aged healthy controls  
37 underwent two serial MRI scans at baseline and 12 months. Tissue  
38 characteristics were captured using two quantitative MRI parameters:  
39 longitudinal relaxation time (qT1) and transverse relaxation time (qT2). The  
40 two groups (AD and controls) were statistically compared using a voxel based  
41 quantification (VBQ) method based on Matlab and SPM8. At baseline,  
42 subjects with AD showed a significant reduction of qT1 and qT2 compared to  
43 controls in bilateral temporal and parietal lobes, hippocampus, and basal  
44 ganglia. This pattern was also observed at follow-up. Longitudinally, in AD we  
45 found a significant increase rather than further reduction of qT1 and qT2 from  
46 the baseline in bilateral hippocampus, thalamus and right caudate nucleus. In  
47 addition, the longitudinal change of qT1 in left hippocampus was negatively  
48 correlated with cognitive decline in AD over the 1-year period, and the general  
49 disease severity significantly predicted the amount of increase of qT1 in  
50 bilateral hippocampus over 12 months. The longitudinal change of qT2 in left  
51 parahippocampus correlated with change in neuropsychiatric features over  
52 time. In summary, quantitative MRI parameters were reduced in AD cross-  
53 sectionally, but increased over time, showing distinct spatiotemporal patterns

54 from the atrophy in AD. We also showed the clinical relevance of quantitative  
55 MRI parameters, indicating their potential promise as new imaging markers in  
56 AD.

57

58 (286 words)

59

60 **Keywords** quantitative MRI, VBQ, Alzheimer's disease, amyloid, relaxometry,  
61 early diagnostics

62

63 **Introduction**

64 One of the main current research focuses on Alzheimer's disease (AD) is  
65 early intervention, thus, there is a pressing need for reliable biomarkers for  
66 early AD detection. Existing biomarkers include cerebrospinal fluid (CSF),  
67 PET/SPECT imaging of neural metabolism and  $\beta$ -amyloid binding, as well as  
68 structural and functional MRI [1]. Among these techniques, MRI is  
69 noninvasive, so provides a powerful and safe tool for research and clinical  
70 diagnosis / screening, both of which are important for investigating the  
71 underlying neuropathology and early detection [2].

72

73 It has been shown that MRI not only reveals changes in brain volume and  
74 macrostructure, but also microstructural alterations in brain tissue integrity  
75 and its biochemical environment [3]. An emerging neuroimaging technique is  
76 quantitative MRI (qMRI) based on the physical measurement of longitudinal  
77 (qT1) and transverse (qT2) relaxation times. (Here we shall refer to these  
78 quantitative measurements as qT1 and qT2 to distinguish them from the more  
79 common qualitative radiological T1 or T2 weighted scans). In general, qT1 is  
80 correlated with water content and the level of myelination, and qT2 is related  
81 to chemically determined brain iron concentration [3].

82

83 Quantitative MRI provides important information about tissue properties in  
84 brain both in normal ageing [4,5], degenerative disorders such as Parkinson's  
85 disease [6], AD and dementia with Lewy bodies (DLB) [7], and neuronal loss  
86 due to brain and spinal cord injury [8]. In our previous study of DLB using  
87 qMRI, we demonstrated that the spatial pattern in the changes of qT1 and

88 qT2 was different from the pattern of atrophy, thus quantitative MRI may  
89 provide incremental benefit over and above that of structural MRI [7]. We also  
90 argue that quantitative MRI may indirectly detect neuronal and molecular  
91 changes in AD that precede the structural brain damage and clinical  
92 impairments decades in time [2]. Although it is well known that those with AD  
93 show a specific pattern and faster rate of atrophy than controls, the precise  
94 spatial and temporal patterns of quantitative MRI alteration in AD are  
95 unknown.

96  
97 In this study, we aimed to investigate neuroimaging correlates of AD using  
98 serial quantitative MRI. In particular, we evaluated maps of qT1 and qT2 in  
99 grey matter comparing these parameters between the AD and the similarly  
100 aged control groups using the voxel-based quantification (VBQ) approach [4]  
101 both cross-sectionally and longitudinally. In addition, we explored the clinical  
102 relevance of quantitative MRI changes over a 12-month period.

103

## 104 **Materials and Methods**

### 105 *Subjects, assessment and diagnosis*

106 Thirty-six subjects with probable AD [9] over the age of 60 were recruited from  
107 a community dwelling population of patients referred to local Old Age  
108 Psychiatry, Geriatric Medicine or Neurology Services. Thirty-five similarly  
109 aged control subjects were recruited from relatives and friends of subjects  
110 with dementia or volunteered via advertisements in local community  
111 newsletters. Twenty-one AD subjects and 32 controls underwent MR imaging

112 at both baseline and follow-up and are included in the analyses reported here.  
113 These subjects underwent clinical and neuropsychological evaluations at  
114 baseline and follow-up at 1 year. (The rest of subjects were not scanned at  
115 follow-up, thus excluded from the analysis.)

116

117 The research was approved by Newcastle & North Tyneside 1 Research  
118 Ethics Committee (No. 05/Q0905/217). All subjects or, where appropriate,  
119 their nearest relative, provided written informed consent. Assessment of  
120 global cognitive measures at both baseline and follow-up assessments  
121 included the Cambridge Cognitive Examination (CAMCOG) [10], which  
122 incorporates the Mini-Mental State Examination (MMSE) [11]. Motor  
123 parkinsonism was evaluated with the Unified Parkinson's Disease Rating  
124 Scale Part III (UPDRS-III) [12]. For subjects with dementia, neuropsychiatric  
125 features were evaluated with the Neuropsychiatric Inventory (NPI) [13].

126

#### 127 *MRI data acquisition*

128 Participants underwent MRI scanning on a 3T Philips Achieva MRI system  
129 with an 8-channel receiver head coil at both baseline and 12-month follow-up.  
130 Structural images were acquired using a T1 weighted volumetric sequence  
131 (3D MPRAGE, sagittal acquisition aligned with the AC-PC line, 1mm isotropic  
132 resolution, matrix 240×240×180, TR=9.6ms, TE=4.6ms, flip angle=8°, SENSE  
133 factor 2). In addition, a B0 field-map using a dual echo 3D GRE (2mm  
134 isotropic resolution, matrix 128×128×72, TR=27ms, TE=2.6/6.1ms) was  
135 acquired.

136

137 Quantitative mapping of tissue qT1 and qT2 relaxation times was performed  
138 using custom designed MRI sequences developed in-house under a research  
139 agreement with Philips Medical Systems.

140

141 Fast qT1 mapping was based on the inversion recovery (IR) methods  
142 originally published by Ordidge et al [14] and expanded by Clare and Jezzard  
143 [15]. The sequence imaged 72 axial slices spanning the brain, which were  
144 grouped into 5 consecutive slabs each of thickness 24mm. For each slab a  
145 slice selective adiabatic (sech) inversion pulse was applied to invert the  
146 magnetisation ensuring that the region of full inversion encompassed the 24  
147 mm thick section of interest. This inversion was followed by slice selective  
148 single shot EPI readout of 12 contiguous slices of 2 mm thickness equally  
149 spaced across the slab. The first slice was imaged 250ms post inversion and  
150 subsequent slices every 205 ms thereafter. During the repetition time (TR) of  
151 15000ms each slab was inverted and imaged with a slab order of 1,3,5,2,4 to  
152 minimise interaction between slices. In this way the time between inverting  
153 adjacent slabs was 6000ms, sufficient to allow full relaxation of brain tissue.  
154 The sequence was repeated 12 times and on each repetition the order of  
155 acquired slices within each slab was permuted by one position, for example  
156 1,2,3,4,5,6,7,8,9,10,11,12  
157 2,3,4,5,6,7,8,9,10,11,12,1  
158 3,4,5,6,7,8,9,10,11,12,1,2  
159 etc  
160 such that the at the end of acquisitions every slice had been imaged at each

161 of the 12 inversion times. Total scan time was therefore 180s for the  
162 complete IR series in 72 slices with isotropic 2mm resolution. This sequence  
163 is shown schematically in Figure 1.

164

165 Insert Figure 1 here

166

167 Calculation of qT1 maps used a purpose written algorithm in Matlab, which  
168 reordered the data into incremental inversion order for each slice and then  
169 performed voxel-wise non-linear least squares fitting to the standard 3  
170 parameter model for the inversion recovery experiment:

171

$$S(T_{IR}) = S_o(1 - \alpha e^{-\frac{T_{IR}}{qT1}})$$

172

173 where  $T_{IR}$  is the inversion time,  $S(T_{IR})$  is the signal value or the data obtained  
174 from the inversion recovery experiment, qT1 is the longitudinal relaxation  
175 time,  $S_o$  is the proton density and  $\alpha$  is the effective inversion efficiency . qT1,  
176  $S_o$  and  $\alpha$  were computed pixel-wise from the fitting process. Ideally  $\alpha$  is  
177 expected to be 2 but it was allowed to be a free variable in the fitting in order  
178 to increase the accuracy of the computed qT1. The 3D images of proton  
179 density  $S_o$ , qT1, and the goodness of fit were saved for evaluation.

180

181 Fast qT2 mapping used a multi-spin echo sequence with segmented EPI  
182 readout based around a Gradient and Spin Echo Imaging sequence. The  
183 sequence collected 8 spin echoes with equal spacing of 20ms and 5 gradient  
184 recalled echoes per spin echo (EPI factor 5) to accelerate image collection.



185 Repetition time was set to 4700ms and 72 slices were collected in standard  
186 interleaved acquisition (2mm isotropic resolution, matrix 128×128). Total  
187 scan time was 120s.

188

189 Calculation of qT2 maps again used a purpose written algorithm in Matlab,  
190 which performed voxel-wise non-linear least squares fitting to the standard 2  
191 parameter model for transverse relaxation.

192

$$S(TE) = S_0 e^{\frac{-TE}{qT2}}$$

193

194 where  $TE$  is the echo time,  $qT2$  is the transverse relaxation time and  $S_0$  is the  
195 proton density.  $qT2$ , and  $S_0$  were computed pixel-wise from the fitting  
196 process. The 3D images of proton density  $S_0$ ,  $qT2$ , and the goodness of fit  
197 were saved for evaluation.

198

199 Scans were collected using both sequences in aqueous and gel based test  
200 objects of known  $qT1$  and  $qT2$  and compared to data collection using a single  
201 slice inversion recovery sequence for  $qT1$  and a single slice Carr-Purcell  
202 Meiboom-Gill (CPMG) sequence for  $qT2$  to validate the method.

203

204 *Statistical Tests of Demographic, clinical, and cognitive measures.*

205 Group characteristics were evaluated with Statistical Toolbox of Matlab  
206 ([www.mathworks.co.uk/products/statistics](http://www.mathworks.co.uk/products/statistics)). Differences in demographic and  
207 clinical data were assessed with use of either t-tests for continuous variables

208 or  $\chi^2$  tests for categorical measures. For each test statistic, a probability value  
209 of  $p < 0.05$  was regarded as significant.

210

### 211 *Voxel-based quantification*

212 MRI data processing was performed in a combined FSL  
213 ([fsl.fmrib.ox.ac.uk/fsl/fslwiki](http://fsl.fmrib.ox.ac.uk/fsl/fslwiki)) and SPM ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) based on  
214 the previously validated voxel-based quantification (VBQ) procedure [4]. At  
215 both baseline and follow-up, raw qT1 and qT2 imaging datasets were  
216 corrected for field inhomogeneities using B0 maps and the PRELUDE/FUGUE  
217 algorithm in FSL [16]. Then, the bias corrected qT1 and qT2 maps were used  
218 for subsequent analysis.

219

220 Volumetric structural T1 weighted images were firstly segmented using  
221 Gaussian mixture model implemented in the VBM toolbox [17], and brain  
222 tissues were classified into grey matter (GM), white matter (WM) and  
223 Cerebrospinal fluid (CSF) for both baseline and follow-up. A conjunction GM  
224 brain mask was generated for each individual subject (in both AD and control  
225 groups) by computing the intersection between the GM probability maps at  
226 the baseline and that at the follow-up of the same subject, and then  
227 thresholded at  $p > 0.5$  in each participant's native space. This mask was used  
228 to select a common area of GM tissue at both baseline and follow-up in order  
229 to ensure there were equal number of voxels tested in both time points, thus  
230 avoiding potential bias in the statistical analysis.

231

232 As in standard longitudinal VBM procedure, only the baseline GM probability  
233 maps were non-linearly normalized to standard MNI space  
234 ([www.mni.mcgill.ca](http://www.mni.mcgill.ca)) using the diffeomorphic registration algorithm (DARTEL)  
235 [17] in SPM, and the resulting parameters were used to normalize the qT1  
236 and qT2 maps in both baseline and follow-up. The qT1 and qT2 maps at both  
237 time points were firstly co-registered with the GM probability maps derived  
238 from structural T1 images at baseline, then the thresholded conjunction GM  
239 brain masks were applied to qT1 and qT2 data at both time points. Finally, we  
240 transformed the GM maps of qT1 and qT2 MRI parameters into standard MNI  
241 space using the participant-specific diffeomorphic parameters estimated from  
242 the baseline scans based on the previous DARTEL procedure. However, we  
243 did not apply modulation to these quantitative MRI parameters in order to  
244 avoid confound of age and disease related GM volume changes. Finally, all  
245 normalized quantitative qT1 and qT2 maps were smoothed with an isotropic  
246 Gaussian kernel of 6 mm full width at half maximum.

247

248 For statistical analysis investigating disease induced regional microstructural  
249 alterations between the AD and control groups, and between the baseline and  
250 follow-up, we used the General Linear Model (GLM) with age and gender as  
251 covariates. Then, two-tailed t-tests were performed at each voxel to detect  
252 voxel-wise difference between the groups or time points. We also tested the  
253 group x time interaction using a mixed model ANOVA. The false positive rate  
254 was controlled using family-wise error (FWE) correction for multiple  
255 comparisons, and thresholded at  $p < 0.05$  at the cluster level.

256

257 *Post-hoc region-of-interest analysis*

258 To explore the clinical relevance of qT1 and qT2, in a post-hoc region-of-  
259 interest (ROI) analysis, we extracted the averaged quantitative MRI values  
260 from significant clusters found in the longitudinal comparison. Here, we used  
261 the unsmoothed maps in order to preserve the original values of the MR  
262 parameters. Then, we correlated cognitive and clinical measures with the  
263 averaged quantitative MRI values extracted from the significant clusters  
264 obtained from the previous group comparisons. These measurements  
265 included the CAMCOG, MMSE, UPDRS III and NPI total scores. Multiple  
266 comparisons were controlled using Bonferroni correction for the number of  
267 ROIs.

268

269 **Results**

270 *Demographic clinical and cognitive measures*

271 As shown in Table 1, there were no significant differences between AD and  
272 control groups for age, sex and educational level. However, as expected, the  
273 two groups significantly different at both baseline and follow-up for UPDRS III,  
274 NPI, MMSE and CAMCOG scores with subjects with AD scoring poorer in all  
275 measures compared to the controls.

276

277 Insert Table 1 here

278

279 *Cross-sectional comparison of qT1: AD vs. controls*

280 As shown in Figure 2A and Table 2A, at the baseline, we found a significant  
281 decrease in qT1 for the AD group compared to controls ( $p < 0.0001$ , FWE) in  
282 bilateral temporal, parietal and occipital lobes, as well as several subcortical /  
283 striatal nuclei. Largest significant clusters were within bilateral hippocampus,  
284 parahippocampus, cuneus, precuneus, caudate and putamen. At the follow-  
285 up, we found a very similar pattern of qT1 changes comparing between AD  
286 and control groups. See Figure 2B and Table 2B. No significant increase in  
287 qT1 was found for the AD group compared to controls at either baseline or  
288 follow-up.

289

290

Insert Table 2 here

291

292

Insert Figure 2 here

293

294 *Cross-sectional comparison of qT2: AD vs. controls*

295 For qT2 at the baseline, we also found a significant decrease for the AD group  
296 compared to controls ( $p < 0.0001$ , FWE) in left superior and right middle  
297 temporal lobes, bilateral hippocampus and left parahippocampus as shown in  
298 Figure 3A and Table 3A. At the follow-up, we found a very similar pattern of  
299 qT2 changes comparing between AD and control groups except for a cluster  
300 covering left caudate, putamen and pallidum, and a right cuneus cluster,  
301 which were not seen at the baseline. See Figure 3B and Table 3B. No  
302 significant increase in qT2 was found for the AD group compared to controls  
303 at either baseline or follow-up.

304

305

Insert Table 3 here

306

307

Insert Figure 3 here

308

309 *Longitudinal comparison of qT1: baseline vs. follow-up*

310 When comparing qT1 between baseline and follow-up in the AD group, we  
311 found a significant increase over 12 months in bilateral hippocampus and  
312 parahippocampus ( $p < 0.0001$ , FWE), thalamus ( $p < 0.0001$  for left and  $p = 0.002$   
313 for right, FWE) and right caudate ( $p < 0.0001$ , FWE) as shown in Figure 2C  
314 and Table 4A. The significant hippocampus clusters in the longitudinal  
315 comparison are more medial to the clusters found in the cross-sectional  
316 comparisons; see Figure 2. We found no longitudinal change for qT1 in the  
317 control group and no group (AD and controls) x time (baseline and follow-up)  
318 interaction.

319

320

Insert Table 4 here

321

322 *Longitudinal comparison of qT2: baseline vs. follow-up*

323 For qT2, we found a significant increase between the baseline and the follow-  
324 up in the AD group as shown in Figure 3C and Table 4B. Significant clusters  
325 are located in left parahippocampus ( $p < 0.0001$ , FWE), right caudate, putamen  
326 and insula ( $p < 0.0001$ , FWE), as well as right middle frontal lobe ( $p = 0.002$ ,  
327 FWE). We found a significant group x time interaction in right insula

328 ( $p < 0.0001$ , FWE) bordering with right putamen and extending to right caudate  
329 at lower threshold ( $p < 0.001$ , uncorrected). We argue that atrophy is unlike the  
330 explanation of this effect because if atrophy were a driver of an increase in  
331 qT2 over time, we would expect AD subjects at baseline to have a greater  
332 qT2 than controls because of their greater atrophy, a result opposite to what  
333 we found here.

334

335 For the control group, we found a significant longitudinal increase in qT2  
336 ( $p < 0.0001$ , FWE) in left superior parietal lobe, caudate head, and  
337 supplementary motor area extending to superior medial prefrontal cortex.  
338 (See Figure 3D.)

339

#### 340 *Correlation between cognitive / clinical measures and quantitative MRI*

341 Here, the main focus is on the relationship between longitudinal changes in  
342 quantitative MRI parameters and cognitive / clinical measures in the AD group  
343 at baseline, follow-up and change over the 1-year period. Thus for qT1, we  
344 have defined three region-of-interest (ROIs) based on the results of the  
345 longitudinal comparison of qT1, i.e. left / right hippocampus and right caudate.  
346 We found a significant negative correlation between the longitudinal changes  
347 in qT1 in left hippocampus and the cognitive decline (i.e. change in total  
348 MMSE score over the 1-year period) in AD ( $r = -0.58$ ,  $p = 0.006$ ). We found a  
349 significant correlation between baseline CAMCOG score and the changes of  
350 qT1 in bilateral hippocampus over the 1-year period. In addition, a significant  
351 correlation was found between baseline UPDRS III score and the longitudinal

352 changes of qT1 in bilateral hippocampus. Both effects were stronger in the  
353 right hemisphere than in the left and only the right hippocampus survived the  
354 correction for multiple comparisons. (See Table 5A.) Nonetheless, this  
355 suggests that baseline CAMCOG and UPDRS scores predict changes in qT1  
356 in these regions, that is, the more severe the dementia in general, the more  
357 qT1 increases.

358

359 We also found a significant correlation between qT1 in right caudate and  
360 UPDRS score cross-sectionally at both baseline ( $r=0.60$ ,  $p=0.004$ ) and follow-  
361 up ( $r=0.51$ ,  $p=0.018$ ) in AD. Moreover, qT1 in right hippocampus was  
362 significantly correlated with the CAMCOG score only at the follow-up ( $r=-0.60$ ,  
363  $p=0.004$ ) but not at the baseline. (See Table 5A.) No other correlation  
364 survived the correction for multiple comparisons.

365

366

Insert Table 5 here

367

368 For qT2, we have extracted qMRI values from four significant clusters found in  
369 the longitudinal comparison: right insula extending to putamen, left  
370 parahippocampus, right caudate and right superior medial frontal cortex  
371 (BA8). We found a significant correlation between the longitudinal changes of  
372 qT2 in left parahippocampus and the changes in NPI score over time ( $r=0.54$ ,  
373  $p=0.012$ ) in AD. (See Table 5B.) No other correlation was found.

374

375 *Individual analysis in longitudinal comparison*



376 Figure 4 shows the individual variability in longitudinal changes in quantitative  
377 MRI within the AD group, and how it relates to disease severity. It can be  
378 seen in Figure 4A that subjects with AD showed a general increase in qT1 in  
379 right hippocampus, and the rate of increase seems to accelerate in moderate  
380 and severe cases of AD, i.e. baseline MMSE score lower than 20. The same  
381 pattern was observed in other clusters found in the longitudinal analysis.  
382 Figure 4B shows the individual changes in qT2 in left parahippocampus over  
383 time. We have found a very similar trend as in qT1 with subjects with AD  
384 generally showing an increase in qT2 over 12 months. We have also found  
385 similar pattern in other significant clusters derived in the longitudinal analysis  
386 in the AD group.

387

388 Insert Figure 4 here

389

## 390 **Discussion**

391 Using novel longitudinal and quantitative MRI method and voxel-based  
392 quantification, we showed that at the cross-sectional level, qT1 and qT2 in AD  
393 were significantly decreased in multiple cortical areas in bilateral temporal and  
394 parietal lobes with the largest changes in medial temporal structures  
395 comparing to similarly aged control subjects at baseline. This pattern is  
396 consistent with previous neuroimaging studies with AD showing significant  
397 volume reduction in these regions such as hippocampal, enthorinal and  
398 parahippocampal cortices [18].

399

400 At follow-up, we found a very similar pattern of quantitative MRI changes in  
401 both qT1 and qT2 comparing AD and control groups with the main affected  
402 brain areas in bilateral hippocampus and associated structures. This  
403 demonstrates the robustness of qMRI in reproducing a consistent pattern of  
404 quantitative MRI parameter changes at two different time points with 12  
405 months gap.

406

407 It is worth noting that both qT1 and qT2 parameters consistently picked up  
408 substantial changes in subcortical regions, e.g. bilateral caudate, putamen  
409 and thalamus. However, comparing to neocortex, in particular the  
410 hippocampus, these basal nuclei and thalamus have received much less  
411 attention in previous research, and conventional volumetric MRI showed a  
412 mixed picture of atrophy in these subcortical areas. For example, atrophy of  
413 putamen was found in DLB but not in AD [19], whereas putamen and thalamic  
414 atrophy were both shown in AD in a different study [20] reflecting a relatively  
415 high level of noise in volumetric measures in these regions and large inter-  
416 study variability. In contrast, quantitative MRI may detect neuronal and  
417 macromolecular changes in tissue environment, which does not rely on  
418 atrophy shown in structural T1 weighted MRI. Based on this, we argue that  
419 qMRI provides incremental benefit over and above that of structural MRI.

420 Quantitative MRI also offers a unique opportunity to detect early brain  
421 changes in AD before observable brain volume reduction can be reliably  
422 shown and cognitive / clinical impairments can be found [2].

423

424 Although we found that AD was characterized by a reduction in qMRI

425 parameters in cross-sectional comparison, in longitudinal comparison, we  
426 found that qT1 was increased rather than decreased in right caudate, bilateral  
427 hippocampus and parahippocampus, as well as in thalamus. We also found  
428 that qT2 was increased in right caudate, putamen and insula in addition to left  
429 parahippocampus in AD. Frontal changes in qT2 over time were also notable  
430 in AD. This spatial pattern is consistent with the development of atrophy in AD  
431 [21]. Increase in qT2 was found in healthy controls in left superior parietal  
432 lobe, left caudate head, and bilateral supplementary motor area extending to  
433 superior medial prefrontal cortex. However, none of these brain regions  
434 overlap with longitudinal qT1 / qT2 changes found in AD suggesting the  
435 increase of qT2 in AD and controls may reflect distinct underlying causes. The  
436 only significant group x time interaction was found in right insula extending to  
437 putamen and caudate nucleus.

438

439 To further understand the increase in qMRI over time in AD, we performed an  
440 individual analysis, which showed the changes of qT1 and qT2 over time in  
441 hippocampus, caudate and other clusters found in the longitudinal  
442 comparison. By sampling individual subjects that were at different stages of  
443 the disease indicated by their severity (i.e. baseline MMSE), we have pictured  
444 an overall trajectory of qT1 and qT2 in clinically diagnosed AD. It is notable  
445 that the rate of qMRI increase was slow at early stages of AD but accelerated  
446 at severe stage of AD. Thus, although qT1 and qT2 decreased in AD  
447 comparing with controls at the cross-section level, it is possible that qT1 and  
448 qT2 may increase over time in AD because plaque burden may stabilize or  
449 even fall in established stages of AD as the disease progresses. In addition,

450 we found that general disease severity (measured by different but related  
451 scales, i.e. CAMCOG and UPDRS) predicts the rate of change in qT1 in  
452 hippocampus showing a faster rate of increase at the severe stage of AD.

453

454 These changes in qMRI followed a trend that mirrors the inverted 'U' shape  
455 trajectory of brain  $\beta$ -amyloid over time [22] suggesting that at the severe stage  
456 of AD the brain  $\beta$ -amyloid (and/or other factors) may undergo a decrease. A  
457 more quantitative treatment of the parallels between the current results and  
458 the findings by Jack et al will require a deeper analysis of how the very  
459 different properties of the two might reflect how brain  $\beta$ -amyloid burden is  
460 captured by each imaging method, and the consequences of this for temporal  
461 trajectory of these variations. This analysis is outside the scope of the current  
462 paper, and the comparisons we provide here should be regarded as  
463 preliminary and illustrative. However, it is clear that such a 'U' shaped  
464 temporal pattern has not been found in atrophy in AD.

465

466 In addition, we showed the clinical relevance of quantitative MRI parameters  
467 in AD, i.e. the change of qT1 at left hippocampus was correlated with  
468 cognitive decline (annual changes in MMSE score) in AD. For qT2, the  
469 longitudinal change in right parahippocampus was significantly correlated with  
470 the annual changes in neuropsychiatry condition (NPI) in AD over time. In  
471 addition, we found a significant correlation between qT2 in right caudate  
472 nucleus and parkinsonism (UPDRS score) at the cross-sectional level (at both  
473 baseline and follow-up), suggesting the AD pathology may be present in basal  
474 ganglia structures, therefore causing motor parkinsonism in AD. Here, it is

475 interesting to see that qT2 in caudate was sensitive to the changes in UPDRS  
476 scores. This finding is also consistent with Wilson et al. [23], which showed  
477 that parkinsonian signs are strong predictors for the progression of AD.

478

479 In our previous cross-sectional study on dementia with Lewy bodies (DLB),  
480 we also found a significant reduction of qMRI parameters in DLB compared to  
481 controls [7]. In addition, we showed that the spatial pattern of qMRI alteration  
482 only partly overlapped with that of atrophy, making the latter an unlikely  
483 explanation for the qMRI decreases. In the current study, we have followed  
484 the similar analysis procedure in order to avoid partial volume effect due to  
485 atrophy. In seeking the possible underlying biochemical interpretation of these  
486 quantitative MRI parameters and their evolution over time, qT1 is  
487 predominantly influenced by water content but also relates to the degree of  
488 myelination [24,25,26]. Both qT1 and qT2 are also sensitive to the level of  
489 iron, which is present in amyloid plaques [27] and in microbleeds.

490

491 Although it is not possible to precisely determine the pathological changes  
492 associated with alterations in qT1 or qT2 in AD, it has been suggested that  
493 decreases in qT1 and qT2 might be associated with increases in amyloid  
494 burden and iron load in the brain based on histologically confirmed animal  
495 model of AD [28,29]. Consistent with our finding, decreased qT1 was found to  
496 be associated with an increase in  $\beta$ -amyloid deposition in 5xFAD transgenic  
497 mouse model of Alzheimer's disease compared with wild type mice [30]. In  
498 addition, decreased qT2 was found in APP/PS1 transgenic mouse model of

499 AD [31,32] probably reflecting a complex interaction between  $\beta$ -amyloid and  
500 iron concentration as well as other factors.

501

502 In a longitudinal study of  $\beta$ -amyloid plaque development in Tg2576 transgenic  
503 mice using qT2 relaxation time [33], it has been found that qT2 decreases  
504 with age (12 - 18 months) following an increase in plaque area, number and  
505 size in the brain. Although it is difficult to match the disease stage of the  
506 mouse model to humans with AD and it is not common to find atrophy in the  
507 animal model of AD, this finding is nonetheless consistent with our results  
508 showing decreased qT2 in medial temporal lobes in AD. It has also been  
509 shown in a study based on APP/PS1 transgenic mice model that qT2 is  
510 modulated by the level of amyloid in subiculum without histochemically  
511 detectable iron in the brain [34], providing promises in detecting the  
512 pathological process in AD at the earliest stages.

513

514 In addition to our previous findings on qMRI changes in DLB [7], as well as  
515 the established literature on animal qMRI correlates, the current data provided  
516 convergent evidence for the potential ability of quantitative MRI in detecting  
517 early changes of tissue property caused by neurodegenerative disease. Being  
518 able to apply this novel technique in human using relatively safe MRI method  
519 enables new means for early detection of AD and tracking its progression  
520 over time. This approach is also likely to close the gap between animal  
521 models of amyloidosis and studies on human AD in the context of drug  
522 discovery.

523

524 **Conclusion**

525 In summary, quantitative MRI parameters were reduced in AD cross-  
526 sectionally, but increased over time, showing distinct spatiotemporal patterns  
527 from the atrophy in AD. Our findings are consistent with animal model of AD  
528 showing quantitative MRI can provide information, which may reflect  
529 pathology such as amyloid burden and iron load. We also showed the clinical  
530 relevance of quantitative MRI, indicating their potential promise as new early  
531 imaging markers in AD. With reduced radiation, MRI is more suited for  
532 longitudinal studies than PET. Thus, longitudinal and quantitative MRI will be  
533 valuable in developing new treatments by tracking brain changes associated  
534 AD in vivo.

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550 **Reference**

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