

# Optic Radiations Microstructural Changes in Glaucoma and Association With Severity: A Study Using 3Tesla-Magnetic Resonance Diffusion Tensor Imaging

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1 **OPTIC RADIATIONS MICROSTRUCTURAL CHANGES IN**  
2 **GLAUCOMA AND ASSOCIATION WITH SEVERITY: A STUDY USING**  
3 **3TESLA-MAGNETIC RESONANCE DIFFUSION TENSOR IMAGING**

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37

38 **ABSTRACT**

39

40 **Purpose:**

41 To compare microstructural changes along the optical radiations and brain structure  
42 volumes between glaucoma and control subjects using in vivo magnetic resonance  
43 imaging and to analyze their association with severity of the disease.

44

45 **Methods:** 50 open-angle glaucoma subjects and 50 healthy age- and sex-matched  
46 controls underwent detailed ophthalmological examinations (including visual field  
47 testing (VF), funduscopy and Spectral-Domain Optical Coherence Tomography) as  
48 well as Diffusion tensor imaging (DTI) using a 3.0-Tesla MRI. Fractional anisotropy  
49 (FA), Mean Diffusivity, Radial Diffusivity (RD) and Axial Diffusivity (AD) were  
50 quantified semi-automatically along the optical radiations. DTI parameters and  
51 volumes of specific brain structures were compared between cases and controls  
52 using conditional logistic regression. Association between DTI metrics and the  
53 severity of the disease was studied using linear mixed regression analyses.

54 **Results:** In glaucoma subjects, optic radiations FA was significantly lower (0.57 vs  
55 0.59;  $p= 0.02$ ) and RD was significantly higher ( $52.78 \cdot 10^{-5} \text{ mm}^2/\text{s}$  vs  $49.74 \cdot 10^{-5}$   
56  $\text{mm}^2/\text{s}$ ;  $p= 0.03$ ) than in controls. Optic radiations FA was significantly correlated with  
57 homolateral functional and structural damage of glaucoma (mean deviation of VF  
58 ( $p=0.03$ ), retinal nerve fiber layer thickness ( $p=0.03$ ), vertical cup/disc ratio  
59 ( $p=0.0007$ )). Volume and DTI parameters of other brain structures (including  
60 hippocampus) were not significantly different between glaucoma and controls.

61 **Conclusion:** We evidenced microstructural modifications along visual pathways of  
62 glaucoma patients and these alterations were correlated with disease severity. The  
63 association of glaucoma with other neurodegenerative alterations would need further  
64 exploration and a prospective follow-up of our cohort of subjects.

65

66 **Keywords:** glaucoma, fractional anisotropy, 3T MRI, optic radiations, DTI

67

68

## 69 INTRODUCTION

70

71

72 Glaucoma affects 64 million people and is the first cause of irreversible blindness,  
73 worldwide<sup>1,2</sup>. It encompasses a group of disorders characterized by progressive  
74 degeneration of the optic nerve head, loss of retinal ganglion cells and a  
75 corresponding pattern of visual field loss<sup>3</sup>. Primary open-angle glaucoma (POAG) is  
76 the predominant form of glaucoma in Western countries. Although some risk factors  
77 for POAG have been identified (high intraocular pressure, age, high myopia, ethnicity  
78 and heredity), several aspects of its pathophysiology remain unclear. As the disease  
79 could also affect intra-cerebral visual pathways in addition to optic nerve head  
80 degeneration, a neurodegenerative hypothesis raises concerns<sup>4,5</sup>

81 Central visual pathway degeneration in glaucoma was first suggested in experimental  
82 and histological studies, which have evidenced that glaucoma is not strictly limited to  
83 the optic nerve<sup>6,7</sup>. In an animal model of ocular hypertension, brain changes were  
84 observed in the lateral geniculate nucleus and superior colliculus, in parallel with  
85 retinal ganglion cells loss<sup>8</sup>. In another study, grey matter of glaucoma patients was  
86 reduced compared to healthy subjects, in the approximate retinal lesion projection  
87 zones in the visual cortex<sup>9</sup>. Moreover, a clinicopathological case in humans  
88 highlighted a neural degeneration in intracranial optic nerve, lateral geniculate  
89 nucleus and visual cortex<sup>10</sup>. This paradigmatic shift is further supported by several  
90 other small-sized clinical studies using brain magnetic resonance imaging, showing  
91 reduced volume of all the visual pathways (optic tracts, optic chiasm, lateral  
92 geniculate nucleus, optic radiations) measured at 1.5T<sup>11-13</sup> or 3T field strength<sup>14,15</sup>.

93 Some other experimental studies may help understand the pathogenesis of the  
94 disease. Using in vivo MRI studies is a way to study metabolic and spatiotemporal  
95 changes in glaucoma<sup>16-18</sup>.

96 In addition, epidemiological studies have also suggested that glaucoma might be  
97 associated with other neurodegenerative disorders, in particular Alzheimer's  
98 disease<sup>19,20</sup>, and a few studies have found a reduced volume of brain structures,  
99 beyond the visual pathways – particularly in the hippocampus – which is well known  
100 to be affected in Alzheimer's disease<sup>14,21,22</sup>. These data have nevertheless been  
101 collected in patients with long standing disease. Whether subtle alterations  
102 suggestive of associated neurodegenerative disease can be captured from the early  
103 stage of glaucoma, prior to atrophy, is unknown.

104 Recent improvements in neuroimaging techniques allow more accurate evaluation of  
105 brain structure volumes and intra-cerebral microstructural damage. By quantifying  
106 microscopic movements of water molecules, Diffusion Tensor Imaging (DTI) – a  
107 functional MRI technique – provides a sensitive evaluation of underlying brain  
108 microstructural changes even prior to atrophy<sup>23</sup>. Therefore, this technique appears  
109 particularly promising in the documentation of intra-cerebral damage in glaucoma.  
110 The most commonly assessed DTI parameters include fractional anisotropy (FA,  
111 which reflects the degree of cellular structural alignment within fiber tracts and the  
112 structural integrity of the fiber tracts) and mean diffusivity (MD, which measures the  
113 average motion of water molecules independently of fiber directionality).

114

115 Several case-control studies have already shown that FA of the optic radiations is  
116 decreased and MD increased in glaucoma patients<sup>24,25</sup>, and some others have  
117 suggested that these changes may be progressive with increasing axon loss of the

118 optic nerve<sup>26</sup>. Whereas these studies provide new insights in the understanding of  
119 glaucoma disease, they were limited in sample size and mainly included advanced  
120 glaucoma patients.

121

122 Therefore, our study aimed at exploring the potential neurodegenerative hypothesis  
123 associated with glaucoma and whether subtle changes could be measurable at the  
124 early stage of the disease. Thus we analyzed both the microstructural changes of the  
125 visual pathway, in relation with glaucoma severity, as well as changes beyond the  
126 visual pathway, in particular in regions affected in neurodegenerative pathologies.

127

128

## 129 **METHODS**

130

### 131 **Patient Population**

132 This study is an observational case-control study performed at the University Hospital  
133 of Bordeaux. Fifty patients with POAG (20 men, 30 women, mean age 61.9 +/- 6.9  
134 years) and 50 age- and sex-matched controls (20 men, 30 women, mean age 61.9 +/-  
135 7.0 years) were prospectively included.

136 This research followed the tenets of the Declaration of Helsinki. Participants gave  
137 written consent for the participation in the study. The design of this study was  
138 approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes  
139 Sud-Ouest et Outre-Mer III) in March 2012. This study was registered on the website  
140 <http://clinicaltrials.gov/> (identifier NCT01621841).

141

### 142 **Ophthalmological Examination**

143 All participants underwent a complete ophthalmic examination including measurement  
144 of best-corrected visual acuity, intraocular pressure (IOP) using Goldmann aplanation

145 tonometry, gonioscopy, slit-lamp biomicroscopy and optic disc examination by  
146 funduscopy. Central corneal thickness and anterior chamber depth were assessed  
147 using interferometry (OCT Visante, Carl Zeiss Meditec, Inc., Dublin, CA, USA), and  
148 axial length measurement using IOL Master (Carl Zeiss Meditec, Inc., Dublin, CA,  
149 USA). All participants underwent a visual field testing (Octopus 101, Haag-Streit, Inc.,  
150 Bern, Switzerland) and only reliable tests (false-positive errors <15%, false negative-  
151 errors <15%, loss fixations <20%) were included. In addition, visual fields (VF) were  
152 reviewed and excluded in the presence of artefacts, such as eyelid or rim artifacts,  
153 fatigue effects, inattention, or inappropriate fixation.

154 A measurement of peripapillary Retinal Nerve Fiber Layer (RNFL) thickness was  
155 performed using Spectral-Domain optical coherence tomography (SD-OCT) (Cirrus,  
156 Carl Zeiss Meditec, Inc., Dublin, CA, USA). All images were acquired and reviewed by  
157 specially trained technicians of the study to control the quality of signal strength and  
158 accurate centration and segmentation of the RNFL circle scan acquisition. Signal  
159 strength lower than 6 or acquisitions with artifacts were excluded from the analysis.

160 Glaucoma subjects and controls received a questionnaire requesting for  
161 cardiovascular risk factors, familial history of glaucoma, ophthalmological diseases  
162 and current medications. Each participant underwent Mini Mental State Examination  
163 (MMSE)<sup>27</sup>.

164

165 Primary open-angle glaucoma was defined by the following criteria: the presence of  
166 glaucomatous optic neuropathy (defined as a loss of neuroretinal rim with a vertical  
167 cup-to-disc ratio [VCDR] of >0.7 or an intereye asymmetry of >0.2, with or without  
168 notching attributable to glaucoma) associated to compatible VF loss. This VF loss was  
169 defined as the presence of at least 3 contiguous non edge test points within the same



170 hemifield on the pattern deviation probability plot at  $P < 0.05$ , with at least 1 point  $P <$   
171  $0.01$ , excluding points directly above and below the blind spot, and the presence of  
172 glaucomatous hemifield test results outside normal limits. Iridocorneal angle opening  
173 was graded 3 or 4 on gonioscopy using Schaeffer classification.

174 Controls were defined as normal optic disc without notching or abnormal thinning of  
175 the neuroretinal rim, no visual field defects, IOP measurement  $< 21$  mmHg and no  
176 family history of glaucoma.

177 Exclusion criteria included any diseases that could affect the visual field, secondary  
178 glaucoma including exfoliative and pigmentary glaucoma, diabetes mellitus, any  
179 neurological or psychiatric disorders, and a score  $< 26$  on the MMSE for global  
180 cognition. We also excluded participants according to standard MRI exclusion criteria  
181 such as claustrophobia, ferromagnetic implants or pacemakers, and inability to lie still  
182 for the MRI acquisition time.

183 Stage of severity of glaucoma was classified according to the Hodapp-Parrish-  
184 Anderson classification<sup>28</sup>. The different stages are:

- 185           • Stage 0: no or minimal defect
- 186           • Stage 1:  $MD \geq -6.0$  dB (early defect)
- 187           • Stage 2:  $-12.0 \geq MD \geq -6.0$  dB (moderate defect)
- 188           • Stage 3:  $-20 \geq MD \geq -12.0$  dB (advanced defect)
- 189           • Stage 4:  $MD \geq -20.0$  dB (severe defect)
- 190           • Stage 5: End-stage disease

191

192

193 **MRI Data Acquisition**

194 MRI examinations were performed on a 3-T Discovery MR750w scanner (GE Medical  
195 Systems, Milwaukee, WI, USA) using a 32-channel phased array head coil within 30  
196 days following the ophthalmologic examinations. The protocol included a DTI  
197 sequence to look for microstructural alterations along and beyond the optic radiations,  
198 a 3D-T1-wi sequence to look for global or focal atrophy. The parameters of  
199 acquisitions were as follows. The DTI sequence consisted in dual echo-planar  
200 imaging: 40 axial slices; repetition time, 12000 ms; echo time, 100.9 ms; slice  
201 thickness, 3.5 mm; matrix, 160x160; field of view, 24 cmx24 cm; b values, 0 and 1000  
202 s/mm<sup>2</sup> applied in 32 non-collinear directions. The 3D-T1 was an inversion recovery  
203 gradient echo sequence: 288 slices; repetition time, 11.4 ms; echo time, 4.3 ms;  
204 inversion time, 400 ms; flip angle, 15°; slice thickness, 0.8 mm; matrix, 384x384; field  
205 of view, 25 cmx25 cm.

206

## 207 **Image Processing**

### 208 **Measurement of DTI metrics along the optic radiations**

209 From the DTI data, the distortions induced by eddy currents were first corrected, then  
210 a diffusion tensor model was fitted at each voxel using Olea Medical<sup>®</sup> software to  
211 generate fractional anisotropy (FA) maps and to investigate the microstructural  
212 integrity of the optic radiations. The optic radiations were identified using deterministic  
213 tractography between two seed-regions of interest (ROIs) over the proximal and distal  
214 optic radiations according to previously published method and landmarks<sup>29</sup>. The  
215 proximal ROI was placed near the lateral geniculate nuclei, while the distal ROI was  
216 placed just anterior to its termination in the visual cortex. Fiber tract propagation was  
217 terminated for FA<0.2 and angle<35° based on agreed-upon thresholds. ROIs were  
218 placed by a specialized neuroradiologist symmetrically, based on color-coded FA maps

219 and trace DTI images on the anterior and posterior part of the expected pathway of the  
220 optic radiations (green boxes on Figure 1). Fibers whose directions did not correspond  
221 to the optic radiations based on anatomic knowledge and DTI-derived atlas were  
222 excluded by adding additional ROIs and a logical “not” function<sup>30</sup> (red boxes on Figure  
223 1). Only fibers that connected the anterior and posterior regions of interest were  
224 selected for further analysis. The analysis was independently repeated for a subset of  
225 cases (n=25 out of the 100 cases) by a specialized ophthalmologist with an inter-  
226 reader agreement of 0.88.

227 The median FA and its sub-component (axial and radial diffusivity, AD and RD  
228 respectively) as well as the mean diffusivity were measured along the reconstructed  
229 optic radiations (green streamlines on Figure 1). Decreased FA and increased MD  
230 values is usually considered as a proxy of axonal disruption<sup>31</sup>.

231 None of the people participating in FA measurements had any access to the  
232 case/control status of the participants, nor to any other clinical data.

233

#### 234 **MRI volumetric measurements**

235 For volumetric analyses, T1-weighted images were processed using the volBrain  
236 system (<http://volbrain.upv.es>). After denoising<sup>32</sup>, images were affine-registered<sup>33</sup> into  
237 the Montreal Neurological Institute space and the total brain volume was estimated  
238 using the Nonlocal Intracranial Cavity Extraction method<sup>34</sup>. Hippocampus was  
239 segmented using patch-based multi-template approach<sup>35</sup> following the international  
240 consortium from the EADC-ADNI Harmonized Protocol for anatomical definitions of the  
241 hippocampus<sup>36</sup>. To control variations in head size between subjects, total brain  
242 volumes and hippocampal volumes were scaled using the volumetric scaling factor  
243 determined through the affine registration to the MNI brain template.

244

245 For DTI analysis within the hippocampus masks, an in-house pipeline (dtiBrain) was  
246 used to process diffusion-weighted images. First, diffusion-weighted images were  
247 affine-registered to the T1w MRI in the MNI space<sup>32</sup>. Then, to compensate for EPI  
248 distortion, a non-rigid registration was performed. Finally, a diffusion tensor model was  
249 fitted at each voxel using FSL 5.031 (fmrib.ox.ac.uk/fsl), generating FA and MD maps.  
250 Mean FA and MD were measured within the hippocampal masks previously generated  
251 on anatomical T1-weighted MRI.

252

### 253 **Statistical analysis**

254 Statistical analysis was performed using SAS 9.3 (SAS Institute Inc, Cary, NC).  
255 Differences of MRI characteristics between glaucoma subjects and healthy controls  
256 were tested using logistic conditional analyses, for parameters both along optic  
257 radiations and outside the visual pathway (globally for white and grey matter and in  
258 hippocampal and amygdala structures). Additionally, within the group of patients with  
259 glaucoma, we used mixed linear regression analyses, adjusted for sex and age (as a  
260 continuous variable expressed in years), to test the associations between optic  
261 radiation DTI parameters (FA, MD, AD, RD) and the parameters of severity of the  
262 disease (VCDR, mean deviation of VF and RNFL). This type of analysis allows taking  
263 into account both right and left sides of each patient, while taking into account the  
264 intra-individual correlation between sides. In particular, this allowed studying the  
265 associations of ocular parameters with homolateral (right optic radiation with right eye  
266 and left with left eye) and contralateral (right optic radiation with left eye and vice  
267 versa) optic radiations MRI parameters. In these regression analyses, both ocular and

268 brain parameters were entered as z-scores. In addition, for RNFL, we also adjusted for  
269 axial length, which is strongly associated with RNFL<sup>37</sup>.

270

## 271 **RESULTS**

### 272 **Demographic and ophthalmological characteristics**

273 As shown in Table 1, cases and controls were similar for age, gender, history of  
274 cardiovascular diseases or risk factors and MMSE. Family history of glaucoma was  
275 reported by 58 % of glaucoma patients, and 0 % of controls (since this was an  
276 exclusion criterion for controls).

277 As shown in Table 2, cases and controls did not significantly differ for visual acuity  
278 (distance and near), intraocular pressure and axial length. As expected, they were  
279 significantly different for central corneal thickness, VCDR, RNFL thickness and visual  
280 field parameters. Similar results were observed for the left eye (Table 3).

281 In our study, 70% of glaucoma patients had an early stage of the disease, 20% a  
282 moderate stage and 10% an advanced or severe stage, according to the Hodapp-  
283 Parrish-Anderson classification.

284

### 285 **Comparison of MRI parameters along optic radiations between glaucoma and** 286 **control subjects**

287 One patient refused to do MRI examination and 3 MRI examinations were insufficient  
288 quality for analysis, leaving 49 glaucoma patients and 47 controls for the comparison  
289 of MRI parameters (Table 4). The optic radiations were similarly reconstructed for  
290 glaucoma and control subjects (similar length and volume and reconstructed  
291 streamlines). Glaucoma patients showed significantly lower FA along the optic  
292 radiations than controls (0.57 vs 0.59,  $p=0.02$ ), which was driven by significant

293 increase in radial diffusivity ( $52.8 \cdot 10^{-5} \text{ mm}^2/\text{s}$  vs  $49.7 \cdot 10^{-5} \text{ mm}^2/\text{s}$ ,  $p=0.03$ ) while axial  
294 diffusivity was unchanged. Mean diffusivity tended to be slightly higher in glaucoma  
295 patients, but this did not reach statistical significance ( $82.4 \cdot 10^{-5} \text{ mm}^2/\text{s}$  vs  $80.6 \cdot 10^{-5}$   
296  $\text{mm}^2/\text{s}$   $p=0.10$ ).

297

298

299 **Associations of homo- and contralateral optic radiation parameters with the**  
300 **severity of the disease in glaucoma patients**

301 Table 5 shows the associations of optic radiation parameters (FA, MD, RD and AD)  
302 with the ophthalmological parameters of glaucoma severity evaluated by visual field,  
303 optic disc cupping and RNFL thickness, only among patients with glaucoma ( $n=50$ ).

304 We tested associations of ophthalmological parameters with MRI parameters on the  
305 homolateral (right eye – right optic radiation and left eye – left optic radiation) and  
306 contralateral (right-left and left-right) sides. For the homolateral side, significant  
307 associations were found between optic radiations FA and mean deviation of the visual  
308 field ( $\beta= -0.22$ ;  $p= 0.03$ ), VCDR ( $\beta= -0.42$ ;  $p= 0.0003$ ) and RNFL ( $\beta= 0.22$ ;  $p= 0.03$ ).

309 The direction of the association is opposite for RNFL, since RNFL decreases with  
310 higher severity of glaucoma, while other parameters increase with severity. Mean and  
311 radial diffusivities increased with the severity of the disease measured by VCDR,  
312 ( $p<0.006$  and  $p<0.0008$ , respectively), but were not significantly associated with mean  
313 deviation of VF or RNFL thickness. By contrast, axial diffusivity, as well as length and  
314 volume of optic radiations were not significantly associated with any of the severity  
315 parameters.

316 With regard to the contralateral side, associations of MRI parameters with glaucoma  
317 severity parameters were much weaker, and reached statistical significance only for  
318 the association of FA and RD with VCDR ( $p=0.01$  and  $p=0.02$ , respectively).

319

320 **Brain volumes analyses between cases and controls**

321 Finally, we did not evidence any statistically significant difference between glaucoma  
322 subjects and controls for volumes and DTI parameters of cerebrum white and grey  
323 matters, hippocampus and amygdala (Table 6).

324

325

326 **DISCUSSION**

327 Our study demonstrates microstructural changes of the optic radiations in glaucoma,  
328 as evaluated by lower FA driven by higher RD, and a correlation between the level of  
329 structural modifications and disease severity.

330 Using MRI at 1.5T<sup>38</sup> or 3T<sup>25,26,39</sup> a few case-control studies have also reported such  
331 modifications of diffusion parameters in optic radiations of glaucoma patients. All these  
332 studies found significantly lower FA in glaucoma patients compared with control  
333 patients. In the present study, which included 70 % of early stages of glaucoma, FA  
334 differences between cases and controls are numerically small (about 0.02 for a mean  
335 of about 0.60, i.e about 3.3 %). However, the standard deviation is also small (about  
336 0.04), showing low inter-individual variability in this parameter, and the difference is  
337 substantial when related to the standard deviation (about 0.5 SD), suggesting a major  
338 effect of glaucoma on this highly conserved parameter. In other studies, the  
339 differences in FA of optic radiations observed between glaucoma patients and controls  
340 were larger, but these studies generally included more severe cases. The study by  
341 Engelhorn et al included 22 severe glaucoma cases, and observed a difference in FA  
342 of optic radiations ranging from 17 % to 30 % according to the localization (anterior,  
343 central, posterior)<sup>39</sup>. The study by Murai et al included 18 severe glaucoma cases, 9  
344 moderate and only 2 mild, and observed a 14 % difference in FA of the optic

345 radiations<sup>38</sup>. The study by Garaci et al included 4 pre-perimetric glaucoma cases, 4  
346 early, 4 moderate and 4 severe cases and observed a 36 % differences in FA of the  
347 optic radiations<sup>25</sup>. Finally, the study by Chen et al included a majority of severe cases  
348 (36 eyes with MD>9.5 dB out of 50) but did not report numerically the averages of  
349 optic radiations FA<sup>26</sup>.

350 Furthermore, we observed higher RD value in glaucoma patients and its correlation  
351 with disease severity whereas AD was not significantly different between glaucoma  
352 and control patients. Although, AD and RD are the two components of FA, these  
353 parameters have been scarcely analyzed in the literature and some studies have  
354 already reported increasing RD in glaucoma subjects compared to controls<sup>24</sup>.

355 Even though the underlying pathological alterations are not specifically known, animal  
356 studies have suggested that higher RD could mainly represent myelin loss while lower  
357 AD could be a more specific marker of neuronal loss. However these considerations  
358 were based on simplistic models and whether alterations of optic radiations truly  
359 predominate on myelin or axon component cannot be formally ascertained for  
360 glaucoma patients, for whom other modifications such as microglia activation may  
361 confound the data.

362 Additionally, we observed a trend towards higher mean diffusivity value in the  
363 glaucoma group without reaching statistical significance. However, we observed a  
364 significant positive correlation between mean diffusivity and disease severity  
365 measured with VCDR. Two studies also showed higher mean diffusivity in glaucoma  
366 patients<sup>25,26</sup>. While FA measures the degree of cellular structural alignment within fiber  
367 tracts and their structural integrity, mean diffusivity measures the average motion of  
368 water molecules independently of fiber directionality and is considered as an additional  
369 marker of axonal disruption. As these studies included patients with advanced



370 glaucoma, our findings might be explained by a lack of statistical power and a lower  
371 grade of disease severity in our glaucoma group. Such converging evidence of loss of  
372 fiber integrity in optic radiations in glaucoma cannot be measured in terms of length  
373 and volume of the optic radiations, which were similar in both groups probably  
374 because our measurements were made prior to fiber loss or major disorganization.

375

376 We also observed an association of diffusivity parameters (mainly FA and RD), with  
377 the severity of glaucoma (assessed by mean deviation of the visual field, VCDR and  
378 RNFL measured with SD-OCT), suggesting that microstructural changes to the optic  
379 radiations is one of the components of the severity that could participate in the  
380 clinical status of the patients and the alteration of the visual field. Although we mainly  
381 included early and moderate glaucoma as defined by the Hodapp-Parrish-Anderson  
382 classification, our findings are consistent with some previous studies, which included  
383 more advanced cases<sup>26,38-40</sup>. All these studies also evidenced significant  
384 associations of optic radiations FA with structural parameters of optic nerve head  
385 degeneration evaluated with VCDR or time-domain RNFL thickness, as well as  
386 functional visual field alterations. For example, Michelson *et al.* found a correlation  
387 between FA and visual field<sup>40</sup>. Thus, all these results illustrate the fact that FA could  
388 be a strong biomarker of glaucoma severity.

389 Our study also assessed the associations of glaucoma severity according to FA of  
390 homolateral and contralateral optic radiations. Interestingly, glaucoma severity  
391 parameters – in particular VCDR – were more strongly associated with homolateral  
392 optic radiations diffusion parameters than with contralateral parameters. As chiasmatic  
393 decussation of optic pathways results in approximately 50% crossing of axons on the  
394 contralateral side<sup>41</sup>, we would expect similar associations of glaucoma severity

395 parameters with homolateral and contralateral diffusion parameters. However, our  
396 findings might also be related to an increased vulnerability of some specific retinal  
397 nerve fiber bundles of the optic nerve head resulting in an atrophy of optic radiations  
398 more predominant on the homolateral side of the decussation than on the  
399 controlateral. Indeed, several studies have demonstrated a specific vulnerability of the  
400 temporal and temporal-inferior sides of the optic nerve head to glaucoma damage<sup>42-44</sup>.  
401 Thus, we could expect an increased atrophy of the corresponding optic radiation  
402 predominant on the homolateral side that could explain our findings. However, even if  
403 temporal and temporal-inferior nerve fiber layers are more vulnerable to glaucomatous  
404 damage, the meaning of our findings should be interpreted with caution and would  
405 need further exploration to be confirmed and to identify the exact underlying  
406 mechanism. Indeed, distribution of RNFL is not homogeneous around the optic nerve  
407 head with superior and inferior sectorial RNFL thicker than nasal or temporal RNFL  
408 sectors. Furthermore the mean optic disc-fovea angle delimitating superior and  
409 inferior nerve fiber layers, is around 8°<sup>45</sup>. Thus the exact distribution of nerve fiber  
410 layers of the retina that decussates to the contralateral optic tract or remains on the  
411 ipsilateral optic tract and finally leads to a vertical delimitation through the fovea on the  
412 hemivisual field test remains unclear. Hence, in our study, the corresponding optic  
413 radiations in the homo or contralateral side could not be accurately matched to specific  
414 sectors of the retina or the optic nerve head.

415 Although high intraocular pressure is the main risk factor of glaucoma, this disease is  
416 increasingly considered as a neuro-ophthalmological and neurodegenerative  
417 disease<sup>46</sup>. Furthermore, there are still controversies on the association between  
418 glaucoma and some other neurodegenerative diseases as Alzheimer's disease. In  
419 particular, in a cohort of elderly subjects followed every 2 years, we observed an

420 association of POAG with incident dementia<sup>19</sup>. Volume changes beyond the visual  
421 system in glaucoma patients have also been reported in several studies but with  
422 inconsistent results. For instance, Frezzotti *et al.* reported that POAG patients had  
423 brain atrophy in some grey matter regions and the visual cortex<sup>21</sup>. By contrast,  
424 Williams *et al.* found five cerebral structures larger in the glaucoma group than in the  
425 control group<sup>22</sup>. Chen *et al.* revealed both a decreasing grey matter volume in some  
426 regions and an increasing grey matter volume in some others<sup>14</sup>. In the present study,  
427 we analyzed brain globally and focused on brain regions that are well known to be  
428 affected in the course of Alzheimer's disease – particularly hippocampus – and did not  
429 evidence any significant difference for any of the studied regions of interest, neither in  
430 volume nor in parameters of diffusivity. However, as we included subjects with MMSE  
431  $\geq 26$  at baseline, the risk of brain structures atrophy was probably limited. Regarding  
432 the hippocampus, results have been particularly inconsistent, since Frezzotti *et al.*  
433 reported decreased hippocampus volume in glaucoma patients<sup>21</sup>, while Williams *et al.*  
434 reported no significant difference of hippocampus volume between glaucoma and  
435 controls, but an increase in hippocampus volume with disease severity in patients with  
436 glaucoma<sup>22</sup>.

437 Such differences between study results may be explained by differences in study  
438 methodology, in particular regarding the selection of subjects and severity of the  
439 disease, MRI sequences used or definition of regions of interest. For instance, in a  
440 recent study by Frezzotti *et al.*, only severe cases of glaucoma (but not early) showed  
441 grey matter atrophy of the visual cortex and hippocampus<sup>47</sup>. The evolution of brain  
442 volume in the course of glaucoma and its association with other neurodegenerative  
443 diseases would need further investigation and prospective follow-up of subjects.  
444 Finally, functional MRI may offer new insights into the brain modifications associated

445 with glaucoma, as suggested by two recent studies, showing functional modifications  
446 of the visual cortex at the earliest stages of the disease<sup>47,48</sup>.

447

448

449 In conclusion, we confirmed microstructural changes of optic radiations in glaucoma  
450 and its association with glaucoma severity. In accordance with several other studies,  
451 DTI appears as an objective measurement for evaluating alterations of the visual  
452 pathways in glaucoma and provides new insight in the pathophysiological process of  
453 glaucoma. A prospective evaluation of our cohort of patients would be of interest to  
454 observe the evolution of these microstructural modifications of optic radiations and to  
455 analyze the evolution of brain volume in association with the evolution of glaucoma  
456 disease. DTI could represent a future way to explore central nervous system of  
457 glaucomatous subjects, leading to a better understanding of the pathophysiology and,  
458 potentially, to help clinical trials evaluate new therapeutic strategies based on  
459 neuroprotection or brain repair.

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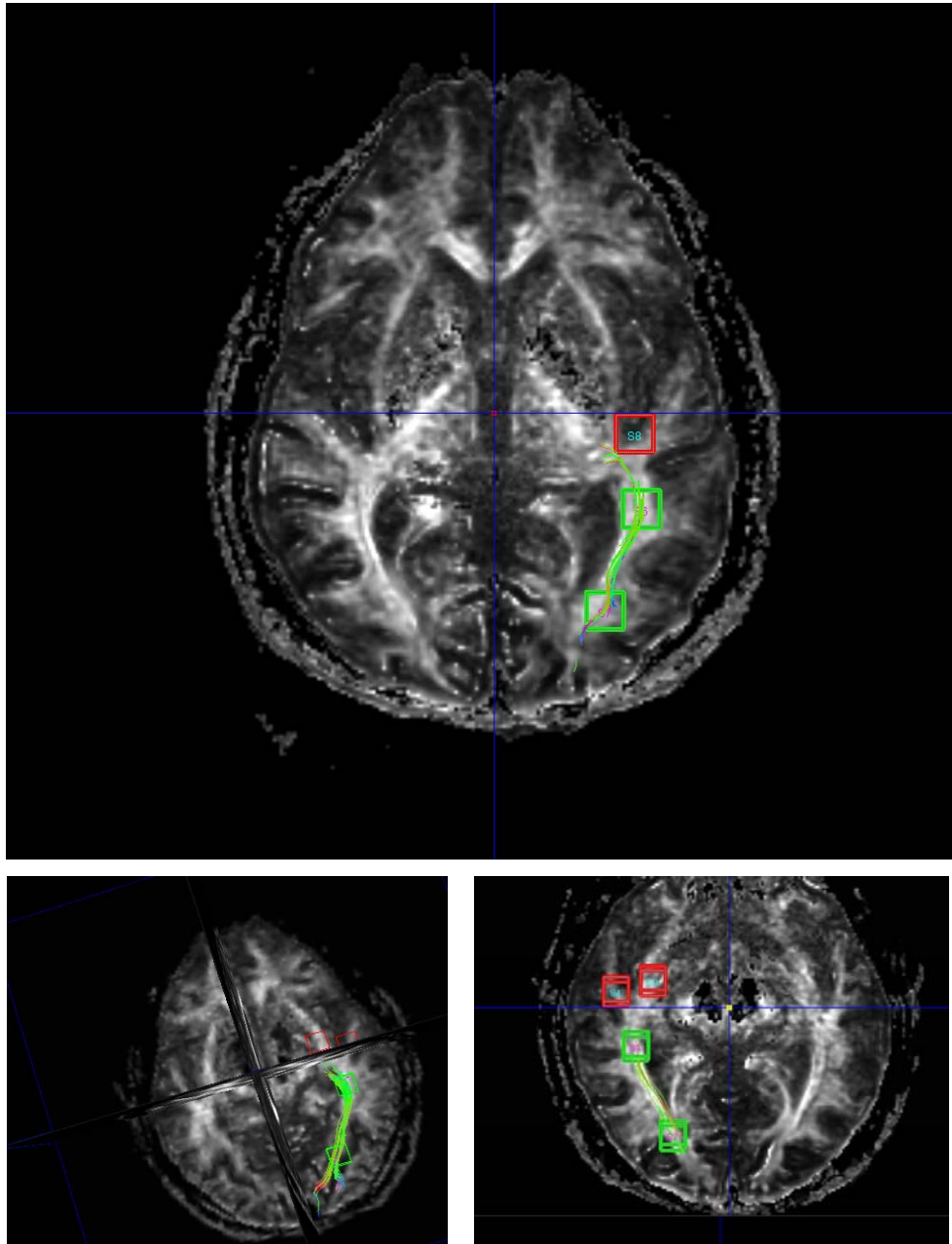
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**Figure 1. Building of optic radiations and measurement of fractional anisotropy, using the Olea Medical<sup>®</sup> software (image: CHU de Bordeaux, department of neuroimaging).**

Green boxes are the markers placed manually on anterior and posterior parts of the optic radiations. Green lines are optic radiations automatically reconstructed by the Olea Medical<sup>®</sup> software. Red boxes are markers manually placed to manually deleted fibers outside the expected area.





**Table 1: General characteristics**

<b>Characteristics</b>	<b>Glaucoma (n=50)</b>	<b>Control (n=50)</b>	<b>p-value*</b>
<b>Age</b> (years, mean(SD))	61.9 (6.9)	61.9 (7.0)	0.98
<b>Sex</b> (n, %)			1
Male	20 (40)	20 (40)	
Female	30 (60)	30 (60)	
<b>MMSE</b> (mean(SD))	29.3 (0.8)	29.4 (0.8)	0.7
<b>Family history of glaucoma</b> (n (%))			<0.0001
Yes	29 (58)	0 (0)	
No	16 (32)	50 (100)	
Unknown	5 (10)	0 (0)	
<b>Self-reported medical history</b>			
<b>Hypertension</b> (n (%))	11 (22)	12 (24)	0.81
<b>Hypercholesterolemia</b> (n (%))	14 (28)	12 (24)	0.65
<b>Hypertriglyceridemia</b> (n (%))	2 (4)	3 (6)	0.65
<b>Myocardial infarction</b> (n (%))	2 (4)	1 (2)	0.56
<b>Smoking</b> (n (%))			0.67
Never	29 (58)	25 (50)	
Former	17 (34)	19 (38)	
Current	4 (8)	6 (12)	

MMSE: Mini Mental State Examination

\* Chi-square for categorical variables and Student t-test for continuous variables

**Table 2: Ophthalmological characteristics; right eye (mean (standard deviation))**

<b>Characteristics</b>	<b>Glaucoma (n=50)</b>	<b>Control (n=50)</b>	<b>p-value*</b>
<b>Best-Corrected Visual Acuity (logMAR)</b>			
Distance	0.03 (0.09)	0.006 (0.02)	0.09
Near	0.19 (0.07)	0.18 (0.00)	0.2
<b>Central corneal thickness (µm)</b>	525.9 (30.9)	544.0 <sup>1</sup> (31.9)	0.005
<b>Axial Length (mm)</b>	24.1 (1.1)	23.7 (1.2)	0.15
<b>IOP (mmHg)</b>	14.6 (3.7)	14.7 (2.5)	0.82
<b>Vertical Cup : Disc Ratio</b>	0.8 (0.2) <sup>1</sup>	0.3 (0.2) <sup>1</sup>	<0.0001
<b>RNFL Thickness (µm)</b>	76.9 (17.7)	93.2 (9.9)	<0.0001
<b>Visual field (dB)</b>			
Mean Sensitivity	21.9 (6.1)	26.6 (1.1)	<0.0001
Mean Deviation	5.0 (6.0)	0.3 (1.0)	<0.0001
Loss Variance	22.6 (26.3)	5.0 (1.8)	<0.0001

RNFL: Retinal Nerve Fiber Layer

IOP: Intraocular Pressure

\* Chi-square for categorical variables and Student t-test for continuous variables

<sup>1</sup> One missing data

**Table 3: Ophthalmological characteristics; left eye (mean (standard deviation))**

<b>Characteristics</b>	<b>Glaucoma</b> (n=50)	<b>Control</b> (n=50)	<b>p-value*</b>
<b>Best-Corrected Visual Acuity</b> (logMAR)			
Far	0.03 (0.08)	0.006 (0.02)	0.08
Near	0.2 (0.1)	0.2 (0)	0.1
<b>Central corneal thickness</b> (µm)	524.6 (30.0) <sup>1</sup>	542.9 (28.4) <sup>2</sup>	0.003
<b>Axial Length</b> (mm)	24.0 (1.0)	23.5 (0.8)	0.003
<b>IOP</b> (mmHg)	14.2 (3.2)	15.2 (2.4)	0.09
<b>Vertical Cup : Disc Ratio</b>			
Funduscopy	0.8 (0.2)	0.3 (0.2)	<0.0001
<b>RNFL Thickness</b> (µm)	70.1 (18.7)	93.1 (9.7)	<0.0001
<b>Visual field</b> (dB)			
Mean Sensitivity: MS	21.8 (4.6)	26.6 (1.2)	<0.0001
Mean Deviation: MD	5.1 (4.5)	0.3 (1.1)	<0.0001
Loss Variance: LV	28.1 (28.0)	4.7 (2.2)	<0.0001

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RNFL: Retinal Nerve Fiber Layer

IOP: Intraocular Pressure

\* Chi-square for categorical variables and Student t-test for continuous variables

<sup>1</sup> One missing data<sup>2</sup> Two missing data

**Table 4: Comparison of MRI parameters along optic radiations between glaucoma and control subjects (mean (standard deviation))**

	<b>Glaucoma</b> (n=49)	<b>Control</b> (n=47)	<b>P-value*</b>
<b>Fractional anisotropy</b>	0.57 (0.04)	0.59 (0.03)	0.02
<b>Mean diffusivity</b> ( $10^{-5}$ mm <sup>2</sup> /s)	82.38 (6.43)	80.62 (4.87)	0.10
<b>Axial diffusivity</b> ( $10^{-5}$ mm <sup>2</sup> /s)	141.59 (7.14)	142.37 (6.74)	0.53
<b>Radial diffusivity</b> ( $10^{-5}$ mm <sup>2</sup> /s)	52.78 (6.74)	49.74 (5.04)	0.03
<b>Length (mm)</b>	63.91 (6.80)	63.72 (7.15)	0.74
<b>Volume (cm<sup>3</sup>)</b>	20.79 (9.13)	24.96 (12.33)	0.11

\*P-value: logistic conditional analyses

**Table 5: Associations of homo- and contra-lateral optic radiation parameters with the severity of the disease in glaucoma patients**

	Visual field Mean Deviation		Funduscopy Vertical Cup/Disc Ratio		SD-OCT examination RNFL thickness	
	$\beta$ [95%CI]	P	$\beta$ [95%CI]	P	$\beta$ [95%CI]	P
<b>Homolateral</b>						
Fractional anisotropy	-0.22 [-0.41;-0.02]	<b>0.03</b>	-0.42 [-0.64;-0.21]	<b>0.0003</b>	0.22 [0.03;0.41]	<b>0.03</b>
Mean diffusivity	0.02 [-0.17;0.21]	0.80	0.29 [0.08;0.50]	<b>0.008</b>	-0.15 [-0.34;0.04]	0.12
Axial diffusivity	-0.11 [-0.31;0.08]	0.26	0.09 [-0.14;0.31]	0.45	-0.06 [-0.26;0.14]	0.56
Radial diffusivity	0.09 [-0.10;0.28]	0.33	0.38 [0.17;0.59]	<b>0.0006</b>	-0.18 [-0.37;-0.007]	0.06
<b>Controlateral</b>						
Fractional anisotropy	-0.17 [-0.37;0.02]	0.09	-0.28 [-0.55;-0.06]	<b>0.01</b>	0.10 [-0.09;0.30]	0.30
Mean diffusivity	0.13 [-0.06;0.31]	0.19	0.20 [-0.02;0.41]	0.07	0.01 [-0.18;0.21]	0.91
Axial diffusivity	0.04 [-0.15;0.24]	0.67	0.07 [-0.16;0.30]	0.55	0.09 [-0.11;0.30]	0.34
Radial diffusivity	0.15 [-0.04;0.34]	0.11	0.25 [0.04;0.46]	<b>0.02</b>	-0.03 [-0.23;0.16]	0.72

CI: Confidence Interval; RNFL: Retinal Nerve Fiber Layer

P-value adjusted for age and gender, with additional adjustment for axial length for RNFL

**Table 6: Comparison of DTI parameters and MRI-based volume between glaucoma and control subjects (mean (standard deviation))**

	Cerebrum white matter			Cerebrum grey matter			Hippocampus			Amygdala		
	Glaucoma	Control	P- value*	Glaucoma	Control	P- value	Glaucoma	Control	P- value	Glaucoma	Control	P- value
	(n=48)	(n=48)		(n=48)	(n=48)		(n=48)	(n=48)		(n=48)	(n=48)	
Volume (cm <sup>3</sup> )	275.28 (115.61)	238.25 (59.03)	0.06	270.19 (59.03)	283.09 (38.17)	0.18	3.95 (0.53)	3.84 (0.46)	0.16	0.60 (0.23)	0.55 (0.18)	0.22
DTI parameters	<b>(n=41)</b>	<b>(n=41)</b>		<b>(n=41)</b>	<b>(n=41)</b>		<b>(n=41)</b>	<b>(n=41)</b>		<b>(n=41)</b>	<b>(n=41)</b>	
FA	0.38 (0.02)	0.37 (0.02)	0.39	0.17 (0.01)	0.17 (0.01)	0.45	0.19 (0.01)	0.19 (0.02)	0.44	0.15 (0.02)	0.15 (0.01)	0.09
MD (10 <sup>-5</sup> mm <sup>2</sup> /s)	78.34 (3.58)	78.19 (3.21)	0.79	121.70 (9.63)	120.01 (8.81)	0.25	98.83 (8.82)	97.72 (5.79)	0.42	80.45 (4.03)	80.56 (3.40)	0.87
AD (10 <sup>-5</sup> mm <sup>2</sup> /s)	110.91 (2.80)	110.35 (2.53)	0.26	138.84 (10.50)	136.96 (9.67)	0.24	116.86 (9.78)	115.46 (6.17)	0.39	92.64 (4.43)	92.21 (3.31)	0.57
RD (10 <sup>-5</sup> mm <sup>2</sup> /s)	62.24 (4.17)	62.27 (3.78)	0.96	113.20 (9.23)	111.60 (8.44)	0.26	90.02 (8.40)	88.90 (5.74)	0.44	74.35 (3.99)	74.73 (3.53)	0.57

Abbreviations: AD: Axial diffusivity; FA: Fractional anisotropy; MD: Mean diffusivity; RD: Radial diffusivity

\*P-value : logistic conditional analyses