

1 **Title: Aberrant visual pathway development in** 2 **albinism: From retina to cortex**

3 **Authors:** Sarim Ather¹, Frank Anthony Proudlock,² Thomas Welton^{3,4}, Paul S. Morgan^{4,5}, Viral
4 Sheth², Irene Gottlob², Rob A. Dineen^{3,4}

5 **Affiliations:**

6 **1.** Nuffield Department of Surgical Sciences, University of Oxford, Level 6, John Radcliffe
7 Hospital, Headington, Oxford, OX3 9DU, UK

8 **2.** University of Leicester Ulverscroft Eye Unit, Robert Kilpatrick Clinical Sciences Building,
9 Leicester Royal Infirmary, Leicester, LE2 7LX, UK

10 **3.** Radiological Sciences, Division of Clinical Neuroscience, University of Nottingham, Queen's
11 Medical Centre, Derby Road, Nottingham, NG7 2UH, UK

12 **4.** Sir Peter Mansfield Imaging Centre, University of Nottingham, Queen's Medical Centre, Derby
13 Road, Nottingham, NG7 2UH, UK

14 **5.** Medical Physics and Clinical Engineering, Nottingham University Hospitals NHS Trust, Queen's
15 Medical Centre, Derby Road, Nottingham, NG7 2UH, UK

16 **Correspondence to:**

17 Professor Irene Gottlob
18 University of Leicester Ulverscroft Eye Unit,
19 Robert Kilpatrick Clinical Sciences Building,
20 Leicester Royal Infirmary,
21 Leicester, LE2 7LX,
22 UK.
23 Email: ig15@le.ac.uk

24 **Running title:** Imaging the visual pathway in albinism

25

26 **Abstract:**

27 Albinism refers to a group of genetic abnormalities in melanogenesis that are associated neuronal
28 misrouting through the optic chiasm. Previous imaging studies have shown structural alterations at
29 different points along the visual pathway of people with albinism (PWA) including foveal hypoplasia,
30 optic nerve and chiasm size alterations and visual cortex reorganisation, but fail to provide a
31 holistic in-vivo characterisation of the visual neurodevelopmental alterations from retina to visual
32 cortex. We perform quantitative assessment of visual pathway structure and function in 23 PWA and
33 20 matched controls using optical coherence tomography (OCT), volumetric magnetic resonance
34 imaging (MRI), diffusion tensor imaging and visual evoked potentials (VEP).

35 PWA had a higher streamline decussation index (percentage of total tractography streamlines
36 decussating at the chiasm) compared to controls ($Z=-2.24$, $p=0.025$), and streamline decussation
37 index correlated weakly significantly with inter-hemispheric asymmetry measured using VEP
38 ($r=0.484$, $p=0.042$). For PWA, a significant correlation was found between foveal development index
39 and total number of streamlines ($r=0.662$, $p<0.001$). Optic nerve ($p=0.001$) and tract ($p=0.010$)
40 width, and chiasm width ($P<0.001$), area ($p=0.006$) and volume ($p=0.005$), were significantly smaller
41 in PWA compared to controls. Significant positive correlations were found between peri-papillary
42 retinal nerve fibre layer thickness and optic nerve ($r=0.642$, $p<0.001$) and tract ($r=0.663$, $p<0.001$)
43 width. Occipital pole cortical thickness was 6.88% higher ($Z=-4.10$, $p<0.001$) in PWA and was
44 related to anterior visual pathway structures including foveal retinal pigment epithelium complex
45 thickness ($r=-0.579$, $p=0.005$), optic disc ($r=0.478$, $p=0.021$) and rim areas ($r=0.597$, $p=0.003$). We
46 were unable to demonstrate a significant relationship between OCT-derived foveal or optic nerve
47 measures and MRI-derived chiasm size or streamline decussation index.

48 Non-invasive imaging techniques demonstrate aberrant development throughout the visual pathways
49 of PWA compared to controls. Our novel tractographic demonstration of altered chiasmatic
50 decussation in PWA corresponds to VEP measured cortical asymmetry and is consistent with

51 chiasmatic misrouting in albinism. We also demonstrate a significant relationship between retinal
52 pigment epithelium and visual cortex thickness indicating that retinal pigmentation defects in
53 albinism lead to downstream structural reorganisation of the visual cortex.

54

55 **Key words:** albinism, magnetic resonance imaging, diffusion tensor imaging, optical coherence
56 tomography, visual pathway.

57 **Abbreviations:** DTI=diffusion tensor imaging, LGN=lateral geniculate nucleus, OCT=optical
58 coherence tomography, MRI=Magnetic Resonance Imaging, ppRNFL=peripapillary nerve fibre
59 layer, PWA=people with albinism, RGC=retinal ganglion cell, ROI=region of interest, RPE=retinal
60 pigment epithelium, VEP=visual evoked potential,

61

62 **Introduction:**

63 Albinism refers to a group of genetic mutations that lead to abnormalities in the melanin synthesis
64 and transport pathway (Montoliu *et al.*, 2014; Kamaraj and Purohit, 2014). The phenotype of PWA
65 includes reduced visual acuity, foveal hypoplasia, nystagmus, increased crossing of nerve fibres at
66 the optic chiasm and changes in the visual cortex on MRI (Levin and Stroh, 2011).

67 In normal foveal development, anti-angiogenic molecules form a molecular barrier to form a “foveal
68 avascular zone” (FAZ) (Provis *et al.*, 2000; Provis, 2001; Provis and Hendrickson, 2008). In albinism,
69 this fails to happen leading to encroachment of the inner retinal layers into the fovea, a deficiency in
70 the formation of the foveal pit and a lack of photoreceptor specialisation (Elschnig, 1913; Naumann
71 *et al.*, 1976; Akeo *et al.*, 1996; Chong *et al.*, 2009). Using spectral domain OCT, our group has
72 previously shown that the degree of incursion of the inner retinal layers at the fovea and foveal
73 photoreceptor specialisation are inversely related and together define the degree of foveal
74 development. In addition, we were able to demonstrate a significant relationship between
75 photoreceptor size and best corrected visual acuity (BCVA) (Thomas *et al.*, 2011; Mohammad *et al.*,
76 2011).

77 The optic nerve comprises of retinal ganglion cell (RGC) axons which form arcuate bundles that
78 travel in the nerve fibre layer before converging at the optic nerve head (ONH). Albino animal studies
79 have shown a reduction in the number of RGCs (Guillery *et al.*, 1984; Leventhal and Creel, 1985).
80 We have previous shown that reduced RGC numbers shown in animal studies translates to thinner
81 peripapillary nerve fibre layer (ppRNFL) thickness (Mohammad *et al.*, 2015). This in turn leads to
82 smaller optic nerves and chiasm which have been demonstrated in PWA using magnetic resonance
83 imaging (MRI) (Schmitz *et al.*, 2003; Mcketton *et al.*, 2014).

84 Additionally, albinism is associated with an abnormally increased chiasmal decussation of the nerve
85 fibres originating from the temporal hemi-retinae (Guillery *et al.*, 1975). Axon guidance at the chiasm
86 is regulated by a number of molecular mechanisms at the retina.(Prieur and Rebsam, 2016)

87 Study of albino mice has shown an increased expression of the transcription factor *Islet2+* which
88 represses the ipsilateral program by reducing the expression of *Zic2* and thus *EphB1* which is a
89 receptor tyrosine kinase important in divergence of axons at the chiasmal midline and plays a key
90 role in stopping axons from crossing at the midline (Garcia-Frigola *et al.*, 2008; Rebsam *et al.*, 2012).

91 Delayed neurogenesis appears to play a key role in the misrouting seen in albinism (Rachel *et al.*,
92 2002; Bhansali *et al.*, 2014). It has been suggested that the time at which the axon reaches the chiasm
93 determines the fate of decussation. During embryological development, the first RGC axons arrive at
94 the chiasm around the fourth week of gestation. In some mammals such as mice and ferrets, it has
95 been shown that axons that reach the chiasm early during development are more likely to stay
96 ipsilateral (Baker and Reese, 1993) Absence of L-Dopa, a pre-cursor of melanin, in the retinal
97 pigment epithelium delays the point at which cells in the developing albino retina exit the cell cycle
98 (Ilia and Jeffery, 1999; Kralj-Hans *et al.*, 2006). As uncrossed RGCs are generated earlier than those
99 that project across the midline, a delay in ganglion cell production means that axons from these cells
100 reach the chiasm at a later point and this increases their probability of projecting to the contralateral
101 hemisphere (Erskine and Herrera, 2014).

102 Diffusion tensor imaging (DTI) is a widely applied quantitative imaging technique for studying white
103 matter anatomy and integrity. By quantifying the magnitude and principle direction of water diffusion
104 within image voxels, DTI data can be used for reconstruction of principle white matter tracts, a
105 technique referred to as tractography (Beaulieu, 2002). Grigorian and colleagues used the technique
106 to study the optic radiation and found that in albinism, fibres from lateral geniculate nucleus (LGN)
107 to the primary visual cortex (V1) are reduced (Grigorian *et al.*, 2016).

108 A number of studies have shown that MRI scanning can detect alterations in visual cortical areas in
109 albinism. Using voxel based morphometry, Von dem Hagen and colleagues found that people with
110 albinism show a reduction in cortical volume at the occipital pole (von dem Hagen *et al.*, 2005), while
111 Neveu and co-workers found that the calcarine fissure is shorter. In addition, the latter study also

112 reported a marked asymmetry in the calcarine sulcus between the left and right hemispheres of the
113 majority of PWA. The authors noted that in the presence of a dominant eye, the calcarine sulcus in
114 the contralateral hemisphere is displaced downwards (Neveu *et al.*, 2008).

115 Surface based analysis provides an alternative methodology to assess cortical differences in the
116 human brain by generating geometric models of the cortical surface (Dale *et al.*, 1999; Fischl *et al.*,
117 1999; Fischl and Dale, 2000). Using this methodology, Bridge *et al.* showed reduced gyrification in
118 the occipital cortex of albinism patients that explains the reduced cortical volume reported by Von
119 dem Hagen *et al.* In addition, they found cortical thickness to be increased at the occipital pole of
120 PWA. This change was more profound in the left hemisphere and cortical thickness was negatively
121 correlated to visual acuity. The authors suggested that these changes are due to a lack of post-natal
122 neuronal pruning as a result of under-development of the fovea seen in albinism and a consequent
123 absence of high-resolution input into V1 (Bridge *et al.*, 2014).

124 In this study, we conduct a holistic assessment of aberrant visual pathway development in PWA by
125 sampling anatomical variation at multiple points along the visual pathway, from the retina to the
126 visual cortex, using various non-invasive imaging techniques. To study the anterior visual pathway,
127 we perform OCT evaluation of the fovea and optic nerve head structure. The post orbital visual
128 pathway was studied using high-resolution T1-weighted MRI imaging to measure cisternal optic
129 nerves, chiasm, optic tracts and V1 cortical thickness. Our aim was to confirm previous reports
130 regarding altered morphology of these structures in PWA.(Schmitz *et al.*, 2003; Bridge *et al.*, 2014;
131 Mcketton *et al.*, 2014)

132 We employ diffusion tractography to study the chiasmal connectivity in albinism for the first time.
133 Structural connectivity at the chiasm was defined by streamline density measurements based on
134 diffusion tractography. We used this to define a decussation index, describing the proportion of
135 crossing fibres at the chiasm and compared it to chiasmal decussation measured using visual evoked
136 potential (VEP), a functional measure of axonal misrouting through the chiasm in albinism.

137 This multimodality data, has allowed us to explore whether the anomalous post-orbital optic nerve,
138 chiasm, tract and visual cortex morphology is related to retinal and optic nerve head abnormalities
139 described in albinism. We investigate the hypotheses that:

- 140 - Alteration in foveal morphology affects the development of the chiasm.
- 141 - Cortical abnormalities in albinism are a result of abnormal visual input due to an underdeveloped
142 fovea.
- 143 - Cortical thickness at the occipital pole is related to the degree of melanin present in the foveal
144 RPE.
- 145 - Optic nerve head morphology is related to the size and connectivity of the optic chiasm.
- 146 - Cortical thickness at the occipital pole is related to optic nerve head morphology in PWA.

147 **Materials and Methods:**

148 **Participants and recruitment**

149 The study was performed in accordance with the tenets of the Declaration of Helsinki and was
150 approved by the local UK National Health Service Research Ethics Committee. All participants
151 provided written informed consent prior to participation.

152 Adult participants with albinism were recruited through the neuro-ophthalmology outpatient clinic at
153 the Leicester Royal Infirmary. Diagnosis of albinism was confirmed by the coexistence of nystagmus,
154 asymmetric VEP responses, foveal hypoplasia and iris transillumination (Gottlob and Proudlock,
155 2014; Papageorgiou *et al.*, 2014).

156 Age, gender and ethnicity matched volunteers were recruited for the control group from within the
157 students and faculty at the University of Leicester as well as healthy visitors to the ophthalmology
158 department. For inclusion, potential control group participants had to have no history of eye disease
159 and have had a best corrected visual acuity (BCVA) of better than 0.0 logMAR. Analyses based on
160 this participant cohort have been reported in a previous publication (Welton *et al.*, 2017).

161 All participants underwent MRI scan using the protocol described below. In addition, the albinism
162 group participants underwent a detailed clinical assessment including assessment of best corrected
163 visual acuity, colour vision, stereo-acuity, ocular movements, slit lamp examination and dilated
164 fundus examination as well as OCT and VEP.

165

166 **OCT**

167 Macular and optic nerve OCT scans were acquired using the SOCT Copernicus HR device
168 (OPTOPOL Technology S.A., Zawiercie, Poland). Foveal layers thickness was measured using
169 ImageJ software (National Institutes of Health, MD, USA). Detailed methodology of this analysis has

170 been previously been described by our group (Mohammad *et al.*, 2011). As foveal development is a
171 combination of processing layer extrusion and photoreceptor lengthening, both these measures were
172 incorporated in the following formula to calculate a foveal development index.

$$173 \quad \text{Foveal Development Index} = 2 - \frac{\text{Processing layer thickness}}{\text{Photoreceptor layer thickness}}$$

174 This index was used for comparison with chiasmal and cortical indices.

175 Work carried out using polarisation sensitive OCT has shown that the reduced melanin in the RPE of
176 patients with albinism alters the reflectivity profile of this layer (Schutze *et al.*, 2014) and therefore
177 the RPE thickness was used as a surrogate for the amount of melanin present.

178 Optic nerve head analysis has been described in detail previously by our group. In summary, custom
179 written macros were used in ImageJ software (National Institutes of Health, MD, USA) to correct
180 nystagmus related motion artefact. Following this, Copernicus SD-OCT software was used to
181 calculate cup, disc and rim dimensions and peripapillary nerve fibre layer thickness (Mohammad *et*
182 *al.*, 2015).

183

184 **VEP**

185 VEP testing was carried out in accordance with international society for clinical electrophysiology of
186 vision standards (Odom *et al.*, 2010). The patients were seated and allowed to wear their full spectacle
187 correction throughout the duration of the test. Five electrodes were placed at 10% intervals in a
188 horizontal chain across the posterior part of the scalp left and right of Oz. In addition, a reference
189 electrode was placed in the midline frontally and a ground electrode placed in the midline over the
190 vertex.

191 The stimulus was a black and white checkerboard pattern, with 100% contrast, a mean luminance of
192 96cd/m² and check size of 1° generated on a 17-inch CRT screen positioned at 46cm distance from

193 the patient, which created a full-field size of 33°. The pattern appeared at a rate of 200ms onset,
194 400ms offset. Patients were asked to fixate a non-illuminated central spot. The responses for the left
195 and right eyes were recorded separately with the other eye completely occluded using an eye patch.
196 The test was performed twice on each eye an average of the two sets of results was used for analysis.
197 VEP asymmetry was calculated by means of an interhemispheric asymmetry index (I_{asym}), based on
198 a methodology described by Apkarian et al. (Apkarian *et al.*, 1983). The initial step is to calculate
199 response lateralization (A.I.) for each eye by plotting the magnitude of response at each electrode
200 against the electrode position and calculating the area under the graph was calculated for each
201 hemisphere (AL and AR).

202 The following formula was used to calculate response lateralisation (A.I.) in each eye.

203 for $A_L > A_R$, $A. I. = \left(\frac{A_R}{A_L}\right)$

204 else $A. I. = 2 - \left(\frac{A_L}{A_R}\right)$

205 The intra-ocular asymmetry index was calculated by subtracting response lateralization in the right
206 eye from the response lateralization in the left eye.

207

208 **MRI**

209 Brain MR imaging was performed using a 3T Philips Achieva MRI scanner with a 32-channel SENSE
210 head coil (Best, The Netherlands). Sequences performed included axial 3D magnetization-prepared
211 rapid acquisition gradient (3D-MPRAGE, TR=7.53 ms, TE=2.22 ms, flip angle=8°, matrix size 320
212 x 320, field of view=256 x 256, 0.8mm isotropic voxels, SENSE factor=1.7, 184 slices; acquisition
213 time 6.5minutes) and diffusion weighted imaging (axial diffusion-weighted echo-planar imaging, six
214 repeats of the b=0 volume, averaged on the scanner, and 61 directional diffusion weighted images
215 with $b=1000 \text{ s/mm}^2$, TE=67ms, TR=8270ms, SENSE factor 3, phase encoding in the anterior-

216 posterior direction, full Fourier, acquisition matrix size 120 x 120, 52 contiguous slices, 1.8 x 1.8 x
217 1.8 mm voxels interpolated to 0.9 x 0.9 x 1.8mm voxels, acquisition time 9.5 minutes).

218

219 **Morphometry of optic nerves, chiasm and tracts**

220 The technique for assessing the optic nerve, chiasm and tract dimensions was based on previously
221 described methodology by manually tracing regions of interests (ROIs) around each structure on the
222 MPRAGE images (Schmitz *et al.*, 2003; Schmitz *et al.*, 2003; Mcketton *et al.*, 2014). This was carried
223 out using a custom written macro in ImageJ software (National Institutes of Health, MD, USA) by an
224 assessor who was blinded to patient demographics and group membership. This allowed calculation
225 of the width and area for each structure (Supplementary figure 1).

226

227 **DTI**

228 DTI data were processed using fMRIB's Diffusion Toolbox in FSL (Behrens *et al.*, 2007). First,
229 "eddy_correct" was used to correct artefacts induced by head motion and eddy currents (Andersson
230 and Skare, 2002). We did not need to exclude any volunteer due to excessive artefact.

231 A binary mask of the brain was created and non-brain structures were removed with the Brain
232 Extraction Tool. The DTIFit tool in FSL's Diffusion Toolkit, was used to fit tensors to the data and
233 determine a variety of values including the fractional anisotropy, mean diffusivity and the three
234 eigenvector and eigenvalues of each voxel. BEDPOSTX (Bayesian Estimation of Diffusion
235 Parameters Obtained using Sampling Techniques) was used to build sampling distributions on the
236 diffusion parameters at each voxel.

237 Masks were manually drawn on the FA maps for optic nerve, chiasm and tract. The first axial slices
238 anterior and posterior to the chiasm where two separate nerves and tracts were visible were used to

239 draw the masks. This is demonstrated in supplementary figure 2. Probabilistic fiber tracking was
240 performed using the streamline tractography algorithm, PROBTRACKX2 (Behrens *et al.*, 2007)
241 contained within FSL to calculate 5000 streamlines per seed voxel with a 0.5mm step length and
242 maximum of 2000 steps, a 0.2mm radius of curvature cutoff and an FA threshold of 0.1.

243 The algorithm propagates streamlines from each voxel in a given seed mask along the path with the
244 largest principal axis of the diffusion tensor until some termination criteria are met (in this case, when
245 the streamline reached the voxels in a termination mask). The number of streamlines generated allows
246 estimation of the strength of connectivity between the seed and target voxels.

247 To increase the signal to noise ratio, the algorithm was run initially with the optic nerve being the
248 seed and the tract the target and then repeated with the seed and target masks reversed. Results from
249 these two streamline counts were then averaged for subsequent analyses. Wilcoxon signed-rank test
250 was carried out to compare the differences between the number of streamlines generated from the left
251 and right eyes and there was no significant difference demonstrated in either group ($p>0.05$). Intra-
252 class correlation coefficient in the albinism group was 0.898 (95%CI=0.754-0.958) and in the control
253 group was 0.821 (95%CI=0.548-0.929).

254 For comparison of chiasmal decussation estimated using DTI and VEP, a “streamline decussation
255 index” was deduced by calculating the percentage of streamlines connecting with contralateral
256 regions of interest through the chiasm using the following formula:

$$257 \text{ Streamline decussation index} = \frac{RN_{LT} + LN_{RT}}{(RN_{LT} + RN_{RT} + LN_{LT} + LN_{RT})} \times 100$$

258 A_B = Number of streamlines between regions of interest A (seed mask) and B (target mask)

259 LN = left nerve, LT = left tract, RN = right nerve, RT = right tract.

260

261 **Cortical analysis**

262 Cerebral cortical thickness and volume were derived using FreeSurfer version 5.0.0
263 (<http://surfer.nmr.mgh.harvard.edu>). Detailed methods have been described previously (Dale *et al.*,
264 1999; Fischl *et al.*, 1999). In summary, the software undertakes a segmentation procedure that
265 identifies white/grey matter interface (white surface) and the grey matter/cerebrospinal fluid interface
266 (pial surface). The distance between these two surfaces is used to calculate cortical thickness and
267 volume. As part of the standard FreeSurfer processing pipeline, an early step in the processing, the
268 0.9mm^3 voxels were resampled to 1mm^3 as part of the co-registration to the MNI305 template.
269 Although the above process is automated, each scan was subject to meticulous manual inspection to
270 check for errors in any of the above steps by an observer masked to the diagnosis. Any inaccuracies
271 were manually corrected and thickness measurements were recalculated. Automatic parcellation of
272 the cortex was performed based on the Destrieux atlas in FreeSurfer (Destrieux *et al.*, 2010).
273 Measurements for the occipital pole region were used for comparison with the foveal and optic nerve
274 head OCT parameters.

275

276 **Statistical analyses**

277 SPSS software version 22 (SPSS, Inc., Chicago, IL) was used to carry out statistical analyses. Due to
278 non-normality of the data, optic nerve, chiasm and tract parameters between and the albinism and
279 control volunteers were performed using non-parametric tests (Mann-Whitney tests). Spearman's
280 rank correlation co-efficient was used to study the relationship between OCT measurements and the
281 MRI derived measurements of optic pathway structure, cortical thickness and functional data (VEP
282 asymmetry and BCVA). Average values were used for comparison of paired structures.

283 As the relationship between structures throughout the visual pathway is being assessed, one of the
284 limitations of the study is the number of comparisons that needed to be carried out. To counter this
285 and ensure our results are biologically plausible, a priori hypotheses were defined based on previously
286 published findings. In addition, where testing a hypothesis required multiple statistical comparisons,

287 a Holm-Bonferroni correction is carried out to account for this (Holm, 1979). The corrected p-values
288 have been labelled p' .

289 **Results:**

290 **Group comparison of albinism patients and controls:** The albinism group (n=23, 17 males)
291 and control group (n=20, 14 males) were matched for ethnicity and age (mean age = 34.0 ± 13.6
292 years, 31.9 ± 10.6 years, respectively $t=0.851$, $p=0.400$). The mean BCVA in the albinism group was
293 0.47 ± 0.21 logMAR. The control group all had a BCVA of 0.0 logMAR or above and normal
294 stereoscopic vision.

295 **OCT Parameters:** We have previously published detailed OCT analysis of foveal and optic nerve
296 (Mohammad *et al.*, 2015) abnormalities in PWA. In this subset of patients, all PWA displayed some
297 degree of foveal hypoplasia with incursion of inner retinal layers through the foveal zone. The mean
298 value for foveal development index was 0.450 ± 0.562 while the RPE complex thickness was $28.6\mu\text{m}$
299 ± 3.28 . In our previous work, we have shown that in healthy controls, the mean FDI = 1.96 ± 0.148
300 and mean RPE thickness = 29.1 ± 5.13 respectively (Mohammad *et al.*, 2011).

301 On OCT analysis of the optic nerve head, eight PWA did not display an optic cup. Mean optic disc,
302 cup and rim areas were $1.82\text{mm}^2 \pm 0.339$, $0.379\text{mm}^2 \pm 0.349$ and $1.44\text{mm}^2 \pm 0.418$ respectively.
303 Mean ppRNFL thickness was $99.3\mu\text{m} \pm 15.2$.

304 Using a threshold of 0.7 defined by Apkarian et al, (Apkarian *et al.*, 1983) all PWA displayed
305 asymmetric VEP response. The mean VEP asymmetry index was 1.43 ± 0.32 . In controls, this has
306 been previously been shown to be -0.047 ± 0.655 .

307

308 **MRI Analysis**

309 **Structural changes to the chiasm region:** Two comparisons each were made for the optic nerve
310 and tracts and three for the chiasm. Optic nerve and tract width as well as the chiasm width, area and
311 volume, were significantly smaller in the albinism group compared to controls (table 1). The optic

312 tract area and the Holm-Bonferroni corrected optic nerve area comparisons were not statistically
313 significant. As the width of the nerve, chiasm and tract were consistently smaller, this was used in
314 subsequent comparisons with OCT measures.

315

316 **Chiasmal connectivity:**

317 Two comparisons were carried out to assess the chiasmal connectivity. Firstly, the total number of
318 streamlines generated between the albinism and control groups were compared, but there was no
319 significant group difference ($p > 0.05$). However, group comparison of the streamline decussation
320 index showed a significantly higher percentage of decussating streamlines at the chiasm in the
321 albinism group (mean = $42.0\% \pm 18.7$) compared to the controls (mean = $27.8\% \pm 17.5$) ($Z = -2.24$,
322 $p = 0.025$, $p' = 0.05$) (Figure 1a). The total number of streamlines did not significantly correlate to the
323 size of the ROIs ($p = 0.217$, $r = 0.197$). Figure 2 provides examples of DTI streamline data from albinism
324 and control volunteers demonstrating higher percentage of contralateral streamlines in the PWA.
325 Receiver operator curve (ROC) analysis of the streamline decussation index yielded an area under
326 the curve of 0.727 (95% CI = 0.575-0.880).

327 **Cortical changes:** Cortical thickness at the occipital pole was 6.88% higher in the albinism group
328 (mean = $2.15\text{mm} \pm 0.16$) compared to controls (mean = $2.01\text{mm} \pm 0.12$) ($Z = -4.10$, $p < 0.001$) (Figure
329 1b).

330

331 **Relationship of Orbital OCT measurements to Post-orbital MRI-derived measures of** 332 **visual pathway structure in albinism patients**

333 Table 2 summarises comparisons between foveal and optic nerve measurements obtained via OCT
334 and foveal development index, RPE thickness, ppRNFL thickness and optic disc, cup and rim areas
335 measured using OCT and post orbital optic nerve, chiasm and tract width, diffusion tensor

336 streamlines, decussation and cortical thickness at the occipital pole measured using MRI. Holm-
337 bonferroni correction was applied based on the comparison of the MRI measures with two foveal
338 parameters and four optic nerve parameters.

339 **Structural changes to the region:** No significant correlation was found between foveal
340 development index and optic nerve ($p=0.160$, $r=-0.303$), chiasm ($p=0.085$, $r=0.367$) or optic tract
341 ($p=0.241$, $r=0.255$) width measured on MRI.

342 The foveal RPE complex thickness was related to optic chiasm width, however, this comparison was
343 not significant once corrected for multiple testing. ($r=0.413$, $p=0.050$, $p'=0.100$). The optic nerve and
344 tract width did not relate to the RPE thickness ($p>0.05$).

345 Significant positive correlations were found between ppRNFL thickness and the optic nerve ($r=0.642$,
346 $p<0.001$, $p'<0.001$) and tract ($r=0.663$, $p<0.001$, $p'<0.001$) width. The chiasm width did not correlate
347 to the ppRNFL thickness. The optic disc area was correlated to optic tract width but this relationship
348 did not survive multiple comparison correction ($r=0.474$, $p=0.023$, $p'=0.069$). The disc cup and rim
349 areas did not relate to any of the other structures in the chiasmal region.

350 **Chiasmal connectivity:** Significant correlations were found between the total number of
351 streamlines and the foveal development index. ($r=0.662$, $p<0.001$, $p'<0.001$, figure 3). There was no
352 significant relationship between the total number of streamlines or the degree of decussation and
353 foveal RPE or optic nerve head measurements.

354 **Cortical thickness:** Mean cortical thickness at the occipital pole was inversely correlated with the
355 mean thickness of the foveal RPE ($r=-0.579$, $p=0.005$, $p'=0.010$) (figure 4). However, there was no
356 relationship of cortical thickness with the foveal development index.

357 Cortical thickness at the occipital pole was also found to correlate with optic disc and rim areas
358 ($r=0.478$, $p=0.021$, $p'=0.042$, and $r=0.597$, $p=0.003$, $p'=0.009$ respectively, figure 5a and b). Cortical
359 thickness did not relate to cup area or ppRNFL thickness ($p>0.05$).

360

361

362 **Relationships between structural MRI and measures of visual function in albinism**
363 **patients**

364 Best-corrected visual acuity was not related to optic nerve, tract or chiasm width or with V1 cortical
365 thickness ($p>0.05$). BCVA was not related to total number of streamlines, but BCVA showed a trend
366 towards significant correlation with streamline decussation index ($r=0.432$, $p=0.051$).

367 Streamline decussation index correlated significantly with inter-hemispheric asymmetry measured
368 using VEP ($r=0.484$, $p=0.042$, figure 6). We did not find any significant relationship between
369 cortical thickness and VEP asymmetry ($p>0.05$).

370

371 **Discussion:**

372 This is the first study that comprehensively investigate the relationship between ocular
373 abnormalities, and post orbital chiasmal and cortical abnormalities seen in albinism. To achieve
374 this, the visual pathway was imaged using OCT, structural MRI and DTI. In addition, the
375 anatomical data were compared to functional measurements such as visual acuity and VEP
376 asymmetry.

377

378 **Chiasmal abnormalities in albinism**

379 Our results agreed with the findings of smaller optic nerve, tract and chiasm in PWA reported
380 by previous studies (Schmitz *et al.*, 2003; von dem Hagen *et al.*, 2005; Bridge *et al.*, 2014). We
381 show for the first time that DTI tractography can be used to demonstrate chiasmal misrouting
382 in albinism. We found that the proportion of tractography streamlines crossing the chiasm (the
383 streamline decussation index) was significantly higher in PWA compared to healthy controls.
384 These findings are validated by the weakly positive correlation between the chiasmal
385 streamline decussation index and VEP asymmetry. It is possible that the significance of this
386 relationship may be improved using the correlation method of VEP asymmetry assessment
387 developed by Hoffmann *et al.* (Hoffmann *et al.*, 2005) rather than the Apkarian method we
388 used. This correlation method uses data from the whole time series of VEP traces rather than
389 point measurements and has been demonstrated as a reliable way of estimating the degree of
390 misrouting (Hoffmann *et al.*, 2005). Time series data were unavailable in the current study to
391 allow the correlation method to be performed. Receiver operator curve (ROC) analysis of the
392 streamline decussation index yielded an area under the curve of 0.727 (95% CI = 0.575-0.880).
393 This indicates that while demonstrate group level differences between albinism and healthy
394 controls, it cannot be used as a diagnostic tool.

395 It had been hypothesized by the authors of these earlier studies that the finding of smaller optic
396 nerves, chiasm and tract in albinism could be due to the underdevelopment of the fovea (von
397 dem Hagen *et al.*, 2005; Mcketton *et al.*, 2014). Although we did not see any relationship
398 between foveal development and the physical size of the chiasm, our DTI data shows
399 significant relationship between foveal development and number of streamlines crossing the
400 chiasm.

401 While this may seem surprising at first glance, a previous study in albino ferrets reported that
402 a delay in the timing of axonal outgrowth from the retina means that there is a disruption
403 in the distribution of large and small diameter axons within the optic nerve with an abnormal
404 thickening of the myelin sheath (Guibal and Baker, 2009). Consequently, a gross measurement
405 of the optic nerve and chiasm might not accurately reflect the number of axons within it. Our
406 data suggests that diffusion tractography may reflect the number of axons crossing the chiasm
407 better than morphometry in people with albinism.

408 We found that the optic nerve size measured using MRI is correlated with the ppRNFL
409 thickness. Using ex-vivo axon tracing studies and through mapping of the visual field to the
410 optic nerve in glaucoma patients, previous studies have indicated that axons from the foveal
411 retinal ganglion cells aggregate in the temporal region of the optic nerve head (Yucel *et al.*,
412 1998; Zangwill *et al.*, 2000; Sihota *et al.*, 2006). Therefore, any variation in the numbers of
413 central ganglion cells would influence the size of the ppRNFL and hence the optic nerve size.
414 Previous animal studies have shown a reduced number of central retinal ganglion cells in albino
415 animals (Stone *et al.*, 1978; Guillery *et al.*, 1984; Leventhal and Creel, 1985; Robinson *et al.*,
416 1987; Donatien *et al.*, 2002).

417 However, in our study, the degree of misrouting did not relate to any foveal or optic nerve
418 head abnormalities. Foveal hypoplasia and misrouting of the optic nerve are two cardinal

419 features of albinism. Aberration in the melanin synthesis pathway are believed to be the cause
420 of both these abnormalities (Jeffery, 1997). However, our findings suggest that there is no
421 direct relationship between these two features. These findings agree with previous suggestion
422 by Neveu et al. who compared the retinal findings in PWA and aniridia. Both these
423 conditions are characterized by foveal hypoplasia but patients with aniridia have normal
424 retino-fugal projections. The authors therefore concluded that optic chiasm formation is
425 independent from foveal development (Neveu *et al.*, 2005). It is more likely that the
426 misrouting in albinism is a function of delayed cell mitosis in albinism, which is a process
427 regulated by L-dopa, a precursor of melanin (Ilia and Jeffery, 1999). The factor determining
428 whether an axon will decussate is thought to be dependent on the timing at which it reaches
429 the chiasm during the development of the optic nerve. In animal models it has been shown
430 that axons originating in the temporal retina, which develop earlier than those originating in
431 the nasal retina, remain ipsilateral as they grow backwards past the chiasm, while the later
432 developing retinally derived axons decussate through the chiasm (Drager, 1985). It is
433 proposed that in albinism, a lack of melanin in the retinal pigment epithelium leads to a delay
434 in the development of the temporal retina and hence a delay in these axons reaching the
435 chiasm leading to increased decussation (Jeffery, 1997; Jeffery, 1998; Ilia and Jeffery,
436 1999). Albinism patients do however retain some normal projection and the degree of this is
437 related to the amount of pigmentation (Hoffmann and Dumoulin, 2015). However, foveal
438 hypopigmentation is not the only cause for chiasmal misrouting and has been shown in
439 normally pigmented individuals and it has been hypothesised that chiasmal misrouting
440 interferes with normal development of the fovea through an anterograde mechanism (van
441 Genderen *et al.*, 2006). This relationship though is more complex as foveal hypoplasia can be
442 present in the absence of chiasmal misrouting (Sloper, 2006; Hingorani *et al.*, 2012).

443

444 Apart from albinism, abnormally increased chiasmal decussation has recently been reported
445 in foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis
446 (FHONDA) syndrome. This is a rare autosomal recessive disorder with 20 reported cases in
447 published literature (Al-Araimi *et al.*, 2013). In 2018, Ahmadi and colleagues studied two
448 affected individuals using ultra-high field fMRI and found that the degree of misrouting does
449 not vary in FHONDA syndrome unlike albinism where misrouting has been shown to vary
450 between 2° and 15° (Hoffmann *et al.*, 2005), and correlates with the pigment deficit (von dem
451 Hagen *et al.*, 2007). Within the visual cortex of FHONDA patients, the normal representation
452 of the temporal retina seen in albinism is also absent. The authors suggest that the misrouting
453 may be due to complete cessation of uncrossed projections at the optic chiasm which points
454 to a different molecular cascade driving misrouting in FHONDA compared to albinism
455 (Ahmadi *et al.*, 2018) .

456 **Cortical abnormalities in albinism**

457 Using surface based analysis, we have been able to shed light on a conflict in previous literature
458 regarding the nature of structural changes within the visual cortex of PWA. Using voxel based
459 morphometry, von dem Hagen et al. found that PWA have a reduction in grey matter volume
460 in the occipital cortex (von dem Hagen *et al.*, 2005). A more recent study by Bridge et al. used
461 surface based analysis to conclude that visual cortex thickness is increased in PWA (Bridge *et*
462 *al.*, 2014). Bridge et al. suggested that the difference in results between the two previous
463 studies were due to the two different analysis techniques being employed.

464 Using a similar technique to Bridge et al., we have found that PWA do indeed have increased
465 cortical thickness at the occipital pole. Bridge et al. found increased gyrification in PWA, which
466 might explain why the earlier voxel based morphometry study may have reported reduced
467 cortical volume in albinism (von dem Hagen *et al.*, 2005; Bridge *et al.*, 2014). We previously
468 reported corroboratory evidence using functional MRI that an increased interhemispheric
469 functional connectivity of the visual processing areas is present in albinism, which may be an
470 adaptation to the upstream structural changes in the visual pathway (Welton *et al.*, 2017).

471 OCT data regarding foveal and optic nerve abnormalities has allowed us to explore possible
472 anterior pathway causes behind cortical changes seen in albinism. We noted several significant
473 relationships of cortical thickness with the fovea, optic nerve and chiasm.

474 Comparison of the visual cortex with the fovea showed that cortical thickness was inversely
475 related to the size of the RPE in PWA. The thickness of the RPE measured on OCT is impacted
476 by the amount of melanin present within the RPE cells. This is due to the optical properties of
477 melanin (Wolbarsht *et al.*, 1981), which mean that the light from the OCT device is scattered
478 when it passes through melanin leading to the thick band like appearance of the RPE seen in
479 OCT images (Chauhan and Marshall, 1999). This indicates that the amount of melanin within

480 the RPE of PWA affects the specialisation of the visual cortex. Von dem Hagen et al. have
481 previously found that level of skin pigmentation is related to the degree of functional
482 reorganisation of the visual cortex (von dem Hagen *et al.*, 2007). Our results suggest that in
483 addition to functional changes, pigmentation defects in albinism also lead to structural changes
484 of the visual cortex.

485 Bridge et al. noted that the thicker visual cortex in albinism is consistent with findings from
486 early blind (Jiang *et al.*, 2009) and anophthalmic (Bridge *et al.*, 2009) individuals and suggested
487 that this is due to a lack of pruning during development. In addition, increased chiasmal
488 decussation means that there is a reduction in binocular competition at V1, which may be
489 another factor in driving axonal pruning. The visual cortex undergoes rapid expansion during
490 foetal and first four months of post-natal life and reaches peak levels (~150% of adult) by 7
491 months gestation (Goswami, 2004). This early post-natal time corresponds with a critical
492 period of foveal (Lee *et al.*, 2015) and visual cortex development (Huttenlocher and de Courten,
493 1987; Leuba and Garey, 1987).

494 The rapid growth phase is followed by synaptic revision with loss of the excess 40% of
495 synapses between ages 8 months and 11 years. Subsequently, these synapse numbers remain
496 stable into adulthood (Garey, 1984). The synaptic elimination has shown to be dependent on
497 visual experience (Bourgeois *et al.*, 1989). In albinism reduced foveal cone density results in a
498 lack of high-resolution input to V1. However, our results showed no relationship between
499 cortical thickness at the occipital pole and foveal development. We were unable to reproduce
500 the negative correlation between V1 cortical thickness and visual acuity demonstrated by
501 Bridge et al. ($r=0.116$, $p=0.606$). This may be due to the fact that the visual deficit in albinism
502 is multi-faceted with factors such as nystagmus, refractive errors, strabismus, iris
503 transillumination, foveal hypoplasia, optic nerve dysgenesis and chiasmal abnormalities all
504 playing a role.

505 While comparing the occipital pole to optic nerve head, we found that the disc and rim areas
506 were positively correlated with cortical thickness at the occipital pole. We have previously
507 shown that the rim size is increased in PWA possibly due to arrest in normal embryological
508 development of the optic nerve (Mohammad *et al.*, 2015). The nasal aspect of the rim appears
509 to be composed of glial tissue, which is remnant of the hyaloid vascular system that has failed
510 to fully regress (JONES, 1963; Renz and Vygantas, 1977; Sheth *et al.*, 2013). This would
511 support the theory that a lack of pruning is responsible for increased thickness of the visual
512 cortex seen in albinism and that it is a phenomenon that affects more than one location in the
513 visual pathway.

514

515 **Limitations of the study**

516 It is important to point out the inherent limitations in our methodology. Diffusion tractography
517 allows non-invasive in-vivo quantification of white matter structure but many factors including
518 anatomical characteristics of the structure being studied, image acquisition parameters and
519 choice of tract reconstruction algorithm can significantly alter the results. The anterior optic
520 pathways are particularly challenging to study with DTI due to the complex convergence,
521 divergence and crossing of axons as they pass through the chiasm. Within each voxel, there
522 may be multiple fibre orientations of axons making it difficult to distinguish between axons
523 that are kissing, crossing, converging or diverging as they all capable of generating a similar
524 diffusion signal. This means the tractography algorithm may jump between two fibre pathways.
525 There is also potential for partial volume effects of cerebrospinal fluid contamination affecting
526 the tractography algorithm in voxels along the surface of the cisternal segments, and the effect
527 of susceptibility distortions due to the adjacent skull base and paranasal sinuses.

528 Prior to commencing the study, we undertook optimisation of the DTI protocol by selecting
529 the maximal resolution achievable (1.8mm isotropic voxel size) while maintaining an
530 acceptable signal to noise ratio and appropriate scan duration. As the structures we were
531 sampling are very small, the mean size of the optic nerve and tract in albinism group for
532 example were 12.3 ± 3.21 and 10.7 ± 3.33 voxels respectively, we remain cautious regarding
533 the interpretation of absolute streamline counts in our data but feel that expressing the
534 streamline decussation as a percentage of the total streamline count provides a plausible
535 measure of fibre crossing at the chiasm given the positive correlation that we found with VEP
536 asymmetry. The ROC analysis of the streamline decussation index indicated that while the
537 technique was able to show group differences, it should not be used for individual patient
538 diagnosis.

539 Since we acquired our data between 2010 and 2012, work on the Human Connectome Project
540 and other similar connectomic projects has significantly advanced the image acquisition
541 techniques with the current standard being multi-shell acquisitions and measures to compensate
542 geometric distortions such as acquisition of field maps or dual phase encoding directions.
543 Further work using state-of-the-art image acquisition and analysis techniques such as sparse
544 fascicle model (Rokem *et al.*, 2015) and filtering of streamlines (Pestilli *et al.*, 2014; Wandell,
545 2016) is warranted to further investigate chiasmal abnormalities and may account for the
546 unexpected results we encounter such as no group differences in the number of streamlines
547 between albinism and control groups and the underestimation of contralateral streamlines in
548 both groups. The mean decussation with DTI in the control group= $27.8\% \pm 17.5$ rather than the
549 expected 50%.

550 In conclusion, our study provides novel insights in to the relationship between retinal, chiasmal
551 and cortical abnormalities in albinism through the use of multiple and complimentary non-
552 invasive imaging modalities. We show for the first time that cortical abnormalities are related

553 to pigmentation levels of the RPE and axonal disorganisation of the optic nerve head. Although
554 the study was not designed as a diagnostic accuracy study, we find that diffusion tractography
555 can demonstrate abnormal chiasmal crossing seen in albinism that relates to VEP asymmetry.
556 Our results suggest that that much like other abnormalities in the anterior visual pathway, the
557 cortical abnormalities in people with albinism represent abnormal embryological and early
558 post-natal development.

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751 **Figure legend:**

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753 **Figure 1:** A - Comparison of the streamline decussation index between albinism and control
754 groups, ($Z=-2.24$, $p=0.025$, $p'=0.05$)

755 B - Comparison of occipital pole thickness averaged across both hemispheres between the
756 albinism and control groups ($Z=-4.10$, $p<0.001$)

757

758 **Figure 2:** Example diffusion tractography streamline data from albinism (left) and
759 control (right) volunteers. Streamlines travelling from the optic nerve to the ipsilateral
760 tract are in orange while streamlines travelling to the contralateral regions of interest
761 are blue. The images were thresholded such that voxels with a streamline density
762 $<10\%$ of the total streamlines are excluded. The images show a variation in the
763 chiasmatal connectivity in both groups.

764

765 **Figure 3:** Comparison of total connectivity at the chiasm estimated using diffusion
766 tractography with foveal development index

767

768 **Figure 4:** Comparison of foveal retinal pigment epithelium (RPE) thickness measured using
769 OCT with the cortical thickness in patients with albinism

770

771 **Figure 5:** Comparison of cortical thickness at the occipital pole with optic disc (A) and rim (B)
772 in patients with albinism

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774 **Figure 6:** Comparison of visual evoked potential and diffusion streamline asymmetry in
775 albinism

776

777 **Supplementary figure 1:** Axial (left) and coronal (right) images of the optic chiasm. The left
778 image demonstrates where the width measurements were obtained while the right image
779 outlines the cross-sectional areas.

780

781 **Supplementary figure 2:** Coronal (left) and axial (right) images of the FA map demonstrating
782 examples of the manual masks drawn for DTI analysis