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

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Running title: Imaging the visual pathway in albinism

Abstract:

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

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

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

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

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

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

Introduction:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Materials and Methods:

Participants and recruitment

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

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

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

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

OCT

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

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

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

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

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

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

VEP

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

The stimulus was a black and white checkerboard pattern, with 100% contrast, a mean luminance of 96cd/m^2 and check size of 1° generated on a 17-inch CRT screen positioned at 46cm distance from

the patient, which created a full-field size of 33°. The pattern appeared at a rate of 200ms onset, 400ms offset. Patients were asked to fixate a non-illuminated central spot. The responses for the left and right eyes were recorded separately with the other eye completely occluded using an eye patch. The test was performed twice on each eye an average of the two sets of results was used for analysis.

VEP asymmetry was calculated by means of an interhemispheric asymmetry index (I_{asym}), based on a methodology described by Apkarian et al. (Apkarian *et al.*, 1983). The initial step is to calculate response lateralization (A.I.) for each eye by plotting the magnitude of response at each electrode against the electrode position and calculating the area under the graph was calculated for each hemisphere (AL and AR).

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

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

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

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

MRI

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

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

Morphometry of optic nerves, chiasm and tracts

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

DTI

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

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

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

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

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

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

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

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

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

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

Cortical analysis

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

Statistical analyses

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

As the relationship between structures throughout the visual pathway is being assessed, one of the limitations of the study is the number of comparisons that needed to be carried out. To counter this and ensure our results are biologically plausible, a priori hypotheses were defined based on previously 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'.

Results:

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

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

On OCT analysis of the optic nerve head, eight PWA did not display an optic cup. Mean optic disc, cup and rim areas were 1.82mm^2 ± 0.339, 0.379mm^2 ± 0.349 and 1.44mm^2 ± 0.418 respectively. Mean ppRNFL thickness was 99.3µm ± 15.2.

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

MRI Analysis

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

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

Chiasmal connectivity:

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

Cortical changes: Cortical thickness at the occipital pole was 6.88% higher in the albinism group (mean=2.15mm ± 0.16) compared to controls (mean=2.01mm ± 0.12) ($Z=-4.10$, $p<0.001$) (Figure 1b).

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

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

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

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

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

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

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

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

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

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

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

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

Discussion:

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

Chiasmal abnormalities in albinism

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

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

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

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

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

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

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

Cortical abnormalities in albinism

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

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

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

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

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

Limitations of the study

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

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

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

In conclusion, our study provides novel insights in to the relationship between retinal, chiasmal and cortical abnormalities in albinism through the use of multiple and complimentary non-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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Figure legend:

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

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

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

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

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

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

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

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

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