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DIAGNOSING HUMAN ALBINISM: STRUCTURAL AND FUNCTIONAL MRI

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DIAGNOSING HUMAN ALBINISM: STRUCTURAL AND FUNCTIONAL MRI

Functional Magnetic Imaging: a precise method to diagnose albinism in comparison with structural and visual evoked potential techniques

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ABSTRACT

Albinism is a genetically determined disorder of melanin synthesis. An abnormal crossing at the optic chiasm of part of the fibers originating in the temporal retina occurs specifically in this condition.

In this study, we test if fMRI provides a time-effective protocol for diagnosis at an individual level and if it's useful in the characterization of abnormal development of visual organization in a pediatric population as a possible advantageous alternative to the classical VEP neurophysiological method. We performed structural analysis of the optic chiasm in 7/8 albino subjects and age-matched controls and compared results between the VEP and fMRI protocols in 8/8 albino subjects. We found significant changes in configuration of the optic chiasm with the albino subjects showing lower chiasmatic width when compared to controls. With the fMRI protocol we were able to clearly diagnose all of our 8 albino subjects in contrast with the VEP protocol only 5 were conclusively diagnosed. We also found that the fMRI method yields more clearcut asymmetric indexes. We conclude that fMRI provides a clear, simple and straight forward strategy for the precise mapping of abnormal decussation and diagnosis of albinism.

KEYWORDS

Albinism; fMRI; VEP; diagnostic method

INTRODUCTION

Albinism is a heterogeneous group of melanin synthesis disorders in which both eyes and high level visual system are severely affected. Prevalence varies worldwide but has been estimated to be 1/17000. The clinical spectrum of oculocutaneous albinism (OCA) ranges in four subtypes: OCA1A being the most severe type with a complete lack of melanin production while OCA1B, OCA2, OCA3 and OCA4 are milder forms that show some pigment accumulation over time [7]. Another type is ocular albinism (OA). The different mutations associated are thought to act through a common pathway involving reduced melanin synthesis in the retina during development to produce the ocular and neuroanatomical abnormalities found. The lack of melanin alters the visual system development which translates into a misrouting of the fibers originating in the temporal retina to the contralateral thalamus and visual cortex. Transgenic animals expressing a functional tyrosinase gene on an albino genetic background display a correction of all these abnormalities, implicating a functional role for tyrosinase in normal retinal development (Giménez et al, 2004 [6]). Another study has proven that albino mutations associated with more severe deficits in melanin, and hence lower pigmentation levels, cause a greater shift in the line of decussation into the temporal retina. Thus a great interindividual variability of the extent in the decussation line shift is known to exist (von dem Hagen et al., 2007 [18]).

The misrouting of the optic nerves fibres causes the visual cortex to receive an abnormal input, which is clinically used to help diagnosing albinism, traditionally assessed by visual evoked potentials (VEP), and also means that albinism can provide a model for investigating self-organising patterns of the human cortex *(*Hoffmann et al, 2006[9]).

The phenotypic evaluation alone is seldom sufficient for definitive diagnosis since there is a wide spectrum of pigmentation levels and other symptoms, like macular hypoplasia; hypopigmentation; iris transillumination; nystagmus; reduced visual acuity; etc, each of which are rather non specific because they can also be present in patients that do not have albinism.

The standard albino-VEP paradigm is based on the rationale that in albinism, the polarities of the interhemispheric difference VEPs obtained for left and right eye stimulation are inverted because each eye predominantly projects to the contralateral hemisphere. Apkarian et al reported a 100% accuracy in albino misrouting detection with zero false positives, detected with the mode of stimulus known as pattern onset.

Since *von dem Hagen et al (2005)[19]* has reported a regionally specific decrease in grey matter volume at the occipital poles in albinism, it would be important to compare the functional and structural changes in each individual. The former can be assessed with functional magnetic resonance imaging (fMRI), another technique that could be potentially be used for means of diagnosis. In this case, it has been suggested that fMRI might be more successful in some cases when the extent of the misrouting is smaller *(von dem Hagen et al, 2008 [17]).*

Our work aims to compare the two different techniques, VEP, structural MRI at the chiasma level and fMRI, in diagnosis and assessment of functional and structural changes in albinism. We did therefore investigate the feasibility and diagnostic yield of structural and visual fMRI in a pediatric population. We seek to prove MRI and/or fMRI can provide time-efficient protocols for individual diagnosis and characterization of abnormal development of visual organization in a pediatric population and yield advantageous alternatives to the classical VEP neurophysiological method.

METHODS

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and all procedures were reviewed and approved by the Ethics Commissions of the Faculty of Medicine of the University of Coimbra (Comissão de Ética da Faculdade de Medicina de Coimbra) and of the Children's Hospital of Coimbra (Comissão de Ética do Centro Hospitalar de Coimbra). Written informed consent was obtained from participants older than 18 years of age and from the parents/guardians in the case of participants younger than 18 years of age. Children and adolescents younger than 18 years of age gave written or oral informed consent.

Subjects

Patients with albinism (ocular or oculocutaneous) were referred to by the Ophthalmology Department of Hospital Universitário de Coimbra. For the control group, participants were recruited from a local school. Twenty-four individuals participated in this study: 8 children with albinism (mean age 10, range: 7-16 years) and 16 control subjects.

Seven (7) children with albinism and 14 age-matched control subjects have undergone structural MRI at 3T, without sedation.

FMRI was performed in the 8 albinos and in 2 control subjects. Those 8 albino subjects also underwent VEP protocol.

The first of our albino subjects has undergone a different fMRI protocol (hemi-field stimulation instead of full-field) and although the albino pattern was evident, the data was not used to compute the asymmetry index of our group analysis.

Stimulus

Stimuli were high-contrast checkerboards, with a central fixation cross. The software used to create the stimulus was Psychophysics MatLab Toolbox .

We uses Pattern-onset stimulus configurations because they are reported in the literature [2;8;11;12;13;17] to overcome nystagmus, a feature present in variable degree in all our albino subjects.

Full-field monocular pattern-onset stimulation was presented as repeating blocks of 16s ON and 16s OFF of counterphasing checks reversing at a frequency of 1Hz. Pattern element size was 60 min.

Luminances of alternating bright and dark sections were chosen such that the mean luminance of the stimulus was the same as that of the neutral gray background. Contrast between the checkers was 98%.

For monocular stimulation the contralateral eye was covered with an eye patch.

Functional Magnetic Resonance Imaging

Scanning was performed on a 3T Siemens TimTrio scanner at the Portuguese Brain Imaging Network, using a 12-channel birdcage head coil. Visual stimulus presented on a projector (Silent Vision Model SV-6011 System, Avotec Inc. Fla, United States).

Sequences included T1-weighted 3D MPRAGE and two visual fMRI runs with monocular stimulation (one run for each eye) for a total scanning time under 12 minutes.

MP RAGE : acquired 160 sagittal slices to cover the whole brain (slice thickness 1.00 mm), with an in-plane image matrix of 256 x 256 voxels, with isotropic resolution of 1x1x1mm3, repetition time (TR) 2.3 s, echo time (TE) 2.98 ms with a 256x256 matrix, flip angle (FA) 9 deg; total acquisition time of 5 minutes and 21 seconds.

fMRI: acquired 26 sagittal slices to cover the occipital lobes (slice thickness 2.50 mm), with an in-plane image matrix of 256 x 256 voxels, with voxel size of 2x2x2.5mm3, repetition time (TR) 2.0 s, echo time (TE) 39 ms, flip angle (FA) 90 deg; total acquisition time of 3 minutes and 8 seconds.

Each eye was stimulated separately, using a full-field pattern-onset checkerboard stimulus, in a block design, shown to generate symmetrical activation in healthy volunteers. Activation maps are calculated online and assessed for the presence of the typical albino pattern. Later, asymmetry indexes are calculated by comparing the size of clusters activated in each hemisphere.

VEP Recordings

Our VEP were recorded with 5 Ag/Cl surface electrodes, positioned posteriorly in a line placed 1/10th of the nasion-inion distance above the inion. The central Oz electrode was placed at the midline, with the other electrodes at lateral spacings of 3 cm to the left and right of the midline. These were referred to Fz reference electrode. A ground electrode was positioned in the forehead.

The VEP protocol was based in a 5-channel Espion E2 Electrophysiology System @ Diagnosys LLC and each participant underwent monocular on-off and pattern reversal stimulation, with a checkerboard stimulus of 60', with far vision refractive correction, when applicable.

Stimuli were presented at a contrast level of 100% on a 18-inch monitor, at a viewing distance of one meter. Voltage range was +/-50 μV and the signal was 1-100 Hz banded-pass filtered. The artifact rejection level was set at 5% below the range mentioned above.

An average waveform of 2 runs of 64 trials each was obtained and peak amplitudes for each recording were determined at the latency (~100ms) of the second voltage peak (C2).

Data Analysis

For the structural analysis, optical chiasm measurements were performed on reformatted images (sliced parallel to the optic quiasm) by two neuroradiology physicians blinded to diagnosis. Fig. 1 exemplifies the measurements: width of the optic chiasm - measured at its smallest aspect (a) ; Angle between the optic nerves - measured by drawing lines along the middle of the optic nerves (α); Angle between the optic tracts - measured by drawing lines along the middle of the optic tracts (β).

Although fMRI asymmetry, as well as VEP asymmetry, can be determined by visual inspection of the left eye response compared to that of the right eye, asymmetry indexes (AI) were used to quantify

the degree of response lateralization and are calculated by comparing the areas activated in each hemisphere.

VEP AI – calculated using peak response amplitude (μV) for each electrode, excluding Oz. We found the peak amplitudes from O1 and O2 electrodes to be more consistent, therefore those were the ones used for statistical analysis (Fig.2).

The peak amplitude for the right hemisphere electrodes (RμV) should be identical to the peak amplitude for the left hemisphere electrodes (LμV), for both right and left monocular fullfield stimulation of control subjects. Meanwhile, the mean peak amplitude should be higher for the left hemisphere electrodes in fullfield stimulation of the right albino eye and higher for the right hemisphere electrodes in fullfield stimulation of the left albino eye. Taking this into account:

- Assymmetry index for right eye (AI_OD) = LμV/RμV+LμV
- Assymmetry index for left eye (AI_OS) = RμV/RμV+LμV

fMRI AI – calculated using cortical activation at the occipital cortex area. We used the Neuro3D tool of the Siemens scanner terminal to address the responsive areas of the occipital cortex for each albino and control subjects. Analysis was performed at a t value threshold of 4 ($p<0.00013$), on 6 slices oriented by the calcarine sulcus, 6mm thick. Using the same paradigm as for the VEP asymmetry indexes:

- Assymmetry index for right eye (AI_OD) = LH/RH+LH
- Assymmetry index for left eye (AI_OS) = RH/RH+LH

AI should be around 0,5 for controls and close to 1 for albino subjects.

Statistical analyses were performed using a standard statistical package (SPSS 17-SPSS, Inc., Chicago, IL), using parametric and nonparametric procedures (when applicable), and ROC curves for the data on chiasmatic structure.

RESULTS

Reformatted images, in albino and control subjects, parallel to the optic nerves and tracts, showed differences in chiasm morphology (Fig.3). This correlates with the findings from Schmitz et al [16] of a X-shaped chiasm in albinos and control chiasms shaped like two back-to-back brackets:)(.

Chiasmatic mean width was lower in albinos compared to control subjects: 10.0±1.2 mm x 12.7±1.4mm, $p=0.002$, Mann–Whitney U test, corroborating and extending the above mentioned study. However, the measured angles were not significantly different between groups.

Admitting that to a lower chiasm width corresponds a higher probability of the albino diagnosis, calculated ROC curve gives an area of 0,929 and a cut-off point at 11.375mm for a sensitivity and specificity of 85,7% and 78,6%, respectively.

With fMRI, in all 8 patients the albino pattern was identified, for both eyes, with variable degrees of miscrossing. Some albino subjects had a less pronounced decussation deviation which lead to some significant activation in the ipsilateral hemisphere (Fig.4), however this activation was in peripheric visual areas and did not affect the identification of the albino pattern nor the AI calculation.

On one of our albino subjects, the first one, we were not able to calculate the fMRI AI in such a way that it could be compared it with the others due to a different protocol. However, the albino pattern was also present (Fig.5), and lead to an unequivocal diagnose, while the VEP was inconclusive (VEP AI= 0,45).

We got a reliable VEP albino pattern in 5 of our albino subjects. This was determined by visual inspection of the left eye response compared to that of the right eye as shown in Figs. 6 and 7. fMRI was able to give a secure diagnose for the 3 subjects in which VEP was inconclusive (Fig.5 and 8). This led to a detection rate in this study of 100% with fMRI protocol and 62,5% with the VEP protocol.

The AI were calculated for each eye and then averaged within individual subjects. Just to confirm data from the literature, we also calculated the AI for controls. Results shown in Table 1.

Control	N	
Mean of IA VEP	2	0,45 +/- 0.01
Mean of IA fMRI	2	0.49 +/- 0.23
Albino		
Mean of IA VEP	8	0.54 +/- 0.10
Mean of IA fMRI	7	0.86 +/- 0.12

Table 1 Asymmetry indexes results for control and albino subjects obtained using VEP and fMRI protocols. Correlation between these methods means for the albino subjects was 0.524 (*ns*).

Wilcoxon Signed Ranks Test showed 7 positive ranks for mean fMRI AI superior to mean VEP AI, for the 7 total comparable cases.

DISCUSSION

As expected by modeled drawing of chiasm morphology based on the crossed temporal optic fibers, the morphologic conformation of the albino optic chiasm shows significant differences from the control. The albino optic chiasm is generally narrower and X-shaped whilst the control is wider and shaped like a two back-to-back brackets.

On what concerns the width of the chiasm our results are congruent with those obtained by Schmitz et al [16]. However, we did not find significant differences in the angles measured. This may have happened because of errors in measurement or differences in individual evaluation of angle insertion. The ROC curve cut-off point found proves this width based method to be useful in identifying albinism in suspected subjects.

In the fMRI study it was easy to discriminate the normal and albino pattern even in online analysis, even though some albino subjects had a less pronounced decussation deviation leading to some significant activation in the ipsilateral hemisphere. In these cases, the activation was in peripheral visual areas and correlated with the existence of some more peripheric temporal fibers with a normal trajectory. This wasn't difficult to discriminate and lead to no doubt in diagnosis.

In the VEP study visual analysis of waves was not a straight forward method to discriminating the albino and control patterns. We used the C2 peak of the pattern-onset experiment output for the average wave of each electrode to create a map of the topographical distribution of amplitudes in the scalp. This way was easy to identify the albino pattern, which required at least one amplitude peak to the contralateral hemisphere of the stimulated eye. The middle electrode (Oz) counted as null. Using this method we were able to diagnose 5 of our 8 albino subjects.

By calculation the AI, fMRI proves to be a more precise asymmetry detection method, which in this case is a good thing to expect from a diagnostic tool. The average AI obtained with fMRI for each subject was always superior to the obtained using VEP protocol. The AI obtained with VEP were not significantly different from the controls AI, probably because it has less spatial resolution.

CONCLUSIONS

Our study supports the observation that the atypical crossing of optic fibers in humans with albinism changes the configuration of the optic chiasm and that these subjects have a lower chiasmatic width when compared to controls. This structural study could be used as a first order assessment, to

evaluate the probability of the albinism diagnosis and the need for further diagnostic investigation or in cases were a functional study is not possible.

Patients refer to be more comfortable and cooperative in the MRI machine than when sitting in from of a VEP monitor. Also we observed that fMRI does not require as much cooperation from the patient to archive a reliable result. fMRI is more expensive but it allows for a complete anatomical, structural and functional study in almost the same time as it takes to run a VEP test. Detection rate with fMRI is superior and more reliable.

Brain MRI and in particular fMRI can be used clinically in a time-efficient protocol for the individual diagnosis and characterization of abnormal development of visual organization of a pediatric albino population. Our study suggests it might be an advantageous alternative to the classical VEP neurophysiological method. When VEP are inconclusive, fMRI gives a clear result, with an immediate diagnosis which can be accessed even while the test is running online. It is sufficient to use fullfield stimulation to reach a result, making it possible to draw a quicker protocol.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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IMAGES

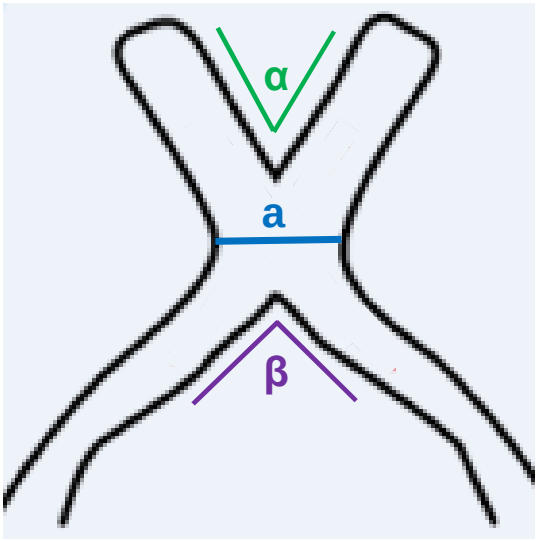

Fig.1 Chiasm measurements. A – chiasm width; α – anterior angle between optic nerves; β – posterior angle between optic tracts

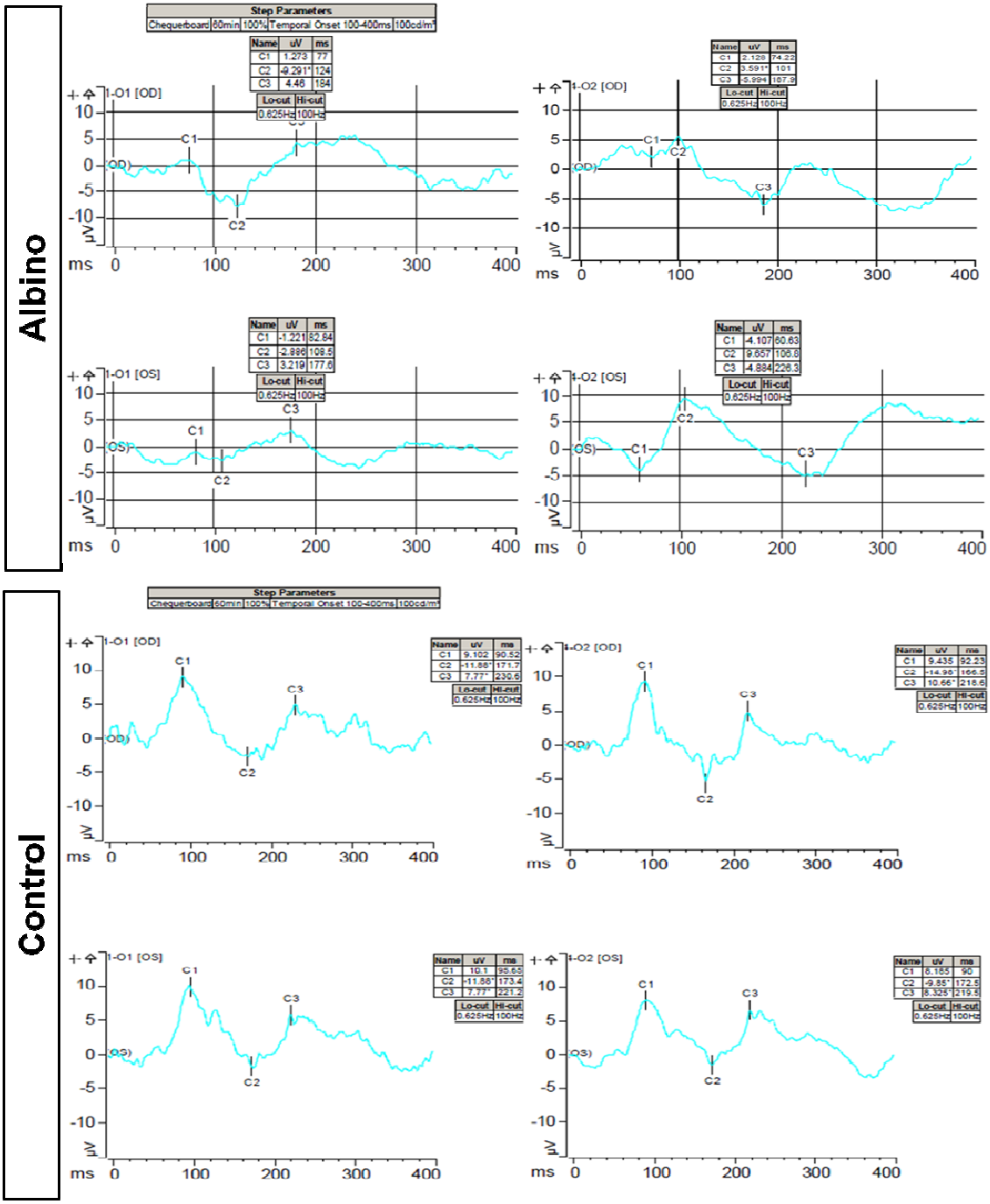

Fig.2 VEP recording of an albino and a control subject. Right eye (OD) and left eye (OS) stimulation. O1 – left side electrode; O2 – right side electrode

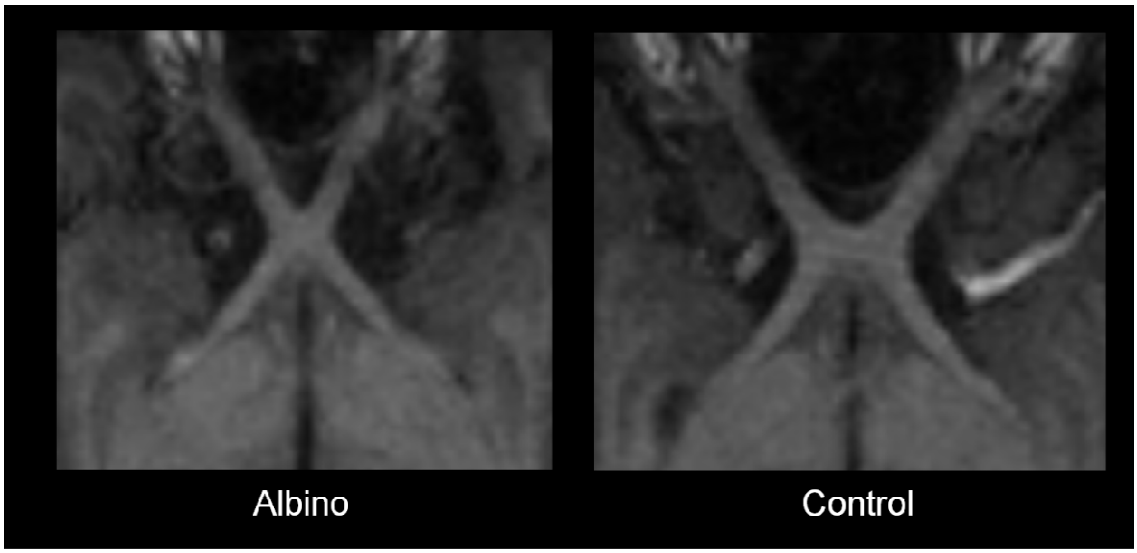

Fig.3 Structural T1 – weighted MRI illustrating morphological difference between the optical chiasm of representative albino and control subjects. Albino chiasm is narrower and X-shaped.

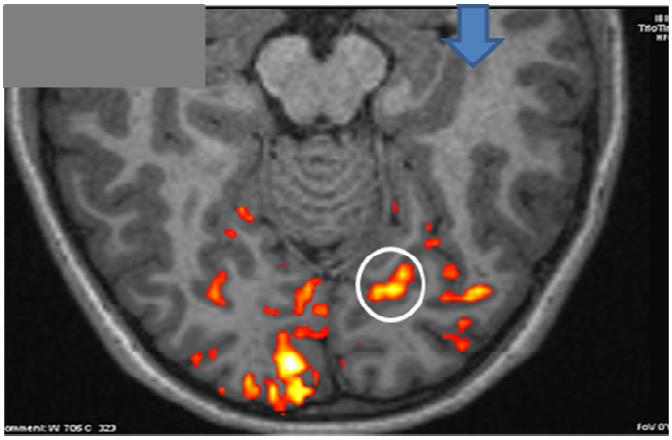

Fig.4 Visual fMRI peripheric ipsilateral activation map to right eye stimulation in albino subject, which correlates with the existence of some more peripheric temporal fibers with a normal trajectory. (white circle identifies the peripheric activation; blue arrow identifies right eye stimulation).

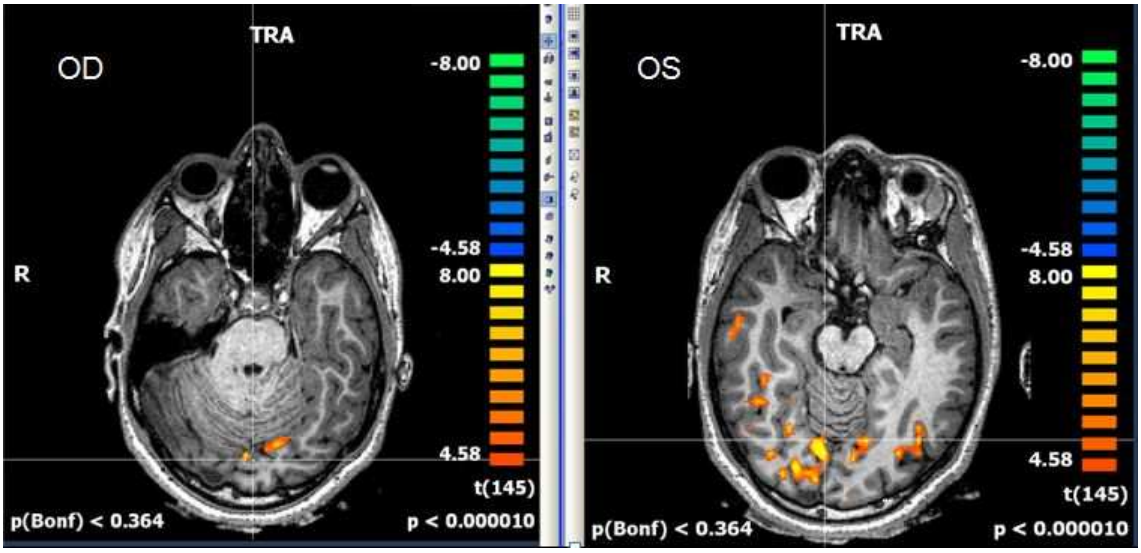

Fig.5 First albino subject who underwent hemifield stimulation. The asymmetry index was not calculated and compared to others because of difference in protocol but the albino pattern is present: right eye stimulation (OD) elicits left hemisphere activation and left eye stimulation (OS) elicits right hemisphere activation. One of the 3 cases were VEP's were inconclusive and the albino diagnosis was clear with fMRI.

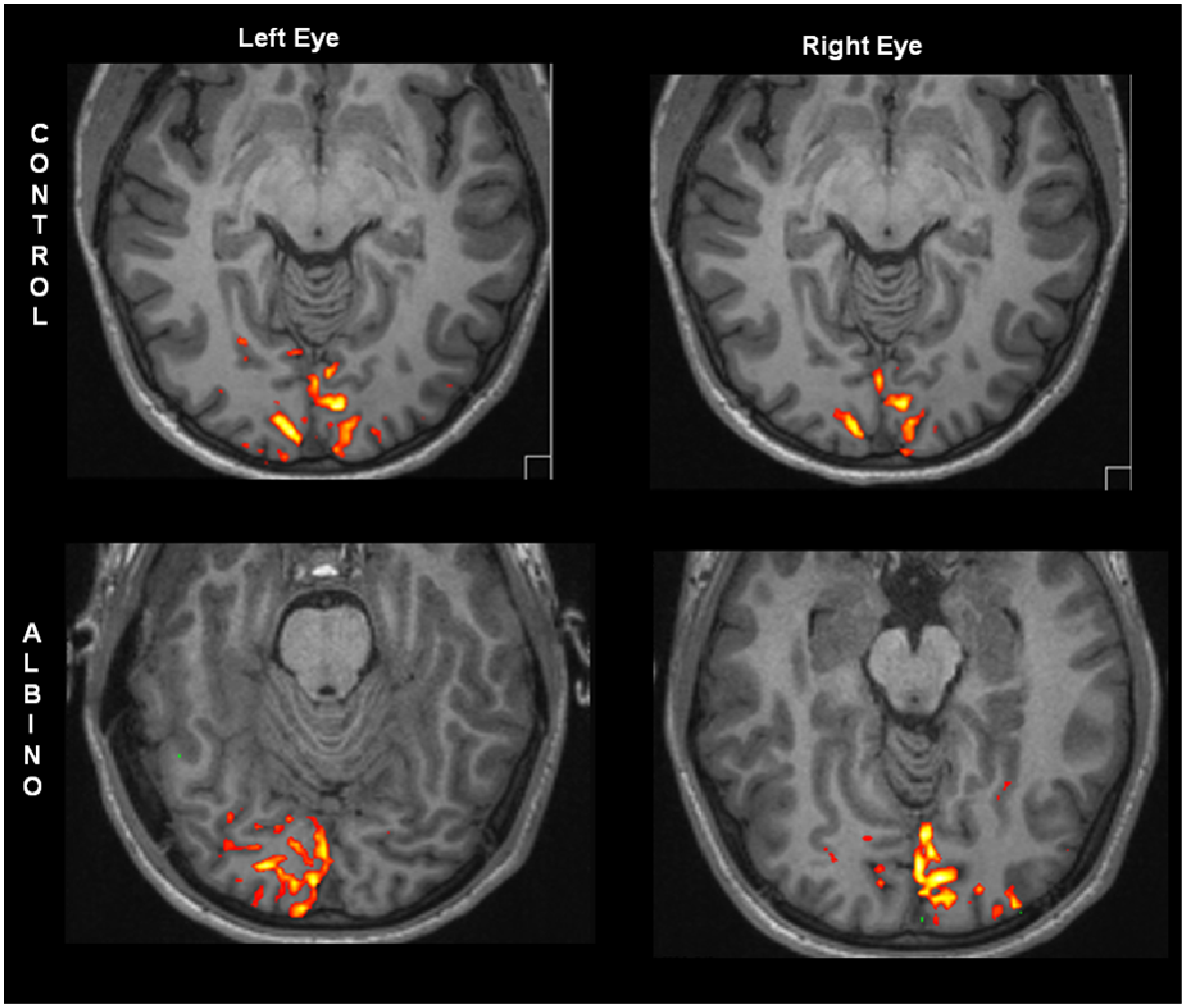

Fig.6 Visual fMRI activation maps in control and albino subject. Control activations are roughly symmetrical, while the albino shows predominant activation in the hemisphere contralateral to the stimulated eye.

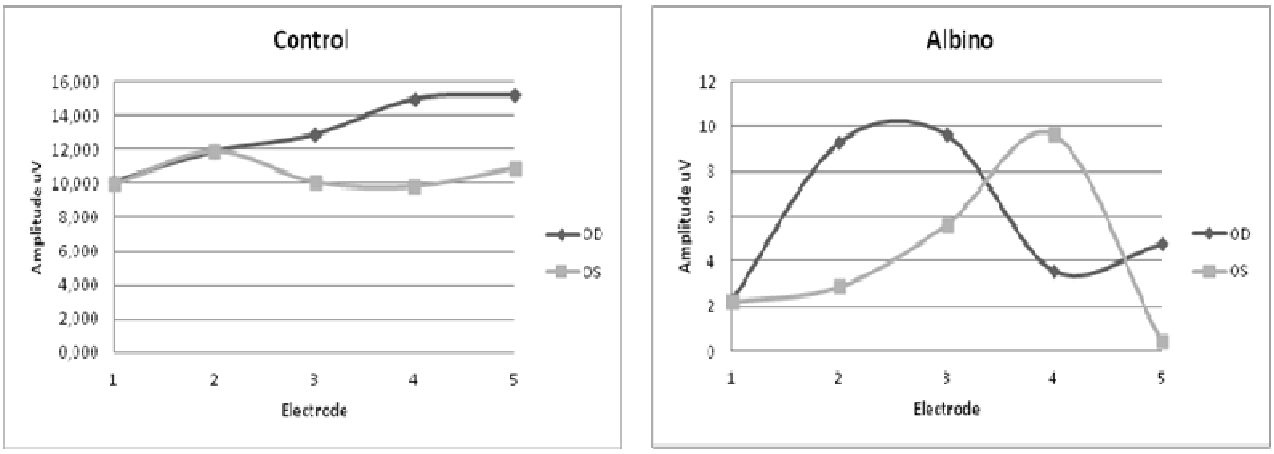

Fig. 7 VEP electrode peak amplitudes in control and albino subject. Control shows a pattern consistent with symmetrical activation in the occipital hemispheres in response to fullfield monocular stimulus. The albino shows a pattern of asymmetric response: right eye (OD) stimulation elicits a left hemisphere (electrode 2) amplitude peak; left eye (OS) stimulation elicits a right hemisphere (electrode 4) amplitude peak. Electrodes are numbered 1 through 5 from their location in the scalp, 1 being the far left, 3 the middle one (Oz), and 5 the far right.

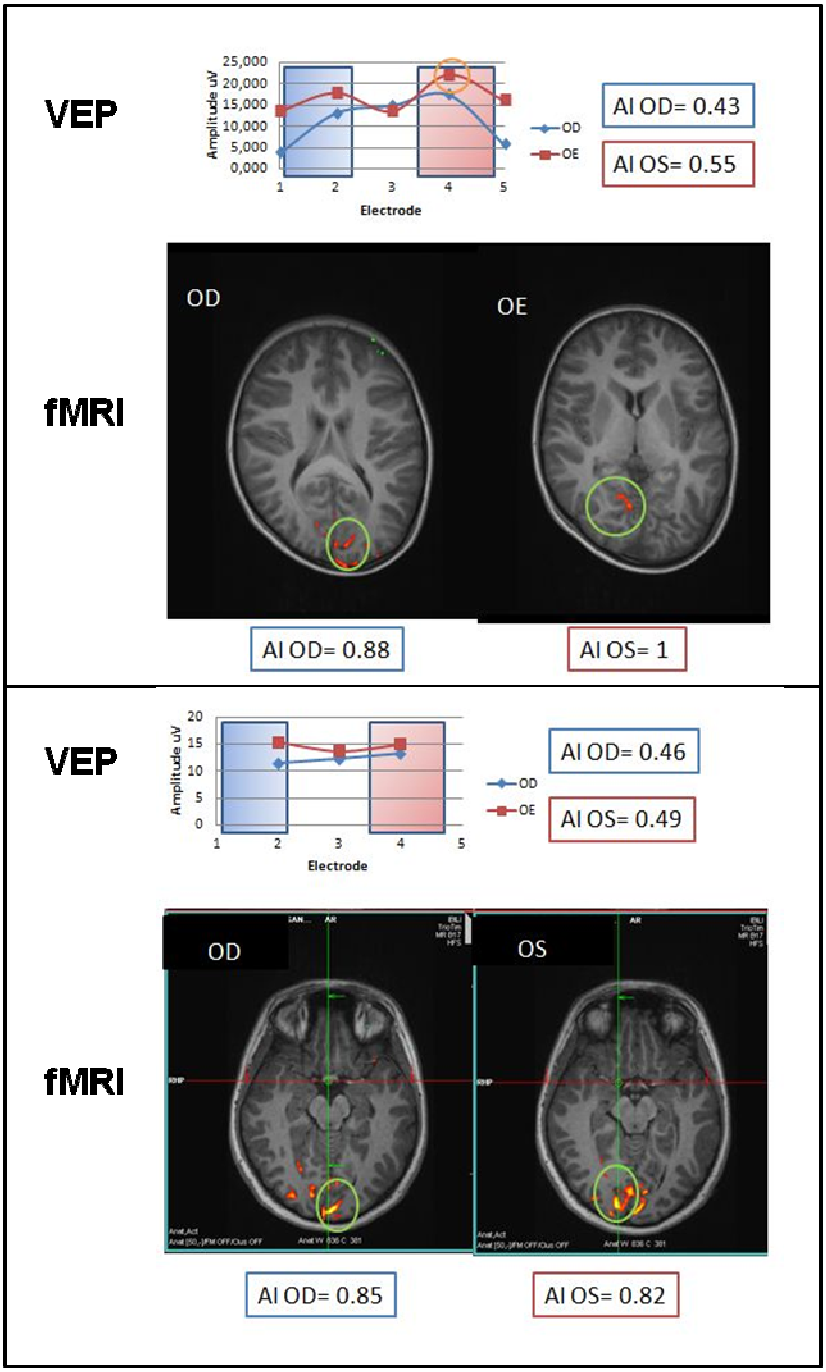

Fig.8 Two of the 3 cases were VEP's were inconclusive and the albino diagnose was clear with fMRI.

APPENDIX

This paper will be submitted for publication with The Journal of Neurology, therefore it was written taking into account the guidelines below.

THE JOURNAL OF NEUROLOGY – INSTRUCTIONS FOR AUTHORS

TITLE PAGE

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The title page should include:
The name(s) of the author(s)
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The e-mail address, telephone and fax numbers of the corresponding author

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Keywords
Please provide 4 to 6 keywords which can be used for indexing purposes.

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Manuscripts should be submitted in Word.
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Use italics for emphasis.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).
Manuscripts with mathematical content can also be submitted in LaTeX.

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Please use no more than three levels of displayed headings.

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Abbreviations should be defined at first mention and used consistently thereafter.

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Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments
Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

SCIENTIFIC STYLE

Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.

REFERENCES

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:
1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published
or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
The entries in the list should be numbered consecutively.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

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