Purpose: To describe the results of the first 12 months of treatment with EPI-743 (alphatocotrienol quinone) in a Brazilian cohort of Leber’s Hereditary Optic Neuropathy (LHON), 11778 haplogroup J mutation.

Methods: Six Brazilian patients with severe visual loss due to LHON were offered the experimental therapeutic EPI-743 as part of an open label trial. Two patients received EPI-743 at time of vision loss and four patients received EPI-743 more than five years after vision loss. Best corrected vision was assessed by Snellen visual acuity (Va). If patients could not detect any target on a standard Humphrey Visual Field (HVF), the mean deviation (MD) was set at a floor value of -35dB. Mean retinal nerve fiber layer (RNFL) thickness was measured using time domain (2007-2009) or spectral domain (2010-2013) Optical Coherence Tomography (OCT).

Results: All six patients showed an initial decline or at best a stabilization in Va in the first 6 months of treatment (mean 1.69 logMAR declined to mean 1.79 logMAR), but then showed an improvement in Va during the following 6 months (mean 1.51 logMAR). The MD of four patients remained at -35 dB, while in two patients who converted >5 years prior to treatment the MD improved to -18.32 ± 6.45 dB by 9 months after the start of treatment. The two patients who were treated at time of conversion showed continued bilateral progression for the first six months, consistent with the natural history of LHON. As expected, all eyes showed a decline in nerve fiber thickness on OCT that then stabilized after treatment.

Conclusions: This is the first report of clinical data for the third generation quinone EPI-743’s use in LHON patients from Brazil. Within 12 months of treatment initiation with EPI-743, all patients demonstrated improvement in visual acuity and two patients who had vision loss over 5 years earlier showed improvement in their visual fields. It is intriguing that improvement in vision occurred years after visual loss and that recovery was delayed by six months from the start of treatment, suggesting that longer duration of therapy may be necessary before a clinical response is observed.

Commercial Relationships: Amitha K. Ganti, None; Edward R. Chu, Edison Pharmaceuticals (F); Rustum Karanjia, None; Jeffery Tran, None; Rubens Belfort, Jr. (I); Milton Moraes, None; Adriana Berezovsky, None; Alfredo A. Sadun, Edison Pharmaceuticals (F), Stealth Peptides (F); Guy Miller, Edison Pharmaceuticals (E), Edison Pharmaceuticals (F), Edison Pharmaceuticals (I); Filipe Chicani, None

Support: Edison Pharmaceuticals and International Foundation for Optic Nerve Disease
Purpose: To evaluate Humphrey Visual Fields (HVF) for subclinical changes among carriers of the 11778 mitochondrial DNA mutation of LHON in a large Brazilian cohort. Previously, we have shown a decrease in multifocal electroretinogram (mERG) signals from the central retina in carriers of LHON from this cohort (Sadun A.A. et al, Trans Am Ophthalmol Soc 2006; 104:51). We aimed to determine whether carriers had an abnormal central perimetry when compared to affected and paternally-related controls.

Methods: The HVFs from the initial assessment of each subject were obtained for both eyes, and were categorized by disease status: affected (n=19), carriers (n=59) and controls (n=147). Subjects were excluded if they had any other ophthalmological problem, such as retinal scars or glaucoma. Observations for each HVF included assessments of mean deviation (MD), foveal threshold (FT), and threshold levels at each of the four central points around the fovea. Each variable was assessed as the dependent variable against disease status and controlled for age and gender in a multivariate linear regression analysis with estimates of significance adjusted using the Bonferroni correction.

Results: Affected patients had lower MD, FT and central thresholds, as expected. Carriers of all ages were not distinguishable from paternally-related controls by any perimetric measure. This finding is in contrast to previous reported findings which showed a decrease in central responses on mERG (Sadun A.A. et al, 2006), suggesting that changes in electrical activity in the retina are not reflected by retinal ganglion cell activity. The MD calculation is based on the HVF database to correct for age-related decline in visual sensitivity. We found that neither controls, nor carriers, demonstrated an association between age and MD (or other perimetric measures), suggesting that the rate of visual decline is normal in all groups. In the affected patients, there were no changes associated with age, presumably due to a floor effect.

Conclusions: Carriers in this large cohort displayed a normal phenotype on HVF perimetry, which is a subjective test of visual loss. There were no associations between MD and age, indicating the rate of perimetric decline in carriers is equal to the widely used database of controls in the HVF, which adjusts for age.

Commercial Relationships: Andrew Pouw, None; Jesse Gale, None; Rustum Karanjia, None; Jeffery Tran, None; Milton Moraes, None; Solange R. Salomao, None; Adriana Berezovsky, None; Filipe Chicani, None; Peter A. Quiros, None; Alfredo A. Sadun, None

Support: International Foundation for Optic Nerve Disease

Program Number: 6202 Poster Board Number: B0121

Presentation Time: 12:00 PM–1:45 PM

Perimetric parameters in unaffected carriers of Leber’s Hereditary Optic Neuropathy (LHON)

Andrew Pouw1, Jesse Gale1, Rustum Karanjia1, Jeffery Tran1, Milton Moraes2,3, Solange R. Salomao1, Adriana Berezovsky3, Filipe Chicani3, Peter A. Quiros3, Alfredo A. Sadun1. Ophthalmology, University of Southern California, Los Angeles, CA; 2Centro Universitario do Espirito Santo, Colatina, Brazil; 3Ophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil.

Purpose: To evaluate Humphrey Visual Fields (HVF) for subclinical changes among carriers of the 11778 mitochondrial DNA mutation of LHON in a large Brazilian cohort. Previously, we have shown a decrease in multifocal electroretinogram (mERG) signals from the central retina in carriers of LHON from this cohort (Sadun A.A. et al, Trans Am Ophthalmol Soc 2006; 104:51). We aimed to determine whether carriers had an abnormal central perimetry when compared to affected and paternally-related controls.

Methods: The HVFs from the initial assessment of each subject were obtained for both eyes, and were categorized by disease status: affected (n=19), carriers (n=59) and controls (n=147). Subjects were excluded if they had any other ophthalmological problem, such as retinal scars or glaucoma. Observations for each HVF included assessments of mean deviation (MD), foveal threshold (FT), and threshold levels at each of the four central points around the fovea. Each variable was assessed as the dependent variable against disease status and controlled for age and gender in a multivariate linear regression analysis with estimates of significance adjusted using the Bonferroni correction.

Results: Affected patients had lower MD, FT and central thresholds, as expected. Carriers of all ages were not distinguishable from paternally-related controls by any perimetric measure. This finding is in contrast to previous reported findings which showed a decrease in central responses on mERG (Sadun A.A. et al, 2006), suggesting that changes in electrical activity in the retina are not reflected by retinal ganglion cell activity. The MD calculation is based on the HVF database to correct for age-related decline in visual sensitivity. We found that neither controls, nor carriers, demonstrated an association between age and MD (or other perimetric measures), suggesting that the rate of visual decline is normal in all groups. In the affected patients, there were no changes associated with age, presumably due to a floor effect.

Conclusions: Carriers in this large cohort displayed a normal phenotype on HVF perimetry, which is a subjective test of visual loss. There were no associations between MD and age, indicating the rate of perimetric decline in carriers is equal to the widely used database of controls in the HVF, which adjusts for age.

Commercial Relationships: Andrew Pouw, None; Jesse Gale, None; Rustum Karanjia, None; Jeffery Tran, None; Milton Moraes, None; Solange R. Salomao, None; Adriana Berezovsky, None; Filipe Chicani, None; Peter A. Quiros, None; Alfredo A. Sadun, None

Support: International Foundation for Optic Nerve Disease

Program Number: 6203 Poster Board Number: B0122

Presentation Time: 12:00 PM–1:45 PM

Although smoking and alcohol are known to increase incidence of Leber’s Hereditary Optic Neuropathy (LHON) only smoking increases severity of LHON

Rustum Karanjia1, Jeffery Tran1, Edward R. Chu1, Jesse Gale1, Starleen E. Frousiakis1, Andrew Pouw1, Christianne A. Wa1, Milton Moraes2,3, Solange R. Salomao1, Valerio Carelli4,5. Ophthalmology, University of Southern California, Los Angeles, CA; 2Centro Universitario do Espirito Santo, Colatina, Brazil; 3Ophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil; 4IRCCS Institute of Neurological Sciences of Bologna, Bellaria Hospital, Bologna, Italy; 5Department of Biomedical and NeuroMotor Sciences (DIBINEM), University of Bologna, Bologna, Italy.

Purpose: To characterize clinical outcome in a previously described pedigree with Leber’s Hereditary Optic Neuropathy (LHON) in relation to environmental toxin exposure.

Methods: Clinical data was collected as previously described (Sadun, F. et al., Am J Ophthalmol. 2004 Feb;137(2):271-7). Patients were characterized by disease status: affected (n=34), carriers (n=150) and controls (n=298). We used an OLS regression to look between and within groups for differences in optical characteristics, namely visual acuity (Va) and color vision (CVa), controlling for age and gender. We then used the regression to look for differences in clinical outcomes within these groups for environmental exposures including smoking, alcohol and occupational exposure to toxic chemicals. As all analyses were based on the same dataset, we adjusted for “multiple looks” using the Bonferroni Correction.

Results: As expected, the affected group had a statistically worse Va compared to the control group. There was no significant difference between the control and carrier groups. Increasing age was found to be correlated with reduced Va in both control and carrier groups. In controls, occupational exposure to toxins did not impact Va. There was no significant difference in Va within the control group between those who smoked regularly or consumed alcohol and those who did not smoke or consume alcohol regularly. However, within both the carrier and affected LHON groups, having smoked regularly was associated with a significantly poorer Va. Notably, using alcohol was associated with a significantly better Va relative to those who did not. This is contrary to our expectations given the published data showing that alcohol consumption increases incidence of LHON in carriers (Sadun, F. et al., 2004 and Kirkman, MA et al., Brain. 2009 Sep;132(Pt 9):2317-26). The explanation might be related to alcohol producing contrary effects at different dosing. Unfortunately, the present data set did not provide accurate quantification of alcohol consumption. CVa was significantly worse in carriers and affected patients when compared to controls.

Conclusions: This is the first study to quantify visual acuity changes relative to toxic exposures in a large pedigree of LHON patients. Increasing age and smoking were associated with decreased visual acuity in both the affected and carrier groups.

Commercial Relationships: Rustum Karanjia, None; Jeffery Tran, None; Edward R. Chu, None; Jesse Gale, None; Starleen E. Frousiakis, None; Andrew Pouw, None; Christianne A. Wa, None; Milton Moraes, None; Solange R. Salomao, None; Valerio Carelli, None

Support: International Foundation for Optic Nerve Disease

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**Program Number:** 6204 Poster Board Number: B0123
**Presentation Time:** 12:00 PM–1:45 PM
**Multifocal bioelectrical cortical responses in Leber Hereditary optic Neuropathy**

*Vincenzo Parisi*, Lucia Ziccardi, Daniela Giannini, Federico Sadun, Anna Maria De Negri, Giacomo Savini, Piero Barbou, Chiara La Morgia, Valerio Carelli. 1Visual Neurophysiology & Neuroophthalmology, GB Bietti Eye Foundation-IRCCS, Rome, Italy; 2Department of Statistical Sciences, “Sapienza” University of Rome, Rome, Italy; 3Ophthalmology, Saint John Evangelist Hospital, Tivoli, Italy; 4Ophthalmology, Azienda San Camillo-Forlanini, Rome, Italy; 5Anterior Segment Unit, GB Bietti Eye Foundation - IRCCS, Rome, Italy; 6Ophthalmology, Ophthalmic Clinic d’Azeeglio, Bologna, Italy; 7Ophthalmology, Scientific Institute San Raffaele -IRCCS, Milan, Italy; 8Neurology, Institute of Neurologic Sciences of Bologna - IRCCS, Bologna, Italy; 9DIBINEM, University of Bologna, Bologna, Italy.

**Purpose:** To assess visual cortical bioelectrical responses in Leber’s Hereditary Optic Neuropathy (LHON) by means of multifocal visual evoked potentials (mfVEPs)

**Methods:** Seventeen patients carrying LHON mutation (mean age 33.35 ± 8.4 years, 17 LHON eyes) and 22 age-matched healthy control subjects (mean age 38.2 ± 6.0 years, 22 Control eyes) were studied by mfVEPs in response to 61 M-stimuli presented to the central 20 degrees of the visual field. MFVEPs P1 implicit time (P1 IT), ms and response amplitude density of the N1-P1 components (N1-P1 RAD, nV/deg2) of the second-order binary kernel were measured for five retinal eccentricities in areas between the fovea and midperiphery: 0-2.5 (R1), 2.5-5 (R2), 5-10 (R3), 10-15 (R4), and 15-20 (R5) degrees.

**Results:** LHON patients showed statistically significant (ANOVA, p<0.01) differences in mean mfVEPs P1 ITs and N1-P1 RADs at all five foveal eccentricities (R1-R5, 0-20 degrees) compared to Controls. In both Control and LHON eyes, mean mfVEPs responses obtained from R1 to R5 showed a progressive shortening of P1 ITs (linear fitting, LHON: R=-0.95; C=R=-0.98) and decreasing of N1-P1 RADs (exponential fitting, LHON: R2= 0.94; C: R2= 0.93). The progressive decay of mean mfVEPs P1 ITs resulted about three times greater in LHON patients than in Controls (LHON: y = -13.33x +182.03; C: y = -4.528x +108.1)

**Conclusions:** Our findings confirm the presence of an impairment of the neural conduction along the visual pathways in LHON. This dysfunction is more evident for the axons driving responses from the central retinal areas with respect to the axons driving responses from the peripheral retinal regions

**Commercial Relationships:** Vincenzo Parisi, None; Lucia Ziccardi, None; Daniela Giannini, None; Federico Sadun, None; Anna Maria De Negri, None; Giacomo Savini, None; Piero Barbou, None; Chiara La Morgia, None; Valerio Carelli, None

**Support:** Italian Ministry of Health grant number: 2006 RF-FGB-2006-368547

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**Program Number:** 6205 Poster Board Number: B0124
**Presentation Time:** 12:00 PM–1:45 PM
**Cardiac Conduction in Leber's Hereditary Optic Neuropathy**

*Starleen E. Frounias*1, Anitha K. Ganti, None; *Thomas Meier*, 1Santhera Pharmaceuticals (Switzerland) Ltd, Liestal, Switzerland; 2Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Germany.

**Purpose:** To evaluate cardiac conduction in affected patients and carriers of Leber’s hereditary optic neuropathy (LHON), 11778 mutation, using electrocardiogram (ECG) from a large Brazilian cohort.

**Methods:** Patient populations were categorized by disease status: affected patients (n=17) and carriers of the 11778 mutation (n=43). Each population underwent ECG testing, performed by a cardiologist, with measurement of PR interval and QTc duration. Control measurements were obtained from a de-identified database and healthy volunteers (n=33). An idealized curve of published data was also obtained from a previously published Italian cohort (Cameli, M. et al. Alcohol Clin Exp Res. 2009 Dec;33(12):2141-6). A Shapiro-Wilks test was used to compare the distributions of PR and QTc to a normal distribution. An unpaired student’s t-test was performed to determine if there was a difference between the groups.

**Results:** There was a significant decrease in the PR interval in the affected and carriers populations compared to controls. Mean PR intervals and standard deviations in control, affected and carrier populations were 151.2±19.0, 140.6±34.4 and 129.8±38.1 milliseconds, respectively. The Shapiro-Wilks test demonstrated that the carrier and affected PR intervals were non-normally distributed when compared to controls. Carrier distribution of PR interval demonstrated a positive skew (p<0.001), with a mode of 120 milliseconds; the lower limit of normal. Similarly, distribution of affected PR intervals demonstrated a positive skew (p<0.001). There was no significant difference detected in the QTc intervals in affected or carrier patients, relative to the control population. The difference between carrier and affected patients was also insignificant.

**Conclusions:** This study determined that affected and carrier populations of the LHON 11778 mutation had a non-normal distribution of PR interval. These findings suggest that there may be subclinical cardiac conduction changes in patients and carriers of LHON.

**Commercial Relationships:** Starleen E. Frounias, None; Rustum Karanja, None; Anitha K. Ganti, None; Andrew Pouw, None; Milton Moraes, None; Solange R. Salomao, None; Rubens Belfort, Jr., None; Peter A. Quiros, None; Filipe Chicani, None; Alfredo A. Sadun, None

**Support:** Edison Pharmaceuticals and International Foundation for Optic Nerve Disease (IFOND)

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**Program Number:** 6206 Poster Board Number: B0125
**Presentation Time:** 12:00 PM–1:45 PM
**Clinical experience with Idebenone (Raxone®) in the treatment of patients with Leber’s Hereditary Optic Neuropathy (LHON)**

*Guenther Metz*, 1Constance Gallemueller, 1Thomas Meier, Thomas Klopopstock, 1Santhera Pharmaceuticals (Switzerland) Ltd, Liestal, Switzerland; 2Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Germany.

**Purpose:** An increasing body of evidence indicates that idebenone has therapeutic potential for the treatment of LHON. Data from a randomized placebo-controlled study (RHODOS) and from a number of case reports and retrospective cohort studies demonstrate that patients with established vision loss may benefit from idebenone treatment and recover visual acuity (VA). This study reports VA outcomes for LHON patients with recent onset of vision loss who received idebenone (Raxone) treatment under an Expanded Access Program (EAP).

**Methods:** LHON patients with recent onset of vision loss were enrolled in a global EAP between November 2011 and October 2013. Treating physicians who wished to enrol patients under Named Patient Programs (Europe, Australia, New Zealand) or treatment IND (USA) were provided with Raxone (idebenone 150 mg tablets). Physicians were requested to report any safety issues and were
encouraged to also report VA outcomes in 3-monthly intervals for patients under treatment. Improvement in VA was defined as (i) improvement in VA by at least 10 letters on ETDRS chart or (ii) improvement from “off-chart” vision to being able to read at least 5 letters on the ETDRS chart.

**Results:** There were 50 LHON patients enrolled in the ongoing EAP of which 42 patients had provided post treatment VA data at submission of the abstract. The average time to treatment from onset was 7 months and average treatment duration was 8 months (range: 3-18 months); patients had disease-specific demographics with respect to age at symptom onset (mean: 32 years), gender (72 % male), and mtDNA mutations (G11778A: 54%, G3460A: 20%, T14484C: 14%, other: 12%). Across all mutations, 19 of 42 (45 %) of patients experienced clinically meaningful VA recovery from nadir with the majority (74%) of these patients showing VA recovery within 6 months of treatment. Treatment with idebenone was safe and well tolerated

**Conclusions:** A high proportion of LHON patients treated with idebenone under a global EAP experienced a clinically meaningful recovery of vision, further demonstrating the therapeutic potential of idebenone in the treatment of LHON.

**Commercial Relationships:** Guenther Metz, Santhera Pharmaceuticals (E); Constanze Gallemueller, None; Thomas Meier, Santhera Pharmaceuticals (E); Thomas Klopotock, Santhera Pharmaceuticals (C), Santhera Pharmaceuticals (F)

**Program Number:** 6207 **Poster Board Number:** B0126

**Presentation Time:** 12:00 PM – 1:45 PM

**LONG PCR BASED ANALYSIS OF WHOLE MITOCHONDRIAL GENOME OF LEBER’S HEREDITARY OPTIC NEUROPATHY (LHON) PATIENTS Bibhuti B. Saikia,1 Jayashree Charmakani,1 Mahesh K. Shanmugam,2 Periasamy Sundaresan.1 1Department of Genetics, Aravind Medical Research Foundation, Madurai, India; 2Neuro-ophthalmology Clinic, Aravind Eye Hospital and Post Graduate Institute of Ophthalmology, Madurai, India.

**Purpose:** Leber’s Hereditary Optic Neuropathy (LHON) patients mostly carry three mitochondrial DNA point mutations, m.3460 G>A, m.11778 G>A and m.14484 T>C, occurs in the genes encoding complex I subunits of the respiratory chain. Over 95% of LHON pedigrees harbor one of these three primary mitochondrial DNA mutations. The aim of this study was to screen whole mitochondrial genome of LHON patients to explore the role of primary mutations and other mtDNA variants in the pathogenesis of disease

**Methods:** Thirty five LHON patients and thirty five age matched controls were recruited for the study from the Neuro-ophthalmology clinic, Aravind Eye Hospital, Madurai, Tamil Nadu, India. Long PCR based nine pairs of primer were used to amplify whole mitochondrial genome of both the cases and controls. Bidirectional sequencing was performed with thirty one different primers. The sequences from patients and controls were compared with the revised Cambridge reference sequence (rCRS NC_012920). The observed variations were compared with mitochondrial databases such as Mitomap (http://www.mitomap.org); mtDB (http://www.genpat.uu.se/mtDB) and HmtDB (http://www.hmtdb.uniba.it:8080/hmdb) for their significance

**Results:** Out of thirty five LHON patients screened, ten patients reported with the m.11778G>A mutation and one with m.14484T>C mutation. We could not identify m.3460G>A mutation in any of the patients. Along with these primary mutations, we have identified nonsynonymous variations in protein coding genes in both the cases and controls. Known disease associated variants were also identified in this study

**Conclusions:** Two primary mutations (m.11778G>A and m.14484T>C), non synonymous mutations and other mitochondrial disease associated variants were also identified in this study. Better characterization of the relationship between mitochondrial DNA mutations background and optic nerve dysfunction will result in improved genetic counseling and development of therapeutic strategies.

**Commercial Relationships:** Bibhuti B. Saikia, None; Jayashree Charmakani, None; Mahesh K. Shanmugam, None; Periasamy Sundaresan, None

**Support:** Department Of Science and Technology

**Program Number:** 6208 **Poster Board Number:** B0127

**Presentation Time:** 12:00 PM – 1:45 PM

**The mt11778/ND4 mutation, in Leber’s hereditary optic neuropathy, is associated with less degeneration in the peripheral nerve compared to the mt3460/ND1 mutation Edward R. Chu1, Fred N. Ross-Cisneros2, Anh H. Pham3, Jesse Gale3, Milton Moraes4, Solange R. Salomao1, Adriana Berezovsky1, Valerio Carelli4, Rubens Belfort, Jr.,1 Alfredo A. Sadun1,2 Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, CA; 3Centro Universitario do Espirito Santo, Colatina, Brazil; 4Optophthalmology, Federal University of Sao Paulo, Sao Paulo, Brazil; 1Neurological Sciences, University of Bologna, Bologna, Italy; 2Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy.

**Purpose:** We previously demonstrated peripheral nerve degeneration in Leber’s hereditary optic neuropathy (LHON) from the mt3460/ND1 mutation, considered to be the most severe of the frequent mutations (Mnatsakanyan et al., J Neuroophthalmol. 2011;31:6-11). Herein, we assessed histologic findings of a branial plexus from a LHON mt11778/ND4 patient also compared to age-matched control nerves.

**Methods:** Brachial Plexus was obtained at necropsy from a 77-year-old man with LHON carrying the mt11778/ND4 mutation, with no known history of peripheral neuropathy and diabetes. This was compared to three age-matched controls obtained from the tissue bank of the National Disease Research Institute (Philadelphia, PA). There was no history of neurological diseases or diabetes in control nerves. All tissues were fixed by immersion in a buffered aldehyde solution and processed for epon blocks. Semi-thin cross-sections were obtained for histological examination and stained with p-phenylenediamine (PPD) for myelin and examined by light microscopy. Morphometry was performed on five different areas of the plexus at 400x magnification. Degenerated axons were manually identified as dark, homogenous, opaque profiles. A custom ImageJ software macro (NIH, Bethesda, Maryland) was used to quantify total axonal counts and average myelin density per axon in both LHON and control peripheral nerves.

**Results:** Peripheral nerves from LHON mt11778/ND4 and controls demonstrated scant nonspecific degeneration, attributable to normal aging. This finding was different from our previous study of mt3460/ND1, in which significant degeneration of the peripheral nerve was present (Mnatsakanyan et al., 2011). Interestingly, total axonal counts were markedly greater in LHON compared to the control group (p<0.05), while average myelin density per axon was significantly less (p< 0.05) in LHON compared to controls.

**Conclusions:** This is the first report to quantitatively describe histologic features of the peripheral nerve in LHON mt11778/ND4 mutation. Unlike mt3460/ND1, there was no appreciable degeneration in this mt11778/ND4, the less severe LHON mutation. However, reduced myelination and increased axonal counts observed
in the peripheral nerve (brachial plexus) might reflect compensatory regeneration.

Commercial Relationships: Edward R. Chu, Edison Pharmaceuticals, Inc. (F); Fred N. Ross-Cisneros, Edison Pharmaceuticals, Inc. (F); Anh H. Pham, None; Jesse Gale, None; Milton Moraes, None; Solange R. Salomao, None; Adriana Berezovsky, None; Valerio Carelli, Edison Pharmaceuticals, Inc. (F); Sigma-Tau (F); Rubens Belfort, Jr., None; Alfredo A. Sadun, Edison Pharmaceuticals, Inc. (F); Stealth Peptides, Inc. (F)

Support: Research to Prevent Blindness, International Foundation for Optic Nerve Disease (IFOND), Struggling Within Leber’s, Eierman Foundation, The Poincnet Family and NIH Grant # EY03040.

Program Number: 6209 Poster Board Number: B0128
Presentation Time: 12:00 PM–1:45 PM
Mitochondrial DNA integration and replication in transgenic mutant ND4 mice
John Guy1, Hong Yu1, Arpit Mehta2, Alfred S. Lewin3. 1Bascom Palmer Eye Institute, University of Miami, Miami, FL; 2Hussman Institute Human Genomics, Miami, FL; 3University of Florida, Gainesville, FL.

Purpose: To evaluate integration and replication of the mutant human ND4 in mitochondria following injection of a mitochondrially targeted AAV into the blastocyst of mice. Last year we showed that these transgenic mice develop optic neuropathy that is vertically transmitted from mother to offspring.

Methods: Mutant human G11778A ND4 responsible for most cases of LHON was inserted into a mito-targeted AAV containing the COX8b leader sequence inserted into the VP2 capsid then microinjected into the mouse blastocyst to generate transgenic mutant ND4 mice. Next generation sequencing was performed on mitochondrial DNA extracted from the retina, brain and muscles of these transgenic mice and the retinas of mice injected with the MTS-AAV as a positive control. We performed alignments with the human and mouse mitochondrial genomes and also with the virus AAV genome looking for reads which align one part to the virus and other to mouse or human mtDNA and determining the depth at each base level to look for variants. To determine whether the AAV transferred mutant human ND4 replicated, we performed in vitro replication of extracted mtDNA.

Results: In each of the samples we identified 0 to 208 reads that mapped to the MTS-AAV containing human ND4. In retinal samples from transgenic mice and retinas directly injected with the MTS-AAV we identified some retention of the first iTR as well as the iTR at the other end of the vector with muscle and brain samples showing deletion of the iTR. Chimera of human ND4 and the mouse mitochondrial genome were absent. No insertions of human ND4 or the transferred viral genome were detected in the mouse mitochondrial genome. In vitro replication revealed a ~2kb band of the vector in transgenic mice, absent in normal B6 mice, with hybridization to a human ND4 probe.

Conclusions: Optic neuropathy detected in transgenic mice generated from blastocyst injections appears to be due to mutant human G11778A ND4 and not due to recombination with mouse mtDNA or deletions of host mtDNA. Replication of transferred viral DNA occurred in the absence of inclusion of the CSB regions of the D loop into our AAV construct suggesting that the vector iTR may be driving replication of the episomal mutant human ND4. These findings suggest that MTS-AAV mediated transfer of a normal human ND4 allele to the human embryo may be a yet unexplored option to prevent transmission of blindness in subjects with mutated ND4.

Commercial Relationships: John Guy, None; Hong Yu, None; Arpit Mehta, None; Alfred S. Lewin, None
Support: EY017141 and EY012355

Program Number: 6210 Poster Board Number: B0129
Presentation Time: 12:00 PM–1:45 PM
Multietnic involvement in autosomal dominant optic atrophy; report of a new gene mutation causing optic atrophy and deafness Dan Milea1,2, Patrizia Amati-Bonneau3, Sharon Tow1, Dominique Bonneau1, Vincent Procaccio2, Pascal Reynier1, Jing Liang Loo1. 1Singapore National Eye Centre, Singapore, Singapore; 2Angers University Hospital, Angers, France.

Purpose: Autosomal dominant optic atrophy (ADOA) is an ubiquitous condition causing bilateral visual loss, most commonly related to mutations in the OPA1 gene, mapped on the chromosome 3q28-q29. Despite recent data suggesting that the condition is more common than previously thought (the estimated minimum prevalence in a population in northern England is 4.07/100,000, or about 1 in 25,000), ADOA is more rarely reported in Asia. As a first step, our study aimed to detect patients with genetically confirmed ADOA in an Asian population in Singapore.

Methods: We conducted a preliminary cross-sectional study at the Singapore National Eye Centre, testing patients (using direct sequencing of the OPA1 gene) who had a clinical picture compatible with ADOA and who were seen in the outpatient clinics at our institution between January and August 2013.

Results: We selected and tested genetically 5 patients among a series of patients with unexplained bilateral optic neuropathy, after ruling out other common causes of optic neuropathies (compression, toxic, Leber’s hereditary optic neuropathy, glaucoma, etc.). Among these, 3 patients had a genetically confirmed ADOA, on exons 8 and 9: c.869G>A (p.Arg290Glu), c.871-1G>A and c.892A>G (p.Ser298Gly). Each patient belonged to 3 different families and 3 different ethnic groups: Chinese, Indian and Malay. All patients had bilateral visual loss occurring during the first two decades of life, associated with paracentral scotomas, color vision loss and optic atrophy. A positive family history of visual loss was found only in one of the patients. One proband harbored a novel heterozygous OPA1 pathogenic variant c.892A>G (p.Ser298Gly) that has not been previously catalogued in the eOPA1 database, causing optic atrophy and deafness in early childhood.

Conclusions: We report the first 3 families with genetically confirmed ADOA in Singapore, belonging to the three main ethnic groups of the country (Chinese, Indian, Malay). Among them, we report a new, previously undescribed mutation causing “dominant optic atrophy plus”, adding deafness to the classical phenotype of optic atrophy. Further epidemiological studies are needed in order to determine the prevalence of ADOA in Singapore.

Commercial Relationships: Dan Milea, None; Patrizia Amati-Bonneau, None; Sharon Tow, None; Dominique Bonneau, None; Vincent Procaccio, None; Pascal Reynier, None; Jing Liang Loo, None

Program Number: 6211 Poster Board Number: B0130
Presentation Time: 12:00 PM–1:45 PM
Macular Sensitivity and Fixation Patterns in Patients with Autosomal Dominant Optic Atrophy Cecilia Ronnback1,2, Michael Larsen1,2. 1Department of Ophthalmology, Glostrup Hospital, Glostrup, Copenhagen, Denmark; 2Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

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Purpose: To test macular sensitivity, fixation stability and fixation location using microperimetry in patients with autosomal dominant optic atrophy (ADOA) and their mutation-free first degree relatives.

Methods: A cross-sectional study of 43 patients with exon 28 (2826 delT) mutation in OPA1 (age 11.7-71.5 years, best corrected visual acuity (BCVA) 20/724 – 20/13) and 49 mutation-free family members (BCVA 20/25 - 20/10) underwent ophthalmic examination including macular 12° eccentricity microperimetry. Stable fixation was defined as 75% or more fixation points being within 2° from the center of the fovea, predominantly central fixation as 50% of fixation points within 2°, while poorer fixation was labelled relatively unstable and predominantly eccentric fixation.

Results: Average sensitivity was significantly reduced in ADOA patients compared to controls, 14.9±4.4 dB versus 19.7±0.42 dB (p<0.0001). In a retinotopic projection, the largest relative sensitivity deficits in ADOA were seen in the nasal (13.0±6.8 dB versus 19.9±0.38 dB), inferonasal (12.8±4.5 dB versus 19.8±0.42 dB) and central (13.2±5.5 dB versus 19.8±0.43 dB) fields of the macula. Average sensitivity decreased with decreasing BCVA in ADOA (r=0.74, p<0.0001). Stable fixation was found in 58% (25/43) of ADOA patients and 86% (42/49) of controls, relatively unstable fixation in 35% (15/43) of ADOA patients and 14% (7/49) of controls, whereas unstable fixation was found only in ADOA, where the prevalence was 7% (3/43).

Conclusions: ADOA was associated with unstable fixation and subnormal microperimetric sensitivity, especially in the central and nasal half of the macula where the ganglion cell layer thickness is the lowest in ADOA.

Commercial Relationships: Cecilia Ronnback, None; Michael Larsen, None

Program Number: 6212 Poster Board Number: B0131
Presentation Time: 12:00 PM–1:45 PM

Ganglion cell complex analysis in toxic optic neuropathy by optical coherence tomography

luisa vieira, Nuno Silva, Arnaldo D. Santos, Rita Anjos, Luís Abegão Pinto, Andre Vicente, Barbara Borges, Joana Ferreira, Duarte Amado, João Paulo Cunha. Centro Hospitalar Lisboa Central, lisboa, Portugal.

Purpose: To analyze the ganglion cell complex (GCC) by optical coherence tomography (OCT) in toxic optic neuropathy and to correlate its thickness and volume with functional damage.

Methods: We conducted a prospective, case-control study, in healthy subjects and in patients with toxic optic neuropathy, observed in Neuroophthalmology section of Centro Hospitalar Lisboa Central. Full ophthalmology examination, OCT (Spectralis ®) and computerized static perimetry were performed. Thickness and macular volume of GCC (retinal ganglion cell layer and inner plexiform layer) were measured after manual segmentation.

Results: The study included 16 eyes (12 healthy subjects) and 16 eyes (8 patients with toxic optic neuropathy). Age and sex did not differ between the two groups. The etiological factors that generated the neuropathy were: ethambutol (4 patients) and alcohol-tobacco (4 patients). A statistically significant decrease in the thickness and volume of GCC, in all quadrants at 2 and 3mm, was detected in the neuropathy group compared to control (TS2 – p<0.001; TT2 – p=0.009; T12 – p=0.001; TN2 – p<0.001; TS3 – p<0.001; TT3 – p<0.001; T13 – p<0.001; TN3 – p<0.001; VS2 – p=0.001; VT2 – p<0.001; VI2 – p=0.001; VN2 – p<0.001; VS3 – p<0.001; VT3 – p<0.001; VI3 – p<0.001; VN3 – p<0.001). A positive correlation between GCC thickness and mean deviation (MD) (T52 – r=0.880 p<0.001; TT2 – r=0.718 p=0.009; T12 – r=0.841 p<0.001; TN2 – r=0.875 p<0.001; TS3 – r=0.718 p=0.009; TT3 – r=0.630 p=0.028; T13 – r=0.729 p=0.007; TN3 – r=0.851 p<0.001; and between GCC volume and MD (VS2 – r=0.770 p=0.003; VT2 – r=0.731 p=0.007; VI2 – r=0.924 p<0.001; VN2 – r=0.838 p=0.001; VS3 – r=0.813 p=0.001; VT3 – r=0.648 p=0.023; VI3 – r=0.729 p=0.007; VN3 – r=0.875 p<0.001) was detected. A negative correlation between MD and time of disease (r=−0.846 p<0.001) and a positive correlation between MD and visual acuity in logMAR (r=0.739 p=0.006) was also obtained. The majority of the structural parameters also correlated negatively with time of disease (p<0.05).

Conclusions: The decreased GCC thickness and volume detected in this study support the described retinal ganglion cells toxicity. GCC analysis may contribute to the diagnosis and management of this pathology.

Commercial Relationships: luisa vieira, None; Nuno Silva, None; Arnaldo D. Santos, None; Rita Anjos, None; Luís Abegão Pinto, None; Andre Vicente, None; Barbara Borges, None; Joana Ferreira, None; Duarte Amado, None; João Paulo Cunha, None

Program Number: 6213 Poster Board Number: B0132
Presentation Time: 12:00 PM–1:45 PM

The electrophysiological characteristics and monitoring of ethambutol toxicity

Anthony G. Robson1, 2, Shiying Li1, Magella M. Neveu1, 2, Zheng Q. Yin1, Graham E. Holder1, 2. ‘Electrophysiology, Moorfields Eye Hospital, London, United Kingdom; 1Institute of Ophthalmology, UCL, London, United Kingdom; 2Southwest Eye Hospital, Third Military Medical University, Chongqing, China

Purpose: To describe and monitor the electrophysiological and ophthalmic characteristics of ethambutol optic neuropathy (EON).

Methods: Thirty patients (median age 59 years; range 30-86 years) being treated with ethambutol for tuberculosis, and with a clinical diagnosis of presumed EON, were identified from the Department of Electrophysiology database at Moorfields Eye Hospital. All had undergone pattern reversal and flash visual evoked potential (PVEP; FVEP), and pattern and full-field electroretinogram (PERG; ERG) testing. Serial data were available in 7 cases monitored over periods of up to 3 years following cessation of ethambutol. The case notes were reviewed and the available clinical data compared with the electrophysiological findings.

Results: Patients presented with blurred vision or reduced visual acuity (VA; median logMAR 0.8; range 0 to 3). PVEPs were delayed in 33 of 45 eyes with a detectable response (median peak time 130ms; range 117-175ms) and were subnormal in 18 eyes including 5 without delay. Comparison between eyes revealed a high degree of inter-ocular symmetry of PVEP peak times (slope =0.92, r2=0.87) and amplitudes (slope= 0.70, r2= 0.68). Pattern ERG P50 component, a measure of macular function, was normal in 45 of 56 eyes and mildly reduced (<33%) in 11 eyes; the N95:P50 ratio was subnormal in 21 of 56 eyes, indicating retinal ganglion cell dysfunction. ERGs were mildly abnormal in 3 patients including two with normal PERG and one case with mild bilateral P50 reduction. The VEP timing and PERG P50 parameters did not correlate with VA. Eleven of 14 eyes showed improvement in VA following cessation of ethambutol (median improvement logMAR 0.5). Pattern VEP timing improved bilaterally in 9 eyes of 7 patients (by 4 to 46ms) including 4 eyes that normalised; PVEP returned in 3 eyes with previously undetectable PVEPs, including 2 with normal N95 components at baseline.

Conclusions: Ethambutol use may be associated with severe optic nerve and retinal ganglion cell dysfunction. Severe PVEP abnormalities may resolve following cessation of ethambutol and the need for early diagnosis is stressed.

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Commercial Relationships: Anthony G. Robson, None; Shivyng Li, None; Magella M. Neveu, None; Zheng Q. Yin, None; Graham E. Holder, None

Program Number: 6214 Poster Board Number: B0133
Presentation Time: 12:00 PM–1:45 PM
Reversible changes in patients with early detected ethambutol induced ocular toxicity: after discontinuation of ethambutol treatment


Purpose: We found out P100 latency delay on pattern VEP and thickening of RNFL on OCT at 4 months after ethambutol (EMB) treatment. This study aimed to determine whether these findings were reversible after discontinuation of EMB treatment and to investigate the duration of time these findings to be restored to the baseline level.

Methods: Twenty-two patients who started 6 month regimen of EMB for treatment of respiratory tuberculosis were recruited prospectively for this study. We performed OCT scan, VEP and visual field test at the beginning of EMB treatment (baseline) and with 1 month intervals to detect early findings of EMB induced ocular toxicity. Among these patients, fourteen patients who showed significantly increased RNFL thickness and delay or prolongation of P100 latency at the end of the treatment compared to baseline were included. RNFL thickness and P100 latency were measured at 3, 6, 12 months after discontinuation of EMB treatment and compared with data at baseline by paired t test.

Results: P100 latency delay was progressed at 3 months after discontinuation of taking EMB (Rt. eye, p < 0.001, L.t. eye, p = 0.005). Thickening of RNFL was also progressed at 3 months after discontinuation EMB treatment (both eyes, p < 0.001). After 6 months, these finding were reversed. After 12 months, P100 latency & RNFL thickness were restored to baseline level (P100 latency, Rt. eye : p = 0.507, L.t. eye : p = 0.750 / RNFL thickness, Rt. eye : p = 0.245, L.t. eye : p = 0.388).

Conclusions: Delay of p100 latency & thickening of RNFL were progressed at 3 months after discontinuation of EMB treatment. And, p100 latency & RNFL thickness were restored to their baseline level at 12 months after discontinuation of taking EMB. So, patient should be observed until 12 months after discontinuation of EMB treatment to detect of EMB induced ocular toxicity during these period.

Commercial Relationships: Kyung Lae Kim, None; Sung Pyo Park, None

Program Number: 6215 Poster Board Number: B0135
Presentation Time: 12:00 PM–1:45 PM
Prevalence and Characterization of Epiretinal Membranes in Patients with Optic Nerve Disease

Nicole De Cair1, Juliet Idiga1, Khushmit Kaur1, Jeffrey Odel1, Gustavo V. De Moraes1, Robert Ritch1, Donald C. Hood1. Columbia University, New York, NY; ‘New York Eye & Ear Infirmary, New York, NY.

Purpose: Because patients with optic nerve disease can exhibit ocular inflammation and it has been hypothesized that inflammation can lead to ERM formation, we compared the prevalence and characteristics of epiretinal membranes (ERM) in glaucoma patients, glaucoma suspects, patients with other optic nerve diseases, and healthy controls.

Methods: ffOCT horizontal cone scans of the macula (Topcon Inc, Japan) were obtained and examined for the presence of ERMs, which were classified as: Simple [a thickened, hyper-pigmented region of the inner limiting membrane (ILM)-see Fig. 1a] or Complex (i.e. a thickened region of the ILM that has a gap separating it from retinal nerve fiber layer, which in some cases appeared distorted-see Fig. 1b). The study population included 54 eyes of 54 control patients (52.8 ± 8.2 yrs), 75 eyes of 75 glaucoma patients (58.1 ± 11.5 yrs), 49 eyes of 49 glaucoma suspects (51.2 ± 13.4 yrs), and 56 eyes of 56 patients with other optic nerve diseases (47.32 ± 17.6 yrs). Glaucoma patients and suspects had glaucomatous optic neuropathy, with abnormal (glaucoma) or normal (suspects) 24-2 visual fields based upon cluster criteria. The patients with other optic nerve disease included patients with MS, ischemic optic neuropathy, and optic atrophy.

Results: Overall, 83.3% of control eyes, 60.0% of glaucomatous eyes (GL), 67.4% of glaucoma suspect eyes (GS), and 82.2% of eyes with other optic nerve disease (OND) exhibited ERMs (Table). Based upon age-adjusted logistical regression, the prevalence of ERMs in the patient groups was not significantly greater than the controls [Odds Ratios: 0.34 (G); 0.41 (GS), 0.83 (OND)]. Of note, 30 (16.7%) of the patients’ eyes, but none of the control eyes, had complex ERMs. However, only age was a statistically significant predictor of complex ERMs. Given that the control group was significantly eyes with temporal hemianopia due to optic chiasmal compression and 80 normal eyes. Any participants with nasal visual field loss were excluded. The average visual field sensitivity (VFS) of the temporal visual field in Humphrey visual field analyzer was expressed by 1/ Lambert. In BA eyes, we analyzed the relationship between cpRNFL thickness in 12-clock sectors and VFS by regression analysis. Floor effect (FE) in each cpRNFL sector was set when temporal visual sensitivity was 0 and compared with the thickness of normal eyes.

Results: In BA eyes, cpRNFL thicknesses in all sectors were statistically reduced compared with normal eyes. While the average cpRNFL thickness was 102.9μm in normal eyes, the average FE in BA eyes was 71.2μm. All cpRNFL sectors showed a significantly negative correlation with VFS. The reduction rates of FE in 12 sectors from normal eyes ranged from 16 to 52μm, among which 1 and 5 clock sectors particularly showed a high reduction rate.

Conclusions: CpRNFL originating from the nasal hemiretina was estimated to be about 30μm of entire cpRNFL. Furthermore, cpRNFL from the nasal hemiretina is considered to enter mainly nasal-upper and nasal-lower area of optic disc, and that might be similar to the distribution of cpRNFL from temporal hemiretina.

Commercial Relationships: Kaori Ueda, None; Akiyasu Kanamori, None; Yoshiko Matsumoto, None; Azusa Akashi, None; Yuko Yamada, None; Makoto Nakamura, None
Clinical Trial: UMIN000006900

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younger than the patient groups, we cannot rule out the possibility that patients are more likely to develop complex ERMs with age. **Conclusions:** The patients with optic nerve disease did not show an increased prevalence of ERMs. Complex ERMs are more likely with age and it is possible that this is even more so in patients. Older controls need to be added to test this hypothesis.

<table>
<thead>
<tr>
<th>ERM</th>
<th>Control (n=54)</th>
<th>Suspects (n=49)</th>
<th>Glaucoma (n=75)</th>
<th>Other Nerve Disease (n=56)</th>
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<tr>
<td>None</td>
<td>16.7</td>
<td>32.6</td>
<td>40.0</td>
<td>30.4</td>
</tr>
<tr>
<td>Simple</td>
<td>82.3</td>
<td>53.1</td>
<td>41.3</td>
<td>58.9</td>
</tr>
<tr>
<td>Complex</td>
<td>0</td>
<td>14.3</td>
<td>18.7</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Table. Percent of eyes.

**Commercial Relationships:** Nicole De Cuir, None; Juliet Idiga, None; Khushmit Kaur, None; Jeffrey Odel, Bayer (C); Gustavo V. De Moraes, None; Robert Ritch, None; Donald C. Hood, Topcon, Inc. (F)

**Support:** NIH/NEI R01-EY02115

**Program Number:** 6217  **Poster Board Number:** B0136  **Presentation Time:** 12:00 PM–1:45 PM

**Ocular findings and handheld optical coherence tomography in microcephaly**

Eleni Papageorgiou1, Helena Lee1, Frank A. Proudlock1, Viral Sheth1, Ravi Purohit1, Pradeep Vasudevan1, Irene Gottlob1. 1Ophthalmology, University of Leicester, Leicester, United Kingdom; 1Clinical Genetics, University of Leicester, Leicester, United Kingdom.

**Purpose:** To describe the ocular and optical-coherence tomography (OCT) findings in patients with microcephaly.

**Methods:** Twenty-two patients with microcephaly (mean age 13.1 years) underwent an ophthalmological examination and handheld OCT (Bioptigen Inc., USA). The tomograms were imported into ImageJ software where manual retinal layer segmentation was performed. The thickness of the central foveal retina, perimacular retina (1 mm temporal and 1 mm nasal to the fovea) and individual foveal layers were quantified and compared to 22 age-matched healthy controls.

**Results:** Six patients had microcephaly, lymphoedema and chorioretinal dysplasia (MLCRD), two patients had possible progressive encephalopathy with edema, hypsarrhythmia and optic atrophy (PEHO-syndrome) and one patient had microcephaly with pontine and cerebellar hypoplasia (MICPCH-syndrome)). In five patients there was an autosomal recessive pattern of inheritance, four patients had isolated, non-syndromic microcephaly and in the remaining four patients the microcephaly could not be classified. Ocular abnormalities were found in fifteen out of 22 subjects (68%). The most common findings were strabismus (45%), and nystagmus (36%). Other ocular anomalies included retinal pigment epithelial changes, optic nerve hypoplasia, amblyopia, significant refractive errors, chorioretinal dysplasia with punched-out lesions and retinal folds. The perimacular retinal thickness was significantly reduced in the microcephaly group compared to the control group: nasal to the fovea (296.98 microns vs. 343.82 microns, p<0.001) and temporal to the fovea (268.41 microns vs. 324.66 microns, p<0.001). There was also thinning of the ganglion cell layer (GCL) nasal to the fovea (41.23 vs. 53.32 microns, p<0.001) and temporal to the fovea (30.72 vs. 46.80 microns). Additionally, the inner plexiform layer nasal to the fovea (40.68 vs. 47.45 microns, p=0.028), the outer nuclear layer temporal to the fovea (51.34 vs. 67.54 microns, p=0.015) and the inner segment of the photoreceptors temporal to the fovea (19.62 vs. 25.98 microns, p=0.0036) were thinner in microcephaly. Five patients had foveal hypoplasia.

**Conclusions:** Ocular anomalies, especially strabismus, nystagmus and retinal abnormalities are frequent in microcephaly. The perimacular retina and the GCL were thinner on OCT. Patients with microcephaly due to heterogeneous aetiology have significant retinal pathology and share specific OCT findings.

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Commercial Relationships: Eleni Papageorgiou, None; Helena Lee, None; Frank A. Proudflock, None; Viral Sheth, None; Ravi Purohit, None; Pradeep Vasudevan, None; Irene Gottlob, None


Program Number: 6218 Poster Board Number: B0137
Presentation Time: 12:00 PM–1:45 PM

Sectorial macular retinal ganglion cell thinning caused by retrograde degeneration of the visual pathway
Bernardo F. Sanchez Dalmau1, 2, Johannes Keller1, Pablo Villoslada1, 1Institut Clin d’Oftalmologia, Hospital Clinic.Seu Maternitat, Barcelona, Spain; 2Center for Neuroimmunology, IDIBAPS, Barcelona, Spain.

Purpose: Retrograde trans-synaptic degeneration of retinal ganglion cell (RGC) neurons of the visual pathway has been demonstrated after various types of injury in humans and monkeys. In humans, this phenomenon can be seen in some but not all patients after stroke when imaging the peripapillary retinal nerve fibre layer with optical coherence tomography (OCT). We set out to test the hypothesis that owing to the anatomy of the visual pathway and refinements in OCT techniques the macula is a better region to demonstrate this finding. Additionally, we explored a potential correlation between sectorial thinning of RGC layer and the sensitivity loss of the corresponding quadrant visual field (VF) defect.

Methods: Retrospective case note review of patients with retrogeniculate lesions studied by OCT of the macula and automated VF analysis. Patients underwent imaging using the Zeiss Cirrus-HD OCT with the macular cube and peripapillary disc protocols. VFs were tested using the Humphrey analyser with the SITA-standard 24-2 protocol. Pattern standard deviations were calculated for each quadrant and correlated with the corresponding macula and peripapillary sector on the OCT images. Correlation coefficients were calculated with the Pearson product moment method. Significance level was set at 0.05.

Results: 8 patients with either hemianopia or quadrantanopia due to brain lesions (stroke =5; surgery = 2; CNS infection =1) were analyzed. A strong correlation was found between the pattern standard deviation of the VF quadrant and the corresponding macular RGC sector for the right (R =0.792, p <0.001) and left eyes (R =0.674, p <0.001).

Conclusions: The correlation between sectorial thinning of the macular RGC and the depth of the VF defect in patients with posterior visual pathway lesions confirms that retrograde trans-synaptic neuronal degeneration occurs after visual pathway injuries even in small lesions. There is a significant functional correlate with the VF loss. This finding may have clinical applications analysing VF defects anatomically as well as an imaging marker for evaluating new neuroprotective therapies.

Commercial Relationships: Bernardo F. Sanchez Dalmau, None; Johannes Keller, None; Pablo Villoslada, Bionure Farma (I), Digna Biotech (C), Heidelberg Engeneering (C), MedImmune (C), Neurotec Farma (C), Novartis (C), Novartis (F), Roche (C), Roche (F), TFS (C)

Program Number: 6219 Poster Board Number: B0138
Presentation Time: 12:00 PM–1:45 PM

Infra-red photography retinal abnormalities and inner nuclear layer microcystic retinal degeneration in eyes with band atrophy of the optic nerve
Mario L. Monteiro1, Rafael Miranda M. Sousa1, Daniel A. Ferraz1, Nithya Rajagopalan2, Mohammad A. Sadiq2, Yasar Sepah2, Quan Dong Nguyen2, Walter Y. Takahashi2, 1Ophthalmology and Otolaringology, University of São Paulo Medical School, São Paulo, Brazil; 2Ophthalmology, University of Nebraska Medical Center Stanley M. Truhlsen Eye Institute, Omaha, NE.

Purpose: To investigate the occurrence of hyporefective areas (HA) of fundus abnormalities on infra-red photography (IRP) and of microcystic retinal degeneration (MRD) in the inner nuclear layer (INL) of eyes with permanent temporal visual field (VF) defects and band atrophy (BA) of the optic nerve from compressive chiasmal lesions. To verify the possible associations between such findings and with the severity of VF loss on standard automated perimetry

Methods: Forty-six patients (29 patients) with temporal VF defects and BA and 20 healthy subjects (38 eyes) underwent 24-2 VF testing (Carl Zeiss Meditec), IR and fd-OCT cube scans (Heidelberg Inc). Forty-nine OCT scans of each patient were analyzed (examiner blinded for diagnosis) for the presence of microcysts in the INL. Another blinded examiner analyzed the presence of HA on IRP of the fundus. Agreement of the presence of MRD on OCT and HA on IRP were assessed. Correlation between the number of scans with microcysts, the area of abnormality on IRP and the severity of VF loss were verified.

Results: HA areas were found in 19 of 46 eyes with BA and in 2 of 38 controls (p<0.001, Chi square test). Microcysts on the INL were found in 23 BA eyes and 2 controls (p<0.001). When at least two scans with microcysts were required for defining the eye as having MRD, the abnormality was then present in 18 of 46 eyes with BA and in no controls. Using two abnormal scans as the definition of abnormality both HA areas and MRD were found in 10 eyes indicating a significant agreement between the two methods (p=0.01, kappa test). There was a significant correlation between the area of abnormality on IRP and the number of scans with microcysts on OCT (r=0.41, Spearman test, p=0.004). No statistically significant correlation was also found between the severity of VF loss and both the area of abnormality on OCT and the number of scans with microcysts on OCT

Conclusions: Hyporefective abnormality on IRP and MRD occur in a large percentage of eyes with BA of the optic nerve and temporal hemianopia from chiasmal compression, probably due to a trans-synaptic degenerative process in the retina. However both abnormalities do not appear to be correlated with the severity of VF loss suggesting that factors other than degeneration may influence its development

Commercial Relationships: Mario L. Monteiro, None; Rafael Miranda M. Sousa, None; Daniel A. Ferraz, None; Nithya Rajagopalan, None; Mohammad A. Sadiq, None; Yasar Sepah, None; Quan Dong Nguyen, Genetech (F), Regeneron (F); Walter Y. Takahashi, None

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant 306487/2011-0, Brasilia, Brazil.
Treatment with sodium methylprednisolone succinate (1000 mg) for 3 days. The therapeutic response of visual acuity was evaluated using Fukado’s classification 1). Good response was defined as an increase in visual acuity by 2 steps or more. A change in visual acuity was represented by the logarithm of the minimum angle of resolution (log MAR). Visual acuity less than 0.01 was converted to log MAR according to the report by Schulze-Bonsel et al 2).


Purpose: To determine the effectiveness of levodopa alone or in combination with allopurinol and tetracycline on visual acuity, visual field, and retinal nerve fiber layer (RNFL) thickness in eyes affected by nonarteritic anterior ischemic optic neuropathy (NAION).

Methods: Retrospective cohort study involving 33 eyes of 33 patients from 119 consecutively evaluated patients with NAION. Patients evaluated within 45 days of NAION onset and who had at least one optical coherence tomography (OCT) within 1 year of NAION onset were enrolled. Patients received treatment with levodopa alone (Levodopa only) or in combination with allopurinol and tetracycline (Levodopa plus). Best corrected visual acuity converted to logMAR, mean deviation (MD) threshold sensitivity on automated perimetry, and RNFL thickness on OCT were recorded at initial visit and follow-up within 1 year of NAION onset. Primary outcome measures were changes in logMAR visual acuity, MD threshold sensitivity, and RNFL thickness.

Results: Improvement in visual acuity by 3 or more lines was documented for 71% (5 of 7) of Levodopa only and 50% (4 of 8) of Levodopa plus eyes having an initial visual acuity of 20/60 or worse. No levodopa treated eye had worsened visual acuity. There was no significant difference (p=0.91) between the change in logMAR visual acuity for the Levodopa only (-0.43 logMAR) and Levodopa plus (-0.4 logMAR) groups. There was no significant difference (p=0.9) between the change in MD threshold sensitivity for Levodopa only (-1.79 dB) and Levodopa combined (1.54 dB) groups. The average RNFL thickness decreased by 23.6% for levodopa treated eyes, in contrast with previously published reports which documented decrease in average RNFL thickness of 35% to 42% for untreated patients.

Conclusions: Treatment within 45 days of onset of NAION with levodopa alone or in combination with allopurinol and tetracycline improved visual acuity but not visual field. Levodopa may promote neuroprotection in NAION by decreasing retinal ganglion cell loss and subsequent RNFL thinning.

Commercial Relationships: Lenworth N. Johnson, None; Deanna P. Harris Lyttle, None; Gregory F. Petroski, None

Clinical Trial: n/a
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Mesoporphyrin IX and the ease of intraperitoneal injections this experimental model may offer advantages for future neuroprotective studies.

Commercial Relationships: Dimosthenis Mantopoulos, None; Athanasios Tsakris, None; Basil S. Pawlyk, None; Michael A. Sandberg, None; Joan W. Miller, None; Joseph F. Rizzo, None; Demetrios G. Vavvas, None; Dean M. Cestari, None
Support: Bacardi Fund, Research to Prevent Blindness Foundation, Lions Eye Research Fund, Onassis Foundation, Fight For Sight Grant in Aid, Harvard Ophthalmology Department Support and National Eye Institute grant EY014104

Program Number: 6223 Poster Board Number: B0142
Presentation Time: 12:00 PM–1:45 PM
Revatio (Sildenafil) protects retinal ganglion cells immediately after optic nerve crush injury, but high dose intraperitoneal may induce sporadic optic nerve stroke

1Ophthalmology, Schneider Childrens Med Ctr, Petach Tikva, Israel; 2Sackler School of Medicine, The Krieger Eye Research Laboratory, FMRC, Tel Aviv University, Petach Tikva, Israel; 3Ophthalmology Department, Assaf Harofeh, Zerifin, Israel.

Purpose: To measure the effect of Revatio on mouse ocular blood flow and RGCs survival with and without injury induction.

Methods: Seventy six mice were used. Right optic nerve crush (ONC) was induced in 36 mice, half of which (n=12 C57Bl6 and n=6 Thy-1-1-CFP) received intravitreal (IVT) injection of Revatio (0.2-4µg/3µl) immediately before injury and identical number of mice received no treatment. The left eyes served as a control. The remaining 40/76 mice were injected intraperitoneally (IP) (24µg/300µl), without ONC injury. FA was performed (day 0), retinal structure was examined histologically, RGCs were counted using H&E, and optic nerve using 2,3,5-Triphenyltetrazolium chloride (TTC) and luxol fast blue (LFB) staining for stroke detection (days 14, 21). Quantitative real-time PCR was used to quantify gene expression of heme-oxegenase-1 (HO-1), superoxide dismutase-1 (SOD-1), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), Bcl-2 and BAX.

Results: Maximal retinal vessels dilatation and increased choroidal effusion were detected by FA immediately after IVT Revatio, and 30 minutes after IP Revatio injection. At 21 days following ONC and IVT Revatio, RGCs were protected relative to ONC without treatment. CFP-Thy1 transgenic mice also showed protection with Revatio. Apoptotic gene expression in the retina had an anti-apoptotic profile, as compared with the untreated ONC. IVT without ONC produced no RGC loss or optic nerve stroke at 14 or 21 days after injection. However, following IP Revatio (n=40) 3 animals showed RGC loss by H&E and optic nerve damage by TTC or LFB staining. In the IVT group, gene expression analysis showed an increase in Bcl-2 on day 1 which reverted to baseline at day 3; no significant change was detected in the other gene levels. All genes measured in the IP group (relating to apoptosis, oxidative stress, and glial scar), increased (2-3 fold) on both days 1 and 3.

Conclusions: Revatio increased choroidal perfusion and mildly dilated retinal vessels. Following IP injection, possible association with optic nerve stroke (3/80 optic nerves) in correlation with an increased apoptosis and ischemic related gene expression. On the other hand, injection immediately before ischemic retinal damage induction, revealed a neuroprotective effect, probably associated with vessels dilatation and reperfusion.

Commercial Relationships: Nitzia Goldenberg-Cohen, None; Mark Viyra, None; James D. Nicholson, None; Dana Morzaev, None; Orkun Muhsinoglu, None; Shirel Weiss, None; David Zadok, None
Support: This study was supported in part by the Zanvyl and Isabelle Krieger Fund, Baltimore, MD (NGC).

Program Number: 6224 Poster Board Number: B0143
Presentation Time: 12:00 PM–1:45 PM
TrkB Neurotrophin Receptor Activation with Pharmacophore as Possible Treatment for Experimental Ischemic Optic Neuropathy M. Ali Sharifi1, Jeffrey Ma1, Frank Longo1, Tao Yang2, Ben Barres1, Chandrani Chakraborty1, Yaping J. Liao1.
1Ophthalmology, Byers Eye Institute at Stanford, Stanford, CA; 2Neurology, Stanford School of Medicine, Stanford, CA; 3Neurobiology, Stanford School of Medicine, Stanford, CA.

Purpose: Anterior ischemic optic neuropathy (AION) is the most common acute optic neuropathy in older adults with no effective treatment. Neurotrophin BDNF (brain-derived neurotrophic factor) binds to TrkB receptors and is retrogradely transported to promote retinal ganglion cells (RGC) survival, leading to the hypothesis that restoration of neurotrophic support may be an effective therapeutic approach. In this study, we tested the effects of LM22A-4, a TrkB receptor small molecule partial agonist with TrkB specificity and nanomolar affinity1 on RGC survival.

Methods: To assess in vitro effects, we cultured purified RGCs using immunopanning, determined RGC survival, and examined TrkB receptor signalling pathway. To measure in vivo effects, we induced optic nerve head ischemia following rose bengal injection using a frequency doubled Nd: YAG laser (400 µm diameter, 50 mW, 1 second duration, 15 spots). Animals were treated with one intravitreal injection and 3-week daily intranasal and intraperitoneal treatments immediately following ischemia. We performed spectral-domain optical coherence tomography (OCT) analysis and quantified Brn3A+ RGCs in whole mount preparations at week-3. Data were analysed with custom macro in Image J and Prism.

Results: In vitro, LM22A-4 treatment significantly increased RGC survival (drug: 27.0 ± 1.5%; negative control: 11.0 ± 3.9%; P <0.0001), similar to the effects of BDNF (27.1 ± 1.2%). This improved survival correlated with significant nuclear and cytoplasmic translocation of MAP kinase (P <0.0001), a molecule downstream of TrkB receptor activation. Following AION, OCT circular scan and manual segmentation of the ganglion cell complex (2 µm) and volume scan with automatic segmentation of the total retinal thickness around the optic disc (10 µm) revealed partial preservation of retinal thickness after LM22A-4 administration. LM22A-4 treatment also led to significant rescue of the RGCs (drug: 2087 ± 65 Brn3A+ cells/mm2; saline: 1827 ± 90 Brn3A+ cells/mm2; P = 0.02).

Conclusions: LM22A-4 promoted TrkB receptor activation and RGC survival in culture similar to the effects of endogenous ligand BDNF. Treatment with LM22A-4 after experimental ischemic optic neuropathy led to partial preservation of retinal thinning on OCT analyses and significantly increased the number of surviving Brn3A+ RGCs, suggesting LM22A-4 may be effective treatment for AION.

Commercial Relationships: M. Ali Sharifi, None; Jeffrey Ma, None; Frank Longo, Founder of PharmatrophiX, a company focused on development of neurotrophin small molecule ligands (C); Tao Yang, None; Ben Barres, None; Chandrani Chakraborty, None; Yaping J. Liao, None
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Optical coherence tomography measurements of the macular area in eyes with optic nerve sheath meningioma

Stanley Darma1, Peerooz Saeed1, Maarten P. Mourits1, Michael D. Abramoff2, Frank D. Verbraak3. 1Ophthalmology, Academic Medical Center, Amsterdam, Netherlands; 2Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA; 3Electrical and Computer Engineering, University of Iowa, Iowa City, IA.

Purpose: To explore optical coherence tomography measurements in the macular area as an objective measure of early visual damage in eyes with optic nerve sheath meningioma (ONSM).

Methods: In 10 patients diagnosed with unilateral ONSM, SD-OCT volume scans were made of the macular and the optic nerve head area, using Topcon 3D OCT-2000 (Topcon Medical Systems). Peripapillary retinal nerve fiber layer (pRNFL), ganglion cell layer (GCL), ganglion cell – inner plexiform layer (GCIP) and macular inner retinal layer (mIRL) thickness were measured with the Iowa Reference Algorithm. Patients also underwent visual acuity, color vision and visual field testing. Measurements of the eyes with ONSM were compared with fellow eyes. Statistical testing was performed with Wilcoxon signed rank tests and Spearman correlation tests.

Results: 40 OCT scans of 10 eyes with ONSM and 10 fellow eyes were used for analysis. pRNFL, GCL and GCIP thickness differed significantly between eyes with ONSM and fellow eyes. All three correlated with visual acuity. Mean pRNFL was 50 um vs 96 um; mean GCL was 15 vs 32 um; GCIP was 48um vs 69um; mean mIRL was 80 vs. 98 um for eyes with ONSM vs. fellow eyes.

Conclusions: pRNFL, GCL and GCIP are significantly thinner in eyes with ONSM, compared with fellow eyes. The thickness measurements correlated with the visual function and might be used as an objective measure of early visual damage in eyes with ONSM.

Commercial Relationships: Stanley Darma, None; Peerooz Saeed, None; Maarten P. Mourits, None; Michael D. Abramoff, IDx LLC (E), IDx LLC (I), University of Iowa (P); Frank D. Verbraak, None

Early axonal damage detection by Ganglion Cell Complex with Optical Coherence Tomography in Nonarteritic Anterior Ischemic Optic Neuropathy

Begona Arana, Barbara Berasategui, Ana Orive, Nerea Martinez Alday, Marta Galdós. Cruces, Barakaldo, Spain.

Purpose: To investigate the ability of Ganglion Cell Complex (GCC) analysis with Optical Coherence Tomography (OCT) to detect an early axonal damage masked by optic disc edema in nonarteritic anterior ischemic optic neuropathy (NAION) and to find the relationship with visual field defect and visual function parameters.

Methods: 24 patients participated in this retrospective observational study. RNFL, GCC average and minimum values, best corrected visual acuity (BCVA), Ishihara test and Humphrey visual field (24-2 SITA fast) were recorded at first month and six months after NAION. Pearson coefficient was used to find relationships between GCC and visual field defects.

Results: Mean RNFL decreased from 151,13 µ to 66 µ and GCC average and minimum from 68,21 µ and 56,08 µ to 56,76,09 µ and 43,18 µ respectively. At acute stage, atrophy of the optic nerve appeared in 8,3% of patients in RNFL, while 58,3% and 79% of patients exhibited an early damage on the GCC average and minimum. Atrophy increased to 87% at 6 months in RNFL and GCC average and minimum to 88% and 100% respectively. Pearson coefficients for correlation between GCC average at acute stage and Visual Field Index (VFI) and Mean Deviation (MD) at acute stage were 0,47 (p=0,047) and -0,46 (p=0,031). Stronger correlation was found between GCC at acute stage and VFI and MD at chronic stage; 0,54 (p=0,013) and -0,56 (p=0,01) respectively. Significant correlation was also found between total deviation of superior and inferior hemifields and its respective hemifields on the ganglion cell complex map: -0,48 (p=0,024) and -0,57 (p=0,006) for correlation between superior GCC at acute stage and inferior hemifield at acute and chronic stages respectively and -0,61 (p=0,002) and -0,59 (p=0,003) for inferior GCC at onset and superior hemifield at acute and chronic stages

Conclusions: GCC analysis with OCT is capable of detecting an early axonal damage in NAION eyes at the acute stage that cannot be detected by RNFL. GCC defect at acute stage shows significant correlation with global visual field and location of the defect, with a stonger correlation with final visual field defect.

Commercial Relationships: Begona Arana, None; Barbara Berasategui, None; Ana Orive, None; Nerea Martinez Alday, None; Marta Galdós, None

Severe Impairment of Axonal Transport in Acute Experimental Anterior Ischemic Optic Neuropathy

Jeffrey Ma1, M. Ali Shariati1, Laura Pisani2, Franco Pestilli3, Bob Daugherty2, Lee Michael Perry1, Gun Ho Lee1, Chris Contag3, Brian A. Wandell3, Yaping J. Liao1. 1Ophthalmology, Byers Eye Institute at Stanford, Palo Alto, CA; 2Stanford Center for Innovation in In Vivo Imaging, Stanford, CA; 3Psychology, Cognitive and Neurobiological Imaging Center at Stanford, Stanford, CA.

Purpose: Although retinal ganglion cells (RGCs) are gradually lost as part of normal aging, they degenerate rapidly following anterior ischemic optic neuropathy (AION), the most common acute optic neuropathy in adults over age 50. Identifying events that occur in RGCs early after acute ischemia may guide new therapies to salvage RGCs. In this study, we investigated the impact of ischemia on axonal transport using histology and in vivo using serial 7-Tesla manganese-enhanced magnetic resonance imaging (MEMRI).

Methods: We induced experimental AION using laser-assisted photochemical thrombosis in adult mice. To assess anterograde transport, we performed bilateral intravitreal injections of AlexaFluor488-conjugated cholera toxin B (CTB-A488) or manganese chloride (MnCl2) 2 hrs after inducing AION in one eye. We tracked transport at 24 hrs after AION by using histology/epifluorescent microscopy or 7-Tesla MRI, respectively, to measure

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Results: Within 24 hrs after AION, there was severe impairment of anterograde axonal transport of CTB-A488 along the optic nerve, as demonstrated by near complete loss of fluorescence in the contralateral superior colliculus (N = 19 mice, P < 0.0001) and lateral geniculate nucleus (N = 10, P < 0.01). Serial activity-dependent MEMRI confirmed a decrease in anterograde transport of manganese 24 hrs after AION (N = 4, P < 0.05). This is similar to the effect of colchicine, a known microtubule inhibitor.

Conclusions: Acute optic nerve head ischemia is associated with severe impairment of anterograde axonal transport within 24 hours, which likely contributes to progressive axonal dysfunction and RGC death. Manganese-enhanced magnetic resonance imaging is a useful in vivo imaging modality for longitudinal visualization of transport defects. We demonstrated for the first time using MEMRI that axonal transport impairment is an important early event after experimental AION.

Commercial Relationships: Jeffrey Ma, None; M. Ali Shariati, None; Franco Pestilli, None; Bob Dougherty, None; Lee Michael Perry, None; Gunho Lee, None; Chris Contag, None; Brian A. Wandell, None; Yaping J. Liao, None
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Program Number: 6228 Poster Board Number: B0147
Presentation Time: 12:00 PM–1:45 PM
Assessment of apoptosis along the optic nerve after low-level repeated blast exposure
Jae Hyek Choi1, Joseph Novak2, Teresa A. Burke1, Brian J. Lund1, Anthony J. Johnson1, Jeffery Cleland1, Heuy-Ching H. Wang1. Ocular Trauma, U.S. Army Institute of Surgical Research, JBSA Fort Sam Houston, TX; 1Pathology, U.S. Army Institute of Surgical Research, JBSA Fort Sam Houston, TX.

Purpose: Visual dysfunction is a common symptom observed in victims of blast-induced TBI. While effects on the brain of primary blast exposure have been extensively studied, there is little research on primary blast effects on the eye or visual system. In particular, little is known about the cumulative effects of repeated low-level blast exposure. The purpose of the present study is to characterize the effects of repeated low-level blast exposure on the optic nerve using a rat model.

Methods: A compressed-air driven shock tube was used to expose Long-Evans rats to blast waves of peak overpressure 68.0 ± 2.7 kPa and positive peak duration 2.8 ± 0.1 msec. For repeated blast exposure (RBE), rats were exposed once daily for five consecutive days then euthanized on day 5, one hour after the last blast exposure. Rats subjected to a single blast exposure (SBE) were euthanized 5 days following the one blast exposure, and rats not exposed to blast were included as controls. Optic nerve tissues were collected and processed for immunohistochemistry to detect activated caspase 3 and glial fibrillary acidic protein (GFAP). Quantification of caspase 3 positive cells was achieved using a morphometric grid on each 1 mm of the optic nerve.

Results: Activated caspase 3 was detectable in the optic nerves from rats exposed to both SBE and RBE. For both SBE and RBE rats, a significantly higher number of caspase 3 positive cells were found in the 1 mm of optic nerve closest to the eye and optic chiasm. However, the presence of caspase 3 positive cells was significantly higher for the RBE rats than the SBE rats. In addition, increased GFAP was detected in the optic nerve from all animals subjected to blast exposure. Tissues from control animals not exposed to blast exposure were negative for activated caspase 3 and GFAP.

Conclusions: Low-level repeated blast exposure lead to an increase in apoptosis in the optic nerve, as indicated by an increase in caspase 3 positive cells. The section of the optic nerve closest to the eye was especially sensitive. This suggests that there is a cumulative effect to low-level blast exposure that may eventually lead to visual dysfunction.

Commercial Relationships: Jae Hyek Choi, None; Joseph Novak, None; Teresa A. Burke, None; Brian J. Lund, None; Anthony J. Johnson, None; Jeffery Cleland, None; Heuy-Ching H. Wang, None
Support: U.S. Army Military Operational Medicine Research Program (MOMRP) and Defense Medical Research and Development Program (DMRP).

Program Number: 6229 Poster Board Number: B0148
Presentation Time: 12:00 PM–1:45 PM
Optic nerve changes after mild traumatic brain injury in mice expressing human Tau
Alexandra Quezada1, Megan Gautier1, Benoît Mouzon1, Joseph Oyo2, Fiona Crawford2, Radouil T. Tzekov3, 2, 1Roskamp Institute, Sarasota, FL; 2VA hospital, Tampa, FL.

Purpose: A role for the tau protein in the pathogenesis of chronic traumatic encephalopathy and the consequences of repeated mild traumatic brain injury (r-mTBI) has received recent attention because of the evidence from high profile autopsy cases and the increased amount of significant health consequences of repetitive mTBI. However data from animal models are limited, and there are no data focusing on effects of tau on the visual system after TBI. Thus, the current study was designed to evaluate the long-term effects of r-mTBI on the visual system of mice expressing human tau protein.

Methods: Male mice expressing human tau protein on a null murine tau background (hTau, Jackson Laboratory, aged 3 months) were used. Single mTBI (s-mTBI; n = 4) was induced according to an established model. According to the same model, repetitive mild traumatic brain injury (r-mTBI; n = 4) was induced by applying 5 impacts with an interinjury interval of 48 hours, while repetitive sham (r-sham; n = 3) received anesthesia of the same duration. Histological evaluation of the optic nerves was carried out at 12 months post injury. Naïve mice (n = 6; 6-9 mo old C57BL/6 retired breeders) served as control.

Results: Slightly increased cellularity (~17%) and a slight disorganization of the nuclear arrangement was observed in optic nerves of mice after r-sham, s-mTBI and r-mTBI mice compared to the optic nerves of naïve control mice. Surprisingly, there was no statistically significant difference in optic nerve cellularity between r-sham and s-mTBI or r-mTBI (p = 0.9779; Kruskal-Wallis test) or between any of these groups and naïve mice (p<0.05). Focal areas of demyelination were observed in some s-mTBI and r-mTBI optic nerves.

Conclusions: We have previously demonstrated increased cellularity in the optic nerve in wild type mice after TBI. Overall, the degenerative changes in the optic nerves after r-mTBI in hTau mice at 12 months post injury were milder compared to changes observed at a similar age (8 months) in wild type mice and, despite the localized myelin loss in some nerves, the overall level of cellularity was not different from naïve mice or between the groups. Further characterization of this model currently underway could shed additional light on the apparent protective effect of human tau expression in r-mTBI.
Commercial Relationships: Alexandra Quezada, None; Megan Gautier, None; Benoit Mouzon, None; Joseph Ojo, None; Fiona Crawford, None; Radouil T. Tzekov, None

Program Number: 6230 Poster Board Number: B0149
Presentation Time: 12:00 PM–1:45 PM

Links between the endoplasmic reticulum protein WFS1/ Wolframin and mitochondrial proteins defined in silico may explain optic atrophy
Rainald G. Schmidt-Kastner1, Tyler Seidman1, Gabriel Quinones-Medina1, Birgit Lorenz1, Markus N. Preising1. 1C.E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL; 2Department of Ophthalmology, Justus-Liebig University, Giessen, Germany.

Purpose: Wolfram syndrome 1 (WFS1, MIM 222300) is caused by recessive mutations of WFS1 (wolframin) that lead to optic atrophy, diabetes mellitus, diabetes insipidus and hearing loss. WFS1 is expressed in the human retina (Schmidt-Kastner et al., Exp. Eye Res. 2009). WFS1 is localized to the endoplasmic reticulum (ER) and associated with ER stress. WFS2 (MIM 604928) is caused by mutations of CISD2, localized to ER and mitochondria. Since optic atrophy is predominantly caused by mutations related to mitochondrial function, we hypothesized that WFS1 in the ER must be closely associated with mitochondrial proteins. In silico studies of protein-protein interactions (PPIs) were used to identify putative mitochondrial partner proteins.

Methods: GeneMANIA was used to retrieve PPIs of WFS1 and CISD2 (150 interactions; human, mouse and rat data combined). PPIs were intersected with a database of mitochondrial proteins (MitoCarta). Nuclear-encoded mitochondrial genes known to cause optic atrophy were collected from NEIBank, OMIM and Mitophenome (n=31); PPIs (n>20 interactions; human) were generated and annotated for mitochondrial functions (MitoCarta; DAVID Bioinformatics).

Results: PPIs of WFS1 contained n=15 mitochondrial proteins, including MRPS31, a mitochondrial ribosome protein previously identified as auto-antigen IMOGEN 38 in type 1 diabetes mellitus. WFS1 and MRPS31 interact with EIF6 (BioGRID) that controls ribosome activity. PPIs for CISD2 had enriched links to mitochondrial proteins (n=20; chi-square p=0.03) including MRPL43 and MRPL51. The only PPI shared by WFS1 and CISD2 was with Rhoa (MIRO-2), an axonal transport protein for mitochondria. PPIs for mitochondrial genes causative in optic atrophy (n=8) contained links to the mitochondrial ribosome (DAVID, p<0.05 Bonferroni). Reverse searches in PPIs of mitochondrial ribosome proteins (n=77) also lead to proteins related to optic atrophy.

Conclusions: WFS1 and CISD2 were tentatively linked to proteins of the mitochondrial ribosome via PPI datasets. In addition, PPIs of nuclear encoded mitochondrial genes causing optic atrophy contained links to the mitochondrial ribosome. ER synthesis of nuclear encoded mitochondrial ribosome proteins and transport from ER into mitochondria are suggested as targets for experimental and translational studies on optic atrophy in WFS1.

Commercial Relationships: Rainald G. Schmidt-Kastner, None; Tyler Seidman, None; Gabriel Quinones-Medina, None; Birgit Lorenz, None; Markus N. Preising, None

Program Number: 6231 Poster Board Number: B0150
Presentation Time: 12:00 PM–1:45 PM

Vitamin A Deficiency as a cause of Optic Neuropathy
Francinia McCartney, Meghan Berkenstock, Jessica M. Ackert. Ophthalmology, Drexel College of Medicine, Philadelphia, PA.

Purpose: Optic neuropathy often presents as gradual, bilateral, painless vision loss. Causes are varied and include medication related etiologies, ingestion of exogenous materials, neoplastic disease, and vitamin deficiencies. The non-specific clinical presentation coupled with diverse causes can lead to diagnostic challenges. Here we present a cohort of 4 patients, all Nepalese females previously housed in one refugee camp in Nepal, who presented with complaints of poor vision. All were found to have a nutritionally derived optic neuropathy.

Methods: Retrospective case review

Results: All patients presented with complaints of painless, bilateral vision loss over the preceding months. Visual acuity at presentation ranged from 20/20 (eccentrically) to 20/100. There were no pupillary abnormalities noted. Ophthalmoscopy revealed variable, and often times subtle, temporal pallor of the optic nerve. OCT imaging showed RNFL thinning in all patients. Humphrey visual field testing consistently revealed bilateral central scotomas. Mean deviations ranged from -4.10 to -6.58. An exhaustive work-up including imaging was done in all patients, and was notable only for low Vitamin A levels.

Conclusions: The diagnosis of optic neuropathy was made based on bilateral vision impairment, impaired color vision, and central scotomas. All patients were found to have low vitamin A levels. Nutritional optic neuropathies are uncommon and this cluster of optic neuropathy is associated with vitamin A deficiency. Our small case series highlights the importance of maintaining a high index of suspicion for nutritionally derived optic neuropathies in at risk populations. Early diagnosis and treatment are necessary to preserve vision and prevent further visual field defects. All patients have been started on Vitamin A supplementation with stability of their vision and visual fields.

Commercial Relationships: Francinia McCartney, None; Meghan Berkenstock, None; Jessica M. Ackert, None

Program Number: 6232 Poster Board Number: B0151
Presentation Time: 12:00 PM–1:45 PM

Effects of Pentoxifylline on Blood Flow in Patients with Non-Arteritic Ischemic Optic Neuropathy

Jesse Gale1, Edward R. Chu1, Starleen E. Froesiskis1, Sowmya Srinivas1, Rustum Karanjia1, Ou Tan3, David Huang3, Srinivas R. Sada1, Alfredo A. Sadun1. 1Ophthalmology, University of Southern California, Los Angeles, CA; 2Doheny Eye Institute, Los Angeles, CA; 3Casey Eye Institute, Oregon Health and Science University, Portland, OR.

Purpose: To determine whether Doppler OCT can be used to measure retinal arteriole-derived ocular blood flow in patients with non-arteritic ischemic optic neuropathy (NAION), who are treated with pentoxifylline.

Methods: Seven affected eyes and two fellow eyes from five patients with NAION (one eye not measureable), all receiving pentoxifylline (a xanthine derivative that affects erythrocyte rheology), were examined with Doppler OCT. This group was compared to our previously published group of untreated NAION eyes (N=8) (Wang et al.; IOVS 52(2): 840, 2011) and normal eyes (n=10), (Wang, et al.; Br J Ophthalmol. May; 93(5): 634, 2009). Doppler OCT was performed using high-resolution Fourier domain-OCT scans and post-acquisition blood flow calculations, using our previously published technique (Wang et al., 2009).

Results: The average blood flow in pentoxifylline-treated eyes with NAION was 29 μl/min, which was comparable to 28 μl/min in the previous untreated cohort of NAION eyes (Wang et al.; IOVS 52(2): 840, 2011). The average blood flow in the fellow eye of treated patients with unilateral NAION was 52 μl/min, which was a small difference from that of normal eyes. The blood flow in the fellow eyes of patients with NAION was significantly lower than normal control values (p<0.001). Pentoxifylline had no significant effect on retinal arteriole-derived ocular blood flow.
increase from somewhat younger control eyes in the previous cohort (45 μL/min; Wang et al., 2009).

**Conclusions:** Doppler OCT may be promising as a technique for comparing treated to untreated eyes. When compared to untreated normative data, pentoxifylline was associated with a small increase in the retinal blood flow of fellow eyes in patients with unilateral NAION. Pentoxifylline did not increase blood flow in affected eyes. Further data collection is ongoing to permit a statistical analysis.

**Commercial Relationships:** Jesse Gale, None; Edward R. Chu, None; Starleen E. Frousiakis, None; Sowmya Srinivas, None; Rustum Karanjia, None; Ou Tan, Carl Zeiss Meditec, Inc (P); Optovue, Inc (F), Optovue, Inc (P); David Huang, Carl Zeiss Meditec, Inc (P), Optovue, Inc (F), Optovue, Inc (I), Optovue, Inc (P); Srinivas R. Sadda, None; Alfredo A. Sadun, None

Program Number: 6233 Poster Board Number: B0152
Presentation Time: 12:00 PM–1:45 PM

Induction of autophagy protects axons against TNF-induced optic nerve degeneration with possible involvement of p62 inhibition

Yasushi Kitaoka, Kaori Kojima, Yasunari Munemasa, Hitoshi Takagi. Ophthalmology, St Marianna Univ School of Med, Kawasaki, Japan.

**Purpose:** To examine the role of p62 and autophagy in TNF-induced optic nerve degeneration.

**Methods:** Eight-week-old male Wistar rats were received intravitreal injection of 10 ng TNF alone or simultaneous injection of TNF and p62 siRNA (50 pmol). Intravitreal injection of rapamycin (2 μl; 1 mM) was also performed. The expression of p62 in optic nerve was examined by immunoblotting. The effects of p62 siRNA or rapamycin on axon were evaluated by axon number counting 2 weeks after intravitreal injection.

**Results:** Immunoblotting showed that there was an increase in p62 protein level in optic nerve after TNF injection compared to the PBS injection and this increase was significantly inhibited by p62 siRNA. Rapamycin also significantly inhibited upregulation of p62 in optic nerve. Morphometric analysis showed that p62 siRNA significantly ameliorated axon loss induced by TNF. Rapamycin which is an autophagy inducer also significantly ameliorated axon loss induced by TNF.

**Conclusions:** Inhibition of p62 may have potential for axonal protection in TNF-induced optic nerve degeneration.

**Commercial Relationships:** Yasushi Kitaoka, None; Kaori Kojima, None; Yasunari Munemasa, None; Hitoshi Takagi, None

Program Number: 6234 Poster Board Number: B0153
Presentation Time: 12:00 PM–1:45 PM

Electrophysiological Monitoring in Children with Optic Nerve Glioma

Valeria L. Fu1, Ellen Mitchell1, Anagha Medsinge2, Kanwal Nischal1. 1Ophthalmology, Children’s Hospital of Pittsburgh, Pittsburgh, PA; 2Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

**Purpose:** To demonstrate value of Pattern Visual Evoked Potential (VEP) as an initial tool for diagnosis and monitoring optic nerve glioma (ONG).

**Methods:** In a retrospective study, all children who had been diagnosed with Neurofibromatosis Type 1 (NF1) were tested where possible with VEP within a mean interval of 4.4 months. Pattern VEP was performed on 23 children (age 1.1 to 13.2 years old; median age = 6.9 years old; mean age = 6.3 years old). Six children had unilateral ONG and 10 children had bilateral ONGs. Seven children had no ONG. Forty eyes were tested. Five children had multiple VEPS. All children had at least one neuroimage (MRI) taken. ONGs were confirmed with MRI scan. Visual acuity (HOTV or matching HOTV) was assessed in children older than 3 years old.

**Results:** In the 16 children (age 1.6 to 13.2 years old; median age = 4.9 years old; mean age = 5.9 years old) with ONGs, seventeen of 36 eyes (47%) showed a delay in VEP latency and 18 of 36 eyes (50%) displayed a decrease in VEP amplitude. All (age 1.1 to 10.5 years old; median age=8.5 years old; mean age = 7.5 years old) but 2 children without ONG had normal VEP responses. These 2 children with abnormal VEP without ONG were found to have optic nerve pallor and anomalous optic nerves.

In children with ONG (26 eyes), only 4 eyes (15%) which had abnormal visual acuity (>0.4 logMAR) showed prolongation in VEP latency and 2 eyes (17%) had abnormal visual acuity showed reduction in VEP amplitude.

In a longitudinal study of 5 children with ONG who had at least 2 consecutive VEP showed that those children either displayed gradual decrease in VEP amplitude (20%) or gradual decrease in VEP amplitude and prolongation in VEP latency (80%) in a period of 2.4 to 14.4 months (average 7.2 months).

**Conclusions:** Most children with ONG displayed abnormal Pattern VEP. However, consecutive VEP revealed a gradual deterioration in VEP responses even when visual acuities remained stable. On the contrary, children with NF1 without ONG showed normal VEP responses. In summary, Pattern VEP can be considered as a helpful initial tool for assessing function of visual pathway in children with ONG.

**Commercial Relationships:** Valeria L. Fu, None; Ellen Mitchell, None; Anagha Medsinge, None; Kanwal Nischal, None

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Program Number: 6235 Poster Board Number: B0154
Presentation Time: 12:00 PM–1:45 PM

Characteristics of Normal Optic Nerve Development Using Hand-Held Ultra-High Resolution Spectral Domain Optical Coherence Tomography in Children and Young Adults

Dr Patel, Ravi Parvot, Helena Lee, Viral Sheth, Gail Macachie, Elieni Papageorgiou, Frank A. Proudlock, Rebecca J. McLean, Irene Gottlob. Ophthalmology Group, University of Leicester, Leicester, United Kingdom.

**Purpose:** Early development of the optic nerve has previously been limited to histology and fundus photography. This is the first study to characterise optic nerve morphology in healthy full term infants and young adults using ultra-high resolution spectral domain hand-held OCT (HH SD-OCT).

**Methods:** 178 infants and young adults aged between 1 day and 18 years of age were recruited to the study. All participants were over 37 weeks gestational age at birth with no known ocular or neurological concerns. Full ophthalmological examination and a HH SD-OCT scan (Biopptigen, 2.6μm axial resolution) were performed without sedation. Images were analysed using Imaged software with the assessor masked. Optic nerve cup, disc and neuroretinal rim parameters along with peripapillary retinal thickness and retinal nerve fibre layer (RNFL) were quantified and correlated with log gestational age (logGA).

**Results:** The optic disc and cup diameters and areas increased linearly with logGA (p<0.001) with the result that cup / disc ratios of diameters did not significantly change with logGA (p=0.08). Nasal and temporal neuroretinal rim areas and nasal peripapillary RNFL also increased linearly with logGA (p=0.04, p=0.01 and p=0.08 respectively). In contrast temporal peripapillary RNFL demonstrated a marked initial decrease in thickness between birth and 90 weeks
gestational age followed by a slow increase. Change in peripapillary retinal thickness was also linear (p<0.001).

**Conclusions:** This is the first study to describe optic nerve development of full-term infants and children using HH SD-OCT.

We have demonstrated the temporal RNFL has a different course of development to other optic nerve parameters. Our results provide a normative database for further studies investigating optic nerve pathology.

**Commercial Relationships:** Dr Patel, None; Ravi Purohit, None; Helena Lee, None; Viral Sheth, None; Gail Maconachie, None; Eleni Papageorgiou, None; Frank A. Proudlock, None; Rebecca J. McLean, None; Irene Gottlob, None

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**Program Number:** 6236 **Poster Board Number:** B0155

**Presentation Time:** 12:00 PM–1:45 PM

Multifocal Photopic Negative Response (mfPhNR) and Ganglion Cell-Inner Plexiform Layer Thickness (GCIPLT) in Patients with Optic Nerve Lesions

Ari Kamei1, Eiichiro Nagasaka2. 1Ari Eye Clinic, Oshu-Mizusawa, Japan; 2Mayo Corp., Inazawa, Japan.

**Purpose:** To evaluate the interrelation of mfPhNR and GCIPLT in all, superior and inferior in patients with optic nerve lesions.

**Methods:** Twelve eyes of twelve volunteers with normal vision and eighteen eyes of nine patients with optic nerve lesions including normal tension glaucoma (NTG) were tested.

The mfPhNR was recorded with the VERIS Science System 5.0.4. The visual stimulus was made up of 37 hexagons in an approximately 40-degree visual field, Pseudo-randomly alternating between black (5cd/m2) and white (200cd/m2) on the CRT monitor. Burian-Allen ERG Electrodes, Adult-bipolar or Pediatric-bipolar, were used for this testing. The recording time was approximately 8 min. with dilated pupils having the best-corrected visual acuity. The band pass filter of the amplifier was set from 1 to 100 Hz. The amplification and stimulus frequency were set to 10000 and 9.41 Hz (8 frames) respectively.

Each trace of the mfPhNR found in all, superior and inferior regions was analyzed in about 10 degrees.

The GCIPLT was measured using the Cirrus HD-OCT. The GCIPLT in average, superior and inferior was applied for analysis.

**Results:** There was a correlation between the amplitude of the mfPhNR and GCIPLT in overall (R=0.385651, P=0.037821) and in the superior (R=0.470078, P=0.011359). On the other hand, there was no correlation between the amplitude of the mfPhNR and GCIPLT in the inferior (R= 0.027809, P= 0.880958).

**Conclusions:** We presented previously that there was a strong correlation between the amplitude of mfPhNR and Retinal Nerve Fiber Layer Thickness. There was less correlation between the amplitude of mfPhNR and GCIPLT.

**Commercial Relationships:** Ari Kamei, None; Eiichiro Nagasaka, None

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Diagnostic ability of macular analysis by SD-OCT for compression optic neuropathy at chiasma


**Purpose:** Chiasmal compression predominantly affects the crossing nerve fibers and inner retinal layer in the nasal hemiretina and shows band atrophy (BA) of the optic nerve head. The purpose of this study was to assess the diagnostic performance in macular parameters by spectral domain-optical coherence tomography (SD-OCT) for detection of BA.

**Methods:** In this study, 49 BA eyes with permanent temporal hemianopia, 89 normal eyes were enrolled. Any patients had nasal visual field loss were excluded. Each participant was imaged by 3D OCT-2000 (Topcon Inc.) and 10×10 grids were automatically divided from the macular analysis. The retinal nerve fiber layer (RNFL), ganglion cell layer (GCL)+ (GCL+inner plexiform layer (IPL)), and GCL++ thickness (RNFL+GCL+IPL) in both nasal and temporal hemiretina were calculated and compared between BA and normal groups. The areas under the receiver operating characteristic curves (AUCs) in these parameters were compared between the nasal hemiretina and temporal hemiretina by bootstrap and Pepe’s method.

**Results:** All parameters in BA eyes were significantly thinner than those in normal eyes. The AUCs for the RNFL, GCL+, and GCL++ thickness in the nasal hemiretina were 0.890, 0.988 and 0.981, respectively. The AUCs for the RNFL, GCL+, and GCL++ thickness in the temporal hemiretina were 0.619, 0.789 and 0.768, respectively. The nasal parameters showed significantly higher AUCs than those parameters in the temporal hemiretina. Also, GCL+ thickness displayed significant higher AUC than RNFL in the nasal hemiretina (p<0.001)The inner retinal parameters at macular in the nasal hemiretina exhibited high abilities for diagnosing BA. GCL+ was more affected than RNFL in nasal hemiretina. Unexpectedly, thinning of inner retinal layer in the temporal hemiretina was exhibited. The uncrossing nerve fibers at chiasma might be damaged in subclinical degree or median strip of overlap at fovea might be involved.

**Conclusions:** The inner retinal parameters at macular in the nasal hemiretina exhibited high abilities for diagnosing BA. GCL+ was more affected than RNFL in nasal hemiretina. Unexpectedly, thinning of inner retinal layer in the temporal hemiretina was exhibited. The uncrossing nerve fibers at chiasma might be damaged in subclinical degree or median strip of overlap at fovea might be involved.

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