Inherited optic atrophy gene discovery using whole exome sequencing

John S. Borchert, Isao Nakata, Daniel Navarro-Gomez, Maria Janessian, Elizabeth Delbono, Janey L. Wiggs

Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.

Purpose: Inherited disorders of the optic nerve cause significant visual impairment in children and adults. Approximately 50% of patients with inherited forms of optic neuropathy have mutations in OPA1, mitochondrial DNA (LHON) or other known genes. The genetic etiology of the disease is unknown for the remaining 50% of patients. Whole exome sequencing (WES) has been shown to be a useful approach for discovery of genes responsible for diseases with mendelian inheritance, such as inherited optic atrophy. The purpose of this study is to use exome sequencing to identify novel inherited optic atrophy genes.

Methods: Genomic DNA purified from optic atrophy probands were initially screened for mutations using our diagnostic panel test that includes 13 optic atrophy genes (including OPA1, OPA3, WFS1) and mitochondria DNA (GEDI test). 10 probands and 5 family members who did not have mutations in any of the known genes included in our diagnostic panel underwent whole exome sequencing (WES) using Sure Select V4+ UTR+ Mito Agilent (71.4 Mb target region) exome capture followed by paired end sequencing on the Illumina Hi-Seq. Sequence data was analyzed by our Ocular Genomics Institute (OGI) pipeline and annotated variants were filtered to select alleles with pathogenic features including: rare (less than .1% in population databases); evolutionarily conserved (GERP > 2.0); nonsynonomous; in silico pathogenicity (Polyphen2 and SIFT); genetic disease model (autosomal dominant, autosomal recessive or mitochondrial) and ocular expression.

Results: High quality whole-exome sequence data (75X mean coverage and 99% with >20X coverage) was obtained for all 10 probands and 5 family members. After filtering, 10 potentially disease causing mutations were identified in 5 different genes: SYDE2, HELZ2, BPIFB3, CLEC5A, and POLR3GL. Further evaluation of candidate variants in other pedigree members (if available) is currently ongoing. Variants of interest will be examined for functional effects using morpholino-mediated knockdown in zebrafish.

Conclusions: Using whole exome sequencing, we have identified novel candidate genes responsible for inherited optic atrophy. Further studies investigating segregation of putative disease-causing alleles in affected families and functional studies in zebrafish will be necessary to establish causality.

Commercial Relationships: John S. Borchert, None; Isao Nakata, None; Daniel Navarro-Gomez, None; Maria Janessian, None; Elizabeth Delbono, None; Janey L. Wiggs

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Figure A depicts the optic nerve of a 3-month-old zebrafish and B depicts the optic nerve of a 12-month-old zebrafish both using a 60x oil immersion lens. The bright green color indicates Gfap labeling and blue color labels for nuclei.

**Conclusions:** With the important caveat that the range of ages we examined does not include older fish, our hypothesis is not supported by these data; however, further experiments are being conducted to quantify the data, as well as to examine older fish (24, 36, 48-month-olds). Additionally, experiments are currently underway to determine if Gfap expression correlates with markers for senescence, such as p16. If both of these indicators for aging and senescence are observed, then zebrafish may be a defensible animal model for understanding aging in humans.

Figure A

Figure B

**Commercial Relationships:** Pedro Gonzalez, None; Dana M. Garcia, None

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**Program Number:** 5065
**Poster Board Number:** B0295
**Presentation Time:** 11:00 AM–12:45 PM

**Atg4A in axonal protection by short-term hyperglycemia in TNF-induced optic nerve degeneration**

Kana Sase, Yasushi Kitaoka, Yasunari Munemasa, Hitoshi Takagi. Ophthalmology, St. Marianna University School of Medicine, Kawasaki Kanagawa, Japan.

**Purpose:** We have recently showed that short-term hyperglycemia protects axons in TNF-induced optic nerve degeneration and that this axonal-protective effect may be associated with autophagy machinery. In this study, we investigated the expression of autophagy-related genes 4A (Atg4A) in this axon process.

**Methods:** Wistar rats were divided into two group. One group remained normoglycemia (NG), the other was rendered hyperglycemia (HG) by an intraperitoneal injection of streptozotocin (STZ). In both groups, TNF was administered intravitreally into the right eyes. PBS alone was administered into the left eyes as a control. In the HG group, 3-methyladenine (3-MA) was also administered simultaneously with TNF into the right eyes. One week after intravitreall injection, Western blot analysis and immunostaining were performed to investigate the Atg4A expression in optic nerve.

**Results:** Western blot analysis showed the existence of Atg4A in optic nerve. The protein level of Atg4A was significantly increased in optic nerve in TNF-treated eyes compared with that in PBS-treated eyes. The short-term HG did not significantly alter the Atg4A protein levels in optic nerve. Treatment of 3-MA did not alter the Atg4A protein levels in optic nerve in HG groups. Immunohistochemical study showed that Atg4A was colocalized with GFAP but not neurofilament.

**Conclusions:** Although we previously observed that LC3 (Atg8) exists inside axon and is involved in the short-term HG axonal...
Ripasudil, a ROCK inhibitor, modulates p62 expression in TNF-induced optic nerve degeneration

Yasushi kitaoka1, 2, Kana Sase1, Yasunari Munemasa1, Hitoshi Takagi1, 1Ophthalmology, St. Marianna University School of Medicine, Kawasaki, Japan; 2Molecular Neuroscience, St. Marianna University Graduate School of Medicine, Kawasaki, Japan.

Purpose: Ripasudil, a ROCK inhibitor, has been shown to decrease intraocular pressure. However, its role in optic nerve axonal degeneration remains to be examined. Thus, we determined whether ripasudil modulates axonal loss induced by tumor necrosis factor (TNF) and affects the expression of p62, also called sequestosome 1, in TNF-induced optic nerve degeneration.

Methods: Experiments were performed on adult male Wistar rats that received an intravitreal injection of 10 ng TNF alone or simultaneous injection of TNF and 2, 20, or 200 pmol of ripasudil. The effects of ripasudil on axons were evaluated by morphometric analysis 2 weeks after intravitreal injection. The expression of p62 in the optic nerve was examined by immunoblot analysis 1 week after intravitreal injection.

Results: Intravitreal injection of ripasudil exerted significant axonal protection against TNF-induced optic nerve degeneration. Immunoblot analysis showed that increased p62 protein level induced by TNF was significantly inhibited by ripasudil. Treatment with ripasudil alone also decreased p62 protein levels in the optic nerve compared with the baseline.

Conclusions: ROCK inhibition may affect autophagy machinery in optic nerve. The modulation of p62 levels by ripasudil may be involved in part in its axonal protection.

Commercial Relationships: Yasushi Kitaoka; Kana Sase; Yasunari Munemasa; Hitoshi Takagi

Program Number: 5066 Poster Board Number: B0296
Presentation Time: 11:00 AM–12:45 PM

Rapamycin ameliorates ethambutol induced toxic optic neuropathy by facilitating mitophagy

YEONJI JANG1, Sang-Mok Lee2, Hyoung Oh Jun2, Jin Hyung Kim3, Jeong Hun Kim4, Byung Joo Lee5, 1Ophthalmology, Seoul National University Hospital, Seoul, Korea (the Republic of); 2Department of Ophthalmology, Hallym University Sacred Heart Hospital, Seoul, Korea (the Republic of); 3Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea (the Republic of); 4Department of Biomedical Science, Seoul National University Graduate School, Seoul, Korea (the Republic of).

Purpose: Ethambutol (EMB) is the most common causative agent of toxic optic neuropathy, the pathogenesis of which is still obscure. In this study, we aimed to determine the exact mechanism of neuronal death in EMB induced toxic optic neuropathy (EON) and the address the role of autophagy in EON.

Methods: LC3 conversion and activation of apoptotic cascade according to EMB treatment were evaluated in Y79, human retinoblastoma cell. Viability of EMB treated Y79 cell was checked with and without rapamycin (RM). Temporal change of mitochondrial membrane potential (ΔΨm) was determined using JC-1 probe after EMB±RM treatment. The effect of EMB on the phosphorylation and mitochondrial translocation of parkin was evaluated. C57BL/6 mice were injected with EMB (intraperitoneal injection, 200 mg/kg/day) for 6 weeks. Retinal LC3 conversion and apoptotic cell death was determined with western blot and TUNEL assay, respectively. The protected effect of intravitreal rapamycin injection on retinal ganglion cell loss was evaluated by retrograde labelling technique.

Results: EMB treatment induced depolarization of ΔΨm, LC3 conversion and cleavage of caspase 3 in Y79 cell. Rapamycin pre-treatment prevented EMB induced depolarization of ΔΨm and rescued Y79 from EMB induced cell death. Phosphorylation and mitochondrial translocation of parkin were detected after EMB treatment and in vitro knockdown of PARK2 elevated the susceptibility of Y79 cell to EMB induced cell death. In the retinal tissue of EON mouse model, EMB induced LC3 conversion and apoptosis of retinal ganglion cell. This selective loss of retinal ganglion cell was significantly alleviated by RM co-treatment.

Conclusions: EMB induces depolarization of ΔΨm and subsequent apoptotic cell death of retinal neuronal cell. RM facilitates mitophagy in retinal neuron, thus protect it from EMB induced apoptosis.

Commercial Relationships: YEONJI JANG; Sang-Mok Lee; Hyoung Oh Jun; Jin Hyung Kim; Jeong Hun Kim; Byung Joo Lee

Program Number: 5067 Poster Board Number: B0297
Presentation Time: 11:00 AM–12:45 PM

Laser-induced optic nerve head injury in mice: challenges in establishing phototherapeutic models to study optic neuropathies

Peggy Bouzika, Joseph F. Rizzo, Joan W. Miller, Demetrios Varvass, Dean M. Cestari, Massachusetts Eye & Ear Infirmary, Boston, MA.

Purpose: In vivo phototherapeutic models have been extensively used to study diseases of the optic nerve (ON) such as non-arteritic anterior ischemic optic neuropathy. In these models photodynamic therapy (PDT) is applied to the ON, leading to optic nerve head (ONH) capillary occlusion, with minimal laser thermal effects on surrounding tissues. The use of mice presents extra difficulties given the animals’ small eye size and unique ONH blood supply. We present here a series of findings arising from the laser treatment itself of mouse ONH, which may interfere with the establishment of proper optic neuropathy models.

Methods: Laser (532nm) was applied to the ONH of C57BL/6 mice for various durations without the prior injection of a photosensitizer. The laser power was set at 50mW and the spot size used was 300um. The laser was focused either on the anterior part of the ONH head, closer to the peak of the hyaloid artery remnant, or slightly deeper into the ONH. Fundoscopy, optical coherence tomography (OCT) and fluorescein angiography (FA) were performed to record any ONH findings. Retinal whole-mounts were also analyzed by immunohistochemistry using the Brn3a marker.

Results: Laser treatment for one second led to slight ONH whitening 24h after application, with ONH late leakage on FA but without retinal edema on OCT. Increasing the duration of laser treatment to three seconds caused increased ONH whitening, and occasional branch retinal vein occlusion. There was loss of retinal ganglion cells one week after laser treatment of 3 seconds, as assessed by Brn3a staining of retinal whole-mounts. Finally, we observed that the position of the laser focus on the ONH also plays an important role on the type of lesion induced; laser applied while focused more anteriorly on the ONH led to more instances of retinal vessel occlusion, as well as to retinal lesions extending well beyond the peripapillary area.

Conclusions: Establishing a photosensitizer model of ONH in mice is challenging due to the fact that even brief application of commonly
available lasers at lowest settings lead to ONH photothermal injury that contributes significantly to the final lesions induced.

Commercial Relationships: Peggy Bouzika, None; Joseph F. Rizzo; Joan W. Miller; None; Demetrios Vavvas, None; Dean M. Cestari, None

Program Number: 5069 Poster Board Number: B0299
Presentation Time: 11:00 AM–12:45 PM
NAION: Light At the End of the Tunnel? Tassos Georgiou1, YAO-TSENG WENG2, Panagiotis Kolovos1, Maria Kalogerou1, Katerina Prokopiou1, Anastasia Neokleous1, CHIN-TE HUANG3, Rong-Kung Tsai1, 1Ophthalmos Research and Educational Institute, Nicosia, Cyprus; 2Institute of Medical Science, Hualien, Taiwan; 3Buddhist Tzu Chi General Hospital, Institute of Eye Research, Hualien, Taiwan.

Purpose: Non-arteritic anterior ischemic optic neuropathy (NAION) is a visually disabling disease due to primary damage of retinal ganglion cells (RGC). Currently there is no effective treatment. High doses of omega-3 (ω-3) fatty acids demonstrated a promising effect in other types of ocular pathologies. Therefore, the purpose of this study was to investigate the therapeutic effect of ω-3 using a rat model of anterior ischemic optic neuropathy (rAION) and examine its potential neuro-regenerative and/or neuro-protective properties on RGCs.

Methods: RAION was induced using laser-induced photoactivation of intravenously administered Rose Bengal in the optic nerve head of adult male Wistar rats. Daily gavage administration of fish oil (1 g/day eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) or phosphate buffered saline (PBS) was given either pre- or post-rAION induction. The animals were assigned to the following treatment groups (n=6/group): PBS treatment, ω-3 pre-treatment (3 days pre-rAION induction), ω-3 immediate treatment (day 0 to day 2 post-rAION induction), ω-3 delayed treatment (day 3 to day 5 post-rAION induction) and ω-3 continuous treatment (day 3 pre-rAION to day 7 post-rAION induction). Gas chromatographic analysis was used to assess the level of blood arachidonic acid (AA)/EPA following treatment on day 0, 3 and 7. Animals used in this study underwent flash visual-evoked potentials (FVEP) to assess visual function and were euthanized 4 weeks post-infarct. Density of RGCs was counted using Fluoro-gold retrograde labeling. Two-tailed Student’s t-test was used for statistical analysis.

Results: The RGCs’ density (1922 ± 361, 1576 ± 313 and 2720 ± 618 vs 744 ± 183, p < 0.05) and P1-N2 amplitude (48.2 ± 2.4, 44.3 ± 6.1 and 54.2 ± 2.1 vs 25.0 ± 1.3, p < 0.05) were found significantly higher in the groups of immediate, delayed and continuous treatment, compared to that of PBS group. Analysis of blood fatty acids showed that the AA/EPA ratio was greatly reduced on day 3 and day 7 post-treatment compared to day 0 (2.88 ± 0.07, 1.69 ± 0.16 vs 5.87 ± 0.22, p < 0.0001).

Conclusions: Our findings suggest that administration of ω-3 fatty acids has a neuro-regenerative effect in the rAION as demonstrated both by the RGCs density and FVEP analysis. This might indicate a remarkable turning point in the current treatment approach of patients with NAION; however further work is needed in order to elucidate the underlying mechanism of this effect.

Commercial Relationships: Tassos Georgiou, Tassos Georgiou (P); YAO-TSENG WENG, None; Panagiotis Kolovos, None; Maria Kalogerou, None; Katerina Prokopiou, None; Anastasia Neokleous, None; CHIN-TE HUANG, None; Rong-Kung Tsai, None

Program Number: 5070 Poster Board Number: B0300
Presentation Time: 11:00 AM–12:45 PM

Postnatal growth of the human optic nerve Moshe Meister1, Steven L. Bernstein1, Jiachen Zhuo2, Rao Guillapalli1. 1Ophthalmology and Visual Sciences, University of Maryland School of Medicine, Baltimore, MD; 2Radiology, University of Maryland School of Medicine, Baltimore, MD.

Purpose: While the average length of the adult human optic nerve (ON) is known, no data actually exists on the length of the living neonatal ON, nor its postnatal speed of anteroposterior (AP) growth. We wanted to evaluate the length of the neonatal ON, as well as the speed of growth from birth to maturity.

Methods: We utilized T1 and T2 axial magnetic resonance image (MRI) images from the Infant Brain Imaging Study (IBIS) network database (www.IBIS.org), which contains brain images from 15 normal infants scanned at birth and at one year. We evaluated average ON length from 5 additional Caucasian age cohorts...
(14 subjects/cohort): 3, 5, 10, 15 and 20y. Male:female ratios were equal, except for the 10y (46.7%; n=15) and 15y (42.9%; n=14) groups. Individuals were excluded if they presented with any visual complaints, growth anomalies or intracranial mass lesions. ONs were measured bilaterally from the posterior of the globe to the middle of the optic chiasm on both using the Siemens Leonardo workstation. Results were averaged. ON size was averaged for both sides from all individuals, with mean length in mm +/- SD reported.

**Results:** The mean newborn ON is 25.3 +/- mm in length, reaching 45.3 mm (adult) by 20 years of age. This is an increase of 80%. Human optic nerve growth is linear in the first three years of life, reaching 80% of the total adult length by this time (Fig 1; area indicated by (1): slope y=4.55x+24.214; r2=0.9991). The ON continues slow growth, until ~15 y/o (Fig 1; area indicated by (2): slope y=0.52x + 36.7; r2=0.8214). AP ON length is stable thereafter through 20y (Fig).

**Conclusions:** This is the first neuroradiological survey of in vivo total ON growth from birth to maturity. Our data reveal that the human ON grows by 80% after birth, with the greatest linear growth occurring within the first three years of postnatal life. ON growth continues at a slower rate from 5-15 years and then stops. This growth pattern likely corresponds to the increase in skull size around the age of puberty, and final skull growth. This data has relevance both to early antimetabolic treatments for cancer, as well as pinpointing the enhanced growth capacity of the juvenile ON, compared with adult ON.

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**Purpose:** Non-arteritic ischemic optic neuropathy (NAION) is caused by a sudden blood insufficiency for the optic nerve and is a major cause of optic nerve dysfunction. A rodent model of NAION (rNAION) was previously developed. However, the evidence that optic nerve head blood flow is reduced in rNAION has not been demonstrated in vivo. Our purpose was to evaluate optic nerve head blood flow in normal and rNAION rats, in vivo, using laser speckle flowgraphy (LSFG).

**Methods:** We used male Sprague-Dawley rats (200–240 g; Kyudou, Kumamoto, Japan). The rats were anesthetized with intramuscular ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively). To induce rNAION, Rose Bengal (RB) (2.5 mM, 1 mL/kg) was injected into the tail vein. After administration of RB, the left optic nerve head was photoactivated using a 514 nm argon green laser (Coherent, Kumamoto, Japan). The rats were anesthetized with intramuscular ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively). To induce rNAION, Rose Bengal (RB) (2.5 mM, 1 mL/kg) was injected into the tail vein. After administration of RB, the left optic nerve head was photoactivated using a 514 nm argon green laser (Coherent Ultima 2000 SE Argon) with an approximate 500 μm spot size for 12 seconds. We measured optic nerve head blood flow in normal rats (n=10) and in acute phase rats 1 day after induction of rNAION (n=6) using LSFG (LSFG-Micro, Softcare Co., Ltd, Fukuoka, Japan). The mean blue rate (MBR) of the vascular area (MV) and mean tissue area (MT) were used as the indicators of blood flow. We compared the MBR between the right eye and the left eye in the normal rats and

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rNAION rats. For statistical analysis, the Mann-Whitney U-test was used. P values <0.05 indicated statistical significance.

Results: In normal rats, there was no significant difference of MV or MT between the right eye and left eye (MV: P=0.473; MT: P=0.140, respectively). In the rNAION rats, the MBR of involved eyes was 78.8% in MV and 81.5% in MT, which was lower than that of unaffected eyes. The ratios of MV and MT in the left eyes of rNAION rats were significantly smaller than those in the right eyes (MV: 0.788, P=0.004; MT: 0.815, P=0.03, respectively)

Conclusions: Our results indicated that optic nerve head blood flow of rNAION rats was reduced in the acute stage at 1 day after induction.

Commercial Relationships: Takako Hidaka; Hideki Chuman, None; Nobuhisa Nao-i, None

Program Number: 5073 Poster Board Number: B0303
Presentation Time: 11:00 AM–12:45 PM

Optical Coherence Tomography Angiography in Ischemic Optic Neuropathy
Emily C. Wright, Sabin Dang, Emily Cole, Carlos E. Mendoza, Thomas Hedges, Laurel Vuong. Ophthalmology, New England Eye Center at Tufts MC, Boston, MA.

Purpose: Non-arteritic anterior ischemic optic neuropathy (nAION) is thought to be a consequence of hypoperfusion of branches of the short posterior ciliary arteries. Optical coherence tomography angiography (OCTA) is a technology that is allowing us to better visualize vascular pathology in ocular diseases. In this study we utilize OCTA to qualitatively depict the changes in the peripapillary vasculature in patients with nAION.

Methods: Patients with a prior or new diagnosis of nAION were recruited. The following were acquired for both eyes in each patient: OCTA of the peripapillary vasculature using the Optovue RTVue XR Avanti AngioVue®, structural OCT images of the retinal nerve fiber layer (RNFL) and ganglion cell layer (GCC) using a Zeiss Cirrus 5000; Humphrey Visual Fields (HVF). OCTA images were qualitatively graded for peripapillary retinal capillary and peripapillary choriocapillaris perfusion by a masked reader who reported whether each quadrant had OCTA changes consistent with low flow. The findings were then compared to HVF findings and RNFL and GCC measurements to assess for correlation.

Results: Five patients were recruited, two of whom had bilateral nAION. Among the 7 eyes with nAION, OCTA of the optic nerve head revealed radial peripapillary capillary dropout that correlate with HVF deficits as well as RNFL and GCC thinning on structural OCT. Additionally, in five of the seven eyes, OCTA of the peripapillary choriocapillaris demonstrated ischemic changes which correlated with HVF deficits as well as RNFL/GCC structural OCT findings. In the remaining two eyes, angiography of the choriocapillaris was unreliable due to overlying optic nerve edema and motion artifact. In patients with unilateral nAION, there was an appreciable difference in the flow signal in the peripapillary choriocapillaris and retinal capillaries between the two eyes, with the affected eye demonstrating a relative decreased flow signal and vascular density.

Conclusions: Our findings suggest that flow to both the peripapillary choriocapillaris and the radial peripapillary retinal capillaries are affected in nAION eyes. This has been difficult to demonstrate with traditional angiography. With the depth encoded angiogram provided by OCTA we are able to visualize segmental hypoperfusion of the these regions. To our knowledge, this is the first report to describe these findings. We plan to further this work through quantification of areas of low flow.

Commercial Relationships: Emily C. Wright; Sabin Dang, None; Emily Cole, None; Carlos E. Mendoza, None; Thomas Hedges, None; Laurel Vuong, None

Program Number: 5074 Poster Board Number: B0304
Presentation Time: 11:00 AM–12:45 PM

Ganglion Cell Complex Thickness in Mitochondrial Optic Neuropathies
Starleen E. Frousiakis1, Jack J. Tian2, Alexandria Lee4, Jeffrey Tran1, Anna Ter-Zakarian1, Kaitlin Kagochi3, Jesse Gale3, Fred N. Ross-Cisneros1, Luis Dimes0, Rustum Karanjia3, Alfredo A. Sadun1. 1University of Southern California, Pasadena, CA; 2David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA; 3Doheny Eye Institute, Los Angeles, CA; 4Ophthalmology, UCLA, Los Angeles, CA; 5Huntington Memorial Hospital, Pasadena, CA.

Purpose: Our lab and others have shown that the ganglion cell complex (GCC) - the sum of the retinal nerve fiber layer, ganglion cell and inner plexiform layers - is reduced in patients affected with Leber’s Hereditary Optic Neuropathy [Balducci, 2015, and Akiyama, 2013]. The purpose of this study was to determine if this finding is consistent in all patients affected by mitochondrial optic neuropathies (MON).

Methods: A retrospective chart review was conducted to identify all patients with mitochondrial optic neuropathies who presented to our clinic over a 1-year period and had both a central scotoma demonstrated on visual field and who had undergone a macular cube scan for GCC analysis (HD-OCT (Zeiss Meditec)). Forty-five subjects affected with LHON (n=41) or dominant optic atrophy (n=4) were identified.

Results: All patients affected with a MON had a reduction in the GCC layer in all quadrants of greater than 2 standard deviations when compared to age-matched controls. The average GCC thickness was 59.5±19.9 μm. There was no significant difference between quadrants. Both LHON and DOA patients showed a similar decrease in GCC thickness.

Conclusions: The pattern of GCC loss and RNFL thinning appears to be a consistent finding in MON. This suggests that GCC may be a useful metric for patients with MON, particularly if used in conjunction with retinal nerve fiber layer thickness.

Commercial Relationships: Starleen E. Frousiakis, None; Jack J. Tian; Alexandria Lee, None; Jeffrey Tran, None; Anna Ter-Zakarian, None; Kaitlin Kagochi, None; Jesse Gale, None; Fred N. Ross-Cisneros, None; Luis Dimes, None; Rustum Karanjia, Stealth; Alfredo A. Sadun, Stealth, Edison (F), Stealth (F)

Program Number: 5075 Poster Board Number: B0305
Presentation Time: 11:00 AM–12:45 PM

Laser Speckle Blood Flow of the Choroid is Significantly Reduced in Patients with Acute Anterior Ischemic Optic Neuropathy caused by Giant Cell Arteritis (GCA)
Randy H. Kardon1,2, Matthew Thurtell1, Michael Wall1,2, Anna Ketcham1,2, Jan M. Full4,4, Cole Starkey1,2, 4Neuro-ophthalmology, University of Iowa, Iowa City, IA; 4Center for the Prevention and Treatment of Visual Loss, Iowa City VA Medical Center, Iowa City, IA.

Purpose: We sought to differentiate patients with acute arteritic anterior ischemic optic neuropathy (AAION) from patients with non-arteritic anterior ischemic optic neuropathy (NAION) using non-invasive quantification of choroidal blood flow using laser speckle flowgraphy (LSFG). Since AAION is due to vascular occlusion of at least one posterior ciliary artery supplying the choroid and optic...
nerve head, it was anticipated that choroidal blood flow would be decreased.

**Methods:** Laser speckle flowgraphy (Softcare, Japan) was used to record relative ocular blood flow quantified by the degree of blur of a laser speckle pattern by moving blood cells in the imaging plane. The dynamics of blood flow during the cardiac cycle were measured in the choroid, major retinal arteries and veins and anterior optic nerve head. Systolic, diastolic, and intraocular pressures were measured to derive relative ocular perfusion pressure. Vascular resistance was quantified by dividing ocular perfusion pressure by mean speckle blur rate of the vascular region of interest. Eyes from the following patient groups were compared: 16 patients with (+) biopsy for giant cell arteritis (four eyes from patients with acute AAION and one eye from 12 patients without AAION), three eyes from three patients with (-) biopsy for giant cell arteritis, and 20 eyes from 20 patients from acute NAION.

**Results:** Choroidal vascular resistance was significantly higher (and blood flow lower) in acute AAION eyes compared to the other groups (ANOVA rank test, nonparametric analysis; p=0.039). The subgroup analysis showed that choroidal vascular resistance was significantly higher compared to eyes from patients with NAION.

**Conclusions:** A significant decrease in choroidal blood flow and increase in vascular resistance was found in patients with acute AAION who had a positive temporal artery biopsy using laser speckle blood flowgraphy. Measurement of choroidal vascular resistance may be an alternative approach to fluorescein angiography for diagnosis and monitoring treatment of giant cell arteritis.

**Commercial Relationships:** Randy H. Kardon, FaceX (I), Novartis (C), University of Iowa Research Foundation (P), MedFace (I), Iowa City VA Research Foundation (S); Matthew Thurtell, None; Michael Wall, None; Anna Ketcham, None; Jan M. Full, None; Cole Starkey, None

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**Program Number:** 5077 Poster Board Number: B0307
**Presentation Time:** 11:00 AM–12:45 PM

**Retinal Structural Changes as A Promising Biomarker of Neuromyelitis Optica Associated Optic Neuritis**

**Poster Board Number:** 5077
**Presentation Time:** 11:00 AM–12:45 PM

**Purpose:** We detected retinal structural changes in neuromyelitis optica associated optic neuritis (NMO-ON) patients compared with multiple sclerosis associated optic neuritis (MS-ON) patients by optical coherence tomography (OCT), and evaluated their correlations to cerebral spinal fluid (CSF) biomarkers to investigate the role of retinal structural markers in diagnosis and monitoring of NMO-ON.

**Methods:** In this cross-sectional study 31 (58 eyes) NMO-ON patients, 38 (38 eyes) MS-ON patients and 40 (80 eyes) healthy controls (HCs) were evaluated. Parapapillary retinal nerve fiber layer (pRNFL) and segmented macular layers were evaluated with Spectralis OCT. Best-corrected visual acuity (BCVA) of all subjects were assessed. We tested the CSF samples from 20 NMO-ON patients and 19 MS-ON patients as well. Anti-aquaporin 4 (anti-AQP4) antibody was detected by indirect immunofluorescence. Interleukin (IL)-6 and glial fibrillary acidic protein (GFAP), a biomarker of astrocytic injuries, were detected by ELISA.

**Results:** Compared with healthy control eyes, both NMO-ON and MS-ON eyes showed significant decrease of pRNFL thickness. Thinning of RNFL and the ganglion cell layer plus the inner plexiform layer (GCL+IPL) at all inner and outer macular regions were also detected in NMO-ON and MS-ON eyes. Besides, NMO-ON patients had a thinner pRNFL and macular GCL+IPL than MS-ON patients. Moreover, the CSF anti-AQP4 antibody, IL-6 and GFAP levels of NMO-ON patients were significantly higher than that of MS-ON patients. Although pRNFL thickness had no significant association with CSF anti-AQP4 antibody titres, it had a correlation to CSF IL-6 (r = 0.57, p < 0.02) and GFAP level (r= 0.63, p < 0.01) in NMO-ON patients. There was a correlation between pRNFL thickness and BCVA (r = 0.57, p < 0.0001) in NMO-ON patients as well.

**Conclusions:** The current study revealed a notable difference in the optic disc RNFL thickness and segmented macular layers among NMO-ON, MS-ON and normal eyes, and demonstrated the correlation between retinal structural changes and CSF biomarkers in NMO-ON patients as well. It indicated that the retinal structural changes detected by OCT may be a promising method to distinguish the aetiology of ON and a novel noninvasive biomarker to observe and monitor central nerve system pathophysiological progress, especially astrocytopathy, of NMO-ON.

**Commercial Relationships:** Lu Cheng, None; Qiuhai Lin, None; Zhenfen Ling, None; Xun Xu, None

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Optic nerve magnetic resonance imaging characteristics in inherited optic neuropathies

Eric D. Gaier1, Katherine Boudreault1, Isaio Nakata1, Dean M. Cestari1, Janey L. Wiggs1,2, Paul Caruso3, Joseph F. Rizzo1,2

Purpose: Inherited optic neuropathies include dominant optic atrophy (DOA), Leber’s hereditary optic neuropathy (LHON), and Wolfram syndrome (DIDMOAD). DOA is the most common of these, and mutations in OP1A account for 40-60% of DOA cases. Few studies have examined or compared findings on neuroimaging of the optic nerves in inherited optic neuropathies. The purpose of this study was to characterize magnetic resonance imaging features of the optic nerves in patients with inherited optic neuropathies.

Methods: Using an updated retrospective database of 111 patients with bilateral optic atrophy referred for genetic testing, magnetic resonance (MR) images were analyzed and compared across genotype groups. Patients were screened using next generation sequencing of 243 genes (including OP1A, WFS1) and the mitochondrial genome (includes all LHON mutations). T2 signal was quantified in MR images (3T) of the orbits and/or brain and normalized to internal standards within each slice. A sample of patients without ocular, central nervous system or visual diagnoses was used to validate the T2 quantification methods.

Results: Eight patients with and 19 without OP1A mutations had MR images available for analysis. There were 2 patients with DIDMOAD and 1 patient with LHON in our sample who also had MR images available. The optic nerves of optic atrophy patients appeared smaller with increased normalized T2 signal compared to controls. There was a wide range of variability in the degree of optic nerve T2 signal among optic atrophy patients.

Conclusions: Increased T2 signal intensity is not a typical feature of neurodegenerative disease and may reflect nuances in optic atrophy specific to this group of conditions. Differences in T2 signal could represent differences inherent to distinct hereditary optic neuropathies and/or the degree of atrophy or gliosis. Further characterization of these differences on MR imaging can help guide diagnostic genetic testing and provide insight into differences in the pathophysiology of hereditary optic neuropathies.

Commercial Relationships: None; Eric D. Gaier, None; Katherine Boudreault, None; Isaio Nakata, None; Dean M. Cestari, None; Janey L. Wiggs, None; Paul Caruso, None; Joseph F. Rizzo, Bionic Eye Technologies (I), Visus Technologies (I), Magic Leap, Inc

Optic Neuritis in a DNMT-1-Related Disease with a Mitochondrial Pattern of Axonal Loss

Fred N. Ross-Cisneros1, Chiara La Morgia1, Vallerio Carelli1, Alfredo A. Sadun1, 2

Purpose: To describe the histopathological features of postmortem ocular tissue from a patient with optic neuropathy carrying a DNMT-1 mutation. Mutations in the DNMT-1 gene impair DNA methylation and have been associated with autosomal-dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN), and hereditary sensory and autonomic neuropathy with dementia and hearing loss type IE (HSAN IE).

Methods: Eyes and optic nerves were obtained at autopsy from a 60 year old male affected with DNMT-1 (with ADCA-DN) and compared with two healthy age and gender-matched controls. Eyes were immersion-fixed in formalin within 17 hours after death. Eyes were dissected at the horizontal meridian at the level of the temporal and nasal regions of the retina and the optic nerve head. Retinal and optic nerves were dissected in cross-sections just posterior to the globe. All tissues were processed for and embedded into paraffin. Tissue sections were cut at 5 μm and placed on glass slides. Retinas were stained with H & E for general morphology and the optic nerves were immunostained for neurofilament protein and myelin basic protein to examine the integrity of the axonal fibers.

Results: The control retinas and optic nerves showed a normal cellular architecture. The DNMT-1 retina demonstrated widespread loss of retinal ganglion cells (RGCs) which appeared to be greatest in the temporal region of the retina, especially those contributing to the papillo-macular bundle. In the DNMT-1 optic nerve, there appeared to be a “diffuse” loss of axons throughout the nerve, but this loss appeared more remarkable in the temporal and inferior regions.

Conclusions: There was a depletion of RGCs in the DNMT-1 retina, most notably those contributing to the papillo-macular bundle. This corresponded to axonal loss predominantly in the temporal and inferior sectors of the nerve in a pattern characteristic of a mitochondrial optic neuropathy. We hypothesize that DNMT-1 mutations may also affect mitochondrial DNA methylation possibly leading to mitochondrial dysfunction producing this classic pattern of mitochondrial optic neuropathy.

Commercial Relationships: Fred N. Ross-Cisneros, Stealth Peptides, Inc. (F), Edison Pharmaceuticals, Inc. (F); Chiara La Morgia, Vallerio Carelli, None; Alfredo A. Sadun, Edison Pharmaceuticals, Inc. (F), Stealth Peptides, Inc. (F)

Support: NIH Grant EY03040 and International Foundation for Optic Nerve Diseases (IFOND)

Non-arteritic anterior ischemic optic neuropathy after recombinant human granulocyte colony stimulating factor treatment

CHIN-TE HUANG1, 2, YAO-TSENG WENG3, Rong-Kung Tsai1, 2

1Department of Ophthalmology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan; 2Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan; 3Institute of Eye Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan.

Purpose: Non-arteritic anterior ischemic optic neuropathy (NAION) is the most common acute optic neuropathy in patients over 50 years of age and most probably results from flow impairment to the anterior optic nerve, which generates optic nerve (ON) ischemia. We tested the probable mechanism of granulocyte colony-stimulating factor (G-CSF) treatment in the rat model of anterior ischemic optic neuropathy (rAION) to rescue retinal ganglion cells (RGCs).

Methods: Our previous report demonstrated that immediate administration of recombinant human G-CSF had neuroprotective effects in a rAION model through the dual actions of anti-inflammation and anti-apoptosis. In order to investigate the mechanism of anti-apoptosis, we compared RNA expression patterns between rAION-induced rats and rAION-induced rats with G-CSF treatment on 7 day after rAION induction by using microRNA
expression array. We also confirmed the RNA expression by using real time RT-PCR and confirmed the protein expression by using western blotting.

**Results:** According to microRNA array analysis, we found that 476 genes were induced and 113 genes were reduced by G-CSF treatment in the rAION model. One G-CSF induced protein, TAF9, was confirmed that RNA expression was increased 3.8-fold and protein expression was increased 2.5-fold in the G-CSF treated group. To investigate the role of TAF9, we evaluated anti-apoptotic effect of G-CSF treatment, G-CSF plus TAF9 siRNA treatment, G-CSF plus scramble siRNA treatment, and PBS treatment in the hypoxic retinal pigmented epithelium (RPE) injury model. We found that inhibition of TAF9 expression can reduce the protective effect of G-CSF in the hypoxic RPE injury model.

**Conclusions:** The expected findings provide much crucial information about neuroprotective effect of the G-CSF-induced TAF9 in response to ischemic stress in the rAION model.

**Commercial Relationships:** CHIN-TE HUANG, None; YAO-TSENG WENG, None; Rong-Kung Tsai, None

**Program Number:** 5081 **Poster Board Number:** B0311
**Presentation Time:** 11:00 AM–12:45 PM

**Features of non-arteritic anterior ischemic optic neuropathy in Japan**

**Program Number:** 5082 **Poster Board Number:** B0312
**Presentation Time:** 11:00 AM–12:45 PM

**Epidemiologic characteristics of giant cell arteritis using a national inpatient database**

Natasha V. Nayak1, Milap Raikundalia2, Jean A. Eloy2.

1Ophthalmology, New York Eye and Ear Infirmary of Mount Sinai, New York, NY; 2New Jersey Medical School, Rutgers University, Newark, NJ.

**Purpose:** Giant cell arteritis (GCA) is a vasculitis associated with significant ocular and systemic morbidity. The possibility of seasonality of GCA, and the role of diabetes in this population are amongst recent topics of interest. Our purpose was to evaluate epidemiologic characteristics of giant cell arteritis using a national inpatient database.

**Methods:** The Nationwide Inpatient Sample database from 2002-2010 was utilized to analyze epidemiological characteristics of patients with giant cell arteritis in the United States. Patient demographics, time/length of stay, hospital charges, incidence of temporal artery biopsy, and concomitant diagnoses were analyzed

**Results:** 5,337 cases of giant cell arteritis were identified (mean age 74.4±11 years; 74% female). The number of cases varied by year, reaching a peak in 2003 (n=633) and a trough in 2010 (n=512). The number of cases by month peaked in August (n=459), and troughed in November (n=331). Temporal artery biopsy was performed in a majority of cases (53% unilateral, 3% bilateral). Diabetic GCA patients (n=1547, 30%) were significantly younger, more likely to be of non-Caucasian ethnicity, and had a longer length of stay (p<0.0001). Inpatient mortality was 7%. The most common noted cranial nerve palsy was of the sixth nerve (0.5%). Retinal artery occlusion was noted in rare cases (2.5%).

**Conclusions:** The Nationwide Inpatient Sample could be utilized to further understand the epidemiologic characteristics of giant cell arteritis.

**Commercial Relationships:** Natasha V. Nayak, None; Milap Raikundalia, None; Jean A. Eloy

**Program Number:** 5083 **Poster Board Number:** B0313
**Presentation Time:** 11:00 AM–12:45 PM

**Assessment of Neutralizing Antibodies Against Adeno-associated virus (AAV)2 Capsids in Serum and Ocular Fluid Samples of Leber’ Hereditary Optic Neuropathy (LHON) Gene Therapy Clinical Trial Patients**


Ophthalmology, Bascom Palmer Eye Institute, Miami, FL.

**Purpose:** Neutralizing antibodies (NAb) against AAV, could interfere with the recombinant AAV vector-mediated gene transfer, thus decreasing the efficiency of the vector administered. We evaluated NAb titers against tyrosine-mutant AAV2 in LHON patients before and after gene therapy injections.

**Methods:** Self-complementary AAV2 (Y444, 500, 730F)-P1ND4v2 was intravitreally injected into the eyes of 9 LHON G11778A participants. The eye with worse acuity was treated. Six of them received low dose vector (5x10^9vg) and three medium dose (2.46x10^10vg). NAbs were assessed in serum and anterior chamber fluid (ACF) samples. The NAb titer was reported as the highest serum or ACF dilution that inhibited scAAV2-smCBA-mCherry transduction by >50%, compared with no serum control. NAb levels were categorized as low (<50), moderate (<5120) or high (>20,480).

**Results:** Serum Results. Strong inhibition to mCherry expression due to high NAbs against AAV2 (>20,480) were found in serum samples of 4/9 (44%) LHON subjects at baseline, 1d, 7d, 3m, 6m and (or) 1yr postinjection (pi). 3 patients showed absent or low NAbs at
baseline (33.3%). One of these patients with NAb=5 at baseline and received a low dose vector showed a transient rise of NAb to 20 at 7dpi, however NAbS decreased to baseline at 3 mpi. Another patient with NAb=80 at baseline, who received a medium dose showed a moderate increase at 1dpi to 320 and a further rise to higher levels at 7dpi (>20,480). At 2mpi this patient developed uveitis in the injected eye that subsided at 3m, however the serum NAb titer remained unaltered. In the third patient, the NAb titer was low to absent (=5) at baseline and did not change postinjection. One of the 4 patients who had high NAbS and received low dose vector also showed uveitis at baseline. Two other subjects (22.2%) had moderate NAb levels (>5120 and >320) at baseline, 1d, 7d and 3mpi. ACF Results. NAbS in all the baseline ACF samples were absent or mild.

Conclusions: Despite the presence of high NAbS to AAV2 in baseline serum samples of LHON participants, the NAb levels detected from ACF were lower, suggestive of high serum NAb levels may not be a barrier to successful ocular gene therapy. Transient uveitis developed in the treated eyes of two patients, might be due to immunopathogenic response to the P1ND4v2 AAV2.

Commercial Relationships: Rajeshwari D. Koilkonda, None; William J. Feuer, None; Joyce C. Schiffman, None; Janet L. Davis, None; Vittorio Porciatti, None; Phillip Gonzalez, None; Byron L. Lam, None; John Guy, None

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Clinical Trial: NCT02161380

Program Number: 5084 Poster Board Number: B0314
Presentation Time: 11:00 AM–12:45 PM

EPI-743 (Quinone) therapy for Leber’s Hereditary Optic Neuropathy: the Brazil experience

Sapanut Apinyawasisuk1, Amitha Ganti2, Rustum Karanjia1, Edward R. Chu1, Tana Wagshal2, Rubens Belfort3, Milton N. de Moraes-Filho4, Solange R. Salomao5, Guy Miller6, Alfredo A. Sadun7

1. Ophthalmology, Doheny Eye Institute, Los Angeles, CA; 2. Ophthalmology, University of Southern California, Los Angeles, CA; 3. Ophthalmology, Universidade Federal de Sao Paulo, Sao Paulo, Brazil; 4. Centro Universitario do Espirito Santo, Colatina, Brazil; 5. Visual Field Reading Center, University of Iowa, Coralville, IA; 6. Edison Pharmaceuticals, Mountain View, CA.

Purpose: To determine the degree of visual improvement, using markers of visual function, in a homogeneous Brazilian cohort of Leber’s Hereditary Optic Neuropathy (LHON) 11778 receiving up to 52 months of therapy with a novel quinone.

Methods: As part of an open label trial, six Brazilian, LHON patients experiencing significant visual loss were offered an experimental therapeutic, alpha-tocotrienol quinone (EPI-743). Two acute patients receiving EPI-743 during acute visual decline and four patients had chronic disease (>5 years) at the start of therapy. Snellen visual acuity (VA) in logMAR represented best correct vision. We utilized an algorithm developed by the University of Iowa Visual Field Reading Center that derives mean deviations (MD) from raw numerical data of STIM III and STIM V Humphrey Visual Fields (HVFs) to obtain participants’ visual field MDs. A change of +5 dB was considered an improved HVF. Retinal nerve fiber layer (RNFL) thickness was measured using time (2007-2009) or spectral (2010-2015) domain Optical Coherence Tomography (OCT).

Results: The two patients who were treated at time of conversion showed continued bilateral progressive loss of vision for the first 6 months, consistent with the natural history of LHON. VA declined or stabilized in the first year of therapy in all patients. Due to issues with agent access, one acute and two chronic patients chose to withdraw from the study. The observed improvement in VA was stable for up to 52 months of EPI-743, though there was a small VA decline after the withdrawal of therapy in those patients who discontinued therapy. HVF improved (>5 dB) in one chronic and both acute patients, while three chronic patients remained stable. HVF remained stable after the withdrawal of therapy in those patients who chose to withdraw from the study. As expected, all eyes showed reductions in RNFL on OCT that eventually stabilized on therapy, not inconsistent from the natural history of the disease.

Conclusions: The visual acuity improvements begun in the first 12 months of EPI-743 described in our previous ARVO abstract, continued for 36-52 months of treatment; remarkably this included even in subjects who had converted over 5 years prior to the initiation of treatment.

Commercial Relationships: Sapanut Apinyawasisuk, None; Amitha Ganti, None; Rustum Karanjia, Stealth Biotechnology (F), Edison Pharmaceuticals (F); Edward R. Chu, Tana Wagshal, None; Rubens Belfort, None; Milton N. de Moraes-Filho, None; Solange R. Salomao, None; Guy Miller, Edison Pharmaceuticals; Alfredo A. Sadun, Edison Pharmaceuticals (F), Stealth Biotechnology (F)

Clinical Trial: NCT02300753

Program Number: 5085 Poster Board Number: B0315
Presentation Time: 11:00 AM–12:45 PM

Treatment of visual impairment in patients with Leber’s Hereditary Optic Neuropathy (LHON) using Idebenone (Raxone®)

Shabir Hashami1, Guenther Metz2, Claudia Catarino3, Thomas Klopopstock4, 1Santhera Pharmaceuticals, Liestal, Switzerland; 2Friedrich-Baur Institut, Klinikum der Universität München, Munich, Germany.

Purpose: LHON is an orphan mitochondrial disorder affecting the retinal ganglion cells leading to permanent blindness from which recovery is rare. More than 90% of patients harbor one of three mitochondrial DNA mutations in the genes coding of complex I of the respiratory chain. Idebenone, a short-chain benzoquinone, is a potent antioxidant and also interacts with the electron transport chain facilitating mitochondrial electron flux. Due to these properties idebenone (Raxone®) has been investigated for the treatment of LHON and we summarize the evidence available for efficacy based on a placebo-controlled trial and from clinical practice.

Methods: Visual acuity (VA) data from a randomized placebo-controlled study (RHODOS), from case reports, retrospective cohort studies, an Expanded Access Program (EAP) and a natural history case report survey have been collected in a database of approximately 500 patients with LHON. The disease progression based on natural history data and from the Placebo treated patients are compared to the outcome for patients treated with idebenone with respect to the prevention of vision loss and recovery of lost vision.

Results: In the RHODOS study, the number of patients experiencing a clinically relevant recovery after 6 months of treatment increased from 10.3% in the placebo group to 30.2% in the idebenone-treated group. Patients in the EAP showed a recovery rate of 30.6% after 6 months of treatment increasing to 49.3% when comparing the final outcome after 15 months (mean treatment) to the VA at nadir. The number of patients experiencing more severe vision loss to above 1.0 logMAR VA was lower in RHODOS and in the EAP when compared to the datasets of untreated patients.

Conclusions: A large body of evidence demonstrates that patients with LHON benefit from idebenone (Raxone®) treatment and that the drug, recently approved by the European Medicines Agency (EMA) as a first treatment of visual impairment due to LHON, was well tolerated.

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LHON Gene Therapy Clinical Trial: Year 2 Results

John Guy1, William J. Feuer1, Janet L. Davis1, Vittorio Porciatti2, Rajeshwari D. Koikonda3, Huijun Yuan4, Byron L. Lam1, William Hauswirth4. Ophthalmology, University of Miami, Miami, FL; 1University of Florida, Gainesville, FL.

Purpose: To determine the safety and tolerability of AAV2(Y444,500,730F)-P1ND4v2 in patients with LHON.

Methods: After obtaining informed consent for participation in this open-label, unilateral single-dose, intravitreal injection of AAV2(Y444,500,730F)-P1ND4v2 per subject in a dose-escalation study designed to investigate the safety of three vector doses (5x109 vg, 2.46x108 vg or 1x107 vg) in subjects with molecularly confirmed G11778A-mutated mitochondrial DNA; six patients with long-standing (>12 months) and bilateral acuity loss down to ≥ 35 ETDRS letters (20/200) received intravitreal injections of low dose study drug (5x107 vg) (n=3) or medium dose (2.46x108 vg) (n=3) into the eye with worse acuity. Three patients with acute (<12 months) and bilateral acuity loss down to ≥ 35 ETDRS letters (20/200) received intravitreal injections of low dose study drug (5x107 vg) (n=3) into the eye with worse acuity. Clinical testing included ETDRS visual acuity, Humphrey visual fields (30-2), OCT, pattern ERG (perg) and neuro-ophthalmic examinations. Blood samples were screened for viral DNA and neutralizing antibodies (Nabs) to AAV2 prior and after intravitreal injection. Postinjection evaluations thus far are 1 year to 2 months.

Results: None of the patients lost vision in the injected eyes. Adverse events were minor, but included low grade trace cell and flare anterior uveitis that was asymptomatic (n=2) and seen 2 months after injection resolving without intervention. Of these 2 patients, serum neutralizing antibodies with a titer of 1:80 preinjection rose to 1:20,480 7 days and 3 months postinjection. The other had a titer of 1:20,480 preinjection that remained unchanged. Vector DNA were not detected by qPCR in serum samples. All 3 patients with visual loss for less than a year who received the low dose had improvements of vision in injected eyes by 3 lines on the ETDRS chart. Of the 6 patients with chronic visual loss only 1 experienced improvement by 3 lines.

Conclusions: Study drug was associated with mild and transient anterior uveitis in 2 of 9 patients. Four of 9 (44%) patients experienced improvements in visual acuity with injection of Study drug relative to 8 patients (18%) who had spontaneous improvement of 3 lines or more out of the 44 patients entered into our prior natural history study (JAMA Ophthalmol. 2014:132(4):428-36). Thus far, study drug appears to be safe at low and medium doses.

Commercial Relationships: Shabir Hasham; Guenther Metz, Santhera Pharmaceuticals; Claudia Catarino, Santhera Pharmaceuticals (R); Thomas Klopopsc, Santhera Pharmaceuticals (C), Santhera Pharmaceuticals (F)

Program Number: 5086 Poster Board Number: B0316
Presentation Time: 11:00 AM–12:45 PM

Risk of Stroke in Patients with NAION: A Nationwide Retrospective Cohort Study

Rong-Kung Tsai1, Yue-Chang Lee1, Jen-Hung Wang4, Tzu Lun Huang4, 5. 1Institute of Eye Research, Buddhist Tzu Chi Medical Center, Hualien, Taiwan; 2Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan; 3Department of Ophthalmology, Buddhist Tzu Chi Medical Center, Hualien, Taiwan; 4Department of Medical Research, Buddhist Tzu Chi Medical Center, Hualien, Taiwan; 5Department of Ophthalmology, Far Eastern Memorial Hospital, New Taipei City, Taiwan; 6Department of Health Administration, Tzu Chi College of Technology, Hualien, Taiwan.

Purpose: To investigate the incidence and prevalence of nonarteritic anterior ischemic optic neuropathy (NAION) and extrapolate the risk of cerebrovascular events following NAION compared to an age- and gender-matched reference group.

Methods: Retrospective cohort study design. The study cohort was comprised of patients diagnosed with NAION seeking ambulatory care from 2000 to 2011. The comparison cohort was extracted from a subset of the National Health Insurance Research Database of Taiwan by randomly selecting two patients for every NAION patient, matched by age and gender. Cox proportional hazard regression analysis was performed to calculate adjusted odds ratios for the two cohorts. Subgroup analyses between subjects without or without comorbidities of NAION and comparison cohorts were conducted.

Results: Four hundred and fourteen patients were included in the study cohort and 789 in the control group. The mean follow-up period was 5.9 years. The incidence of NAION was 3.72/100,000 person-years, and prevalence of NAION was 48.18/100,000 persons in Taiwan. The NAION group was more likely to have any stroke (adjusted hazard ratio (aHR) = 1.93, p = 0.002) and ischemic stroke (aHR = 2.03, p = 0.003), but not hemorrhagic stroke (aHR = 1.24, p = 0.696) than the comparison group. Among the subgroup with comorbidities, the odds of ischemic stroke among the subjects with NAION were 3.35 times higher than for those without NAION (95% CI: 1.67, 6.70).

Conclusions: The incidence of NAION in Taiwan is 3.72/100,000 person-years. The NAION group had an increased risk of ischemic stroke and all stroke. Physicians should refer all patients with NAION for systemic vasculopathy examinations and control of modifiable risk factors to prevent irreversible neurological sequelae.

Commercial Relationships: Rong-Kung Tsai, None; Yue-Chang Lee, None; Jen-Hung Wang, None; Tzu Lun Huang, None
Support: Research grant from Buddhist Tzu Chi General Hospital, Hualien, Taiwan (TCRD103-21)

Program Number: 5087 Poster Board Number: B0317
Presentation Time: 11:00 AM–12:45 PM

Oxygen, Atmospheric Pressure and Non-arteritic Ischemic Optic Neuropathy

Anna Ter-Zakarian, Rustum Karanjia, Alfredo A. Sadun. Doheny Eye Institute, Glendale, CA.

Purpose: To assess variations in aircraft pressurization for potential high altitude dangers in patients predisposed to non-arteritic ischemic optic neuropathy (NAION). As an airplane ascends, pressurization of the cabin partially offsets and limits the fall of air pressure. The United States Federal Aviation Administration (FAA) requires that the cabin pressure on commercial airplanes be maintained at levels equivalent to the atmospheric pressure below 8,000 feet – the maximum allowed. Most aircraft are pressurized to 6,000 and 8,000 feet, depending on the type of aircraft and composite materials of the fuselage. While this has little effect on most passengers, patients with...
a history of NAION may not tolerate a reduction of inspired oxygen pressure and may be at increased risk of a recurrent ischemic event.

Methods: As part of this study, altimeters were used to measure commercial aircraft pressurization at cruising altitude. A total of 13 planes were measured for pressure, including 6 narrow-body planes and 7 wide-body planes manufactured by Boeing and Airbus. Single-aisle aircraft with a fuselage cabin diameter of 10-13 ft. were considered narrow-body, while twin-aisle aircraft with a fuselage width of 16-20 ft. were qualified as wide-body.

Results: Larger aircraft maintained a lower internal pressure altitude than the smaller aircraft, with average cruising altitude pressures of 5910 ft. and 7020 ft., respectively.

Conclusions: Consequences of high altitude include hypoxic depression of cerebral function and cerebral edema, manifesting as retinal hemorrhages and papilledema. NAION may also be exacerbated by several mechanisms induced by low oxygen pressure, leading to hyperperfusion of the optic nerve head. Patients with an underlying history of NAION may be at increased risk of fellow eye involvement, especially in unpressurized flights and narrow-body planes, which based on our study are on average 1110 ft. less pressurized relative to wide-body aircraft. Consequently, there may be a greater danger of optic neuropathy development in commercial flying in smaller aircraft for patients predisposed to AION.

Commercial Relationships: Anna Ter-Zakarian, None; Rustum Karanjia; Alfredo A. Sadun, None

Program Number: 5089 Poster Board Number: B0319
Presentation Time: 11:00 AM–12:45 PM
Therapeutic window of systemic methylprednisolone in a rat model of anterior ischemic optic neuropathy (rAION)

Tsun Huang†, Rong-Kung Tsai†. †Ophthalmology, Far Eastern Memorial Hospital, New Taipei, Taiwan; ‡Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan; §Institute of Eye Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan.

Purpose: Controversy exists regarding the methylprednisolone (MP) treatment for in non-arteritic anterior ischemic optic neuropathy in human (NAION). We tested the hypothesis that systemic MP treatment had significantly neuroprotective effects on RGC survival in immediate treatment after insult using a rat model of ischemic optic neuropathy (rAION).

Methods: After rAION induction, the rats were randomly assigned to either the MP-treated group (n = 36) or the PBS-treated ischemia-only (n = 12) or sham group (n = 12). MP injections were given at immediately (MP-0d, n = 12), 7 days (MP-1W, n = 12) or 14 days (MP-2W d, n = 12) after rAION. Outcome measurements included density of RGCs with retrograde Fluoro-Gold labeling, flash visual-evoked potentials (FVEP), TUNEL assays of the retinal sections and ED1 staining of the optic nerve. Optic nerve vascular permeability was quantified in the amount of Evan’s blue dye extravasation. Statistical analysis was performed with IBM SPSS 19. We used Kruskal-Wallis test and Mann-Whitney U test to evaluate differences between the groups.

Results: At the 4th week post-infarct, MP treatment significantly rescued the RGCs (mm2) in the central retinas (2050 ± 160) and mid-peripheral retinas (1040 ± 240) in 0D-MP compared with other treated groups (all p<0.05, n=6 each group). Functional assessment with IVEP demonstrated that amplitude improvements of P1 (55 ±UV) were significantly found in 0D-MP treated group comparing with other groups (all p<0.05, n=6 each group). TUNEL assays showed a significantly decrease in the number of apoptotic cells in the RGC layers of 0D- MP and 1W-MP treated retinas compared to the PBS-treated group (6 ± 1.9 cells/HIF, 9.9 ± 3.0 cells/HIF, p ≤ 0.05), compared to other groups (all p<0.05). Results of vascular permeability showed that re-establishment of BRB and infiltration of microglia into ON before day 2 post rAION.

Conclusions: Immediately MP treatment may play a role in rescue RGC survival rate and electrophysiologic visual function improvement in a rAION model. The rescue effects may be through the multiple actions on anti-apoptosis of RGCs as well as anti-inflammation in optic nerves.

Commercial Relationships: Tzu Lun Huang, None; Rong-Kung Tsai, None

Program Number: 5090 Poster Board Number: B0320
Presentation Time: 11:00 AM–12:45 PM
A Novel Animal Model for Traumatic Optic Neuropathy (TON)

Wensi Tao, Brian Tse, Galina Dvoriantchikova, Dmitry V. Ivanov, David T. Tse, Daniel Felaez. Ophthalmology, Bascom Palmer Eye Institute, University of Miami, Miami, FL.

Purpose: Traumatic optic neuropathy (TON) is a devastating cause of permanent visual loss following blunt injury to the head. The indirect injury occurs as a concussive force to the orbit is transmitted at a distance through the skull to the optic nerve. Several animal models have been used to simulate TON—all of which can produce similar end-results of retinal ganglion cell (RGC) loss, but fail to reproduce the clinical scenario of closed head indirect injury to the nerve and subsequent neurodegeneration. Thus, we developed a clinically-relevant animal model for TON using a novel ultrasonic pulse injury modality.

Methods: To trigger Traumatic Optic Neuropathy (TON), 3 month-old C57BL/6 mice were anesthetized, and a micropipet probe (3 mm) of a laboratory sonifier was placed on the supraorbital border directly above the insertion of the optic nerve into the optic canal. A 500 μsec ultrasonic pulse at 35% amplitude (80J) was then delivered to the orbit. After the injury, mice were followed for up to 1 month and assessed for development of optic neuropathy. Whole retina flat-mounts were stained for retinal ganglion cell (RGC) quantification, optic nerves were harvested for immunohistochemistry and real-time PCR was performed. Nerve fiber layer thickness was measured by Spectral Domain Optical Coherence Tomography (SD-OCT). RGC function was assessed by pattern electroretinogram (pERG).

Results: The number of RGCs in the retina steadily decreased over a 2 week period with significant loss of RGCs in the injured retinas after 1 week. In the optic nerve, we found RNA and immunohistochemical expression of inflammatory markers such as TNF-alpha as early as 6 hours after injury. Immunohistochemistry showed activation of microglia (Cd11b) and infiltration of CD45-positive macrophages in the optic nerve and initiation of a glotic response. One month after the injury, the RGC function measured by pERG was decreased significantly by 19.3% over baseline and the contralateral eye.

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Conclusions: This ultrasonic pulse delivery method is capable of delivering a non-invasive concussive injury to the optic nerve and induce unilateral TON. Our results suggest that the traumatic event initiates a cascading sequence of metabolic events that can exacerbate the injury, and accounts for the progressive nature of neurodegeneration seen in the human manifestation. After the injury, we observed a decrease of RGC survival and function with an intact tissue ultrastructure.

Commercial Relationships: Wensi Tao; Brian Tse, None; Galina Dvoriantchikova, None; Dmitry V. Ivanov, None; David T. Tse, None; Daniel Palcuet, None

Program Number: 5091 Poster Board Number: B0321
Presentation Time: 11:00 AM–12:45 PM

Dexras1 Deletion Attenuates Retinal Ganglion Cell (RGC) Loss and Preserves Vision in Experimental Optic Neuritis
Reaas Sulaimankutty1, Kimberly Dine1, Bailey Baumann1, Ying Song1, Alyssa B. Cwanger1, Sangwon F. Kim2, Joshua L. Dunaiief1, Kenneth S. Shindler1,5093
Purpose: Optic neuritis is an inflammatory optic neuropathy associated with multiple sclerosis (MS). Prior studies suggest oxidative stress mediates RGC loss during optic neuritis in the EAE model of MS. Elevated iron found in the brain of MS patients as well as in EAE mice, may contribute to oxidative stress. Dexras1, a small G protein, is activated by S-nitrosylation by nitric oxide (NO) produced by inducible nitric oxide synthase (NOS) in activated microglia/macrophages, or by neuronal NOS, leading to iron import via divalent metal transporter 1. Thus, we hypothesized that Dexras1 exacerbates oxidative stress induced neurotoxicity via iron entry in experimental optic neuritis, and examined whether dexras1 deletion reduces RGC damage in EAE.

Methods: EAE was induced in Dexras1 KO and wild-type mice by immunization with myelin oligodendroglial glycoprotein peptide. One wild-type EAE group was treated daily with oral iron chelator deferiprone (DFP). Visual function was assessed by optokinetic responses (OKR) at baseline and weekly until sacrifice, 6 weeks post-immunization. Retinas and optic nerves were isolated. Inflammation was assessed by H&E and IBA1 staining, demyelination by Luxol Fast Blue staining, and axon loss by neurofilament staining of optic nerves. RGCs were immunolabeled with Brn3a antibodies to quantify RGC survival.

Results: Progressive decreases in OKR occurred in wild-type EAE mouse eyes; whereas, Dexras1 KO and DFP treated EAE mice had significantly less vision loss (p<0.01; N=8-10/treatment group). Dexras1 KO (p<0.001) and DFP treated (p<0.05) EAE mice also had significant attenuation of RGC and axonal loss compared to wild-type EAE mice. Dexras1 KO and DFP treatment did not significantly reduce inflammation, but showed a trend toward decreased demyelination in EAE mice.

Conclusions: The preservation of vision and attenuation of RGC loss in Dexras1 KO mice suggests that the NO activated Dexras1 signaling cascade which drives iron entry into cells is a potential mechanism of neuronal death in experimental optic neuritis. Improved vision and RGC survival with DFP treatment further suggests a role of iron overload exacerbating neuronal damage. Results suggest modulation of Dexras1 signaling and iron chelation are potential novel treatment strategies for optic neuritis that warrant further investigation.

Commercial Relationships: Reas Sulaimankutty, None; Kimberly Dine, None; Bailey Baumann, None; Ying Song, None; Alyssa B. Cwanger, None; Sangwon F. Kim, None; Joshua L. Dunaiief, None; Kenneth S. Shindler, None
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Program Number: 5092 Poster Board Number: B0322
Presentation Time: 11:00 AM–12:45 PM

Therapeutic effect of melatonin in an experimental model of optic neuritis

Purpose: We have developed a new experimental model of optic neuritis (ON) through the microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve, which reproduces central features of human ON. The aim of this work was to analyze the effect of melatonin (MEL) on the optic nerve axoglial alterations induced by experimental ON.

Methods: For this purpose, LPS (1 μl, 4.5 μg) was injected in one optic nerve from adult male Wistar rats, while the contralateral optic nerve was injected with vehicle. One group of animals received a subcutaneous pellet of MEL (20 mg) one day before LPS or vehicle injection which was replaced at 15 days, and another group was submitted to a sham procedure. In another set of experiments, the pellet of melatonin was implanted at 4 days post-injection of LPS or vehicle. The effect of melatonin was analyzed at 21 days post-injection in terms of: i) visual pathway function (visual evoked potentials (VEPs)), ii) anterograde transport from the retina to the superior colliculus (intravitreal injection of cholera toxin β-subunit), iii) pupil light reflex (PLR), iv) microglia/macrophages (by Iba-1 and ED1 immunoreactivity), v) astrocytes (by glial fibrillary acidic protein-immunostaining), vi) axon number (by toluidine blue staining), vii) demyelination (by luxol fast blue staining), viii) retinal ganglion cells (RGCs) number (by Brn3a immunoreactivity), and iv) optic nerve lipid peroxidation (TBARS).

Results: LPS induced a significant decrease in VEP amplitude and PLR, a reduction in retinal anterograde transport, an increase in Iba-1 and ED1 immunoreactivity, astrocytosis, demyelization, an increase in lipid peroxidation, and RGC loss. The pre-treatment with MEL significantly prevented all these alterations. The post-treatment with MEL significantly preserved VEP amplitude and PLR.

Conclusions: The treatment with melatonin prevented functional and histological alterations and diminished the vulnerability of RGC to the deleterious effects of experimental ON, probably through an antioxidant mechanism. Therefore, these results indicate that melatonin could be a promissory resource in the management of ON.

Commercial Relationships: Marcos Luis Aranda, None; Damian Dorfman, None; Hernan H. Dieguez, None; Pablo Sande, None; Monica S Chianelli; Ruth E. Rosenstein, None
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Program Number: 5093 Poster Board Number: B0323
Presentation Time: 11:00 AM–12:45 PM

Rapamycin Treatment Inhibits the Progression of Optic Neuritis Mediated Visual Loss in Experimental Autoimmune Encephalomyelitis (EAE) Mice
Vena Taila, Vittorio Porciatti, John Guy. Ophthalmology, Bascom Palmer Eye Institute, Miami, FL.

Purpose: To study the effect of the mTOR inhibitor rapamycin on retinal ganglion cell axonal degeneration, visual loss and optic neuropathy in EAE mouse model.

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Methods: EAE was induced in Female DBA1J mice by subdermal injection of 0.1 ml homologous spinal cord emulsion in complete Freunds adjuvant. Mice injected with complete Freunds adjuvant and age-matched normal mice were used as controls. Visual function was assessed by pattern electroretinograms (PERGs) at 1, 4 and 7 month post sensitization (MPS). Upon the decrease in PERG, mice were randomly divided into two groups. Group one received intraperitoneal injection of rapamycin (8.0 mg/Kg body weight) everyday for 30 days, whereas group two received only vehicle. One month later visual function was assessed by recording the PERG.

Spectral domain OCT evaluated thickness of the inner plexiform layer to nerve fiber layer. Mice were euthanized at 9MPS, retinal ganglion cell (RGC) and mitochondrial numbers were evaluated by Tuj1 and GRIM19 staining on retinal whole mounts. The regenerative ability of the RGC axons were evaluated by GAP43 staining.

Results: PERG analysis at 7MPS indicated decrease in amplitudes by 27% in EAE mice compared to CFA control (p=0.0002). There was no significant change in PERG amplitude of pretreatment verses post rapamycin treated EAE mice. However, PERG amplitude in vehicle treated EAE mice was decreased by 31% (p=0.01) compared to pretreatment. Rapamycin injections prevented the loss of amplitudes by 100% compared to EAE vehicle treatment. OCT showed rescue of inner retinal thickness in rapamycin treated group that was comparable to CFA control whereas, it is thinner by 14% in vehicle treated EAE mice (p<0.0001). RGCs were preserved in rapamycin treated EAE mice and comparable to CFA control whereas, there was a decrease by 21.4% (p<0.001) in vehicle treated EAE mice. Mitochondrial numbers were found to be higher in rapamycin treated group (p=0.03) whereas, they were comparable to CFA control in EAE vehicle group.

Conclusions: Rapamycin may be useful for reducing neurodegeneration associated with permanent visual loss in optic neuritis and multiple sclerosis patients.

Commercial Relationships: Venu Talla, None; Vittorio Porciatti, None; John Guy, None

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Program Number: 5094 Poster Board Number: B0324
Presentation Time: 11:00 AM–12:45 PM

Role for Myopia in Determining Measurements of Retinal Nerve Fiber Layer (RNFL) and Ganglion Cell Layer (GCL) Thinning in Multiple Sclerosis (MS)
Diana Laura, Rachel Nolan, Mengling Liu, Lisa Park, Steven Galter, Laura Balcer. New York University School of Medicine, New York, NY.

Purpose: RNFL and GCL/inner plexiform layer (GCL+IPL) thickness, as measured by optical coherence tomography (OCT), are reduced in MS and correlate with visual acuity (VA). Myopia, as measured by spherical equivalent (SE) or axial length, is associated with peripapillary RNFL and macular thinning. We conducted a secondary analysis using a case-control study to examine the contribution of myopia to RNFL/GCL+IPL thinning in MS.

Methods: Study participants with MS (n=447, mean age 46.3±10.4, 72.7% female) and disease-free controls (n=230, mean age 34.4±10.6, 69.1% female) with SEs of 0.5 to 16.0 diopters (D) underwent spectral-domain OCT and VA testing. Exclusion criteria included recent history (≤ 3 mos.) of optic neuritis (ON) or, for controls, eye disease other than refractive error or BCVA<20/20. Thickness of the peripapillary RNFL and GCL+IPL, average macular thickness and volume, high-contrast VA, low-contrast letter acuity at 2.5% and 1.25% contrast, and 25-item National Eye Institute Visual Function Questionnaire (NEI-VFQ-25) scores were obtained. The relation of SE to retinal thickness and VA were examined with generalized estimating equation models, accounting for age and within-patient, inter-eye correlations.

Results: Within the control cohort, more myopic SEs were a significant predictor of thinning of the RNFL (P<0.001) and GCL+IPL (P=0.005), average macular thickness (P=0.011), macular volume (P=0.012), monocular high-contrast VA (P<0.001) and 2.5% low-contrast acuity (P=0.04). Binocular VA, 2.5% and 1.25% low-contrast acuity (P<0.001), and NEI-VFQ-25 scores (P=0.005) were worse among controls with more myopic SEs. In the MS cohort, SE was also a significant predictor of lower RNFL thickness (P<0.001), though each negative D corresponded to a smaller thickness reduction than observed in controls. This relation was significant among MS eyes with and without ON (P=0.001-0.008).

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Conclusions: Higher degrees of myopia contribute significantly to RNFL thinning among both MS patients and controls. The relation between SE and RNFL thickness was stronger among controls, reflecting the known role that MS has in RNFL and GCL+IPL thinning. The potential influence of myopia is noteworthy when interpreting OCT data in MS patients. Furthermore, even among controls without MS history, higher degrees of myopia predict worse visual function and quality of life.

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Program Number: 5096 Poster Board Number: B0326
Presentation Time: 11:00 AM–12:45 PM

Evolution of optic neuropathy in a patient with breast cancer: a personal journey

Friederike Mackensen1, Viviane Grewing2, Stephan Schulz3, Christina Beisse1, Frederik Marmé1, 2, 3. 1Ophthalmology, University of Mainz, Mainz, Germany; 2Ophthalmology, University of Freiburg, Freiburg, Germany; 3Ophthalmology, University of Heidelberg, Heidelberg, Germany; 2Gynecology, University of Heidelberg, Heidelberg, Germany; 3National Center of Tumor Diseases, Heidelberg, Germany; 4Heidelberg Engineering, Heidelberg, Germany.

Purpose: Triple negative breast cancer (TNBC) is the least frequent type of BC. It can metastasize among other organs to the choroid. There are only two to three cases of unilateral carcinomatous optic nerve (ON) involvement published. We want to present a case of bilateral blindness that presented as ON swelling in cranial hypertension (CH).


Results: A 39 year old female (FM) was diagnosed with TNBC, BRCA2 positive, regional lymph nodes positive in 08/13. Chemotherapy was followed by surgery and radiation with a satisfying response. 06/14 bone metastasis were discovered. Immune therapy (IT) (Nivolumab, Ipilimumab) was initiated but was not able to prevent bone marrow carcinomatosis and stopped in favor of chemotherapy with eribuline until 12/14 with good partial remission. Upon progression of disease the patient was treated with four cycles of PEI (Cisplatin, Ifosfamid, Etoposid) again resulting in a good partial response. In 06/15 another form of IT was started (Pembrolizumab) and tumor vaccination. In 07/15 the patient developed symptoms of CH including attacks of blurry vision. OCT and fundoscopy showed ON swelling. Lumbar punction revealed leptomeingeal disease. MRI findings did not show solid meningeosis. Treatment with intrathecal MTX was started and led to improvement. IT was continued. After four intrathecal cycles, vision decreased to light perception. Impairment of left nerve XII and nerve III were seen. Funduscopy showed vital ON and OCT normal nerve fiber layers (NFL). MRI showed solid meningeosis, ON compression by tumor cells, ON inflammation and ballooning of the pituitary. Steroids were given with no result. High dose systemic MTX (3mg/ m²) and Olaparib were started. This led to clinical (nerve III, XII) and radiologic improvement but vision stayed unchanged. OCT showed no more NFL on the left eye and only rest in the right. Funduscopy mirrored this by ON pallor in the left eye and partial vitality in the right.

Conclusions: ON compression by metastasized breast cancer cells is rare and bilateral simultaneous involvement has not been published.

We think treating doctors have to be aware of the possibility to diagnose it early and maybe treat more aggressively at a time point when vision could still be preserved.

Commercial Relationships: Friederike Mackensen, None; Viviane Grewing, None; Stephan Schulz, Christina Beisse, None; Frederik Marmé, None