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SIREV-OFTARED Joined Congress Abstracts

Madrid (Spain), June 29–30, 2018

About SIREV and the 2018 Congress

The Society for Research in Retina and Visual Sciences (SIREV; former SIRCOVA) was first established in 2009 by professionals interested in ophthalmology and research that all together built this society. SIREV was born from a multi-disciplinary viewpoint, with the challenge of encouraging investigation, innovation and biotechnological development in retina and visual sciences.

SIREV is a non-profit organization that is in keeping with the member fees and altruist donations. The objectives of the society are: to promote knowledge concerning the retina and the visual sciences based on the highest standards of research in our countries, to promote the exchange of information, education and training in all aspects of retina and visual sciences investigation, to nurture and evolve the links between the ophthalmologists and ophthalmic researchers, and to advise other scientific societies, professionals, patient associations or anybody interested in advancing knowledge in eye care through outstanding ophthalmic research, innovation and biotechnology. Since 2011, colleagues engaged in these matters from Italy and Portugal joined SIREV. For further information please visit our site at HYPERLINK "<http://www.sirev.es>" as well as the major active social networking websites, where you will find information concerning our society, its membership, scientific partnerships, interesting education courses, masters, conferences, donations, etc. together with other resources and relevant news.

The President of SIREV and the President of the Organizing Committee for the 2018 SIREV-OFTARED joined Congress welcome you all to Madrid for this event. Main purpose for our Congress is to provide a forum at which ophthalmologists, scientists, optometrists, students, health care professionals and industry representatives can meet, learn, investigate and create new ideas, the outcomes of which have to be translated to the ophthalmological practice to better manage the ophthalmologic patients. In this meeting we deal with highlighting the most relevant topics on the prevention, diagnosis and treatment of ocular disorders, ranging from basic pre-clinical research to clinical and population studies. These activities will be accomplished in various plenary lectures, monographic symposiums, as well as express free communications (3 slides/3 minutes), and other activities that can be discovered throughout the 2018 SIREV-OFTARED program (HYPERLINKS: "<http://www.sirev.es>" "<http://www.oftared.es>"). A panel of international experts has selected a winner and two honor mentions among the oral communications (basic pre-clinical research and clinical research), the Final Master's work, and the award to the best Doctoral Thesis defended in 2016–2017. The Board of SIREV and the Organizers of the 2018 SIREV-OFTARED Congress want to thank specially the Faculty of Medicine and the Faculty of Optics and Optometry of the Complutense University (Madrid) for providing help and administrative support to this event. Thanks to the Committees members for excellent work. We specially thank our major sponsor for this congress, **The Fundacion Valenciana de Estudios Avanzados (FVEA)** by means of Professor Santiago Grisolia, as well as other outstanding collaborators: **Bloss, Brudylab, Palex, Thea, and Zeiss** for great support.

Thank you indeed to everyone for joining us in Madrid for the **VI SIREV-OFTARED Congress**.

Maria Dolores Pinazo-Durán President of SIREV

Jose Manuel Ramirez-Sebastián President of the Organizing Committee for the 2018 SIREV-OFTARED Congress

Oral Communications

Correlation Between the Geometric Analysis of Corneas Treated with Crosslinking and the Quality of Vision

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Purpose: To diagnose changes in the morphology of the cornea due to crosslinking treatment and to investigate its correlation with the clinical results obtained.

Methods: A retrospective observational case series study was carried out including patients without any previous ocular surgery and submitted to the Dresden crosslinking treatment. For each eye, the optical, visual and tomographic data were analyzed preoperatively as well as 2 months and 9 months after the crosslinking treatment. For each eye a 3D model reconstruction of the cornea was performed. 23 morphogeometric variables were defined and correlated with the patient quality of vision.

Results: A total of 22 eyes from 22 patients aged 14–41 years were included. Nine morphogeometric variables defined in the patient-specific 3D modeling have shown a statistically significant ($p < 0.05$) decrease between the preoperative visit and the 2 months post-operative visit. Five of these variables have shown a statistically significant ($p < 0.05$) increase between the 2 months and the 9 months post-operative visits. A very high correlation has been detected between the Posterior apex deviation variable and the RMS Coma ($Rho = 0.900$ ($p < 0.037$)).

Conclusions: This accurate characterization of the cornea enables to get new evaluation criteria to optimize the therapeutic effects of the crosslinking procedures.

Evaluation of Corneal Local Volume Parameters of Subclinical Keratoconus Using a 3D Virtual Model

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Purpose: To evaluate the efficacy of corneal local volume parameters measured with the virtual model in discriminating subclinical keratoconus corneas and normal corneas.

Methods: This study included 195 subjects divided into two groups according to: 89 (40.1 years ± 12.1) classified as subclinical keratoconus and 106 normal patients (32.7 years ± 10.9) as the control group. For each cornea, was carried reconstruction of 3D virtual model from data of tomographer (Sirius system, CSO, Firenze). Corneal volume parameters were defined as: VOLmct, defined by the points of minimal thickness; VOLaap/VOLpap, defined by the anterior/posterior corneal apex. All volumes were calculated for different radius (0.1–1.5 mm). On based on parameters obtained, had proposed a new model predictive that the detection of subclinical keratoconus.

Results: There were significant differences in all parameters ($p < 0.001$). A logistic regression analysis revealed that the detection of early keratoconus was related to spherical equivalent, coma root mean square (RMS), spherical aberration, spherical-like RMS and VOLaap (R^2 Nagelkerke: 0.879). The overall percentage of cases correctly classified by the model was 96.03%.

Conclusions: Local volume parameters measured by 3D model effectively discriminate subclinical keratoconus corneas from normal corneas, and the combination with optical data allows an accurate detection of incipient cases.

Optical Coherence Tomography for Differentiation of Pterygium and Corneal Intraepithelial Neoplasia

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Purpose: To assess if anterior-segment optical coherence tomography (AS-OCT) is a non-invasive diagnostic method suitable to differentiate benign from premalignant corneo-conjunctival lesions.

Methods: An observational cross-sectional study was conducted in 22 eyes with conjunctival lesions clinically suspicious for pterygium ($n = 18$) and corneal intraepithelial neoplasia (CIN) ($n = 4$), between 2015 and 2017. All patients underwent a

complete eye examination and AS-OCT. Morphology, epithelial thickness (EE) and extension over corneal surface (GIC) were compared. Diagnosis was confirmed histologically after surgical excision.

Results: We found statistical significance ($p < 0.021$) between mean age of patients with pterygium (52.67 ± 15) and CIN (74 ± 12). Typical features in AS-OCT of pterygium were: normal, thinning or slightly thickened EE (mean: $77.5 \pm 26 \mu\text{m}$), and an increased wedge-shaped subepithelial tissue. CIN had a mean thickened EE ($262.5 \pm 124 \mu\text{m}$) and hyperreflective, with abrupt transition between normal and pathological epithelium. Analysis of EE between both groups revealed statistical significance ($p < 0.002$). ROC curve showed 100% sensitivity and specificity of AS-OCT in the differentiation between CIN and pterygium, using $141 \mu\text{m}$ of EE as cutoff point.

Conclusions: AS-OCT is a useful tool to distinguish between pterygium and CIN able to provide typical morphological characteristics. An EE greater than $141 \mu\text{m}$ in AS-OCT presents a sensitivity and specificity of 100% for the diagnosis of CIN.

Incidence and Risk Factors of Dry Eye

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Purpose: To estimate the incidence of dry eye (DE) and to evaluate risk factors in an adult cohort in Spain.

Methods: The Salnés Eye Study (SES) was a cross-sectional population-based study of 654 subjects conducted from 2005 through 2006. After eleven years, 264 (65.8% response rate) individuals participated in Salnés Eye Study 2 (SES 2). The incidence cohort consisted of 209 subjects not diagnosed of DE in SES 1 (mean age (SD) 67.6 years (± 10.1), range: 51–92, women 69.4%). DE was defined as the simultaneous presence of symptoms and at least one sign.

Results: The 11-years incidence of DE was 25.4% (95% CI, 19.5–31.3), and that of symptoms was 31.6% (CI, 25.4–37.8). DE incidence was significantly associated with age ($P < 0.05$). After adjusting for age secondary or university studies were protective for incident DE; taking anxiolytics or antidepressants, and angiotensin-converting enzyme inhibitors increased the risk of symptoms; and a history of hypertension, chronic obstructive pulmonary disease or autoimmune diseases increased the risk of signs.

Conclusion: Annual incidence rate of DE, symptoms and signs, was 2.3 per 100 person-years. Incidence of symptoms was higher than in similar studies.

Meibomian Glands Integrity and its Relationship with the Ocular Surface

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Purpose: To assess the meibomian gland integrity and study its relationship with the ocular surface.

Methods: One-hundred sixty-one random volunteers (mean age, 42 ± 17 years, 91 women and 70 men) participated in this study. Non-contact infrared meibography was performed using Keratograph 5M (K5M; Oculus, Germany) and meibomian gland loss (MGL) assessed using the grading system by Arita *et al.* Association between upper (UL) and lower (LL) eyelid regarding MGL was analysed. Once it was performed, clinical parameters as symptoms assessment (OSDI and SPEED), tear film osmolarity (TearLab Corp, USA), non-invasive anterior eye assessment (K5M) corneal and conjunctival integrity, lid margin abnormalities and tear film volume were studied for each MGL group.

Results: Fair association (k value = 0.2; $p = 0.3$) between both eyelids was found. In addition, a fair and statistically significant correlation was found between UL and LL ($r = 0.308$; $p < 0.001$). No statistically significant differences were found in OSDI and SPEED questionnaires among different MGL groups. Regarding tear film and ocular surface parameters, statistically significant differences were found among groups in tear film osmolarity, corneal staining, conjunctival staining and bulbar redness ($p < 0.05$).

Conclusions: Our findings suggest that a MGL $> 33\%$ could be related to changes on the ocular surface parameters.

Glaucoma-Like Phenotypes in a *Cyp1b1* Knockout Zebrafish Line

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Purpose: Congenital glaucoma is an important cause of visual loss in children. Loss-of-function *CYP1B1* mutations are the main known genetic cause of this type of glaucoma by an unknown molecular mechanism. It has been proposed functional alteration of this gene produces an abnormal embryonic development of the iridocorneal angle. Our main purpose was to establish and characterize a *cyp1b1* knockout zebrafish line to assess its value as a novel glaucoma animal model.

Methods: The CRISPR/Cas9 was employed to generate the *cyp1b1* knockout zebrafish line. Mutant phenotypes were analyzed *in vivo* with a Nikon SMZ18 microscope. Gelatin embedded adult eye histological sections were stained with hematoxylin-eosin and examined with a Nikon Eclipse-Ti microscope. Fluorescent immunohistochemistry was carried out using an anti-*CYP1B1* antibody in a LSM710 Zeiss confocal microscope.

Results: Ocular phenotypes include an increased volume of the eye anterior chamber of 144hpf larvae and structural changes in

the ciliary margin zone, anterior segment vasculature, the photoreceptor layer and cornea of adult fishes.

Conclusions: These results suggest that this *cyp1b1* knockout zebrafish line might be an animal genetic model for congenital glaucoma.

Ocular Characterization of a *lrp2a* Mutant Zebrafish Line

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Purpose: *LRP2* encodes a transmembrane protein of high molecular weight, belonging to the family of LDL receptors, involved in the transport and internalization of multiple molecules. *LRP2* has two orthologs in zebrafish: *lrp2a* and *lrp2b*. Loss-of-function *lrp2a* mutations have been previously described to produce an enlarged eye phenotype (Bugeye) in adult fishes (>6 months). Retinal damage and several non-neuronal cell populations, like Müller Glia, may have relationship with the pathogenesis of glaucoma. The main purpose was to characterize the ocular phenotypes of a *lrp2a* knock-out mutant zebrafish line.

Methods: The zebrafish line was obtained from ZIRC repository. Gelatin embedded adult fishes were used for hematoxylin-eosin staining. Fluorescent immunohistochemistry using TUNEL and anti-glial fibrillary acidic protein (GFAP) antibody. The tissue sections were visualized in a LSM710 Zeiss confocal microscope.

Results: The histological observation of hematoxylin-eosin stained sections showed structural retinal alterations in the internal nuclear layer and in photoreceptors. The presence of apoptotic cells in the corneal epithelium were revealed by the TUNEL analysis. Finally, IHC showed retinal glial reaction supporting the existence of retinal injury.

Conclusions: These results show corneal and retinal alterations associated with *lrp2a* loss-of-function that may contribute to investigate the role of this gene in glaucoma.

Generation and Preliminary Characterization of a *Cpamd8* Knock-Out Zebrafish Line: Role in Anterior Segment Dysgenesis and Congenital Glaucoma

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Purpose: *cpamd8* mutations have been identified in a few patients with glaucoma and anterior segment dysgenesis. This gene encodes a member of the protease inhibitor I39 (alpha-2-macroglobulin) protein family and its biological function and pathogenicity remains largely unknown. The goal of this study was to gen-

erate an animal model to analyze the role of this gene in ocular development and in glaucoma.

Methods: CRISPR/Cas9 genome editing was used to generate a zebrafish *cpamd8* knockout line. Early-phenotypes were characterized *in vivo* using a SMZ18 Nikon binocular microscope. Gelatin embedded larvae 96 hour post fertilization histological sections were used for hematoxylin-eosin staining. The tissue sections were visualized in a Nikon eclipse Ti microscope.

Results: Approximately 50% of the microinjected embryos with CRISPR/Cas9 showed normal phenotypes and 50% exhibited serious head and ocular alterations that were classified into four phenotypes characterized by increasing gross abnormalities consisting mainly of variable degrees of pericardial, yolk sac and periorbital edema and microphthalmia.

Conclusions: We have generated a useful genetic tool to study the function of *cpamd8*. Our preliminary data indicate that *cpamd8* play an important role in zebrafish ocular development.

Production and Characterization of a *Sparcl1* Knockout Zebrafish Line as a Genetic Tool to Evaluate its Putative Role in Glaucoma

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Purpose: Monogenic glaucomas, e.g., congenital and juvenile glaucomas, are rare and severe ocular neuropathies believed to be produced by an abnormal development of the iridocorneal angle. However, the genetic and molecular basis of the disease remains largely unknown. *MYOC* alterations are the main known cause of dominant juvenile glaucoma. This gene encodes an extracellular protein which is characterized by its interactions with different extracellular proteins including fibronectin and HEVIN (SPARC-like 1), a matricellular protein with an unclear function that modulates cell shape and cell adhesion. The goal of this study was to obtain a *sparcl1* knockout zebrafish model to study its possible role in glaucoma.

Methods: The CRISPR/Cas9 genome editing technology was employed to generate the *sparcl1* knock-out zebrafish line. *Sparcl1* mRNA stability was evaluated using RT-qPCR. Early-phenotypes were characterized *in vivo* using a SMZ18 Nikon microscope. Gelatin embedded sections of zebrafish were used for initial histological characterization.

Results: CRISPR/Cas9 technique generated a zebrafish line carrying the *sparcl1* c.1296_1301delGTGGT mutation in homozygosis. RT-qPCR showed decreased *sparcl1* mRNA expression, supporting a loss-of-function for this gene. Preliminary phenotype characterization showed several ocular and craniofacial alterations.

Conclusions: The preliminary data suggest that this gene plays an important role in zebrafish ocular and craniofacial development.

A Fingerprint for the Pre-Clinical Phase of Primary Open-Angle Glaucoma versus Ocular Hypertension Using MicroRNAs in Tears

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Purpose: To evaluate the expression profile of microRNAs in tears of patients with primary open-angle glaucoma (POAG) or ocular hypertension (OHT), to find molecular biomarkers for a better management of OHT and, to succeed the preclinical POAG diagnosis.

Methods: Case-control study carried out in 24 POAG and 24 OHT patients. Next Generation Sequencing (Illumina NextSeq500 Technology) was used to analyse the expression profile of microRNAs, finally bioinformatic analyses were performed.

Results: Outstanding differential microRNAs fingerprint ($p < 0.05$) were found, 8 up-regulated and 2 down-regulated, in the glaucomatous tears. Most of them were implicated in glycosylation processes.

Conclusions: Our findings provided evidence enough to suggest important roles for the microRNAs in glaucoma care in foreseeable future.

Molecular Biomarkers in Glaucoma? From Discovery to Validation

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Purpose: Molecular biomarkers of glaucoma in readily available biological fluids may be extremely beneficial in the diagnosis and management of this eye disease. To this end, we aimed to identify and validate glaucoma biomarkers using a multidisciplinary and high-risk approach.

Methods: Differential proteomics-based techniques (i.e., 2D-DIGE) have been used in the discovery of new biomarkers. Qualitative immunoassays (ELISA screening) confirmed the alteration in individual protein levels. Pre-clinical validation was carried out by next-generation quantitative proteomics, using LC-MRM-MS.

Results: We have identified in serum, by differential proteomics, the over-expression of 35-candidate glaucoma biomarkers with high degree of specificity. We confirmed by ELISAs that the top-17-ranked proteins were present in altered levels in glaucoma patients when compared to controls. We developed and implemented a new methodology based on labeled proteotypic peptides and tandem

mass spectrometry for the simultaneous absolute quantification of three serum proteins proposed as candidate biomarkers (APOA4, C3 and VTN), obtaining a diagnostic efficiency of 56%.

Conclusions: Systemic biomarkers of neurodegenerative eye diseases may offer a new clinical tool to facilitate diagnosis, predict disease prognosis, and monitor treatment responses by a non-invasive test in serum of patients. Current trends and limitations observed along our research in glaucoma will be discussed.

Clinical Utility of Microperimetry and Standard Automated Perimetry of the Macular Area in Glaucoma

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Purpose: To evaluate the diagnostic capability of the microperimetry (MP) compared with the gold standard functional test in glaucoma, the standard automated perimetry (SAP).

Material and Methods: 44 eyes without glaucoma (22 healthy/22 ocular hypertensive) and 84 with glaucoma (26 early/20 moderate/38 severe) underwent macular MP and SAP with strategies 10-2 and 30-2. Sensitivities were averaged in different sectors, anatomically equivalent in MP and SAP 10-2 and the duration of each test was also considered. The results were compared between the general groups (with/without glaucoma) and the five subgroups and diagnostic capabilities were calculated using receiver operating characteristics (ROC) curves.

Results: Comparison of the parameters obtained between the different subgroups showed significant differences in all the subgroups and groups with the exception of the healthy versus hypertensive and early versus moderate subgroups. ROC curve analysis showed that the best parameters to distinguish among glaucoma stages were pattern standard deviation (PSD) and duration. MP showed no better areas under the curve than SAP 10-2.

Conclusions: MP does not seem superior to SAP. PSD and duration of both tests (this latter variable had never been studied previously as a diagnostic criterion of glaucoma) seem useful parameters to differentiate between groups and subgroups.

Does Fovea-Disc Alignment Affect Macular Ganglion Cell Layer Thickness Results Measured by Posterior Pole Grid? A Study in Healthy, Hypertensive and Glaucomatous Patients

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Purpose: The posterior pole 8x8 grid of the Spectralis SD-OCT is automatically aligned with the fovea-disc axis. We aim to know whether this alignment is important for the determination of macular ganglion cell layer (mGCL) thickness.

Methods: 436 eyes (141 from 82 healthy subjects, 130 from 74 ocular hypertensive and 165 from 104 primary open-angle glaucoma, POAG) were examined using the posterior pole 8x8 grid examinations. Thickness values were obtained on the 64 cells of the grid for mGCL (aligned with the fovea-disc axis). Then all grids were horizontalized and the thickness values of the 64 cells were obtained again. Comparisons of thickness between corresponding cells of these two grids (automatically aligned and horizontalized grids) were performed using the paired t-test and differences were represented using color-coded maps (heatmaps).

Results: There were significant differences in mGCL thickness between the aligned and the horizontalized grid, more marked in healthy (46 out of 64 cells were different) and ocular hypertensive subjects (45 out of 64 cells) than in POAG patients (36 out of 64 cells).

Conclusions: Determination of the mGCL thickness differs between the aligned and the horizontalized exams more in healthy and ocular hypertensive subjects than in POAG patients.

Risk of Obstructive Sleep Apnea Syndrome and Retinal Layer Thickness in Glaucomatous and Non-Glaucomatous Patients

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Purpose: To investigate the association between two underdiagnosed diseases: obstructive sleep apnea syndrome (OSAS) and glaucoma.

Methods: 157 patients (99 with glaucoma and 58 without glaucoma) were included. The risk (low or high) of OSAS was evaluated with the Berlin test questionnaire. The visual field (30-2 SITA Fast, Humphrey) and the thickness of the macular layers and of the peripapillary retinal nerve fiber layer (pRNFL; SD-OCT Cirrus, Zeiss) were also explored. Only one eye (the eye with worse mean deviation -MD-) per patient was included.

Results: Glaucomatous patients showed increased high OSAS risk than non glaucomatous patients. Glaucomatous patients with high OSAS risk had thinner macular nerve fiber layer (mRNFL), ganglion cell-inner plexiform macular layer (GCIPL) and pRNFL than low OSAS risk glaucomatous patients. There were no significant differences in retinal layer thickness between high and low OSAS risk non glaucomatous patients.

Conclusions: Glaucomatous patients with high risk for OSAS have diminished thickness of the mRNFL, GCIPL and pRNFL. Thus, high risk OSAS patients are at higher risk for glaucomatous retinal ganglion cell damage.

Bilateral Increase of Microglial Soma Size, at Different Time-Points After Unilateral Laser-Induced Ocular Hypertension

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Purpose: To analyse quantitatively, a retinal sign of microglial activation (soma size) at different time points after laser-induced ocular hypertension (HTO) in OHT- and their contralateral-eyes.

Methods: Swiss mice were assigned to two groups: Naïve and lasered. Animals were sacrificed at 24 h, 3 d, 5 d, 8 d, or 15 d. Anti-Iba1 was used in retinal whole-mounts to analyse microglial soma size in the plexiform and nerve fiber-ganglion cell layers.

Results: At all time-points, both in OHT- and contralateral-eyes, the soma size was significantly increased ($P < 0.01$) with respect to naïve in all retinal layers analysed. In OHT-eyes larger somas were found at 24 h and decreased significantly at 5 d, 8 d and 15 d after OHT-induction ($P < 0.01$). In contralateral-eyes larger somas were found at 3 d decreasing significantly at 8 d and 15d ($P < 0.05$).

Conclusions: Signs of microglial activation were found at all time-points and in all retinal layers analysed in both OHT- and contralateral-eyes.

Whole-Exome Sequencing of Congenital Glaucoma Patients Reveals Hypermorphic Variants in *GPATCH3*, a New Gene Involved in Ocular and Craniofacial Development

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Purpose: To identify new disease genes involved in congenital glaucoma (CG).

Methods: We performed whole-exome sequencing of 26 unrelated CG patients and a posterior functional analysis of the candidate gene found. We performed *in vitro* analysis of identified variants and then we used zebrafish as animal model to characterize the expression patterns of candidate gene and to analyze the ocular phenotypes derived from gene down regulation with morpholinos.

Results: We identified two rare, recessive and hypermorphic coding variants in *GPATCH3*, a gene of unidentified function, and 5% of a second group of 170 unrelated CG patients carried rare variants in this gene. The recombinant *GPATCH3* protein activated *in vitro* the proximal promoter of *CXCR4*, a gene involved in embryo neural crest cell migration. The *GPATCH3* protein was detected in human tissues relevant to glaucoma (e.g., ciliary body). This gene was expressed in the dermis, skeletal muscles, periorbital mesenchymal like cells and corneal endothelium of early zebrafish

embryos. Morpholino-mediated knockdown and transient over-expression of *gpatch3* led to varying degrees of goniodysgenesis and ocular and craniofacial abnormalities, recapitulating some of the features of zebrafish embryos deficient in the glaucoma-related genes *pitx2* and *foxc1*.

Conclusions: Our data suggest the existence of high genetic heterogeneity in CG and provide evidence for the role of *GPATCH3* in this disease. We also show that *GPATCH3* is a new gene involved in ocular and craniofacial development.

Functional and Morphometric Alterations Following Acute Ocular Hypertension

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Purpose: To analyse the effects of acute ocular hypertension (AOH) in the adult rat retina.

Methods: In adult Sprague-Dawley rats, the intraocular pressure of the left-eye was elevated during 90 minutes to 75 mm Hg with a hydrostatic system connected to the anterior chamber. Retinas were analysed in vivo with Ocular Coherence Tomography (OCT) and full-field Electroretinograms prior to and at 1, 3, 7, 15 and 30 days (n = 6–8). Cryostat radial sections of the retinas were examined for immunohistochemistry using antibodies which recognize horizontals, amacrine and retinal ganglion cells (RGCs) and total thickness of the retina was analysed with the microscope.

Results: Full field ERG showed a significant diminution in the amplitudes of the main b-waves and scotopic threshold response (STR) as early as 24 h after AOH. A significant reduction of the internal retinal thickness (NFL-OPL) was found 3 days (20% measured with OCT) and 30 days (30% measured in radial cryostat sections) after AOH. Furthermore, there was a significant loss of calbindin⁺, parvalbumin⁺ and RBMPS⁺ 30 days after AOH.

Conclusions: AOH results in permanent functional and morphological alterations of the retina.

Ultrastructural Characteristics of the Outflow Pathway in Primary Congenital Glaucoma with CYP1B1 Mutation

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Purpose: To correlate the ultrastructure of trabeculectomy samples from patients with Primary Congenital Glaucoma (PCG) and *CYP1B1* mutation.

Methods: i) genomic DNA analysis from peripheral leucocytes from CPG patients and; ii) transmission electron microscopy of surgical pieces (n = 5) and control tissues from cadaver donors (n = 4).

Results: Ultrastructural findings in the aqueous humor pathway were classified into three groups: 1) no visualization of Schlemm's canal (SC); 2) presence of the SC and no display of the collector channels (CC); and 3) SC and CC visible. Patients from group 1 and 2 had null CYP1B1 enzymatic activity and more dysgenesis and trabecular alterations than the patient from group 3 (60% of enzymatic activity) which had better tissue development and less cell necrosis.

Conclusions: The alterations of the outflow pathway seem to correlate with the enzymatic activity of CYP1B1. This could help to plan treatment strategies in these patients.

Macular Structural-Function Relationship with Microperimetry, Standard Automated Perimetry and Optical Coherence Tomography in Glaucoma

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Purpose: To study the morpho-functional correlation of glaucomatous damage on its evolutionary stages.

Material and Methods: 44 eyes without glaucoma (22 healthy/22 hypertensive ocular) and 84 with glaucoma (26 early/20 moderate/38 severe) underwent microperimetry (MP), standard automated perimetry (SAP) 10-2 and macular OCT. Sensitivities were averaged in order to correspond anatomically with the OCT sectors. In the OCT the thickness of the retinal nerve fiber layer (mRNFL), the sum of ganglion cell layer and inner plexiform layer (GCL IPL) and the outer retina (OR) were measured. Correlations between structural and functional parameters were calculated considering sensitivities in decibels and linear scale.

Results: The analysis determined a good association in almost all sectors between functional tests. Structural and functional tests showed more intense and more frequent correlations as the severity of the disease was higher regardless of the scale used for sensitivity. mRNFL and GCL IPL showed better associations with functional tests than OR. MP and SAP showed similar associations with OCT parameters.

Conclusions: There is a higher association between macular structure and function as the severity of glaucoma increases. The conversion of the sensitivity to linear scale does not seem useful to improve the morpho-functional correlation. MP does not seem superior to SAP in terms of structure-function relationship.

Generation and Characterization of a *Guca1c* Knock-Out in the Zebrafish

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Purpose: GUCA1C is a Guanylate Cyclase activator involved in the regulation of phototransduction, with a significant role in regulating aqueous humour secretion in the ciliary body and the control of the cellular volume of trabecular meshwork and Schlemm's canal cells. The main purpose of this study was to generate a genetic tool to study the function of this gene and to evaluate its possible role in glaucoma.

Methods: CRISPR/Cas9 was used to generate a zebrafish knock-out line. mRNA stability was evaluated using RT-qPCR. Gelatin embedded sections of larvae and adult were used for Nissl staining and fluorescent immunohistochemistry (IHC), which were visualized in confocal microscope.

Results: We generated a zebrafish line carrying the *guca1c* p (Thr47ArgfsTer63) mutation in the homozygous state. RT-qPCR showed 63% decreased *guca1c* mRNA expression. Nissl staining and mesenchymal cell IHC showed morphological alterations of the ventral iridocorneal angle in larvae. Glial fibrillary acidic protein IHC revealed the existence of Muller cell activation in adult fish retina.

Conclusions: We have generated a useful genetic tool to study the function of *GUCA1C*. Our data suggest that this gene is involved in the development of zebrafish tissues involved in aqueous humour dynamics and that its functional loss produces retinal damage.

Bilateral and Simultaneous Nonarteritic Anterior Ischemic Optic Neuropathy Related to Sildenafil Intake

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Purpose: To describe bilateral and simultaneous presentation of nonarteritic anterior ischemic optic neuropathy (NAION) after the ingestion of sildenafil citrate for erectile dysfunction.

Method: Observational case report.

Results: A 58-year-old man with a medical history of post-traumatic cervical myelopathy and spastic tetraparesis presented a sudden loss of visual acuity in both eyes after sildenafil intake. Visual acuity was counting fingers at 50 cm in the right eye and CF at 1 m in the left eye with left afferent pupillary defect. Fundoscopy, revealed bilateral papillary pallor and visual field showed an inferior altitudinal defect in both eyes. OCT exhibited a diffuse and bilateral decrease of peripapillary retinal nerve fiber layer thickness. After a complete neuro-ophthalmological and systemic study, the diagnosis of exclusion of bilateral NAION was made and the patient was treated for five days with methyl-

prednisolone 1 g/day intravenously. The final visual acuity did not improve.

Conclusion: On suspicion of bilateral simultaneous NAION it is important to keep in mind the possible use of drugs that predispose its appearance such as sildenafil.

Clinical Case: Ischemic Optical Neuritis Caused by Hypertensive Anterior Uveitis

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Purpose: To report a case of ischemic optical neuritis caused by hypertensive anterior uveitis.

Methods: A 58-year-old woman, went to the emergency room referring pain and blurred vision of her left eye since 4 days ago. She presented a hypertensive anterior uveitis with intense anterior chamber cellularity (AC) and an intraocular pressure (IOP) of 56 mm Hg. There was a corneal epithelial edema and pupil had a medial mydriasis. Her papilla had sharp edges. A few minutes later, a 360° papillary edema occurred. Analysis were performed to rule out Horton's syndrome, which were negative. An anterior ischemic optic neuritis (AION) was diagnosed. After starting hypotensive and optical corticosteroid treatment, an IOP of 16 mm Hg was achieved, three hours later. Months later she presents a mydriatic pupil, the papillary edema had disappeared, but a quarter of her papilla is pale with an ischemic appearance.

Results: In response to the increase in IOP caused by uveitis, a vasodilatory response occurred in the papilla to increase blood flow. Ocular perfusion pressure (OPP) pushes blood flow. Ocular perfusion pressure (OPP) pushes blood into eye tissues. The OPP is the difference between ophthalmic artery pressure and IOP (PPO=OAP-IOP). Since the IOP is very high, the OPP would fall considerably, and a AION could happen.

Conclusions: AIONs caused by acute angle-closure glaucoma have been observed, however, in this case the AION seems to have been originated by an acute IOP increase due to an anterior uveitis.

Identification and Characterization of Adult Neurogenesis in the Human Retina

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Purpose: Adult neurogenesis in the peripheral retina has been identified in amphibians, fish and birds through life. Nevertheless, there is a controversy about the existence of *in vivo* adult neurogenesis in the mammalian retina. Here, we try to clarify if a neurogenesis process occurs in normal conditions in the adult human retina.

Methods: Immunohistochemistry using neural and retinal progenitor markers and proliferation markers was performed in

adult human and monkey retinas. Posteriorly, confocal microscopy imaging and analysis were done.

Results: In the peripheral retina, close to the *ora serrata*, cells co-express neural and retinal progenitor markers (Sox2, Pax6, Chx10, Rx, Nestin), and display an undifferentiated morphology that becomes more mature towards the central retina. Besides, cells expressing the proliferation markers Ki67 and BrdU have been found in the same region in all the analyzed retinas.

Conclusions: The morphological maturing process that occurs in the peripheral retina together with the presence of proliferating cells suggests that a stem cell niche similar to that described in other species can also exist in the mammalian adult retina. The identification and characterization of this niche would be helpful for the development of new therapies for retinal neurodegenerative diseases.

Adipose-Derived Mesenchymal Stem Cells Intravitreal Transplant: Survival, Toxicity, and Neuroprotection after Axotomy

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Purpose: Mesenchymal stem cell (MSC) transplantation is emerging as an ideal tool for central nervous system repair. We purpose to analyze: i/ the toxicity of an allogeneic intravitreal transplant of adipose-derived MSC (AdMSC), ii/ the survival of the transplanted cells; and iii/ whether they confer neuroprotection on axotomized retinal ganglion cells (RGCs).

Methods: AdMSC-GFP were isolated from the abdominal fat of C57/BL6Tg(CAG-EGFP) adult mice and transplanted into the left vitreous chamber of C57/BL6 mice right after crushing the left optic nerve (10,000 cells/eye). Animals were sacrificed 5 and 21 days after the surgery. ONC+vehicle matched groups were used as controls. Transplant toxicity was assessed in intact retinas. AdMSC-GFP were followed *in vivo* using OCT-SD. Retinas were flat-mounted, RGCs immunodetected and automatically quantified.

Results: AdMSC-GFP remained in the vitreous up to 21 days after the transplant, without forming tumors, integrating in the retina, or causing RGC loss. RGC survival after the axotomy did not differ between vehicle- or cell-treated retinas.

Conclusions: At the dose and time points studied here, AdMSC-GFP intravitreal transplant does not neuroprotect axotomized mice RGCs. However, they are not toxic for RGCs, do not produce tumors, and survive long time after their administration.

Neuroprotective Effect of Statins in Optic Nerve in a Rabbit Model of Hypercholesterolemia

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Purpose: To evaluate optic nerve (ON) changes in hypercholesterolemic rabbits after a low-dosage statins treatment and to analyze their possible neuroprotective effect.

Methods: Three groups of New Zealand rabbits: Control (GO; n = 10) fed with a standard diet; Hypercholesterolemic (G1, n = 8) fed with a 0.5% cholesterol-enriched diet for 8 months; and Statins (G2; n = 8) fed with a 0.5% cholesterol-enriched diet for 8 months plus administration of fluvastatin sodium or pravastatin sodium at a dose of 2 mg/Kg/day each. ON were processed by transmission-electron microscopy.

Results: Cholesterol levels in G1 and G2 were similar. ON in G1 had: i) axonal and myelin degeneration; ii) necrosis in vascular endothelial and muscle cells and basal membrane thickening; iii) astrocytes in necrosis or reactive with increased organelles, abundant glial filaments and myelin debris. The ultrastructural damage in G2 were intermediate between G1 and G0.

Conclusion: Low-dosage statins exert a neuroprotective effect, diminishing the ultrastructural ON damage associated to hypercholesterolemia.

A Macrophage Cell Line (J774.A1) as a Tool to Evaluate the Cytotoxicity of Ophthalmic Excipients and Hypotensive Drug Formulations

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Purpose: High intraocular pressure is the primary risk factor for glaucoma progression. One of the problems associated with topical administration of hypotensive drugs is damage to the ocular surface. This work aimed to evaluate the tolerance of hypotensive drugs solutions and excipients in a cell line of macrophages (J774.A1).

Methods: Macrophages were exposed to solutions of hypotensive agents (Brimonidine 0.2%, Latanoprost 0.005% and Timolol Maleate 0.5%) and different excipients (Trehalose-Borates buffer, Hydroxypropylmethylcellulose and Sodium Hyaluronate) for 15 m, 1 h and 4 h. To select the positive control, different concentrations (0.001%, 0.002%, 0.003%, 0.004% and 0.005%) of benzalkonium chloride (BAK), the most commonly used preservative in eye-drops, were evaluated.

Results: Brimonidine and Latanoprost solutions and excipients showed viability values near or higher than 80% after 15 m, 1 h and 4 h of exposure in J774.A1 cells. Timolol maleate resulted cytotoxic after 4 h of exposure. BAK 0.02% was selected as the pos-

itive control with viability values of $60.4 \pm 1.8\%$, $20.2 \pm 4.1\%$ and $5.9 \pm 0.3\%$ at 15 m, 1 h and 4 h respectively.

Conclusions: The J774.A1 macrophages cell line resulted sensitive for cytotoxicity studies. This cell line can be used to evaluate the tolerance of preparations of topical ophthalmic formulations.

Laponite Clay as a Drug-Controlled Delivery System to Treat Intraocular Diseases

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Purpose: To evaluate the potential use of Laponite as a biological well tolerated controlled release system for ocular drugs.

Methods: Sixteen New Zealand rabbits were intravitreally injected with a 100 microliters Laponite suspension each. Quantification of Laponite in vitreous gel was performed at 1, 7, 28, 84 and 100 days (14 weeks) post-administration by complexometric titration with ethylenediaminetetraacetic acid (EDTA). Clinical parameters including intraocular pressure (IOP) measurements and funduscopy as well as anatomopathological studies were carried out to evaluate biological tolerance.

Results: Progressive diminution of Laponite levels in vitreous humor were observed until 100 days post injection (about 33% of the total initial Laponite dose administered), but was not detected in other ocular tissues such as retina, choroid, lens or sclera. No adverse effects as intraocular inflammation, infection, ocular hypertension (IOP range from 9 to 15 mm Hg) or retinal degeneration were detected by ophthalmological or histological examination; even a significantly higher cell counts in the outer nuclear layer of the retina was observed, possibly due to high contents of magnesium ions into Laponite.

Conclusions: Laponite clay could be considered a potential matrix to serve as a vehicle for sustained delivery drugs for intraocular administration, due to its proven ocular biocompatibility.

Sustained Release of GDNF and Osteopontin from PLGA Microspheres

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Purpose: Neurotrophic factors osteopontin (OPN) and glial cell line-derived neurotrophic factor (GDNF) have shown photoreceptor preservative effect on animal models for retinal degeneration *in vitro*. Single injections of the neurotrophins have provided only transient neuroprotection *in vivo*, likely due to their

limited bioavailability and the complex pathomechanisms of retinal degenerative diseases. To achieve long-term neuroprotection, sustained delivery and combinatory therapies are needed.

Methods: Primary organotypic retinal cultures prepared from neonatal murine eyes retain the retinal architecture with retinal development comparable to *in vivo*, allowing the evaluation of therapeutic effect of neurotrophins *in vitro*. Solid GDNF dispersed in vitamin E was encapsulated in poly (lactic-co-glycolic acid) (PLGA) microspheres using a novel S/O/W emulsion solvent evaporation technique.

Results: Retinal organ cultures prepared from animal models of retinal degeneration treated with GDNF and OPN showed improvement in retinal architecture and photoreceptor cell viability. Sustained release of biologically active GDNF from PLGA/Vitamin E microspheres was possible up to six months after intravitreal injection in rabbits.

Conclusions: We aim to further evaluate the effect of combinatory therapy of GDNF and OPN using organotypic retinal cultures, in addition to formulation and characterisation of GDNF/OPN/PLGA/Vitamin E microspheres.

Controlled Delivery of Tauroursodeoxycholic Acid from Biodegradable Microspheres Slows Retinal Degeneration and Vision Loss in P23H Rats

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Purpose: To assess the efficacy of intravitreal controlled delivery of tauroursodeoxycholic acid (TUDCA) encapsulated in PLGA-microspheres (MSs) in a rat model of retinitis pigmentosa.

Methods: MSs containing TUDCA were prepared according to the extraction-evaporation technique from an O/W emulsion and characterized (morphology, encapsulation efficiencies and *in vitro* release studies). HPLC-MS was performed for TUDCA quantification. Homozygous P23H (line 3) rats received a treatment of intravitreal injections of TUDCA-MSs. Retinal function was assessed by electroretinography at P30, P60, P90 and P120. The density, structure and synaptic contacts of retinal neurons were analyzed using immunofluorescence and confocal microscopy at P90 and P120.

Results: MSs ($22.89 \pm 0.04 \mu\text{m}$) were spherical with porous surfaces. TUDCA ($12.5 \pm 0.8 \mu\text{g}$ TUDCA/mg MSs) was released *in vitro* in a controlled fashion up to 28-days. Scotopic electroretinographic responses showed increased ERG a- and b-wave amplitudes in TUDCA-MSs treated eyes as compared to those injected with unloaded particles. Treated eyes showed more photoreceptor rows than controls. The synaptic contacts of photoreceptors with bipolar and horizontal cells were also preserved in rats treated.

Conclusions: Controlled delivery of TUDCA from MSs has potential neuroprotective effects to prevent neurodegeneration and visual loss in retinitis pigmentosa.

Correlation Between Choroidal Thickness and Systemic Microangiopathy in Diabetic Patients

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Purpose: To compare choroidal thickness (CT) and the presence of systemic microangiopathy in patients with type 2 diabetes (T2D).

Methods: 200 eyes of 100 T2D patients were included after applying the exclusion and inclusion criteria. Choroid was imaged using swept-source optical coherence tomography (Triton, Topcon) and an Early Treatment Diabetic Retinopathy Study (ETDRS) macular grid was automatically obtained. Data from ischemic cardiopathy (IC), cerebrovascular accident, peripheral artery disease (PAD), nephropathy and peripheral polyneuropathy (PPN) were recorded. Correlations between CT and systemic microangiopathy were calculated.

Results: 60 eyes had no diabetic retinopathy (DR), 46 eyes had mild DR, 64 eyes had moderate DR, 20 eyes had severe DR and 10 eyes had proliferative DR. Mean age was 67.38 ± 8.15 years old and mean spherical equivalent was 0.79 ± 1.95 D. IC was moderately correlated with a thinner CT in horizontal ETDRS sectors (γ between -0.29 and -0.38). The type of nephropathy was moderately correlated with a thinner CT in the outer inferior and horizontal sectors (γ between -0.32 and -0.40). The degree of DR was correlated with PAD, nephropathy and PPN.

Conclusions: In contrast to DR, CT showed a moderate correlation with systemic microangiopathy.

Chronic Diabetic Macular Edema in Vitrectomized Patients – Comparative Analysis of Two Intravitreal Implants

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Purpose: To evaluate the functional, anatomical and safety of fluocinolone acetonide (FAC) (ILUVIEN®) and dexamethasone (Ozurdex®) implants in diabetic macular edema (DME) treatment in vitrectomized eyes.

Methods: Retrospective analysis of 29 eyes treated with an intravitreal injection of 0.2 µg/day FAC implant (group 1) and 17 eyes with a 0.7 mg dexamethasone implant (group 2). Best-corrected visual acuity (BCVA), central foveal thickness (CFT), additional intravitreal injections, topical glaucoma therapy drugs and intraocular pressure (IOP) were analysed. Patients with a minimum follow-up of 6 months were included, analysis was performed until the twelfth month.

Results: No differences in baseline characteristics were noticed between the groups. At the sixth month group 1 have a change of BCVA of +13 (0;+40) vs -2 ETDRS letters (-13;+24) in group 2 –

$P < 0.001$; with a significant CFT reduction of $-140.5 \mu\text{m}$ ($+75, -494$) vs $-38 \mu\text{m}$ ($+307, -231$) in group 2 – $P < 0.05$. The need of additional intravitreal injections was higher in group 2: 76.5% vs 6.9% ($p < 0.001$) with an average time until treatment of 7.3 months. No significant differences between the groups were noticed in IOP or topical and surgical glaucoma therapy.

Conclusion: FAC implant demonstrate longer and more significant results in visual acuity and chronic DME in vitrectomized eyes.

Choroidal Thickness in Diabetic Choroidopathy: A Useful Parameter?

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Purpose: To evaluate the variation of measurements of choroidal thickness (CT) between visits, looking for any correlation with the retinal disease progression.

Methods: Diabetic patients treatment naïve were included. Data from baseline (V1) and a follow up visit (V2) was collected for OCT, ophthalmological exam and metabolic analysis.

Results: Seventy-eight eyes were analyzed. CT increased in the V2 in 31 eyes where Retinal thickness (RT) was: equal on 67.7%, increased on 19.4% and decreased on 12.9%. CT decreased in 26 eyes where RT was: equal on 50%, increased on 15.4% and decreased on 36.6%. The CT changes had no correlation with blood pressure, glycosylated hemoglobin or diabetic retinopathy stage.

Conclusion: Choroid has a great individual and inter-visit thickness variation, independent of the retinopathy state, and thickness should be used carefully and correlated to morphological findings as a parameter of DC.

Diabetic Retinopathy-Like Lesions in Heterozygous TRPV2 Knockout Rats

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Purpose: To investigate whether heterozygous (+/-) TRPV2 rats induces diabetic-like vascular pathology in the retina.

Methods: Rat retinas from TRPV2 WT (+/+) and TRPV2 +/- animals at P21, P90 and P360 were dissected and immunolabelled to study the vascular, neuronal and glial components using confocal imaging. Systemic blood pressure was measured and vasopermeability assessed using the Evan's blue dye method. Retinal neurophysiology was examined by electroretinography.

Results: Blood glucose and blood pressure were within normal ranges for both TRPV2 WT and TRPV2 +/- animals. There was a significant increase in vasopermeability ($P < 0.05$) and acellular capillary formation in TRPV2 +/- animals at P90 and P360 ($P <$

0.05), when compared to their WT counterparts. A decrease in the mean number of retinal ganglion cells ($p < 0.001$) and an increase in the proportion of activated Müller glia ($p < 0.0001$) was also observed. In addition, microglial cell numbers were increased at P90 and P360 TRPV2+/- retinas ($P < 0.05$ & $P < 0.01$ respectively), suggesting pro-inflammatory changes. TRPV2+/- animals showed progressive neurophysiological dysfunction, manifesting as a reduction in the amplitude of both the ERG A & B-waves at P360 ($P < 0.01$).

Conclusions: TRPV2 downregulation alone can cause microvascular pathology without changes in blood glucose or hypertension and may contribute to the pathogenesis of diabetic retinopathy.

Telemedicine Diabetic Retinopathy Screening Program: First 3 Months

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Purpose: To analyze the results of our telemedicine diabetic retinopathy (DR) screening program that integrates the Retmarker software.

Methods: Retrospective analysis of the first 3 months of our telemedicine-based DR screening program in a region of Lisbon.

Results: In the first 3 months, 3573 patients with diabetes mellitus were enrolled, of which 1638 (45.9%) attended DR screening. Of this group, 630 (38.5%) were automatically classified by Retmarker as having no signs of DR. The remaining retinography images of 1008 patients were analyzed by ophthalmologists. A total of 464 patients (28.3%) were referred for ophthalmology consultation by DR, non-diabetes ophthalmological pathology or non-classifiable retinographs. The retinography images of eighty-one patients (4.9%) presented signs of referable DR with a need for follow-up and eventual treatment.

Conclusions: Retmarker is a useful tool since it eliminated automatically 38.5% of patients. From the analysis of the retinographs by ophthalmologists, 33.2% of the patients were excluded and sent for a new annual screening. In these 3 months of screening it was possible to exclude 71.7% of the patients, without the need for evaluation in ophthalmology consultation. This program thus allows a reduction in the number of consultations as well as an effective and early referral of diabetic patients.

Long-Term Clinical Outcomes of Fluocinolone Acetonide Implant for the Treatment of Chronic Diabetic Macular Edema (DME)

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Purpose: To evaluate the visual, anatomical and safety outcomes following the long-acting sustained release fluocinolone acetonide implant, 0.2 µg/day (ILUVIEN®), in chronic DME in patient with ≥12 months follow-up.

Methods: Retrospective, clinical data review of chronic DME eyes treated with a single ILUVIEN®. Only patients with ≥12 months follow-up were included. Functional response was assessed by BCVA in ETDRS letters and CFT was evaluated by OCT examinations. Safety outcome measures included mean change IOP. Mean follow-up was 20.8 ± 7.1 months.

Results: 36 eyes/28 patients met the inclusion criteria. At baseline all eyes had presented insufficient response to prior anti-VEGF and/or short-acting steroids. The mean BCVA was 41.5 ± 17.9 ETDRS letters and mean CFT was 557.0 ± 194.5 µm. Following ILUVIEN®, mean BCVA increased by $+11.4 \pm 4.4$ ETDRS letters ($p = 0.005$) and mean CFT decrease by -219.2 µm ($p < 0.001$). IOP increased in 43.5%, in only one case controlled with surgery.

Conclusions: Our results show that both visual and anatomical improvements were achieved with a single ILUVIEN®. These results were sustained and maintained for ≥12 months demonstrating that ILUVIEN® is an effective long-term option when treating chronic DME.

Clinical and Molecular-Genetic Data from the Valencia Study on Diabetic Retinopathy (VSDR) Cohort

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Purpose: To outline type 2 diabetic (T2DM) patients characteristics, risk factors, precise blood-based biochemical parameters and typical diabetic retinopathy (DR) features, to identify novel candidate biomarkers for DR.

Methods: A 38-month follow-up interventional open-label multicenter case-control study (VSDR) was done. Participants were divided as: T2DM ($n = 110$) with (+DR) and without (-DR) retinopathy and 84 healthy controls (CG). Ophthalmologic examination, classic plasmatic parameters, oxidative stress markers and candidate gene expression were assayed.

Results: Significantly lower vitamin B9/B12 availability, antioxidant capacity and SLC23A2 gene expression as well as higher pro-oxidant status in T2DM vs the CG were observed ($p < 0.05$).

Conclusions: We suggest that variations in the SLC23A2 gene as well as precise biochemical parameters can be associated with DR risk in T2DM patients. In addition, an oral supplementation with "antioxidants/w3 fatty acids" could potentially exert additional benefit in diabetics by reducing the oxidative load.

Pterostilbene, a Brake on Development of Diabetic Retinopathy

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Purpose: The aim of this study was to analyze the potential benefits of pterostilbene, a natural polyphenol, in the treatment and/or prevention of diabetic retinopathy (DR) and its possible mechanisms of action.

Methods: We performed *in vitro* assays to evaluate the cytoprotective capability of the stilbene and its effects on vascular endothelial growth factor (VEGF) release using human microvascular endothelial cells and pericytes in culture. To assess the effect of pterostilbene on experimental early DR, three groups of rabbits were studied: Non-diabetic; untreated diabetic animals (alloxan 85 mg/Kg); treated diabetic rabbits with water-soluble pterostilbene (50 mg/kg/day, 6 weeks). Tortuosity of retinal microvessels was evaluated from retinographies by image analyses. Retinal parameters of oxidative stress and antioxidant enzymes activities were assessed by spectrophotometry and apoptosis was detected by TUNEL assay.

Results: Pterostilbene reduces the high glucose cytotoxicity, the release of VEGF and the formation of endothelial cell networks in culture. *In vivo*, protects the retina against early oxidant damages and reduced apoptosis induced by chronic hyperglycemia.

Conclusions: Pterostilbene protects the retina against preclinical and early clinical DR in rabbits with diabetes type 1. The molecular mechanisms underlying the protective effect involves an increase in the retinal antioxidant defenses and reduction of VEGF release.

Portuguese Retinal Practices – PRP Survey

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Purpose: To audit the standards of treatment for retinal conditions is relevant to identify opportunities for improvement and to establish international guidelines on the best management options. We aimed to survey and assess preferred retinal practices (PRP) among Portuguese retinal sub-specialists.

Methods: An online questionnaire containing a total of 72 questions was anonymously answered by Portuguese retinal specialists. These questions were adapted from the PAT survey (American Society of Retina Specialists), and focused on current topics of debate in medical retina. A sub-group and comparative analysis were performed. STATA v.13 was used as statistical package.

Results: A total of 93 responses were obtained. Similarly to the trends observed in the US, most Portuguese physicians (79%) would choose aflibercept to treat a new-onset wet AMD patient in

the hypothetical scenario that all the anti-angiogenic cost the same. Also, when questioned if anti-VEGF injections accelerated macular atrophy progression, about a third of the respondents think so. PRP respondents acknowledged the lack of long-acting agents which would reduce the actual treatment burden.

Conclusions: Both similarities and differences were identified between PRP and PAT survey results. This pioneer study in the European reality provides an overview of the current retinal practices and disclose areas in which clinical guidelines would be more useful.

Microglial Cell Migration and Proliferation in Two Animal Models of Retinal Degeneration

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Purpose: To study the changes in retinal microglial cells in two animal models of retinal degeneration with different aetiologies.

Methods: Homozygous albino P23H-1, pigmented Royal College of Surgeons (RCS), Sprague-Dawley (SD) and pigmented Piebald Virol Glaxo (PVG) rats of different postnatal (P) ages (10, 15, 21, 28, 33, 45 and 60 days) were used. Six retinas per time and strain were cross-sectioned and immunodetected with anti-Iba-1 and anti-PCNA (Proliferating cell nuclear antigen) antibodies to label microglial and proliferating cells and counterstained with DAPI. The numbers of microglial cells were quantified, and the number, location and identity of proliferating cells were investigated.

Results: Microglial cell activation was observed at P15 in P23H-1 and at P21 in RCS rats. In both dystrophic models the numbers of microglial cells increased in the whole retina, decreasing in the inner layers and increasing in the outer layers, but the RCS rat was more inflammatory. Microglial proliferation was also observed in both models, but the initiation and characteristics of the microglial response differed between them.

Conclusions: Retinal degeneration results in microglial cell activation, proliferation and migration in both dystrophic models, but there are differences between them that may be used in the future for therapeutic interventions.

Two Different Outcomes of Punctate Inner Choroidopathy

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Purpose: To report two cases of Punctate inner choroidopathy (PIC) with different endings.

Methods: A retrospective and descriptive case report based on data from clinical records, patient observation and ancillary diagnostic tests.

Results: Case Report 1: A 40-year-old woman presented with unilateral blurred vision in her left eye (LE). In the past, she suf-

ferred two self-limiting unilateral vision loss with complete visual recovery. Best corrected visual acuity (BCVA) was 1.0 in right eye (RE) and hand movement in LE. The fundus aspect of her RE showed white and pigmented spots and LE showed presence of abundant subretinal fibrosis. Treatment with oral corticosteroids and cyclosporine was established improving slightly her LE visual acuity (VA) up to 0.2. Case Report 2: A 52-year-old woman reported at hospital due to metamorphopsia in her LE. BCVA was 0.5 in LE. Fundus examination revealed small, rounded, atrophic-like lesions in the posterior pole. Optical coherence tomography evidenced choroidal neovascular membranes starting with aflibercept intravitreal. There were good anatomical results and stabilization of the initial VA.

Conclusions: PIC remains a challenge despite the emergence of new imaging devices. A high suspicion is still required to arrive at an early diagnosis and a prompt treatment.

Development of an *In-Situ* Crosslinked Hydrogel as Transfection Vector in a Retinal Cell Line

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Purpose: Development of a biodegradable *in-situ* crosslinked hydrogel as a transfection agent for the treatment of retinal cell pathologies.

Methods: The hydrogel was constituted by two different polymers: thiolated hyaluronic acid and hyperbranched poly-amine-ester (HPAEs) (ratio 1:1). The HPAEs, constituted by PEG diacrylate and ethylenediamine, are novel polymers able to transfect cells in a safe manner. The HPAEs was characterized in terms of Mw and branching degree through GPC and molecular structure by NMR. The hydrogel was characterized in terms of degradation rate, tolerance and transfection capacity in a retinal cell line (hTERT-RPE1).

Results: The molecular weight of HPAEs was 31338 with a Mark-Houwink alpha value of 0.17. The NMR data indicated the presence of vinyl groups. The hydrogel took 300 hours until complete degradation (PBS, 37°C). It resulted well tolerated in the hTERT-RPE1 cell line with values close to 100% after exposure times of 24 hours. The hydrogel showed DNA internalization in the hTERT-RPE1 cell line after 96 hours of exposure.

Conclusions: The biodegradable hydrogel presented high tolerance in the retinal cell line of hTERT-RPE. This novel polymer combination can be a good candidate to transfect retinal pigment epithelium cells.

Normative Database for the Macular Ganglion Cell Layer Thickness Measured with the 8x8 Posterior Pole Protocol of the Spectralis SD-OCT

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Purpose: To establish normative thickness values for the macular ganglion cell layer (mGCL) in a healthy Caucasian population using the 8x8 posterior pole protocol (PPP) of the Spectralis SD-OCT.

Methods: One hundred and forty five healthy Caucasian patients aged from 30 to 89 year-old were examined with the 8x8 PPP grid examination of the Spectralis SD-OCT. One eye per patient was randomly selected. mGCL thickness values were obtained for the 64 cells of the standard grid. Patients were divided into three age groups: 30–49 year-old, 50–69 year-old and 70–89 year-old. Mean, standard deviation, 1st, 5th and 95th percentiles were calculated for each cell of the grid and topographic color-coded thickness maps (heatmaps) were obtained.

Results: Mean age of patients was 61.92 ± 12.20 years. Mean mGCL thickness was 31.94 ± 9.48 microns (range 20.28 to 54.17 microns). The paracentral cells showed maximum mGCL thickness while the most peripheral cells showed minimum thickness and the four central cells presented intermediate thickness values.

Conclusion: To the best of our knowledge this is the first normative database for thicknesses of the mGCL layer in healthy adults obtained for 8x8 PPP grid.

Symptomatic Vitreomacular Traction: Resolution Associated with Self-Applied Ocular Massage

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Purpose: To report clinical and OCT features of Vitreomacular traction (VMT) release associated with ocular massage (OM).

Methods: A 70-year-old woman complained about blurred vision and metamorphopsia in her right eye for one month. Her best-corrected visual acuity (BCVA) was 20/50. Macular OCT showed focal VMT in this eye. Moderate intensity, digital OM was performed. However, the traction was still present. The patient was instructed to perform the same OM every 8 hours at home herself.

Results: Four days later she indicated disappearance of metamorphopsia, her BCVA increased to 20/25 and OCT showed VMT release with 39-micron foveal thinning.

Conclusions: As a spontaneous resolution of VMT is highly improbable in few days, we conclude that self-applied OM may be useful for focal VMT release.

Long-Term Visual Acuity Outcomes of Treatment of Age-Related Macular Degeneration

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Purpose: To analyze long-term outcomes of VEGF-inhibitors therapy for treating naïve eyes with neovascular age-related macular degeneration (nv-AMD).

Methods: Database observational study including 48 treatment-naïve eyes with nv-AMD. Visual acuity (VA) was analyzed at 0.5, 1, 3, and 5 years.

Results: Mean VA improved from 61.04 to 65.83 letters after 6 months, being -1.14 , -16.88 and -27.50 letters lower than baseline after 1, 3 and 5 years. Eyes with VA loss lower than 15 letters were 50% (3 years) and 33.3% (5 years). Eyes with VA of 70 letters (20/40) or better were 41.7% (baseline), 22.92% (3 years) and 10.42% (5 years). After the 1st to the 5th year, mean injections received was 4.81, 2.21, 1.73, 1.17 and 0.9 and mean number of visits was 7.19, 4.88, 4.02, 3.40 and 2.85, respectively. Older age, lower VA score and pseudophakic eyes at baseline were associated with lower long-term visual outcomes.

Conclusions: Long-term visual outcomes of the VEGF-inhibitors therapy found in this study are lower than previous reports, may be because of a smaller number of injections were given. Furthermore, follow-up time for all eyes identified was 5 years, although treatment was discontinued because of lack of efficacy.

Comparative Study Between Retinal Ganglion Cell Markers

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Purpose: To compare and assess the specificity and coverage of current retinal ganglion cell (RGC) markers in intact and injured mouse retinas.

Methods: In intact and injured retinas (5 days after optic nerve crush, ONC), nuclear markers (Brn3a and NeuN) and cytoplasmic/axonal markers (β III tubulin, BM88, ER β , NFL, RBPMS and v-Synuclein) were immunoidentified in whole-mounts. As internal control of the total RGC population, the number of positive RGCs for each marker was compared to RGCs retrogradely labelled from the superior colliculi.

Results: In the GC layer all of these proteins, but NeuN, are RGC-specific. NeuN is expressed as well by 15% of displaced amacrine cells. Brn3a, NeuN, RBPMS, and v-Synuclein mark >98% of traced-RGCs, while β III tubulin, BM88, ER β , or NFL only detect less than 50% of them. None of these markers change their expression pattern after ONC.

Conclusions: The nuclear staining of Brn3a and NeuN facilitates RGC quantification, although NeuN is not RGC specific. Cytoplasmic/axonal markers are useful to analyse the anatomical features of RGCs in health or after an injury, but quantitative analyses in the central retina may be inaccurate.

Microspheres Loaded with Norgestrel for the Treatment of Retinitis Pigmentosa. Sterilization Studies

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Purpose: To evaluate the effect of gamma sterilization in PLGA microspheres (MSs) loaded with Norgestrel (NG) for injection in an animal model of retinal degeneration (RD10 mice).

Methods: PLGA-MSs loaded with NG were prepared according to the O/W emulsion-extraction-evaporation solvent (2:10 NG:PLGA). MSs were sterilized by gamma irradiation (25kGy). Size of Norgestrel microspheres was determined by Dual Light Scattering (DLS). Morphological studies were performed by SEM. Encapsulation efficiency (EE) and release of Norgestrel from MSs were determined before and after sterilization. NG was quantified by HPLC-UV ($\lambda = 240$ nm).

Results: The MSs-NG (23.02 ± 0.18 μ m) presented a spherical structure with high EE ($76.4 \pm 10.62\%$). The release rate of NG was characterized by an initial burst ($11.57 \pm 0.68\%$) followed by a sustained delivery (3.26 ± 0.34 μ g NG/mg MSs*Day) during 31 days. At the end of the study almost 100% of NG was released. The release rate of Non-sterilized NG particles was 3.43 ± 0.54 μ g NG/mg MSs*Day.

Conclusions: Norgestrel loaded MSs can deliver the drug in a controlled fashion and their technological properties were not altered by gamma irradiation exposure (25kGy).

Course of Retinal Ganglion Cell Death in Organotypic Mouse Retinal Explants

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Purpose: Organotypic explants are inherently a model of optic nerve transection (ONT). We purpose to assess the temporal course of retinal ganglion cell (RGC) death in organotypic mouse retinal explants and to compare it with the loss of axotomized-RGCs *in vivo* (PMID 21354138; PMID: 26780312).

Methods: Retinas from adult C57Bl/6 mice were fresh dissected and cultured in supplemented neurobasal medium at 37°C/5% CO₂. Retinas were fixed at 24 h intervals up to 8 days (n = 4/day), RGCs immunodetected with mouse anti-Brn3a (Millipore) and quantified by sampling the medial/central retina. As baseline controls fresh dissected retinas were processed immediately for immunodetection.

Results: Compared to baseline retinas, there is an abrupt decrease (30%) of RGCs from day 0 to day 1 *in vitro* (IV). Thereafter,

the death of RGCs is slower. Thus, considering day 1IV 100%, at day 5IV 50% of RGCs are lost, and by day 8IV, 30% of them survive. This course of death follows the trend of RGC loss observed *in vivo*. Finally, tissue integrity is worst *in vitro* than *in vivo*.

Conclusions: Organotypic explants cause a sudden loss of RGCs during the first 24 h. Thereafter, RGC course of death is similar to that observed after ONT *in vivo*.

The Dipeptidyl Peptidase-IV Inhibitor Sitagliptin Slows Down Retinal Neurodegeneration in a Mouse Model of Retinitis Pigmentosa

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Purpose: Retinitis pigmentosa (RP) is a disease characterized by progressive loss of photoreceptors. Sitagliptin, a dipeptidyl peptidase-IV inhibitor (DPP-IV), has demonstrated neuroprotective effects in diabetic rats. The aim of this work was to evaluate the neuroprotective effect of sitagliptin in a mice model of retinitis pigmentosa.

Methods: The rd10 mice were treated orally with sitagliptin (10 mg/kg/day) from P18 to P25. Visual responsiveness was determined by electroretinography (ERG) and optomotor test. Retinal cell morphology and synaptic connectivity were evaluated by hematoxylin staining and immunohistochemistry.

Results: In sitagliptin-treated animals, the reduction of DPP-IV content was confirmed by Western blot. Higher a- and b-waves amplitudes, as well as better visual acuity were found in treated animals. These animals also had a higher number of photoreceptor rows that correlates with functional findings. The photoreceptors of treated animals presented well-defined axon terminals and better-preserved outer and inner segments. Furthermore, presynaptic and postsynaptic elements, as well as synapses between photoreceptors and bipolar or horizontal cells were preserved.

Conclusions: Sitagliptin treatment improves retinal function and preserves photoreceptor number and morphology, as well as their synaptic contacts. The results support sitagliptin as an effective option for RP treatment.

Short-Term High-Fat Diet Accelerates Retinal Degeneration in a Rd10 Mice Model of Retinitis Pigmentosa

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Purpose: High-fat diet induces metabolic syndrome and has been related with diabetic retinopathy. The aim of this study was to investigate acute effects of high-fat feeding on retinal dystrophic diseases.

Methods: Rd10 and C57BL/6 mice 19 days old were fed either a normal chow (5.5% fat kcal) or high-fat diet (HFD, 61.6% kcal) for 2 or 3 weeks. Visual function was assessed by electroretinography and optomotor test. Hematoxylin stains and immunohistochemistry were performed to quantify photoreceptor row number and analyze retinal cell morphology and connectivity.

Results: HFD-fed mice gained more weight and developed reversible glucose intolerance. An increase in the deterioration of retinal function with lower amplitudes in a- and b-waves and worse visual acuity were observed in rd10 mice. HFD-fed dystrophic animals also presented higher loss of photoreceptor rows and shorter outer and inner segments, as well as decreased density of pre-synaptic photoreceptor terminals and retraction of bipolar and horizontal cell dendrites.

Conclusions: Short-term high-fat feeding accelerates pathology progression in retinal degenerative diseases. The results suggest that in patients suffering from ocular neurodegenerative diseases the consumption of high-fat diet could accelerate the progress of the disease.

Degeneration of Retinal Ganglion Cells Following Unilateral Optic Nerve Axotomy Performed at Different Distances from the Optic Disk

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Purpose: Optic nerve axotomy causes the death of retinal ganglion cells (RGCs) in two phases, the first one lasts up to 9 days in mice and 14 days in rats and kills approximately 85% of the RGC population. Thereafter, RGCs die slowly and steadily. In rats, the course of RGC loss is slower the farther from the optic disk the axotomy is inflicted. We aimed to evaluate whether in mice, in which RGC degeneration is faster, the lesion distance had a similar effect.

Methods: The left optic nerve of C57BL6 adult mice was crushed at 0.5 mm or 2 mm from the optic disk. Intact retinas were used as controls. Animals were sacrificed from 3 to 90 days after the lesion, retinas dissected as flat mounts, RGCs immunodetected and automatically quantified.

Results: At all times points the loss of RGC was the same whether the optic nerve was crushed at 0.5 or at 2 mm. At both distances, 50% of RGCs have died at 5 days and 97% at 90 days.

Conclusions: Contrary to rats, in mice the distance at which the axotomy is performed does not influence the course of RGCs loss, at least up to 2 mm from the optic disk.

Taurine Depletion Induces Glial Cell Activation and Photoreceptor Loss

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Purpose: To analyze the course of photoreceptor death and the macro and microglial reactivity caused by taurine depletion.

Methods: Sprague-Dawley albino rats (n = 20) were divided in two groups: the experimental group (n = 10) was treated with β -alanine (3%) in the drinking water to induce taurine depletion, while the control group (n = 10) received regular water. One month later, half of the animals in each group were exposed to white light (3000 lux) continuously for 48 hours. All the animals were processed 2 months after the beginning of the experiment.

Results: Taurine depletion and phototoxicity caused separately photoreceptor degeneration that was characterized by changes in photoreceptor outer segment morphology, a decrease in the outer nuclear layer thickness, microglial cell activation and GFAP over-expression in retinal macroglial cells. The combination of taurine depletion and phototoxicity exacerbated the retinal degeneration.

Conclusions: Both taurine depletion and phototoxicity cause photoreceptor degeneration and increased glial cell reactivity, but their effects are additive. Taurine is thus essential for photoreceptor survival, especially in increased light exposure.

Functional Evaluation of Visual Impairment in an Animal Model of Retinal Excitotoxicity

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Purpose: To characterize the degree of visual impairment in an animal model of retinal excitotoxic by means of pattern Electroretinogram (pERG) and the optomotor test.

Methods: Retinal cell damage was induced by injection of 1 μ l of PBS containing different doses of KA/NMDA into the right eye. Binocular recordings were performed by the pERG technique prior to, immediately after and one week after the induction of excitotoxic retinal damage. Different spatial frequencies were tested. In addition, we performed optomotor test in the same animals by using different spatial frequencies and light contrasts.

Results: The pERG recordings from eyes injected with KA/NMDA showed a significant decrease in amplitudes of the P50 and N95 trace components one week after retinal damage. The pERG showed that at increasing concentrations of KA/NMDA, the amplitude of the P50 and N95 components were diminished, being almost null for highest excitotoxic concentrations. Results from the optomotor test confirmed all data obtained by pERG. At high

concentrations of excitotoxic agents, the visual acuity of the animals showed a significant decrease when the optomotor stripes moved in counterclockwise direction, indicating the damage of the right eye.

Conclusions: The pERG and optomotor test are effective for evaluate induced visual impairment of the retina.

Microglial Reaction after Cone-Photoreceptor Phototoxicity Induced by Focal Light Emitting Diode (LED)

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Purpose: To analyse the microglial reaction after focal light emitting diode (LED)-induced photoreceptor phototoxicity.

Methods: In dark adapted anesthetized adult Sprague-Dawley rats, a blue (400 nm) LED was placed perpendicular to the cornea (10 sec, 200 lux) of the left eye and both retinas were examined 1, 3, 7 or 14 days (n = 6–8) after light exposure (ALE). Retinal whole-mounts were immunoreacted for S-opsin and Iba-1 to identify S-cones and microglia, respectively.

Results: Fellow unexposed retinas showed typical S-cone distribution in the adult rat retina without signs of reactive microglia. LED-exposed retinas showed a focal lesion in the superotemporal quadrant, with loss of S-cones that was clearly delimited at 1d. In the LED-exposed retinas Iba-1⁺ cells resembling reactive microglia were observed in the centre of the lesion within the photoreceptor outer segment layer as early as 1d ALE and persisted until the end of the study. At 1d ALE microglial cells showed a dendritic morphology, by 3d ALE became amoeboid and returned slowly towards a dendritic form by 14d ALE.

Conclusions: Focal LED induced cone photoreceptor phototoxicity results in focal loss of photoreceptors and a microglial reaction in the outer segment photoreceptor layer.

Central Serous Retinopathy Associated with Chronic Inflammatory Disease

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Introduction: Ulcerative colitis is an inflammatory bowel disease (IBD) that can develop ocular manifestations in up to 10% of cases, and usually coexists with other extraocular manifestations. Conjunctivitis, scleritis and episcleritis occur more frequently. Anterior uveitis occurs in around 17% of patients and retinal complications have an incidence of less than 1%.

Case Report: A 47-year-old man who came to refer several episodes of vision loss. Personal history of several episodes of central serous choroidopathy (CSC) since 2007 and ulcerative colitis. Vi-

sual acuity of 0.4 in right eye and 0.3 in left eye (LE). Fundoscopy shows pigmentary alterations of the posterior pole and macular edema. In optical coherence tomography (OCT) we found a neurosensory retina separation and epiretinal membrane in LE. After treating the episode with oral corticosteroids and adding Mesalazine, the patient achieves a complete visual recovery and normality in the OCT, with remission of the episode until now.

Conclusion: Retinal complications and IBD are related in a low percentage of cases, so it is advisable to consider a diagnosis of IBD after a chronic and recurrent CSC. On the other hand, further studies would be required to establish a relationship between the resolution of the CSC and the Mesalazine, since associations have already been found between the resolution of the CSC and the taking of NSAIDs.

Progression in Visual Function and Hepatic Oxidative Stress Markers in an Animal Model of Retinal Degeneration

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Purpose: Increased oxidative damage is observed in retina, aqueous humor, and plasma of Retinitis Pigmentosa (RP) animal models and patients. Hepatic oxidative status may also be affected in RP due to oxidative macromolecules exiting in retina or to alterations in the melanopsin system that negatively alters the antioxidant defense system.

Methods: P23H rats were crossed with pigmented Long Evans rats. We measured hepatic malondialdehyde and 4-hydroxyalkenal concentrations as oxidative stress markers; nitrite level as a total nitrosative damage marker; total antioxidant capacity; and activities of catalase, superoxide dismutase (SOD), and glutathione S-transferase. Retinal visual function was assessed by optomotor and electroretinogram responses.

Results: P23H transgenic rats exhibited diminished visual acuity, contrast sensitivity, and electroretinographic responses corresponding with retinal degeneration. P23H rats at 30 days of age demonstrated only 47% of the hepatic total antioxidant capacity of wild-type animals. Hepatic catalase and SOD activities were also reduced in P23H rats after 120 days, but we detected no difference in glutathione S-transferase activity. P23H rats had increased hepatic oxidative and nitrosative damage markers.

Conclusions: The liver is under increased oxidative stress in P23H rats. Further studies are required, however, to clarify the contribution of systemic oxidative damage to the pathogenesis of RP.

Retinal Microglia Response to a Peripheral Nerve Lesion

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Purpose: To characterize the response of retinal microglia to an experimental peripheral nerve injury.

Methods: In adult C57BL/6J female mice the tibial and common peroneal branches of the left sciatic nerve (SCN) were exposed and crushed for 1 minute (injured), or simply exposed (sham). At 1, 3, 5, 7, 10 or 14 days, injured, sham, or naïve mice were processed, both retinas were prepared as whole-mounts and morphological or quantitative changes in the distribution pattern and appearance of microglial cells identified with Iba-1 antibodies was performed.

Results: Morphological signs compatibles with microglia activation in the innermost retinal layer were evident early after injury and along the time course of study. Quantitatively the number of Iba-1 immunoreactive cells peaked at 3 days post-lesion and decreased thereafter. By 5 days post-lesion typical activated amoeboid microglial cells were found increased. Sham or naïve animals did not show significant morphological features of microglia activation.

Conclusions: Experimental peripheral nerve injury evokes a significant quantitative and qualitative change in the retinal microglia population.

Chaperone Mediated Autophagy as a Cytoprotective Mechanism During Retinal I

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Purpose: Chaperone-Mediated Autophagy (CMA) is a selective autophagic pathway essential for the maintenance of energy homeostasis and cytosolic quality control that is downregulated during ageing in most tissues. Conversely, our previous data shows that CMA becomes up-regulated during retinal aging, suggesting a differential role of CMA in the retina. Currently we are investigating the consequences for retinal homeostasis of manipulating this pathway.

Methods: We are characterizing the retina phenotype in mice deficient for LAMP-2A, a lysosomal membrane protein essential for CMA; and in a mice conditional model overexpressing human LAMP-2A (KI mice) in adulthood. Retinal morphology was assessed by ONL thickness and TUNEL and photoreceptors immunostaining of retinal sections.

Results: LAMP-2A deficient mice display a decrease in the ONL thickness, a shortening in the cones outer segment and increased cell death. On the other hand, LAMP-2A KI mice do not display a major phenotype although we have seen a reduction in the reactive gliosis during ageing.

Conclusions: These data support the role of CMA in the retina as a protective mechanism for photoreceptors. Here we showed how CMA deficiency might affect photoreceptor fitness and how its up-regulation might ameliorate aging related gliosis.

MicroRNA Modulation in Early Stages of Retinal Degeneration in a Mouse Model of Retinitis Pigmentosa

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Purpose: To identify differentially expressed microRNAs (miRNAs) that might play important roles in the etiology of retinal degeneration in a mouse model of retinitis pigmentosa (rd10 mice) at initial stages of the disease, and to analyze the therapeutic potential of the modulation of differentially expressed miRNAs.

Methods: microRNAs-mRNA interaction networks were generated for analysis of biological pathways involved in retinal degeneration. miRNA modulation was achieved via subretinal injections of AAV containing sequence-specific inhibitors. Contralateral eyes were used as controls, which received the same AAV vector solution expressing scramble miRNAs sequences. Evaluation of the effect of miRNA modulation was performed by electroretinography and histological analysis.

Results: Out of more than 1900 miRNAs analyzed, we selected 19 miRNAs based on: 1) a significant differential expression in rd10 retinas compared to control samples, and; 2) an inverse expression relationship with predicted mRNA targets involved in biological pathways relevant to retinal biology and/or degeneration. By inhibiting one miRNA, we were able to achieve a significant preservation of the visual function and the number of photoreceptors.

Conclusions: This study contributes to our understanding of the etiology and progression of retinal degeneration and might help to identify new potential miRNA-based therapeutic approaches.

Pituitary Adenylate Cyclase-Activating Polypeptide and Melanopsin-Expression in Retinal Ganglion Cells of Rat

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Purpose: Here we have examined the topography of the RGC population expressing Pituitary adenylate cyclase-Activating polypeptide (PACAP) and Melanopsin (ML) in naïve adult albino Sprague Dawley (SD) rat retinas.

Methods: Retinas from adult female SD rats were prepared as wholemounts, labelled with antibodies recognizing PACAP and

ML and examined under an epifluorescence microscope. The entire population and its retinal distribution was quantified automatically.

Results: Total numbers of ML⁺RGCs and PACAP⁺RGCs were 2,277±256 and 1,403 ± 136 (mean±SD; n = 5) respectively. Approximately 30% and 50% of the ML⁺RGCs and PACAP⁺RGCs population respectively, co-expressed both proteins. Both populations were distributed as an arciform-like area in the superior retina with the highest densities in the temporal quadrant, where the co-expression of both proteins was greater.

Conclusions: The SD rat shows in the superior retina, above the visual streak, and arciform area that contains the highest densities of ML⁺RGCs and PACAP⁺RGC densities. A large proportion of these populations showed co-expression of both proteins.

Analysis of Visual Function and OCT in Multiple Sclerosis Patients

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Purpose: To review the literature to know how visual function and retinal structure is affected in patients with Multiple Sclerosis (MS).

Methods: Bibliographic search was carried out in different scientific databases (Google Scholar, PubMed, Medline). We have selected articles from the last 10 years.

Results: Visual impairment is a common feature in people with MS and it is the cause of a severe disability in these patients. The high contrast visual acuity (HCVA) is affected during optic neuritis (ON) episode but it is recoverable. However, low contrast visual acuity (LCVA) is reduced similarly in patients with/without ON event. Dyschromatopsias are described in MS patients and are strongly associated with axonal damage. The thickness of retinal nerve fiber layer (RNFL), measured by Optical Coherence Tomography (OCT), is a reliable marker of axonal damage, which also show a positive correlation with Visual Fields (VF).

Conclusion: Although the assessment of visual function is often simplified to the measure of HCVA, other psychophysical tests such as contrast sensitivity test and color vision are more sensitive parameters to analyze the vision of MS patients. Besides, VF and OCT can be used as supplementary tests for the diagnosis and follow-up.

Different Patterns of Retinal Involvement in Patients with Multiple Sclerosis and Neuromyelitis Optica Spectrum Disorder Measured by OCT

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Purpose: To evaluate different patterns of retinal involvement between multiple sclerosis (MS) or neuromyelitis optica spectrum disorder (NMOSD) and to compare with age-matched healthy controls (HC), by optic coherence tomography (OCT) in optic neuritis (ON) and non-optic neuritis (NON) eyes.

Methods: Ninety-six eyes of 48 MS, 18 eyes of 9 NMOSD and 58 eyes of 29 HC patients were included. Evaluation criteria were best-corrected visual acuity (BCVA), macular thickness (MT), ganglion cell complex (GCC) and retinal nerve fiber layer (RNFL). Statistical significant (SS) was considered when $p < 0.05$.

Results: First, ON vs NON, both MS and NMOSD showed SS differences in BCVA, MT, GCC and RNFL. Moreover, ON vs HC, similar affected areas were observed in both diseases but thinner in NMOSD, BCVA was also worse in NMOSD. Furthermore, NON vs HC did not show changes in MT. GCC of MS showed a SS thinning respect to HC which was not observed in NMOSD. RNFL in MS showed changes compared to NMOSD.

Conclusions: In ON eyes BCVA could be a helpful measure to distinguish between MS and NMOSD. The different patterns of affection are better seen in the contralateral eye. The greatest differences were shown in GCC and RNFL.

OCT Changes in the Progression of Alzheimer's Disease

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Purpose: Alzheimer's Disease (AD) is characterized for the β -amyloid and tau accumulation in central nervous system, even in visual brain areas and in the retina. The aim of this study was to investigate the use of optical coherence tomography (OCT) as a tool for the AD diagnosis and follow-up.

Methods: 32 mild AD patients, 20 moderate AD and 35 age-matched controls were analyzed. All subjects underwent macular and peripapillary OCT.

Results: Macular OCT thickness in mild AD patients presents a significant ($p < 0.05$) thinning in the fovea and in the inner macular ring (with the exception on nasal area) with respect to both control and moderate AD patients. In outer macular ring, there was a significant ($p < 0.05$) thickening in the superior, nasal and temporal area in moderate AD patients with respect to mild AD patients. The peripapillary thickness in moderate AD patients was significantly ($p < 0.05$) thinner in the inferior quadrant with respect to moderate AD and control group.

Conclusions: In the macula, mild AD had a thinning but when the disease progresses we found a significant thickening that can be explained by an active gliotic process. On the other hand, changes in the peripapillary region are only evident when the disease progressed.

Retinal Vascular Parameters on Alzheimer's Early Diagnosis and the Promising OCT Angiography

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Purpose: Alzheimer's disease (AD) is the most common cause of dementia and a major public health and dependence issue. It is generally preceded by mild cognitive impairment (MCI). We are now aware that the vascular and neurodegenerative alterations that characterize AD are initiated long before the symptoms' onset. Here we review the changes in retinal vasculature that precede not only AD, but also MCI, by using Optic Coherence Computerized Tomography Angiography (OCTA).

Methods: the search engines used were PubMed and Google Scholar. Three search terms were used: Optical Coherence Tomography and Alzheimer's Disease, Retina and Dementia, Neurovascular coupling in Alzheimer's disease. A total of 532 results were obtained, selecting 32 relevant papers about AD's diagnosis by OCT.

Results: Multiple vascular retinal parameters vary among AD, MCI and healthy controls, such as vascular diameter, venous flow, flow rate, fractal dimensions, choroidal thickness, diameter of the avascular area and the retinal superficial plexus blood vessel densities, among others.

Conclusions: Advances in retinal imaging (OCTA) bring us closer to finding the ideal biomarker. In order to do so, much more research is yet to be conducted regarding these new techniques, and the alterations that have been found.

Retinal Ganglion Cell Degeneration and an Optic Nerve Remodelation in Patients with Parkinson Disease

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Purpose: Parkinson disease (PD) patients suffer visual impairment as one of the first signs of the pathology. The aim of this study was to determine if there are structural changes in the optic nerve (ON) and the ganglion cell layer (GCL) in PD.

Methods: Control and PD human eyes were used. A morphometric analysis of optic nerve cryostat sections stained with hematoxylin was performed. Morphology and distribution of Iba1⁺ cells

in ON and retina were analyzed by immunohistochemistry. In whole-mount retinas, ganglion cell density was evaluated using antibodies against RBPMS.

Results: In PD optic nerves, the number of the smallest bundles and the total area increased. The number of ganglion cells decreased in PD retinas compared with controls. In optic nerve, Iba1⁺ cell density was maintained but their typical ramified microglia morphology changed to an amoeboid morphology. Also, they modified their location: in controls, they were only disposed around the bundles, but in PD they infiltrated and occupied all the bundle area.

Conclusions: In PD, there is a ganglion cell degeneration and an ON remodeling event. This correlates with the GCL thinning described in OCT and could explain visual dysfunctions found in this disease.

Optometric Exam in Autism Spectrum Disorder People

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Purpose: Autism spectrum disorder (ASD) is a neurological and developmental disorder that begins in childhood and lasts during all of a person's life. Social relationships, communication and behavior are the most important areas affected. People with autism present visual problems that must be treated to avoid more difficulties in their daily life.

Methods: Case history is necessary before a visual examination to know the characteristic of the subject and to prepare the convenient procedures. It will help to obtain more cooperation during the exam. Visual acuity should be assessed with an appropriate test (letters, numbers, symbols or preferential looking) depending on the skills of the patient. Refractive errors, accommodation and binocularity must be measured too. Ocular health is necessary to detect any pathology.

Results: Suitable procedures will contribute to obtain reliable answers. Adequate treatment should be adapted to the needs of each patient and to the possibility of compliance.

Conclusions: People with autism due to their features have more difficulties to follow the ordinary protocols in a visual evaluation. That is why professionals must modified the procedures individually to diagnose and to treat visual anomalies in people with autism. Good visual function will give them a better quality of life.

Poster Presentations

Parinaud's Syndrome: How to Get an Early Diagnosis Through the Pupil

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New Retinal OCT Outer Bands Interpretation using Immunohistochemistry

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Diagnosis of an Atrial Myxoma After an Obstruction of the Central Artery of the Retina

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Panuveitis, Hypermature Cataract and Retinal Detachment as Presentation for Juvenile Idiopathic Arthritis

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Tobacco Smokers and Ex-Smokers Show Reduced Peripapillary Retinal Nerve Layer Thickness

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Autophagy Markers in the Retina of a Retinitis Pigmentosa Animal Model

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Red Eye: Differential Diagnosis in Pharmacy Office with and without Optometry Section

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Association Pilot Study of Lipid Metabolism Polymorphisms with Age-Related Macular Degeneration in a Northern Spanish Population

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The Effects of Thioredoxin in an Animal Model of Retinal Degeneration

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Macular Layer Segmentation with Optical Coherence Tomography in Glaucoma

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Quality of Angio OCT Images Related to Acquisition Factors

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Martínez de Aragón Calvo Severe Ocular Affection in an Infant with Langerhans Cell Histiocytosis (LCH): A Clinical Case

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Ocular Surface Temperature in Patients with Evaporative and Aqueous-Deficient Dry Eye Relative to Normals

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Brain Volume as a Biomarker for Visoperceptive Damage in Premature Children

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Retinopathy of Prematurity Incidence and Associated Factors in a Tertiary Hospital

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Comparison of the Ocular Surface Temperature and Evaporation Among Different Lipid Layer Patterns

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Exploring Cellular Interactions Mediating Retinal Degeneration in Retinitis Pigmentosa

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Pars Planitis in Childhood: Epiretinal Membrane as the First Clinical Sign

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Natural Strategies to Increase Mitochondrial Function by Oral Supplementation of R-Lipoic Acid

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New Markers for Glaucoma Progression in Smokers

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Management of Bilateral Retinal Detachment After Laser Photocoagulation in Retinopathy of Prematurity. Case Report

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