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SIREV Congress Abstracts

Murcia (Spain), June 24-26, 2021

Our Society for Research in Retina and Visual Sciences (SIREV) is mainly composed of professionals with a common research interest in ophthalmology and visual sciences. During the next days, June 24-26, we celebrate our VII congress, which was cancelled last year due to the Sars-CoVid pandemic. This year, the meeting has been organized on line, but for those that may be able to come to Murcia, we have the auditorium of the "Hemiciclo de Letras" at the "Campus de la Merced" in Murcia to follow and interact with the meeting.

The congress has been organized with the cooperation of the board of SIREV together with all members of the Spanish Cooperative Networks of Research in Ophthalmology; OFTARED and RETIBRAIN. The main objective of this meeting is to foster participation of every research group within these cooperative networks as well as with other research groups interested in vision sciences and with all members of SIREV.

As in previous editions, the meeting has a number of plenary lectures and special interest symposiums with participation of well known local and international scientists. In addition, we have a large number of free communications sessions devoted to presentation of basic or clinical research work by young scientists (Master or PhD students, post-Doctoral fellows or Ophthalmology residents in training). The abstracts of the accepted free communications will be published in this special issue of Ophthalmic Research that will be available online to all congress participants, at the beginning of the meeting.

Several panels of experts have evaluated and ranked the PhD Thesis, the Master Thesis and the free oral basic or clinical research communications, and corresponding Prizes will be awarded at the end of the meeting to recognize the effort of our young scientists.

We would like to thank all the committee members for their magnificent work, as well as the collaboration of SIREV, OFTARED and RETIBRAIN in the organization and elaboration of the program. Our thanks to the institutions that have sponsored the meeting: Fundación Séneca (Agencia de Ciencia y Tecnología de la Región de Murcia), Universidad de Murcia Campus Mare Nostrum de Excelencia Internacional, Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca (IMIB). Special thanks to the Sponsors of the Prizes of the meeting: PhD Thesis Prizes Santiago Grisolía sponsored by Clínica Rahal and SIREV; Master Thesis Prizes MD Pinazo-Durán sponsored by Dr. MD Pinazo-Durán and SIREV; Clinical and Basic Research Free Communication Prizes sponsored by the Instituto

Murciano de Investigación Biosanitaria-Virgen de la Arrixaca. We would also like to thank our outstanding collaborators: Medicalmix, Alcon, Brudylab, Théa, Esteve, Zeiss, Visufarma, Rayner, Zeiss, Angelini, Johnson & Johnson Surgical Vision, and Horus Pharma Iberica, for their great support.

Thank you and Welcome to the SIREV 2021 meeting.

Manuel Vidal-Sanz *President of the Organizing Committee for the 2021 SIREV Congress*
Jose Manuel Ramírez-Sebastián *President of SIREV*

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Oral Presentations

1

Intravitreal Administration of Adalimumab Delays Retinal Degeneration in *rd10* Mice

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Purpose: Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies characterized by the progressive and irreversible loss of vision. We previously found that intraperitoneal administration of Adalimumab, a monoclonal anti-TNFα antibody, slowed down retinal degeneration in the murine model of RP, the *rd10* mice. **Methods:** We analyze the effect of a single intravitreal injection of Adalimumab on retinal degeneration in rd10 mice at postnatal day (P) 23. We treat control and *rd10* eyes with different dose of Adalimumab in order to select the best dose to achieve the best protective effect. We analyze the effect of the selected dose of Adalimumab on retinal degeneration and inflammation by histological and biochemical techniques. We also study the involvement of different cell death mechanisms by enzymatic and histological techniques. **Results:** Our studies suggest that the photoreceptor cell death is mediated by the activation of PARP and NLRP3 inflammasome, and, to a lesser extent, by caspase dependent mechanisms at postnatal day 23 in rd10 mice. Treatment with intravitreal Adalimumab reduces retinal degeneration, decreasing the activation of PARP, microglia and NLRP3 inflammasome. **Conclusions:** Adalimumab prevented from retinal degeneration without affecting caspase -dependent mechanisms but decreasing PARP activation, microglia activation as well as NLRP3 inflammasome.

2

Neurodegeneration and Neuroinflammation as Key Points in the Retina of a Mouse Model of ALS

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Purpose: To establish the changes in retinal ganglion cells (RGC) and microglia in a SOD1G93A (SOD1) mouse model of amyotrophic lateral sclerosis (ALS) compared to wild type (WT). **Methods:** In retinal whole mounts of 120-day-old animals, the number of retinal ganglion cells (RGC) (with anti-Brn3a+), and signs of microglial activation (with anti-Iba-1) in the different retinal layers were quantified, including: (i) number of Iba-1+ cells in the outer segments (OS), outer plexiform layer (OPL) and inner complex layer (ICL); (ii) area occupied by Iba-1+ cells in the OPL and IPL; and (iii) arbor area of Iba-1+ cells in the OPL and IPL. The expression of anti-IFN-γ and anti-IL-1β (M1 phenotype markers) and anti-arginase-I and anti-IL-10 (M2 phenotype markers) was also studied. **Results:** The retinas of SOD1 mice compared to WT mice showed i) Migrations and reorientation processes of some Iba-1+ cells; ii) a significant increment in the area occupied by each microglial cell in the total retinal area; ii) a significant increment in the area of arborization in the inferior sector of the OPL; iii) presence of cells with retracted processes; iv) areas of cell clustering in some sectors; v) no significant increase in the number of microglial cells; vi) expression of IFN-γ and IL-1β; vii) non-expression of IL-10 and arginase-I; v) a decrease in the number of RGCs. **Conclusions:** In an advanced stage of the disease in an ALS model with SOD1, retinal microglial activation takes place, resulting in a proinflammatory M1 phenotype, which could be related to the loss of RGCs. Although ALS is a disease of motor neurons, it can also affect retinal tissue, where an inflammatory process and death of retinal neurons occurs.

3

Long-Term Study of the Outer Retina after Optic Nerve Transection

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Purpose: To examine the long-term functional and morphological effects of intraorbital optic nerve transection (IONT) on outer retinal layers. **Methods:** In adult albino rats, IONT was performed on the left eyes while the right-fellow eyes served as controls. Functional *in vivo* longitudinal studies included full field electroretinogram (ERG) recordings (n=8) at 90, 150, 300 and 365 days (d), of the pSTR-, a- and b- waves, and SD-OCT measurements of the inner (from nerve fiber to inner nuclear layer), outer (from outer plexiform to outer segment layer) and total retinal thickness at 15, 30, 60, 150, 300 and 365 d. *Ex vivo*, frozen cross sections were analyzed at 90, 150 or 365 d (n=8 per group) for retinal thickness and numbers of L-, S-cone or horizontal cells identified with antibodies anti L or S-opsins or calbindin and manually counted in microphotographs of four different areas (central and periphery areas from dorsal and ventral retina). **Results:** Longitudinal *in vivo* studies showed a diminution of the pSTR wave ≈80% (90-150 d), a reduction of the a-wave ≈25% at 90 d that further reduced ≈75% at 300d, and a reduction of the b-wave ≈35% from 90-150d that further reduced ≈60% at 300d. SD-OCT measurements showed a ≈20% reduction of inner retinal thickness that remained unchanged, while outer retinal thickness showed a reduction of ≈42% from 150 to 300d. *Ex vivo*, at 150d, the total retinal thickness, S- and L-cones were comparable to control while at 365d: i) total retinal thickness diminished ≈38%, mainly due to ≈56% thinning of the outer retina; ii) S- and L-cones diminished (63%) compared to their fellow retinas, and; iii) horizontal cells remained stable. **Conclusions:** IONT induces protracted alterations of outer retinal function that preceded severe thinning of outer retina and loss of cones.

4

Effect of Peripheral Nerve Grafting on the Surviving of Brn3a and Melanopsin Retinal Ganglion Cells

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Purpose: To study the effects of intraorbital optic nerve transection (IONT) alone or with a segment of peripheral nerve (PNG) grafted to the ocular stump on the survival of: i) melanopsin(m) expressing retinal ganglion cells (m^+RGCs), and; ii) Brn3a expressing RGCs ($Brn3a^+$RGCs). **Methods:** In adult Sprague-Dawley (SD) rats, the left ON was IONT or grafted with a PNG (IONT+PNG), while the right eyes served a control. At 4 weeks (w), retinas were dissected, whole-mounted, immunolabeled for Brn3a and m, examined and photographed under epifluorescence microscopy. The entire $Brn3a^+$RGC or m^+RGC population was automatically or manually dotted, respectively, and counted. Their retinal spatial distribution was examined with isodensity or neighbor maps constructed with a computer routine developed in the laboratory. **Results:** The total number of the $Brn3a^+$RGC and m^+RGC population in control retinas of both experimental groups (IONT or IONT+PNG) was (90,659±5,131, n=12; or 2,368±173, n=6, respectively) (mean±SD). At 4w, in the IONT group, the number of $Brn3a^+$RGCs and m^+RGCs were 2,570±718 and 665±147, respectively, representing a 3% and 28% survival of their fellow retinas. In the IONT+PNG group the numbers of $Brn3a^+$RGCs or m^+RGC populations were 9,159±2,445 or 627±157, respectively, and represent a 10% or 24% survival of their fellow retinas. While the surviving $Brn3a^+$RGC population was significantly greater in the IONT+PNG group compared to the IONT group ($p<0.001$; Mann-Whitney Test), there were no differences in total m^+RGCs surviving in both experimental groups. Both populations of surviving RGCs ($Brn3a^+$RGCs and m^+RGCs) showed a diffuse distribution within the retina. **Conclusions:** IONT results at 4w in severe $Brn3a^+$RGCs loss but in a significantly more resilient survival of m^+RGCs. IONT+PNG protects $Brn3a^+$RGCs but not m^+RGCs against ON injury. Overall, this suggests a different response in these two RGC populations against ON-injury and neuroprotection.

5

Risk factors associated with retinal hemorrhages in SARS-CoV-2 patients.

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Purpose: Describe risk factors associated retinal hemorrhages in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) active infection. **Methods:** Observational case-control study. Sixty-two patients, with SARS-CoV-2 active infection, presenting moderate and severe illness, with systemic risk factors for respiratory and cardiovascular disease and 35 controls with SARS-CoV-2 active infection, with similar characteristics without these risk factors. Inclusion criteria were patients older than 18 years old, with SARS-CoV-2 confirmed by RNA detection through reverse transcriptase polymerase chain reaction (RT-PCR), with moderate and severe respiratory symptoms and obesity (BMI > 30 Kg/mts2), arrhythmia and dyslipidemia. The Institutional review board of the hospital approved this study and was performed under the international principles of the declaration of Helsinki. The image acquisition method was smartphone and 28 diopters fundus image and a review of the medical records of all selected patients. **Results:** 124 eyes of 62 patients, 35 controls without obesity, arrhythmia or dyslipidemia and 27 patients with BMI > 30 Kg/mts2, of which 4 patients, 7 eyes in total, presented superficial and deep hemorrhages and VonRoth´s Spots. Comparisons between groups were made using Fisher's exact analysis (95% CI,p= 0.0039) probing statistical significance. None of the patients with BMI < 30 Kg/mts2 presented retinal findings. There was a relation between obesity and other comorbidities like arrhythmia (p= 0.0063) and dyslipidemia (p=0.0051). **Conclusions:** Patients with SARS-CoV-2 who are obese, has arrhythmia or dyslipidemia have an increased risk of developing severe disease; we demonstrate a relation between obesity, arrhythmia, dyslipidemia and the presence of retinal hemorrhages. We must always consider that most of these patients have comorbidities and is possible that the retinal findings are developing, due to the influence of SARS-CoV-2 and the coexistence of diverse hemodynamic factors over the eye.

6

Early Changes in Choriocapillaris Flow Voids as an Efficacy Biomarker of Photodynamic Therapy in Central Serous Chorioretinopathy.

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Purpose: To assess the early changes produced in the choriocapillaris (CC) and choroidal (CH) vasculature using swept-source optical coherence tomography angiography (SS-OCTA) in patients with chronic central serous chorioretinopathy (CSCR) as predictors of the efficacy after photodynamic therapy (PDT). **Methods:** Prospective observational study. A cohort of 52 eyes of 52 patients with chronic CSCR and persistent subretinal fluid (SRF) was included. SS-OCTA scans of the 6x6mm macular region were assessed before; and 2-3 days, 1 month and 3 months after half-fluence PDT. Best-corrected visual acuity (BCVA), height of SRF and vessel occlusion as flow signal voids (FSV) in the CC and CH was measured. Main outcome measures were early increase in FSV in the CC and CH after PDT, and the recanalization after treatment. **Results:** BCVA before PDT was 75.3 ±12.0 letters in the ETDRS scale, which improved to 81.3 ±11.0 after 3 months ($p<0.001$). A 3.67 ±4.12 and 2.76 ±3.63 fold increase in CC and CH FV, due to vessel occlusion, was observed at 2-3 days after PDT versus baseline. There was less SRF at 3 months in patients with an increase in FSV (≥1-fold) compared to those without this increase (<1-fold) after PDT ($p\leq0.003$). An association between the increase in CC and CH FSV at the early control (2-3 days) and the amount of SRF at 1 month was found ($R= -0.405$; $p=0.002$ and $R= -0.356$; $p=0.008$ respectively). At 3 months, recanalization was achieved in the CH versus the baseline ($p=0.619$), but there was a persistent increase in the CC FSV ($p=0.008$). **Conclusions:** Early vessel occlusion by OCTA after PDT in CSCR was associated with excellent treatment response. Therefore, an increase in FSV immediately after PDT could be a robust biomarker to predict SRF resorption.

7

Comparative Analysis of Reconstruction Geometric Methods of Corneal Surfaces from Scheimpflug Tomography Elevation Data

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Purpose: Comparative study of the adjustment capacity of modal mathematical models to represent corneal surfaces with variable complexity. **Methods:** 30 topographies of 30 patients (5 healthy/ 25 keratoconus) obtained from the Sirius tomographer were used. Keratoconus were classified into G-I (6), G-II (6), G-III (6) and G-IV (6) respectively according to the Amsler-Krumeich. We compared the reconstruction models of the surfaces based on the base geometries type biconic, toric, spherical, elliptic and polynomial, from their residues that represent the global irregularities of each topography with respect to each model selected type. The quality of the adjustments for each model was determined by the sum of the squares of the residuals (SSE). **Results:** The polynomial models had a better fit in healthy corneas (SSE= 0.00345), the other models had worse adjustment values but all of them were very alienated (SSE= 0.333±0.086). In diseased corneas, the polynomial model presented the best fit among all grades G-I (SSE= 0.00746), G-II (SSE=0.143), G-III (SSE=0.195), G-IV (SSE=0.196), with the spherical model presenting the worst fit in grades G-I (SSE=29.26), G-II (SSE= 25.07) and G-III (SSE=39.70), and the elliptical model in grade G-IV (SSE=49.5). Regarding the residue of the polynomial model of healthy corneas, the residue grew by 21.6% in the G-I, 44.44% in the G-II, 56.52% in the G-III and 56.81% in the G-IV. **Conclusions:** Polynomial models are a reliable and robust method of surface reconstruction, both in a healthy and pathological scenario, due to the number of parameters that define it, so they are able to capture the deformations of the corneal surfaces. In addition, the residues obtained for each geometric model give an order of magnitude of the deviation of each topography from the healthy model based on the degree of severity of the disease.

8

Study of Morphogeometric and Biomechanical Variables to Improve the Diagnosis in Corneal Ectasia

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Purpose: To characterize the cornea in a morphological and biomechanical way, determining the correlation of biomechanical parameters of the Ocular Response Analyzer (ORA) in keratoconus (KC), using a 3D model. **Methods:** A retrospective case series study was carried out, including 129 subjects divided into two groups: 51 patients classified as KC according to Amsler-Krumeich and 78 healthy. Each cornea was reconstructed from raw data obtained with tomographer, and afterwards an analysis of the 3D model was made. Corneal hysteresis (CR) and corneal resistance factor (CRF) provided by the ORA were also used. Correlation coefficients were calculated to assess relationships among all parameters. **Results:** Regarding the morphogeometric variables, statistically significant differences among KC subgroups were found for most of morphogeometric parameters ($p \leq 0.05$). Only $A_{apexant}$ ($p=0.051$) and PAD ($p=0.079$) did not differ significantly between severity KC subgroups. Regarding the biomechanical parameters, significant differences were found between severe KC subgroups in CH and CRF ($p<0.001$). More specifically, significant differences were found in CRF for the comparisons between grade I-III ($p=0.011$) and grade I-IV ($p<0.001$). Concerning CH, only significant differences were found between grades I and IV ($p<0.001$). On the order hand, a statistically significant correlation of CH ($r \geq 0.59$, $p<0.001$) with volumetric data was found. CH was also found to be significantly correlated with CV ($r=0.48$, $p<0.001$), A_{ant} ($r=-0.387$, $p<0.001$) and A_{post} ($r=-0.36$, $p<0.001$). Similarly, CRF was significantly correlated with the same parameters: CV ($r=0.44$, $p<0.001$), Aant ($r=-0.46$, $p<0.001$), A_{post} ($r=-0.47$, $p<0.001$). **Conclusions:** Significant correlations found among the majority of parameters evaluated confirm the importance of the morphological profile of the cornea in the measurement of corneal biomechanics, specifically the relevance of the asymmetric profile of the cornea during the disease. This would explain the limitation of ORA parameters as diagnostic tools for the detection of KC in incipient cases.

9

Oculocutaneous Albinism and Keratoconus. A Rare and Challenging Association

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Purpose: Oculocutaneous albinism (OCA) underlies a disorder of melanin synthesis that manifests itself as congenital hypopigmentation of ocular/cutaneous tissues. The range of manifestations include congenital nystagmus, iris hypopigmentation and translucency, reduced pigmentation of the retinal pigment epithelium, foveal hypoplasia, reduced visual acuity and refractive errors, according to the OCA1 (tyrosine-negative: complete lack of melanin synthesis) and OCA2 (tyrosine-positive) clinical types, being the latter the most frequently observed. We present two siblings (13 and 22 years of age) who developed early keratoconus (KC) associated with OCA1. Data regarding OCA and its association with keratoconus is still limited, and any new cases are important. **Case Report**: Two patients aged 13 and 22 years, with diagnosed cases of OCA1, presented to our ophthalmology department with a reduction in their visual acuity over the last 2 years. On physical examination, both patients were found to have a best corrected visual acuity of 20/40. Light skin, blond hair, poliosis, heterochromia iridum, and horizontal nystagmus were present. Slit lamp examination showed conical protrusion of the cornea, with thinning of the central stroma, iris translucency, foveal hypoplasia with absence of the foveal pit, and generalized lack of pigment. A corneal topography was performed, revealing features compatible with corneal ectasia. Initial treatment consisted of intracorneal ring segment implantation, which were later extracted due to a total inversion of the astigmatism. Further surgical interventions were deemed unsuitable in view of possible complications. Conservative management was continued until the present day. **Conclusions:** Steep cornea-keratoconus should be ruled out in OCA patients complaining of visual impairment. An extensive genetic study is needed to provide an early diagnosis and treatment of OCA patients at high risk of serious scarring of the cornea and vision loss.

10

Angio-OCT in Idiopathic Macular Telangiectasia Type 1

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Purpose: To describe a series of diagnosed cases of idiopathic macular telangiectasia type 1 (MacTel type 1) with multimodal imaging. The description of microvascular abnormalities using optical coherence tomography angiography (OCTA) is highlighted**. Methods:** Six patients were included: six eyes with MacTel type1 and their respective healthy eyes as controls. Retinography, Optical Coherence Tomography (OCT), Fluorescein Angiography (FA), and Optical Coherence Tomography Angiography (OCTA) were used in all patients. Focal microvascular dilations were identified with FA and OCTA 3x3mm images using commercial platforms: Angiovue (Optovue) and Angioplex 5000 (Zeiss) **Results:** Six patients were diagnosed, 3 men and 3 women, with an average age of 59.1 (14 to 72 years), 2 patients had controlled hypertension, the rest had no important antecedents. Five patients presented with decreased vision in the affected eye, all of them presented cystoid macular edema (CME). Telangectasias with FA and OCTA were identified in all patients. In 5 of the 6 cases, the microvascular abnormalities were located in the deep capillary plexus (DCP) and in 1 patient exclusively in the superficial capillary plexus (SCP). Capillary plexus rarefaction and abnormal microvascular morphology were better identified with OCTA than with FA. **Conclusions:** OCTA is a non-invasive imaging modality that identified abnormalities in the capillary network in greater detail than FA. Microvascular alterations were observed exclusively in the deep capillary plexus in most of the patients.

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Discarded Corneal Endothelial Tissues: A Source of Viable Endothelial Cells Usable for other Therapies

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Purpose: Our local tissue bank is discarding between 40-60 corneas per year. Moreover, endothelial rings are also discarded after their use in different corneal keratoplasties. The purpose of this study is to demonstrate that these discarded tissues are a source of viable human corneal endothelial cells (hCECs) that could be used in Cellular Therapy or Tissue Engineering techniques. **Methods:** hCECs were obtained from 3 types of tissues (n=4): 1- Tissues discarded for their use in a DMEK surgery due to the poor quality of the endothelium ``No DMEK´´ 2- tissues discarded due to not comply transplantation criteria, break during endothelium preparation or failure to find a recipient ``No Transplantation´´ and 3- corneal endothelial rings. Furthermore, 4 native endothelia were used for the studies. Briefly, Descemet´s membrane was dissected and maintained for 2-7 days at 37°C in culture medium. Corneal endothelium was digested with trypsin/EDTA 0.25% for 2 hours at 37 °C. After that, the loosened cells were centrifuged and seeded on a 1 cm^2 culture plate or on an insert transwell®, treated with FNC coating mix®. Cellular growth was assessed by phase-contrast microscopy and confluent cultures of hCECs were used in immunocytochemistry and qPCR studies for Na^+/K^+ ATPase, zonula occludens-1 (ZO-1), Vimentin and Connexin-43 markers. In addition, Transendothelial Electrical Resistance (TER) was also measured and compared. **Results:** hCECs obtained from all discarded tissues were able to attach and proliferate until confluence showing positive staining for the markers. qPCR results showed differences between hCECs cultures and the native corneal endothelium in vimentin and ZO-1 expression. No differences were found in the expression of the markers between the different hCECs cultures. Finally, TER values of hCECs cultures were similar to the native corneal endothelium (30 Ωcm^2), indicating a preserved endothelial barrier function. **Conclusions:** Discarded corneal endothelial tissues provide a source of hCECs for its use in Cell Therapy or Tissue Engineering.

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CRB2 is Involved in the Apicobasal Polarization of RPE Cells by Participating in Tight Junction Maintenance and Cell Cycle Arrest

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Purpose: Apicobasal polarity is essential for the precise performance of epithelial cell functions. It is established thanks to the asymmetric distribution of Par, Crumbs and Scribble polarity complexes. Par and Crumbs constitute the apical polarity machinery, while the Scribble complex operates on the lateral membrane. The Crumbs complex is typically composed of PALS1, PATJ and CRB proteins. To better understand the highly regulated process of polarization, we have studied the expression and role of one of the CRB polarity proteins, CRB2, in retinal pigment epithelium cells (RPE) during differentiation *in vitro* and in mature murine RPE cells *in vivo*. **Methods:** Sequential expression and localization of proteins related to junctional complexes (β catenin, Claudin-19, Occludin) and proteins involved in polarity (PAR3, PALS1) was analyzed during differentiation of human RPE cells (hRPE) and in cultured RPE cells knocked down for CRB2 (shCRB2#1-#2). In CRB2 knockdown cells, cell density and proliferation rates were analyzed; transepithelial electrical resistance (TER) was measured and severe disruption of junctional complexes was performed with acute Ca^{2+} depletion (calcium switch assay). Finally, the role of CRB2 in adult mouse RPE cells was analyzed *in vivo* by knocking down CRB2 with subretinal injections of lentiviral particles containing a shRNA directed against mouse CRB2 mRNA. **Results**: CRB2 is the last of the whole set of polarity proteins to be located in the cell membrane. The absence of CRB2 protein leads to a delay in the formation of cell-cell junctions and an increase in cell proliferation in hRPE. CRB2 knockdown in RPE cells *in vivo* affects the distribution of different apical polarity proteins and disrupts the retinal homeostasis manifested by the invasion of activated microglial cells into the subretinal space. **Conclusions:** CRB2 is a key protein for the development and maintenance of a polarized epithelium.

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Role of *Lrp2b* in Eye Enlargement of Lrp2a Knock-out Zebrafish

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Purpose: Loss of function (LoF) mutation c.69T>A of *lrp2a* zebrafish gene results in characteristic adult-onset enlarged eyes with variable expressivity. L*rp2a* presents an ortholog (*lrp2b)* in zebrafish, and currently *lrp2b* participation in the described phenotype is unknown. The main objective of this study was to evaluate the possible role of *lrp2b* on the molecular mechanisms underlying eye enlargement associated to lrp2a LoF. **Methods:** *Lrp2b* mRNA levels were evaluated by RT-qPCR in zebrafish larvae and in isolated eyes from adult *lrp2a* KO animals. Genomic edition mediated by CRISPR/Cas9 was employed to obtain a double *lrp2a/lrp2b* KO zebrafish. *Lrp2a/b* single and double KO adult fishes (>1 year) were anesthesized and morphologically characterized under a stereomicroscope. Histological defects in ocular tissues were analyzed by Hematoxylin/Eosin staining of eye sections. **Results:** Sanger sequencing revealed the introduction of the mutation c.2424_2435del in the *lrp2b* gene. RT-qPCR analysis confirmed a remarkable reduction of either *lrp2a* or *lrp2b* mRNA levels in the corresponding adult KO zebrafish. Interestingly, *lrp2b* mRNA showed a 23-fold increment in enlarged eyes of *lrp2a* KO adult fishes, but no significative differences were observed neither in contralateral unaffected eyes nor in larval stages. Morphological analysis of single and double KO lines for *lrp2a* and *lrp2b* revealed that eye enlargement was observed in *lrp2a*$^{-/-}$ mutants (8% of fishes) and was completely absent both in *lrp2b* and double *lrp2a/b* KOs. Histological analysis showed phenotypical amelioration of retinal defects observed in *lrp2a* KOs by simultaneous *lrp2b* LoF. **Conclusions:** We hypothesize that *lrp2b* overexpression might underlie eye enlargement in adult *lrp2a* KO zebrafish, which could be a mechanism to compensate *lrp2a* LoF.

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Role of Imaging Tests in the Clinical Follow-up of Patients Treated with Low-Fluence Photodynamic Therapy in Patients with Chronic Central Serous Choroidopathy

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Purpose: To characterize the response to low-fluence photodynamic therapy (PDT) in patients with chronic central serous choroidopathy (CSC) in functional (visual acuity; VA) and anatomical terms using swept-source optical coherence tomography (SS-OCT) and OCT angiography (OCT-A). **Methods:** Retrospective study of 42 eyes with chronic CSC more than 8±1.2 months treated with low-fluence PDT and assessed at 1, 3, 6, 12 and 36 months after treatment by OCT and OCT-A. Patients with previous ocular pathologies, refractive errors greater than 3 dioptres or who had received previous intraocular treatment were excluded. **Results:** Forty-two eyes with chronic CSC belonging to 38 patients (77.7% male and 22.3% female) with a mean age of 49.5 ± 8.0 years and a mean VA according to the decimal scale of 0.64 ± 0.22 were studied. Mean VA improved to 0.85 ± 1.2 after 1 month of treatment and remained stable until the end of the follow-up period ($p<0.05$). Foveal retinal thickness was significantly lower after 1 month of PDT treatment and remained stable until 36 months ($p<0.05$). Foveal choroidal thickness was reduced after 1 month of treatment and maintained similar values until the end of follow-up ($p>0.05$). Perifoveal macular retinal and choroidal macular sectors improved after PDT and remained stable until the end of follow-up. Only two patients needed a second PDT after 12 months. At 36 months, two patients (4.7%) presented choroidal neovascularization by OCT-A and two other patients had subretinal fluid again. **Conclusions:** Low-fluence PDT showed a rapid (first month) and long-lasting (sustained 36 months follow-up) improvement in the treatment of chronic CSC, both in terms of VA and macular thickness (retinal and choroidal). Only 4.7% of the sample showed choroidal neovasularization with OCT-A after 36 months of follow-up.

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Degeneration of Corneal Innervation by Light-Induced Oxidative Stress

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Purpose: Corneal sensory innervation is a key regulator of the homeostasis of the cornea. Degeneration of corneal nerves derives into disease estates such as dry eye disease or neurotrophic keratitis. Trigeminal corneal afferents contain a large number of mitochondria along their axons, making them especially susceptible to oxidative stress. In this work we study the effect of oxidative stress on the sensory innervation of the cornea. **Methods**: Oxidative stress was induced in trigeminal ganglion cell cultures from adult Wistar rat by exposure to an intense blue light source (470 nm and 500 lux, 24 hours). In parallel, rats were exposed to blue light (4 hours per day for 10 days) and corneas and trigeminal ganglia were collected. Control groups were kept in the dark. Fluorescent immunocytochemistry was performed to detect Hemoxygenase-1 (HO-1), Tomm20, Cytochrome C oxidase (CitC), β-tubulin III, α CaMKII, Tau and CGRP on cultured cells. Whole mounted cornea preparations were also immunolabeled with β-tubulin III and imaged under a Leica SP8 confocal microscopy. FIJI software (ImageJ 1.49d, NIH) was used to analyze nerve loss on the subbasal plexus. ARVO statements for the use of animals were followed. **Results**: Blue light exposure caused a significant increase in HO-1 expression (inducible by oxidative stress) and CitC cleavage of the mitochondrial membrane. Also, increased expression of phosphorylated tau protein and activated αCaMKII accounted for the breakdown of sensory axons observed by β-tubulin III discontinuities. Extensive axonal degeneration was observed without severely affecting neuronal survival in culture. Furthermore, distal degeneration was also observed *in vivo* as a reduction in the density of the corneal subbasal nerve plexus. **Conclusion**: Degeneration of sensory nerves in the cornea may be caused by a photo oxidative environment and could be related with the origin of ocular surface diseases.

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Intrasession Repeatability of Physiognomy Parameters Using Device for Foveal Fixation Axis Measurement

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Purpose: Ophthalmic lenses adaptation require accurate measurements of facial physiognomy. Traditionally, nasopupillary distance (NPD) is the mainly used measurement for ophthalmic lenses prescription and they are collected with a frame ruler. But NPD considers the pupil's centre, as the measurement reference, ignoring real foveal fixation axis (FFA), and consequently the point which patient looks trough to achieve a clear vision. FFA represents the imaginary line which links directly the fixation point with the fovea. For these reasons, a new measurement paradigm that takes FFA as a reference has been proposed to ophthalmic lenses prescription. The purpose of this study was to analyze the intrasession repeatability of FFA measurement. **Methods:** Twenty-one healthy volunteers (9 women and 10 men) with a mean age of 52.95±4.35 years participated in the study in accordance with the Declaration of Helsinki. Three consecutive measurements of FFA distance were taken at far and near distance with the prototype designed for this study (figure 1). This prototype consists of two slits which move until the patient is able to see the fixation point in the centre of the slit. Intrasession repeatability was calculated following British Standards Institute and the International Organization for Standardization recommendations. **Results:** Table 1 summarizes the mean, standard deviation (SD) and repeatability coefficients of FFA measurements. Acceptable values of repeatability (CV<5%) were found in far and near distance in right eye and in far distance in left eye. Slightly worse repeatability was found in near distance in left eye. Within-subject standard deviation (Sw) is close to 1 mm in both distances and eyes. **Conclusions:** FFA Measurements collected with new prototype shows an acceptable repeatability for its clinical use. Further research is necessary to compare traditional measurements of NPD with FFA values and to determine if FFA use improve ophthalmic lenses prescription and users' satisfaction.

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Comparative Metabolomic Signature of Human and Dolphin Tears

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Purpose: To compare tear metabolomic signature of humans and dolphins to improve knowledge on tear composition and ocular surface disorders (OSD). **Methods:** Tears from healthy humans (n=10) aged 55±5years, and aquarium dolphins (n=5) aged 28±7years, housed at the Madrid Zoo Aquarium, were collected, frozen and stored at -80ºC until processing by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) or high-resolution magnetic angle spinning (HR-MAS) metabolomic platforms, respectively. 20μL of human tears and 50μL of dolphin tears from each participant were used and spectra were acquired on a spectrometer. Resonances between 0.50–5.26 particles per million (ppm) were considered and quantified using previous knowledge from academic and proprietary spectral databases (Chenomx NMR Suite-4.5) to metabolite assignment. SPSS-26.0 program was used to statistical analysis. **Results:** 32 metabolites were identified in human tears by ^{1}H-NMR (being the most abundant glycoproteins, leucine, N-acetylglucosamine, fatty acids and lipids/cholesterol). Nineteen tear metabolites (including glucose, glycoproteins, fatty acids, and mobile lipids) were identified in dolphin tears by HR-MAS. Importantly, no cholesterol fraction was observed in dolphin tears, Specific amino acids and glycerol were detected in humans, but did not appear in dolphins. Even more, significantly higher levels of lactate, mobile lipids and glucose, and lower levels of glycoprotein, fatty acids, citrulline, acetate, carnitine, creatine phosphate, acetate and choline were detected in dolphins when compared with human tears. **Conclusions:** The discovery of a differential tear metabolomic fingerprinting between human and dolphins indicated for the first time, fascinating metabolites which may help improving our understanding of tears composition and how these metabolites play a role in the pathogenesis of OSD. We suggest that the above findings may lead to ground-breaking diagnostic biomarkers and new biotherapies for advancing eye and vision care.

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Retinal Image Quality with Multifocal, Edof and Accomodative Intraocular Lenses as Studied by Pyramidal Aberrometry

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Purpose: To study and compare the clinical optical image quality following implantation with different premium IOLs, by the analysis of the point spread function (PSF) Strehl ratio using a Pyramidal WaveFront based sensor (PWS) aberrometer. **Methods:** This study included 194 eyes implanted with: A) 19 AcrySof SA60AT (control group); B) 19 Miniwell; C) 24 LENTIS Mplus LS-313 MF30; D) 33 LENTIS Mplus LS-313 MF15; E) 17 AkkoLens Lumina; F) 31 AT LISA tri 839MP; G) 20 Precizon Presbyopic; H) 20 AcrySof IQ PanOptix; I) 11 Tecnis Eyhance. Main outcome measures were PSF Strehl ratio, PSF Strehl ratio excluding second order aberrations (PSFw2), total root-mean-square (RMS), low and high order aberrations RMS. **Results:** AT LISA Tri had the highest significant PSFw2 Strehl ratio at both 3- and 4-mm pupil size (0.52±0.14 and 0.31±0.1), followed by SA60AT (0.41±0.11 and 0.28±0.07) and PanOptix (0.4±0.07 and 0.26±0.04). AT LISA Tri was found to provide a significant better retinal image quality than PanOptix at both 3.00 mm ($p<.0001$) and 4.00 mm ($p=.004$). MPlus MF15 was found to be significantly better than MPlus MF30 at both 3.00 mm ($p<.0001$) and 4.00 mm ($p=.002$). Total RMS, LOA RMS, HOA RMS, PSF Strehl Ratio and PSFw2 varied significantly between the studied groups ($p<0.001$). **Conclusions:** Clinical image quality parameters differed significantly according to the technology of the implanted lens. AT LISA Tri, SA60AT and PanOptix showed the highest values of retinal image quality, while the lowest PSFw2 Strehl ratio was showed by Miniwell, MPlus MF30 and Precizon Presbyopic.

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Morphometric Analysis of Corneal Innervation in Patients Diagnosed with Fibromyalgia

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Purpose: To examine the innervation of central corneal subbasal nerve plexus in patients with fibromyalgia and compare to normal values of healthy patients. **Methods:** A prospective, observational study was conducted analyzing 15 right eyes of 15 patients with fibromyalgia (12 women and 3 males) aged 49.83 ± 12.55 years (range 34 to 70 years). Nineteen eyes of 19 healthy patients (12 women and 7 males) aged 40.26 ± 10.86 years (range 23 to 54 years) was selected as control group. Subjects underwent microscopy confocal in vivo (IVMC) with the Rodstock cornea Module attached to Heildelberg's HRT3. At least 3 images of each eye were selected and the following subbasal central nervous plexus parameters were measured with ACC Metrics software: corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL), corneal nerve fiber total branch density (CTBD), corneal nerve fiber area (CNFA), corneal nerve fiber width (CNFW), corneal nerve fractal dimension (CNFrD). Data analysis was performed with SPSS® software for Windows 22.0 (SPSS ® Inc, Chicago, IL.). The normality of the sample was checked with the Saphiro Wilk test and the results were compared to the T test or the Man-Whitney U test based on the distribution of the data. The differences were considered statistically significant for $P<0.05$. **Results:** The mean and standard deviation were CNFD: (13.407 ± 2.027 fibers/mm^2) vs (23.353 ± 1.778 fibers/mm^2) ($p<0.05$); CNFL: (10.108 ± 1.016 mm/mm^2) vs (13.850 ± 0.768 mm/mm^2) ($p<0.05$); CNFrD: (1.440 ± 0.174) vs (1.475 ± 0.007) ($p<0.05$). These values were significantly reduced in patients with fibromyalgia compared to control group. **Conclusions:** Patients with fibromyalgia show a significant decrease CNFD and CNFL. CNFrD may be a diagnostically able parameter in fibromyalgia patients as has already been observed in peripheral neuropathies.

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Literature Review of Retinal Microvasculature Changes in Preclinical Stages of Alzheimer Disease

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Purpose: Alzheimer's disease (AD) is the most common type of dementia. Signs of the disease may appear even decades before clinical symptoms. Ocular vascular changes have been reported in patients with established disease. The aim of this work is to perform a literature review of vascular changes in the retina of patients in very early stages of the disease, such as the preclinical and prodromal forms of AD, as well as MCI using optical coherence angiography (OCT-A). **Methods:** A literature review of the last 10 years was carried out using "MESH" terms in PubMed and Google Scholar. We used the following keywords: "OCTA", "Alzheimer disease", "Vascular changes", "MCI", "Preclinal stages". **Results:** We have found that many authors have described 3 stages in the evolution of AD these being the preclinical stage, mild cognitive impairment -defined as a phase between healthy patients and AD symptomatic patients-, and AD. They have found using OCT-A an increased retinal microvasculature in preclinical stages, which leads to an overall decreased blood flow in further stages. Functional retinal alterations binded to the physiopathology of the disease, such as neurovascular coupling changes, give place to morphological modifications. These include lower vascular density initially in the superficial vascular plexus and later on in the deep vascular plexus. Furthermore, it has been shown correlations between these morphological modifications and cognitive symptoms. However, we must consider that previous authors have found an increased retinal blood flow in early clinical stages. **Conclusions:** OCT-A is the technique most widely used in the study and detection of ocular vascular changes in very early stages of the AD. This non-invasive technique could be used for monitoring and screening at very early onset of the disease.

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Comparative Effect of Glutamatergic Receptors Agonists N-Methyl-D-Aspartate and Kainate on Mouse Inner Retinal Cells

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Purpose: Due to the heterogeneous sensitivity of the different retinal neurons to glutamate agonists, our objective is to compare the effect of two glutamate agonists, N-methyl D-aspartate (NMDA) and kainate (KA) on inner retinal cells and to assess whether there is a synergic interaction between both. Recently, our group has developed a mouse model of inner retina degeneration induced by a combined intravitreal injection of those two agents, but their separate effect needs to be clarified. **Methods:** C57BL6/J mice were intravitreally injected into one eye with 1µL of PBS containing NMDA 10 mM or KA 3mM (1µL PBS was inoculated into the contralateral eye). The effect on retinal function was evaluated 3-4 and 7-8 days post-injection by optomotor test and electroretinographic (ERG) recording. The structural damage was assessed by immunohistochemical labelling on retinal sections. **Results:** Intraocular injection of NMDA/KA (10/3 mM) into the eye induces the abolition of optomotor response and strong decrease of ERG b-wave at one-week post-treatment but does not show any functional nor structural alterations in photoreceptors. Both excitotoxic agents, when injected independently, caused a complete loss of optomotor response just 3 days post-injection. The sole KA (3 mM) treatment produced the loss of b-wave ERG components, both in scotopic and photopic conditions. Conversely, NMDA-treated eyes preserved these ERG components, but a marked decrease in their amplitude was observed. Nonetheless, no significant changes on the "a" wave amplitude were found after the injection of both agents. Immunohistochemical labeling showed no effects of NMDA nor KA on the outer nuclear layer, but a moderate damage on the inner retinal layers in NMDA-injected eyes, and a deleterious effect in KA-injected eyes. **Conclusion:** Retinal damage induced by KA shows stronger effect than NMDA. However, differences observed between NMDA and KA injection could be caused by the different sensitivity of the retinal neurons, maintaining the possibility of inducing a synergic interaction with lower concentration of KA.

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New Concept of Dome-shaped Macula and Ridge-shaped Macula Definition Based on the SS-OCT. Differences in Clinical Features

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Purpose: To study highly myopic patients with a macular inward convexity, differentiating those who present that convexity only in some meridian across the fovea (Ridge-shaped macula; RSM); vs. those with a complete dome-shaped macula (DSM), basing the classification on the 12radial SS-OCT. **Methods:** Retrospective, observational study including 49 highly myopic eyes from 31 patients who underwent swept-source optical coherence tomography (SS-OCT) 9mm 12radial equal meridian scans centered on the fovea. RSM was defined as a macular inward convexity in some meridians whereas the opposite perpendicularly oriented meridians were flat. DSM was defined as a complete round inward convexity ≥50μm in the 12 OCT scans. Age, refraction, axial length (AL) and best corrected visual acuity (BCVA) were collected. Height of the macular bulge, and scleral and choroidal thicknesses at the fovea and at 4 parafoveal locations 1500μm from the foveal center were measured. Bruch´s membrane defects and the presence of perforating scleral vessels were recorded. **Results:** Thirty-seven (75.5%) eyes were classified as RSM and 12(24.5%) as DSM. Twenty-six (53.0%) eyes showed macular elevation only in the horizontal direction. Mean AL showed statistically significant differences (28.8±2.7vs.30.5±1.5mm, in RMS vs DSM respectively) and the presence of Bruch´s membrane defects was more frequently seen in DSM ($p<0.001$). Mean age, spherical equivalent, BCVA logMAR, height of the inward convexity, foveal retinal and foveal scleral thickness, subfoveal choroidal thickness and the presence of perforating scleral vessels did not show significantly differences. **Conclusions:** This study proposes to extend the use of term “ridge” for those oval-shaped domes that do not show a complete round elevation in all (12) OCT meridians, whether horizontally or vertically oriented and regardless of age, and it demonstrates the utility of the 12-radial OCT for the classification of this myopic maculopathy. Patients with RSM compared to DSM showed differences in AL, being longer in DSM, and regarding the presence of Bruch´s membrane defects, being more common in DSM.

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Optimization of Stimulation Parameters for Pattern Electroretinography Recording

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Purpose: To optimize stimulation parameters for pattern electroretinography (pERG) recording, in terms of color or wavelength (white-black, red-green, blue-yellow), contrast (99%, 60%), shape (checkerboard, vertical bars or horizontal bars), spatial frequency (0.08 / 0.12 / 0.17 / 0.35 cycles per degree [cpd]) through the analysis of the amplitudes of the P50 and N95 waves of the pERGs. **Methods:** The study was carried out in 10 healthy volunteers of 25.1 ± 5.2 years of age. Signals were recorded through a corneal active electrode, a reference electrode on the temporal side of the right eye, and a ground electrode on the forehead, while stimuli of different contrast, shape, color and spatial frequency were applied from a monitor using RETIsystem software. The pERG responses were stored using the commercial RETIcom registration system. The amplitude of the P50 and N95 waves of the pERG was analyzed of line. **Results:** We did not find significant differences in the amplitudes of P50 and N95 waves registered before stimuli with spatial frequencies of 0.08 to 0.35 cpd. The checkerboard and bar stimulus responses did not show statistically significant differences. The white-black stimuli elicited significantly higher amplitudes of the P50 and N95 waves than the red-green chromatic stimuli (5.29 ± 0.44 vs 2.49 ± 0.17 uV, P50 wave, $p < 0.05$) and blue-yellow (5.29 ± 0.44 vs 2.67 ± 0.31 uV, P50 wave, $p < 0.05$). However, the results obtained in response to chromatic stimuli did not show significant differences ($p > 0.05$; t test). Likewise, it was shown that stimuli with higher contrast could elicit higher amplitudes of P50 and N95 waves in the pERG test. **Conclusions:** Regarding the pERG test as a direct indicator of ganglion cell functionality, we propose to use stimuli consisting of checkers or bars, with 99% white-black contrast at a spatial frequency between 0.08-0.35 cpd, since it seems the optimal stimulus.

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Phenotype Characterization of a Mice Model of Visual Blindness

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Purpose: To characterize of the visual functions of a murine genetic model of absolute blindness. The animal model is based on the combination of a mutation in the Pde6b^{rd10} gene, which results in a photoreceptor degeneration, together with a mutation in the Opn4$^{-/-}$ gene, responsible of the melanopsin synthesis in intrinsically photosensitive retinal ganglion cells. **Methods:** The characterization of the visual functions of a double mutant Opn4$^{-/-}$ x Pde6b^{rd10} (OxRd) murine model has been carried out, applying a battery of behavioral tests, as well as *in vivo* electrophysiological recordings, which allowed us to know the degree of functionality of the retina and visual pathways. A structural characterization of the retina was also carried out using immunohistochemical labeling on retinal sections. The results were compared with wt mice and murine animal models that present both mutations separately. **Results:** The OxRd animals showed a total suppression of all visual abilities. The different behavioral tests showed that characteristic physiological visual reflexes and visual behavior of these animals, such as the rejection of illuminated spaces or the pupillary reflex, were totally inhibited. A complete decrease in visual acuity was observed by the optomotor test, as well as the absolute disappearance of the various components of the waves of the full-field and pattern electroretinogram (ffERG, pERG), indicating the functional loss of the different cellular components of the retina. Likewise, no visual evoked potential (VEP) could be recorded in these animals. Immunohistochemical labeling support these data, showing a marked degeneration of the outer retinal layers, due to the Pde6b^{rd10} mutation, as well as the absence of melanopsin labeling. **Conclusions:** The combination of the mutations in the Opn4 $^{-/-}$ and Pde6b^{rd10} genes has allowed us to generate an animal model that does not show any photosensitive element in its retina. This animal is unable to recognize light stimuli, which makes it a potential tool for the study of new therapeutic agents such as optosensitive agents.

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Generation and Characterization of a *Myocilin* Knock-out Zebrafish Line

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Purpose: Myocilin is a matricellular protein present in ocular structures and it is mainly known as the first glaucoma gene. However, both its normal function and the pathogenic mechanism remain largely uncharacterized. Our main objective was to explore the normal role of this protein *in vivo* by the generation of a *myoc* knockout (KO) zebrafish line. **Methods:** *Myocilin* knock-out line was generated using the CRISPR/Cas9 genome editing system. Myocilin expression was evaluated by immunohistochemistry analysis with wild-type and mutants cryosections. The specificity of the detection was evaluated by antigen competition. The histological phenotypes were studied with hematoxylin-eosin staining. Cell death was evaluated with TUNEL assay with larvae and juvenile KO mutants. Finally, the differentially expressed genes in the mutant animals were studied by transcriptomic analysis. **Results:** *Myoc* KO line carries a homozygous variant (c.236_239delinsAAAGGGGGGAAGGGGA) which is predicted to result in a loss of function of the protein.Immunohistochemistry analysis showed the presence of the protein in ocular structures of the anterior segment and caudal muscles of wild-type embryos. The protein was also immunodetected in different adult ocular and non-ocular tissues. No macroscopic or microscopic alterations were identified in KO zebrafish, but notably, we observed absence of females among adult KO animals and apoptosis in the immature juvenile gonad (28 dpf) of these animals. Transcriptomic analysis showed that adult KO males overexpressed key genes involved in male sex determination. **Conclusions:** This is the first study of myocilin expression in zebrafish. *Myoc* KO zebrafish line can be used to study the function of this protein, and it provides evidence for the unexpected function of myocilin as a key factor in zebrafish sex determination.

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Ciliary Muscle Dimensions Measured by Swept-source Optical Coherence Tomography in Eyes with Primary Open-angle Glaucoma and Healthy Eyes

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Purpose: To compare in vivo swept-source optical coherence tomography (SS-OCT) measurements of the ciliary muscle (CM) in patients with primary open- angle glaucoma (POAG) and healthy subjects, **Methods:** Cross-sectional study of the right eyes of 181 subjects: 89 POAG patients and 92 healthy subjects. Using the Triton SS-OCT device (Topcon, Tokyo, Japan), CM length (CML), area (CMA) and thickness measured 1000 lm (CMT1), 2000 lm (CMT2) and 3000 lm (CMT3) from the scleral spur were determined in the temporal and nasal quadrants. CM dimensions were then assessed for correlation with visual field (VF) mean defect (MD), mean retinal nerve fiber layer (RNFL) thickness and intraocular pressure (IOP). **Results:** Mean CMLs were 4325 ± 340 lm and 4195 ± 843 lm for the healthy subjects and POAG patients, respectively ($p = 0.17$). No significant differences were detected between Mean CM thicknesses (all $p \geq 0.25$). In the temporal quadrant, mean CMA was 1.12 ± 0.29 mm^2 and 1.15 ± 0.24 mm^2 for the healthy and POAG subjects, respectively ($p = 0.45$). No correlations were observed between CM measurements and RNFL thickness ($p \geq 0.15$), IOP or VF MD ($p \geq 0.14$) in POAG subjects irrespective of glaucoma severity ($p \geq 0.19$). **Conclusions:** While SS-OCT proved useful to measure CM dimensions in vivo, these dimensions did not differ between healthy individuals and POAG subjects. In the patients with POAG, no correlations were detected between CM dimensions and VF, RNFL or IOP.

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Punctiform and Polychromatic Pre-Descemet Corneal Dystrophy: Clinical Evaluation, Identification of the Genetic Basis and Literature Review

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Purpose: To report the clinical features and genetic basis of three previously unreported families with punctiform and polychromatic pre-Descemet corneal dystrophy (PPPCD). **Methods:** Full ophthalmic assessment was performed for members of three unreported families with PPPCD. Structural and biomechanical alterations of the cornea were screened. Whole-exome-sequencing (WES) was performed on the first family. Novel or rare variants that segregated with the affected status were screened for in the other two families with Sanger sequencing. Identified variants that segregated with the affected status in all families were characterized using in silico prediction tools and/or in vitro splice assays. Additionally, two previously reported PPPCD families were screened for variants identified in the three unreported PPPCD families. **Results:** Twelve of 21 examined members of the three unreported families were diagnosed with PPPCD. The only refractive, topographic or biomechanical abnormality associated with PPPCD was a significantly increased corneal stiffness. WES and Sanger sequencing identified two variants that segregated with the affected status in the all three families: a rare intronic PDZD8 c.872+10A>T variant and a novel missense PRDX3 c.568G>C (p.Asp190His) variant. The same PRDX3 variant was identified in the previously reported PPPCD family expressing the common PPPCD phenotype and is predicted by in silico prediction tools to be damaging to protein function. **Conclusions:** PPPCD is associated with an alteration of corneal biomechanics and a novel missense variant in PRDX3. Screening of additional families will determine whether all families demonstrate a PRDX3 variant, or whether locus heterogeneity may exist for PPPCD.

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Multi-Loaded Microparticulate Drug Delivery System as a Potential Neuroprotective Treatment in an Ocular Hypertension Model of Glaucoma.

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Purpose: To address the neuroprotective capacity (*in-vitro* and *in-vivo)* of multi-loaded drug delivery poly (lactic-co-glycolic) acid (PLGA) microspheres (MSs) after intravitreal injection in a glaucoma animal model of ocular hypertension. **Methods:** Dexamethasone (DEX), melatonin (MEL) and Coenzyme Q10 (CoQ10) were selected as neuroprotective active ingredients (N-APIs) to be released from the microparticles. PLGA-MSs were manufactured encapsulating the three drugs through an oil in water (O/W) emulsion solvent extraction-evaporation technique. The co-loaded MSs were characterized physiochemically in terms of morphology, encapsulation efficiencies and *in vitro* release of the N-APIs. The *in vitro* bioactivity of the encapsulated substances was assessed in a retinal cell line (R28). In order to determine the *in vivo* neuroprotective properties of the MSs, a well-established rodent model of ocular hypertension was used. **Results:** MSs (20–30 μm) presented spherical shapes rendering them suitable for intravitreal administration using 25G–32G needles. Encapsulation efficiencies resulted higher than 60% for the three neuroprotective drugs being released in a controlled fashion for up to 30 days. The multiloaded MSs promoted a significant rescue of R28 cells from glutamate excitotoxicity ($p<0.05$) compared to non-loaded MSs. The novel MSs were capable to significantly ($p<0.05$) reduce retinal ganglion cell loss per integral intraocular pressure (IOP) compared to non-treated animals. **Conclusions:** The novel multi-loaded biodegradable microspheres showed *in vitro* and *in vivo* neuroprotective activity.

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The Stimulation of the Optic Nerve with a Blue Optical Fiber Modifies Dopamine and Melatonin Levels in Rabbits

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Purpose: To quantify the changes in dopamine and melatonin levels in the eye of rabbits after the stimulation of the optic nerve with blue light by using an optical fiber. **Methods:** An experimental, short-term prospective and randomized study was carried out. Fifteen male New Zealand white rabbits were evaluated. The dopamine and melatonin levels in the tears, aqueous humour, vitreous body, and retina were quantified by high-performance liquid chromatography after the stimulation of the optic nerve with a blue optical fiber (450-500 nm) for periods of 1 min (n = 5), 10 min (n = 5), or with no stimulation as a control (n = 5). Each rabbit was evaluated on a different day, randomly. The left eye was operated with a self-developed technique to introduce the optical fiber on the optic nerve excavation, while the contralateral eye was used as an internal control. **Results:** In the control rabbits, there were no statistical differences ($p > 0.05$) in dopamine and melatonin levels between the operated and contralateral eyes. After the stimulation of the optic nerve with blue light for 1 min, there was an increase of dopamine concentration in the vitreous body of the operated eye compared with the contralateral eye ($p = 0.015$). Finally, the stimulation for 10 min produced an increase of dopamine concentration in both the aqueous humour and the vitreous body of the operated eyes, accompanied by a decrease of melatonin concentration in the vitreous body. **Conclusions:** The stimulation of the optic nerve with a blue optical fiber produced a biochemical response associated with the activation of the intrinsically photosensitive retinal ganglion cells. Besides, this self-developed technique for stimulating the optic nerve is proposed to study the ocular physiology by selectively illuminating other retinal regions.

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Differential Expression of Fibrosis-related Genes after Filtering Glaucoma Surgery

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Purpose: Filtration surgery is the most effective strategy to achieve sustained intraocular pressure reduction in glaucoma patients when pharmacological treatment is not effective enough. However, excessive subconjuntival scarring can lead to surgical failure due to ocular fibrosis. Our main goal was to determine the expression changes of specific genes related to the development of conjunctival fibrosis. **Methods:** We have determined gene expression pattern in conjunctival-tenonian samples from glaucoma filtering surgical procedures. Total RNA has been extracted from the ocular specimens and cDNA has been synthetized by the reverse transcription reaction. Expression analysis of a selection of genes potentially associated with fibrosis such as VEGF, TGF-α, TGF-β, IL8, IL18, C-Myc, TSP1, CDKNA1, CDKNA2, CTGF and 18S as internal control (housekeeping gene) has been performed by quantitative real-time PCR. **Results:** We have found an altered expression of TGF-β and TSP-1 genes in the subconjuntival tissue of glaucoma patients with a fibrotic phenotype (who have had to be re-operated) versus control patients (operated once). Other genes associated with the development of fibrosis like VEGF, IL-8, C-MYC and CDKNA1 show a tendency to be overexpressed in patients with fibrosis. Interestingly, these specific genes are substantially induced in glaucoma patients with hyper-fibrotic phenotype (who had been re-operated more than twice in a short period of time). **Conclusions:** We have identified a correlation between the intensity of expression changes of certain fibrosis-associated genes and the degree of conjunctival fibrosis of a group of glaucoma patients. We propose that characterization of the expression pattern that contribute to conjunctival fibrosis could allow, in the near future, to improve the surgical outcome by modulating expression of pro-fibrotic genes.

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Altered Gut Microbiome is Associated with Retinitis Pigmentosa

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Purpose: The homeostasis of the gut microbiome is critical for maintaining human health, and imbalances in the microbial composition of the gut profoundly influence critical features of the host physiology. Recently, a gut-retina axis has been associated with retinal neurodegenerative diseases. Thus, the purpose of this work was to analyze the gut microbiome changes associated with retinitis pigmentosa (RP), a neurodegenerative disease of the retina. **Methods:** One-month-old healthy C57BL/6J and dystrophic rd10 mice (RP) were used to study both retinal degeneration and gut microbiome composition linked to RP. Electroretinographic recording and optomotor test were used to study retinal function, and immunohistochemistry was performed to analyze retinal structure and integrity. Additionally, the gut microbiome was analyzed by 16S rRNA gene sequencing. **Results:** Decreased retinal responsiveness was found in rd10 mice compared to age-matched C57BL/6J mice. This decline was correlated with a significant loss of photoreceptors, which showed abnormal morphology. Photoreceptor death was associated with inflammation of the rd10 mice retina, exhibiting a higher number of microglial cells and reactive gliosis of Müller cells. Furthermore, the analysis of the gut microbiome evidenced differences in alpha and beta diversity at the genera, species and amplicon sequence variants (ASV) levels. Notably, four common ASV in healthy gut microbiome belonging to *Rikenella spp*., *Muribaculaceace spp*., *Prevotellaceae* UCG-001 spp., and *Bacilli spp.* were absent in the gut microbiome of RP mice, while *Bacteroides caecimuris* was significantly enriched in rd10 mice. **Conclusions:** Our results show that exists a relationship between the degenerative process of the retina in RP and changes in the gut microbiome. The findings suggest that microbiome shifting could be considered as potential biomarker and therapeutic target for retinal degenerative diseases.

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Expression and Localization of Polarity Proteins of the Crumbs Complex in *Macaca Fascicularis* Retina

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Purpose: Polarity protein complexes play a major role in establishing polarity in cells and, therefore, facilitate functionality in diverse tissues. Mutation of genes encoding for CRUMBS polarity proteins are proved to cause different pathologies in mice. Since the distribution and functions of the polarity complexes in the retina of primates are still widely unknown, the main goal of this study is to characterize the distribution of CRB proteins and other associated polarity complexes in the primate retina to correlate their localization with that observed in other experimental models. **Methods:** *Macaca fascicularis* retinas were fixed and immunofluorescence labeling of diverse proteins was performed in cryosections. Expression and localization of specific proteins characteristic of the different retinal cell types (Calbindin, Glutamine synthetase, Recoverin, CRALBP) and proteins related to the establishment of polarity, specifically those of the crumbs polarity complex (CRB2 and CRB3) were studied. **Results**: Calbindin was present throughout the entire length of cone photoreceptor cells, glutamine synthetase in Müller cells, recoverin labeled the photoreceptor outer segments and CRALBP is a protein present in the entire length of Müller cells, pigment epithelium and photoreceptors. CRB3 was found throughout the entire length of Müller cells. It was prominent in the soma of these glial cells and it was distributed throughout their processes. In addition, the labeling for this protein was especially intense in both the inner and outer limiting membranes. CRB3 was also located on the apical side of the retinal pigment epithelium. CRB2 was also found in the apical side of the retinal pigment epithelium and in the outer segments of photoreceptor cells. **Conclusion**: CRB3, which belongs to the Crumbs apical polarity complex, is expressed in the macaque retina and shows an expression pattern similar to that found in mice. Proper arrangement of polarity proteins is essential for the successful functioning of tissues as complex and specialized as the retina.

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Ly6c, a New Marker of Retinal Vasculature

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Purpose: One of the main fields of study within neuroscience deals with the relationship between the circulatory system and the central nervous system (CNS). The circulatory system, responsible for providing oxygen and nutrients to all cells in the body, has a crucial a roll, proof of this is that most neurodegenerative diseases are associated with blood supply disorders. We describe here Lyc6, a common monocyte/macrophage cell differentiation antigen as a new marker to detect the CNS vasculature. **Methods:** Brains and retinas from C57Bl/6 mice were dissected, brains were cryostated, retinas flat mounted and immunodetected with Ly6C antibody and IB4 isolectin (a common marker of retinal vessels) for comparative purposes. Retinal and brains photomontages were reconstructed from individual images acquired using an epifluorescence microscope. **Results:** Ly6c is expressed in arteries and veins in the retina and the brain. We also observed that Lyc6 signal is brighter than that of IB4, leading to better images and allowing to differentiate and analyze the vasculature from the 3 retinal plexuses. In addition, while Ly6c marks all blood vessels with the same intensity, IB4 staining is different between veins and arteries. This is due to a lower expression of the terminal α-gal residue in venules, which is the IB4 binding residue. **Conclusions:** Ly6c is a new marker of the retinal and brain vasculature. Compared to the traditional IB4 isolectin presents two main advantages: brighter signal and homogeneous staining, which in turn improves imaging and eases quantitative analyses.

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PRGF Promotes Cell Protective Mechanisms in an *In Vitro* Retina Phototoxicity Model

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Purpose: Plasma rich in growth factors (PRGF) is a serum extracted from the patient's blood with antioxidant capacity, that has been proven to be a promotor of cell proliferation and regeneration in several eye diseases. The aim of this study is to test the interaction between PRGF and some protective pathways, such as autophagy, in order to reduce the damage caused by a phototoxic insult. **Methods:** ARPE-19 cell line was used to analyze the effect of PRGF treatment in a phototoxicity experimental model. Several markers related to cell death and cell renewal pathways, such as autophagy, were included in this study. Western blot and quantitative PCR techniques were used for the analysis of TNFR1, p62, ATG5, IL1b and IL18. **Results:** Up-regulation of TNFR1 gene expression was detected when cells were exposed to the phototoxic stimulus. In addition, a reduction in the expression of the marker was observed when the cells were treated with PRGF, showing values close to control. Along with the reduction of cell death with PRGF treatment, some autophagy-related markers changed their expression pattern. The expression of p62/sqstm1 was increased by the action of the noxious agent compared to the control group or the group treated with PRGF alone. However, cells exposed to the combined treatment of PRGF and blue light showed an enhanced effect on the expression of p62/sqstm1. Similar results were detected in protein analysis of ATG5 marker. Moreover, IL1B protein expression was increased in response to PRGF when given alone or in combination with the insult. However, opposite pattern was observed with IL18, as it showed an overexpression in light-exposed cells that was reduced when PRGF was applied. **Conclusions:** Results suggest that PRGF promotes cellular renovation processes, such as autophagy, stimulating protective cell mechanisms under stress conditions.

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Development of an Osmoprotective Liposomal Formulation Containing Acetazolamide for Glaucoma Treatment

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Purpose: Glaucoma is one of the leading causes of irreversible blindness, considering the raise in intraocular pressure (IOP) the main risk factor. The aim of this work was to develop a liposomal formulation containing acetazolamide and osmoprotective substances to decrease IOP and to preserve the ocular surface integrity. **Methods:** The liposomes were composed of phosphatidylcholine, cholesterol, vitamin E and acetazolamide (8:1:0.08:0.3 mass ratio) by the lipid-film hydration method. Erythritol and trehalose were incorporated as osmoprotective agents in the formulation containing acetazolamide (0.7 mg/mL) and incorporation of HPMC 0.3% was also studied. Physicochemical characterization was carried out by studying the pH, size distribution, osmolarity, viscosity and surface tension. *In vitro* tolerance was evaluated in J774 macrophages and HeLa cells. *In vivo* assays were carried out in normotensive New Zealand white rabbits. **Results:** Liposomal formulations presented particle sizes of 157.3±4.9 nm and 169.7±6.1 nm, in addition to neutral pH values and osmolarity within isotonicity. The surface tension of 30.6±0.9 mN/m ensured a correct extensibility on the ocular surface. The viscosity resulted higher in the formulation containing HPMC and cell viability assays rendered values higher than 80% in the liposomal formulations without HPMC. *In vivo* studies showed good ocular tolerance after repeated administration for 6 hours. After a single administration of 25 µL, maximum reduction in intraocular pressure of 25% and 18% was observed, for more than 7 hours. The addition of polymer resulted in a 1.5-fold increase in the AUC_{0-8h}. **Conclusions:** The liposomal formulation can be considered as a potential strategy for glaucoma treatment.

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Ocular Surface Microbiota: Protocol for its Characterization in Patients with Meibomian Gland Dysfunction

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Purpose: Meibomian gland dysfunction (MGD) is leading cause of evaporative dry eye disease. The microbiota on the ocular surface is thought to play an important role in both forms of blepharitis, (anterior and posterior blepharitis). Recent studies have reported a relationship between the ocular surface microbiome and posterior blepharitis (the most common form of MGD). However, no standardized protocols for the characterization of the ocular surface microbiota in MGD patients have been established which is the aim of this study. **Methods:** The ocular surface exam included the eye lid and Meibomian gland check with slit lamp and keratograph 5M in order to determine which patients had MGD. Some questionnaires for dry eye were also conducted. Ocular samples were collected with a sterile swab moistened with saline solution both from the free margin of the upper eyelid before and after expression of Meibomian glands, and from the tarsal conjunctiva. Samples were used to inoculate and streak blood agar plates and then incubated in aerobic and anaerobic conditions at 37ºC for 48 to 96 h. The number of cell colonies on the plates was counted, and their morphologies were annotated. **Results:** After setting-up and comparing different methods, it was found that samples collected from the upper eyelid showed more biodiversity and a higher number of bacterial colonies than those from the tarsal conjunctiva. Microbial colonies could not totally recover with a higher volume of dissolution after vortex-shaking. A higher volume of saline solution to moisten the swab improved microbial recovery. A higher rate of microbial biodiversity was observed incubating the agar-plates in both conditions even if the vortex-shaking time was reduced. **Conclusions:** This study shows a useful and efficient protocol for futures studies about ocular surface microbiota in MGD patients.

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Pharmacogenetics, Pheno and Genotypic Response to Prostaglandin Analogues in a Population of Glaucoma Patients.

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Purpose Variations in glaucoma treatment response among patients are often observed in daily clinical practice. Individual genetic differences underline at the base of this different clinical response. Several single nucleotide polimorphism (SNPs) have been studied in association with glaucoma pharmacological response. SNPs of the prostaglandin F2α receptor (PTGFR) gene were found to correlate with the response to short-term latanoprost treatment. **Methods** 99 eyes from 53 patients affected with glaucoma under PGA treatment (solo or combo) were included in a prospective study. SNPs PTGFR (rs3766355, rs3753380**)** were analysed using real-time PCR assays, and medical records of patients were reviewed. Baseline and treated introcular pressure (IOP) data was collected. According to treated IOP the patients were classified as PGA-responders and PGA solo and PGA combo non-responders. **Results** From a total of 99 eyes, 28 were clinically identified as PGA responders (25.9%), 27 as PGA solo-non responders (25%) and 40 as PGA combo non-responders (37%). Mean baseline IOP was 26.92±7.99mmHg and mean treated IOP was 21.35±5.22mmHg. SNP rs3766355 analysis identified 4 eyes as wildtype homozygous (HZ) 3.7%; 24 heterozygous (HT) 22.2% and 69 eyes mutated HZ 63.9%. Likewise for rs3753380: 44 eyes were wildtype HZ 40.7%; 48 were HTs 44.4% and 5 eyes were mutated HZ 4.6%. Comparing mean baseline IOP in rs3766355 HT and mutated HZ (27.21± 8.15) with wildtype HZ (20.5± 2.64) significant differences were elucidated (p= 0.046) and also between treated IOP in rs3766355 HT and mutated HZ (21.49± 5.22) with wildtype HZ (16±2.16) (p= 0.021). **Conclusions** We found significant differences regarding baseline and treated IOP in HZ and HT patients carrying a mutated rs3766355 allele with regard to HZ wildtype patients. Therefore, poor response to treatment may be associated with being a carrier of mutated allele.

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Evaluation of Changes in Corneal Morpho-Geometry Introduced by Topographic Cross-Linking Using a 3D Virtual Model

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Purpose: Using 3D virtual reconstruction models to determine the effectiveness of landmark techniques in the evaluation of corneal morphogeometry, as well as to determine the structural changes of the cornea in specific topographic regions as response to the application of the crosslinking treatment. **Methods:** Discrete sets of data from corneal tomographer were used to generate virtual 3D models of the cornea. The surface morphology of 12 corneas was measured in two scenarios: geometrical alterations experienced when advanced corneal ectasias were present and as response to the process of structural stabilization experienced by the cornea after the application of CXL for one year. The asymmetric structure of the cornea was evaluated using linear, surface, and volumetric parameters, only for specific topographical locations. **Results:** All corneas showed significant local variations in their geometry previous to CXL. The local distribution of corneal response post-crosslinking changed significantly, with a decrease in the magnitude of displacement of singular points such as the anterior/posterior apices or the anterior/posterior minimum thickness points, ranging between 16%-50%, depending on the location of the crosslinked region. In addition, reductions in the total corneal area/volume were found, with some levels of regression after 12 months in certain volumetric parameters. **Conclusions:** 3D modelling of the cornea and its subsequent morphovolumetric analysis in a virtual environment are effective tools for analyzing the deformation of the corneal surface as response to both the structural alterations and the geometrical regional changes introduced by crosslinking. Because of the significant regional variability in the corneal geometric response, these properties need to be quantified to allow the determination of the specific local effects of topographic crosslinking, so that its application can be adapted to achieve optimal refractive results.

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Confocal Microscopy of the Cornea in a Clinical Model of Corneal Stromal Expansion Using Adipose Stem Cells and Corneal Decellularized Laminas in Patients with Keratoconus

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Purpose: Recently, we described a new surgical approach based on advanced regenerative therapy, using autologous adipose-derived adult stem cells (ADASCs) and decellularized/recellularized human corneal tissue into the cornea with advanced keratoconus. We report herein the corneal confocal microscopy evolution in vivo of the cellularity along one year following the implantation of ADASCs alone or using a decellularized lamina. We also evaluate the presence of fibrotic tissue. **Methods:** A confocal microscopy study was performed in an interventional prospective, consecutive, randomized, comparative series of cases. Fourteen keratoconic patients were randomized into 3-groups and were the subject of 3 surgical interventions: Group-1: ADASCs implantation, Group-2: Decellularized human corneal stroma, Group-3: ADASCs + Decellularized human corneal stroma. A novel method of quantitative cell counting nuclei was used to evaluate the evolution of cell density, the morphological implanted cells, and the decellularized/recellularized laminas. **Results:** A significant increase (P-value<0.001) was observed in the cell density in the anterior and posterior corneal stroma with all the groups one year postoperatively, the mid stroma in G-1, the anterior, posterior surfaces, and within the laminas in G-2 and G-3. Results at the anterior surface and within the laminas were also statistically significantly higher (P=0,011), as well as at the posterior surface (P=0,029) in G-3/G-2. We did not find a direct nor significant association between recellularization and the presence of fibrotic tissue. **Conclusion:** Confocal corneal microscopy shows to be an essential tool in the assessment "in vivo" of the corneas implanted with ADASCS for corneal regeneration purposes. Using corneal confocal microscopy, we were able to observe a significant increase in cell density up to one year at the corneal stroma following the implantation of ADASCs, with decellularized, or recellularized laminas. The increase in the corneal cell density was not significantly correlated with the presence of fibrotic tissue.

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Evaluation of the Vision-Related Quality of Life in Patients with Wet Age-Related Macular Degeneration Treated with Intravitreal Injections in a Routine Clinical Setting.

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Purpose: evaluation of the impact of chronic treatment with intravitreal anti-vascular endothelial growth factor (VEGF) injections on the vision-related quality of life (VRQoL) of patients with neovascular age-related macular degeneration (AMD). **Methods:** A total of 166 eyes from 166 Caucasian patients ≥55 years old were recruited for this observational study; 78 were identified as normal macular health eyes, early or intermediate AMD and 88 patients suffering from neovascular with at least one year of routine anti-VEGF intravitreal injections. VRQoL was assessed using the composite score of the Spanish version of the 25-item National Eye Institute-Visual Functioning Questionnaire (NEI VFQ-25). Secondary assessments included NEI-VFQ-25 subscale scores. Changes in NEI VFQ-25 composite and subscale scores were analyzed using Student's t-test. **Results:** The mean age was 76.96 years (74.3 years [±7.1] and 79.3 [±8.6] by groups), with statistically significant differences between both groups ($p<0.001$). 64.45% of the patients were female. The overall NEIVFQ-25 composite score was 92.81 [±5.23] in control group and 8159 [±16.37] in patients with exudative AMD. A statistically significant difference ($p<0.05$) compared with the control group was reported in NEI VFQ-25 composite score and most of the NEI VFQ-25 subscales: general vision, near activities, distance activities, social functioning, mental health, role difficulties, dependency, driving, color vision and peripheral vision. There were no statistically significant differences between both groups in general health ($p=0.089$) and ocular pain ($p=0.099$) subscales. **Conclusions:** Vision-related quality of life is decreased in patients treated during more than one year with intravitreal injections in a real-world setting, affecting general vision, near activities, distance activities, social functioning, mental health, role difficulties, dependency, driving, color vision and peripheral vision.

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Ocular Irritancy Prediction Based on Non-invasive Cell Membrane Capacitance Measurement

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Purpose: Cell membranes display the ultrastructure of a natural capacitor due to the dielectric properties of the phospholipid bilayer. Cell capacitance can therefore be related to the cell membrane integrity that could be altered after chemical exposure and might lead to ocular irritation.
In this work, changes in cell membrane capacitance in reconstructed human corneal epithelia (RhCE) were evaluated using the Standardized Operational Procedures detailed in OECD TG 492 for identifying chemicals not requiring classification and labelling for eye irritation. **Methods:** RhCE were prepared from limbal cells cultured on 1.12 cm^2 Transwell insert and differentiated for 7 days under air-lift conditions. Cell membrane capacitance was evaluated using coupled electrodes connected to a U2817A LCR Meter prior to chemical exposure at different frequencies (from 100Hz to 100kHz). Next, 30 chemicals (15 irritants and 15 non-irritants) including liquids and solids were applied in duplicates for 30 minutes or 6 hours respectively. After PBS rinse, RhCE were incubated at 37ºC for 2 hours for liquids or 18 hours for solids. Finally, cell capacitance was evaluated again and cell viability was assessed using the MTT assay. **Results:** Cell membrane capacitance values were normalized as final/initial and a prediction model was developed based on changes of cell membrane capacitance and compared to standard classification obtained by the MTT assay. Standard classification according to MTT resulted in 93% sensibility (14/15), 67% specificity (10/15) and 80% accuracy (24/30). Cell capacitance prediction model using normalized values were in accordance with OECD TG 492 at frequencies ranging from 300Hz-3kHz and resulted in 93% sensibility (14/15), 67% specificity (10/15) and 80% accuracy (24/30) at appropriate cut-off values. **Conclusions:** Our results show that by evaluating cell capacitance, ocular irritation potential of chemical products could be assessed in a non-invasive assay while complying with OECD TG 492 requirements.

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Reduction of Bacterial Load in Monthly Soft Contact Lenses after Application of Good Wear and Maintenance Guidelines

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Purpose: Some bacteria, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Streptococcus pneumoniae*, can cause keratitis. These corneal infections are often associated with poor use and hygiene of contact lenses (CLs) and contact lens cases (CLCs). The aim of this study was to investigate the influence of CL handling and disinfection on the bacterial load in monthly replacement soft CL wearers. **Methods**: A prospective study was conducted involving a total of 30 monthly replacement soft CL wearers. The study consisted of two visits in two consecutive months. Before and after applying the recommendations explained by the optometrist on the hygiene and maintenance to be followed with their CLs and CLCs. During the two visits, clinical tests were carried out to assess the condition of the ocular surface using the slit lamp, as well as microbiological tests to isolate and quantify the microbial load of bacterial origin present in the CLs and CLCs from participants. **Results**: There was a significant reduction in the bacterial load of the CLs (median, 0 vs 12 CFU/mL; range, 0-3-105 vs 0-3-107 CFU/mL; $p < 0.001$) and their CLCs (median, 0 vs 10 CFU/mL; range, 0-106 vs 0-9-109 CFU/mL; $p < 0.001$) in the second month compared to the first month. The incidence of bacterial contamination was higher in CLCs than in CLs. However, a higher bacterial contamination of CLs in the first month was related to those participants with lower visual acuity and increased conjunctival staining. Higher numbers of colonies were also isolated from second-month CLs and CLCs among subjects who had lower tear meniscus and greater ocular symptomatology. **Conclusions**: Proper use and maintenance of CLs and CLCs reduces their bacterial contamination and improves the condition of the ocular surface.

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Quantification of M4 Intrinsically Photosensitive Retinal Ganglion Cells in Pigmented Mice

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Purpose: Intrinsically photosensitive retinal ganglion cells (ipRGCs) express the photopigment melanopsin (m) and constitute a subpopulation of RGCs that are mainly responsible for non-image forming visual functions, although they may also contribute to image formation. Of the six types of ipRCGs (M1-M6) described, only M1, M2 and M3 express melanopsin in sufficient amounts to be readily identified with classic immunohistochemical techniques. Here we propose to quantify the M4 subtype, also known as the ON-sustained alpha (α)RGC, one of the four subtypes of αRGCs. **Methods**: In adult pigmented male C57Bl/6 mice, retinas were prepared as whole-mounts and immune-stained with antibodies against non-phosphorilated high molecular weight neurofilament subunit (SMI-32) (expressed in all αRGCs; ON and OFF, sustained or transient), Osteopontin (OPN) (expressed in all αRGCs), Calbindin (expressed in ON sustained αRGC, and amacrines), T-box transcription factor T-brain 2 (Tbr2) (a key transcription regulator for the development and maintenance of ipRGCs, expressed in ipRGCs and GABAergic displaced amacrine cells) and m. We dotted manually cells positive for each antibody (n=7) and those triple-labelled with SMI-32$^+$-Calbindin$^+$-OPN$^+$ (n=7) or with OPN$^+$-Calbindin$^+$-Trb2$^+$ (n=4). **Results**: The mean total number of SMI-32$^+$RGCs, OPN$^+$RGCs, Calbindin$^+$ or m$^+$RGCs cells was 2280±363, 2337±180, 3309±252 or 1088±109, respectively. The mean total number of triple-labelled SMI-32$^+$-Calbindin$^+$-OPN$^+$ or OPN$^+$-Calbindin$^+$-Trb2$^+$ was 805±65 or 808±77, respectively. None of these triple labelled RGCs was detected with melanopsin antibodies. The distribution of these triple-labelled cells was predominantly in the hemi-temporal retina. **Conclusions**: Co-expression of SMI-32-Calbindin or OPN-Calbindin is a useful tool to identify ON-sustained αRGC, an abundant M4 subtype of ipRGC with low levels of melanopsin expression. The co-expression of OPN and Calbindin with Trb2, a specific transcription factor regulator of ipRGCs, supports and validates our findings.

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Genetic and Environmental Factors Related to the Development of Myopic Maculopathy in Spanish Patients

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Purpose: This study aims to verify if the genetic factors that have been most related to myopic maculopathy (MM) and high myopia (HM) in Asian populations are also associated in Spanish patients, and also to study the demographic, ophthalmic and environmental factors related with these pathologies. **Methods:** A case-control prospective study with 365 highly myopic Spanish patients and 177 non-myopic controls were recruited from various centers of OFTARED (RD16/0008). Genomic DNA was extracted from oral swabs. A set of 8 SNPs in 6 genes were genotyped by Real-time PCR: *COL8A1* (rs669676 and rs13095226), *SCO2* (rs74315511 and rs8139305), *CCDC102B* (rs11873439) *Chromosome 15q14* (rs634990), *PAX6* (rs644242) and *BLID* (rs577948). Demographic, ophthalmic and environmental factors were also analyzed. **Results:** The allelic and genotype frequencies of all studied SNPs did not show statistical differences between MM group and control groups. The genetic analysis showed that *COL8A1* SNP rs13095226 was associated with myopic choroidal neovascularization (mCNV) and also seems to play an important role in the increase of axial length (AL). The SNP rs634990 of *chromosome 15q14* also showed a significant association with MM, although this was lost after the Bonferroni correction. Age, sex, smoking habit, pregnancy history, refractive error, AL and posterior staphyloma, were also associated with MM and mCNV in this population. **Conclusions:** None of the studied SNPs associated with HM and MM in Asiatic populations showed any relation in this European cohort. Some demographic and ophthalmic factors were related with MM and mCNV.

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Regulation of Inflammasome Complex in Mouse Retinas after a Traumatic Unilateral Damage

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Purpose: To analyze the implication of the inflammasome complex in the course of axotomy-induced retinal ganglion cell (RGC) death and to study the expression of several inflammasome components, as well as molecules downstream its activation. **Methods:** The left optic nerve of adult WT and ASC$^{-/-}$, NLRP3$^{-/-}$ or Caspase-1/11$^{-/-}$ C57BL/6 mice was intraorbitally crushed (ONC). As controls, intact animals were used. RGC survival was assessed in whole-mounted retinas (5, 9 and 21 days post-lesion, dpl). NLRP3, NLRP6, NLRC4, ASC, Caspase-1, Caspase-11, IL-1β, IL-18, IL6, TNFα, GSDMA, GSDMC, GSDMD, GSDME and PJVK expression was measured by qRT-PCR in retinal extracts of WT animals at 1 and 5 dpl. **Results**: The course of RGC loss was similar in WT, ASC$^{-/-}$, and NLRP3$^{-/-}$ mice, but it was significantly lower in Caspase-1/11$^{-/-}$ mice at all time points. NLRP3, NLRC4, Caspase-1, Caspase-11, GSDMD, IL-6 and TNFα were up-regulated after ONC compared to intact retinas. **Conclusions:** Together with the prevention of RGC degeneration showed in deficient Caspase-1/11 mice, the regulation of several genes of inflammasome complex suggests the implication of this pathway in retinal degeneration and neuroinflammation triggered by a traumatic unilateral damage.

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Changes in the Retinal Ganglion Cell Population in Experimental Glaucoma in Old and Adult Mice

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Purpose: Experimental models of glaucoma are usually carried out in adult mice and not in old mice which are more representative of human glaucoma. We compared retinal ganglion cell (RGC) populations in experimental glaucoma (EG) with sustained moderate intraocular pressure (IOP) increase in old and adult mice. **Methods**: Old (24-years) and adult (3-month) Thy1-YFP-line-H mice was used. EG was induced by in-situ cross-linking hydrogel injected in the anterior chamber of the left eye. Mice were imaged with in vivo optical coherence tomography (OCT) and fluorescence confocal scanning laser ophthalmoscopy (CSLO) and at 7, 14, 21 and 28 days after IOP elevation. We analyzed the ganglion cell complex (GCC) thickness (with OCT) and the dendritic arbor of the YFP+ RGCs (with CSLO) longitudinally. Immediately after the last imaging session, the retinas processed and immunoreacted against RBPMS to quantify RGC density and loss in the EG compared to the normal contralateral eye. **Results**: In the EG eyes, IOP increased by a mean (±SD) of 11.3 (±4.7) mmHg in old mice and 12.0 (±3.5) mmHg in adult mice. In EG eyes there was a mean RGC loss of 22.4 (±8.5)% and 20.8 (±7.6)% loss in old and adult mice, respectively. After 4 weeks of IOP elevation, there was a decrease of 11% and 9% of the GCC thickness in old and adult mice. Finally, there was a 60% and 20% reduction in dendritic arbor complexity at 4 weeks compared to the baseline in old and adult mice, respectively. **Conclusions**: Sustained IOP elevation caused a loss of RGCs and a decrease of the GCC thickness which was marginally greater in older animals. However, loss of RGCs dendritic arbor complexity was much more notable in older animals indicating the sensitivity and value of single RGC analysis.

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Role of Dietary Patterns and Food Intake in Age-Related Macular Degeneration.

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Purpose: To examine the best scientific evidence available on the role of dietary patterns and food intake in the prevention of age-related macular degeneration (AMD). **Methods:** A systematic literature review was conducted. Sixteen high-quality studies published in the last 5 years, whose main objective was to assess this possible association, were identified. **Results:** Higher adherence to a Mediterranean diet (determined by aMeDi score ≥6) was significantly associated with a reduced risk of progression to advanced AMD after confounder-adjusted analysis. Other major pattern like Oriental diet had decreased association with AMD prevalence (at any stage). Whereas, Western pattern were strongly associated with both early and advanced AMD, increasing their relative risk significantly. The foods that have shown the highest level of evidence for the prevention of AMD, in advanced stages, are oily fish and green leafy vegetables. Likewise, certain nutrients such as omega-3 fatty acids (DHA, EPA), lutein, zeaxanthin and zinc, have shown this effect individually. **Conclusions:** There are multifactorial influences of diet and food intake on the incidence and progression of AMD. Our work as health professionals should not be limited only to the treatment of this pathology, it has to start much earlier, from its prevention, where nutrition plays a fundamental role as a modifiable risk factor. In our environment, adherence to the Mediterranean diet should be strongly recommended for all patients at risk of advanced AMD progression.

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CEP290-Related Cone-Rod Dystrophy: Clinical Characteristics, Imaging Findings and Genetic Results

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Purpose: To describe the clinical characteristics, the imaging findings and the genetic results of a patient with cone-rod dystrophy (CORD) related to mutations in CEP290. **Methods:** A case report of atypical CEP290-related CORD. Ophthalmological examination was performed, including best-corrected visual acuity (BCVA), fundus photography, fundus autofluorescence (FAF) imaging, optical coherence tomography (OCT), a visual field test and electroretinography testing. The genetic test was performed by Next Generation Sequencing (NGS)-based panel test containing 336 genes. **Results:** A 57-year-old female who had reported a visual loss for five years. BCVA was 20/100 in both eyes. The fundus examination revealed a hypopigmented halo around the fovea, showing a paracentral hyperautofluorescent ring on FAF. OCT demonstrated the presence of atrophy in the outer retinal layers. The genetic test identified the probably pathogenic variants c.4028delA and c.5254C>T in compound heterozygosis in CEP290. **Conclusions:** This is the first report to present the clinical characteristics, imaging findings and genetic test results of a patient with CEP290-related CORD. Our case contributes to expanding the clinical involvement of CEP290 pathogenic variants. This study indicates that CEP290-related CORD may have a mild phenotype with late-onset dystrophy, making these patients interesting candidates for innovative treatments such as genetic therapeutic approaches.

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New Mouse Model of Central Areolar Choroidal Dystrophy Generated by CRISPR Reproduces a Human Prph2 Mutation Dystrophy

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Purpose: Central choroidal dystrophies are retinal diseases characterized by a progressive retinal degeneration and an atrophy of the choriocapillaris. Patients suffering from these dystrophies usually present a single mutation in the PRPH2 gene. The aim of this work was to generate a mouse model with the same p.Arg195Leu mutation that was described in diagnosed human patients. **Methods:** The Prph2$^{KI/WT}$ and Prph2$^{KI/WT}$ mouse models have been designed and generated using the CRISPR system to introduce the Arg195Leu mutation. The functional state of the retina was studied from 1 to 12 months using electroretinography and the optomotor test was used to determine visual acuity. The structural state of retinal layers was assessed *in vivo* by optical coherence tomography imaging. In addition, the state of retinal cells was evaluated by immunohistochemistry in cryosections. **Results:** Genetic sequencing of the Prph2$^{KI/WT}$ and Prph2$^{KI/WT}$ mouse models confirmed the same codon mutation found in humans suffering from this dystrophy. Importantly, mice presented a degeneration pattern comparable to patients. From 3 months of age, the mouse optomotor response was significantly reduced indicating a visual acuity decrease. At 6 months, mice presented reduced ERG responses, with smaller a-wave and b-wave amplitudes, and the number of photoreceptor rows was decreased as well as the retinal thickness and presented an increased inflammation with activation of microglia and Müller cells. **Conclusions:** The new Prph2$^{KI/WT}$ and Prph2$^{KI/WT}$ mouse models present a similar degeneration pattern than the one observed in patients and therefore, they will facilitate the analysis of the pathophysiological process, being a suitable model for evaluating different therapeutic strategies.

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Persistent Retinal Microvascular Impairment in COVID-19 Bilateral Pneumonia at 6-Months Follow-up Assessed by Optical Coherence Tomography Angiography

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Purpose: The aim of this study was to evaluate the long-term evolution of retinal changes in COVID-19 patients with bilateral pneumonia. **Methods:** A total of 25 patients with COVID-19 bilateral pneumonia underwent retinal imaging 14 days after hospital discharge with structural optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA) measurements and were compared with a cohort of age- and sex-matched controls. Vessel density (VD) and foveal avascular zone (FAZ) area were evaluated in the superficial and deep capillary plexus (SCP, DCP). A total of 17 of these COVID-19 patients underwent retinal imaging at 6-months follow-up. **Results:** The parafoveal retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) were significantly thinner in COVID-19 patients at 6 months compared to 0 months ($p = <0.001$ in both cases). In the optic nerve analysis, a significantly thinner RNFL was observed ($p = 0.006$) but persisted significantly thickened, compared to controls ($p = 0.02$). The vascular density (VD) at 6 months persisted significantly decreased when compared to the control group, and no significant differences were found with the 0 months evaluation; in addition, when analyzed separately, women showed a worsening in the VD. Moreover, a significantly greater foveal area zone (FAZ) ($p = 0.003$) was observed in COVID-19 patients at 6 months, compared to 0 months. The cotton wool spots (CWSs) observed at baseline were no longer present at 6 months, except for one patient that developed new ones. **Conclusions:** This study demonstrates that some of the microvascular alterations from COVID-19 previously reported by our group persist over time and are still evident 6 months after hospital discharge in patients who have suffered from bilateral pneumonia.

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Intraocular Injection of PLGA Microspheres to Produce an Animal Model of Glaucoma with Progressive Neuroretinal Degeneration

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Purpose: To produce progressive degeneration of neuroretinal tissue through sustained elevated intraocular pressure (IOP) by intracameral injection of PLGA microspheres (MSs) in rat eyes. This glaucoma animal model was compared to the episcleral vein sclerosis (EPI) model. **Methods**: PLGA-MSs (2µL suspension; 10% w/v) were injected to the right eye of 39 rats: 38–20µm particles were injected into 14 eyes (MSs38/20 model) and 25 eyes received 20-10µm particles (MSs20/10 model). Moreover, 25 animals received hypertonic saline solution (1.8M) through episcleral vein injections. Administrations were performed at baseline, 2, 4 and 6 weeks. Clinical signs, IOP, retina and optic nerve thicknesses (optical coherence tomography; OCT), and histological studies were performed. **Results:** IOP increased in all groups resulting in lower values from the PLGA-MSs injection (Ms38/20 produced widest fluctuations). EPI and MSs20/10 models showed no significant differences at 8 weeks. A significant decrease was observed in OCT parameters and in histological ganglion-cell count in all models throughout the 8-week follow-up. Better preservation of the ocular surface was observed for animals receiving MSs. **Conclusions:** Injection of PLGA-MSs (38–20µm and 20-10µm) into the anterior chamber of rats increased IOP, with the MSs20/10 and EPI models producing similar values at 8 weeks. In all cases, progressive degeneration of the retina, retinal ganglion cells and optic nerve (simulating chronic glaucoma) was detected by OCT and histological study.

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Morphological and Functional Characterization of Mouse Amacrine Cells Expressing TRPM8 Ion Channel

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Purpose: Novel transgenic mice expressing fluorescent reporters associated to TRP channels have improved morphological studies of protein location. Here we report the expression of TRPM8 cold-transducing ion channel in a subset of retinal neurons. This study also aims to study the function of TRPM8 in the retina. **Methods**: Retinas from TRPM8-EYFP and TRPM8-KO mice were used to detect by immunofluorescence Calretinin, Calbindin, Parvalbumin, Tyrosine hydroxylase, Choline acetyl transferase, GAD 65/67 and mGluR2. Prior to morphological analysis, WT and KO mice were examined by electroretinography (ERG) and optomotor tests to evaluate the functional role of TRPM8 in the retina. ARVO statements for the use of animals were followed. **Results**: We described a population of TRPM8-positive amacrine cells with cell bodies distributed in the internal nuclear layer (INL) and ganglion cell layer (GCL), forming two densely packed neuropil layers coincident with ChAT+ sublayers 2 and 4 of the inner plexiform layer (IPL). Retinal amacrine cells from TRPM8-KO mice did not express TRPM8 protein or mRNA and were less numerous than in WT mice. Electroretinographic analysis showed alterations in oscillatory potentials and b-wave in KO mice. Optomotor test showed impaired movement discrimination in KO mice compared with WT. **Conclusions**: A subtype of amacrine cells expressing TRPM8 channels has been identified. It appears unlikely that TRPM8 channels expressed by these neurons are involved in cold detection. In contrast, the expression of ChAT and the alterations in oscillatory potentials strongly suggest that about 60% of these cells may be starburst-like cells and could be involved in directional selectivity.

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Evaluation of the Axial Symmetry at the Corneal Vertex of Bilateral Normal Corneas

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Purpose: To investigate interocular symmetry using canonical representations of global corneal topography between fellow eyes. **Methods:** Scheimpflug topographical images of 32 eyes from 15 healthy patients taken with Sirius tomographer were used. Raw elevation data were fitted to the canonical quadratic model of Navarro using as reference a real Cartesian coordinate system of the tomographer. Typical corneal shape intrinsic symmetry axes were obtained by algebraic operations. Comparison between fellow corneas were statistically analysed using shape (Rs, Ry, Qx, Qy), translation (x0, y0, z0) and rotation (α, β, γ) parameters. **Results:** The quadratic model provided a mean RMS fitting error for anterior/posterior surfaces of the right cornea (1.14* 10-13 ± 7.033 * 10-14/1.21* 10-13 ± 5.26 * 10-14) and the left cornea (1.59* 10-13 ± 1.14 * 10-14/1.82* 10-13 ± 1.31 * 10-13). Significant differences were found between anterior surface of fellow eyes for x0, y0, y β parameters; and for posterior surface, significant differences were found for γ, with a prevalence of negative values in left eye. Regarding translation, the vertex of both eyes is more frequent in nasal-inferior region, referred to the intrinsic symmetry axes. Regarding rotation, angles α y β distribution is alike in both corneas. **Conclusions:** The analysis of axial symmetry in corneal vertex has demonstrated a strong specular symmetry referred to plane YZ (translation) and a strong direct symmetry referred to plane XY in nasal region (rotation). These characteristics could help in the diagnosis of corneal diseases.

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Morphological Changes of Microglial Cells: Comparison between Retinal Organotypic Cultures and *In vivo* Axotomized Retinas

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Purpose: The objective of this study was to compare quantitative and qualitatively the population of microglial cells (MCs) in two models of neuronal degeneration in the mouse retina: organotypic retinal cultures, an inherent model of axotomy and retinal detachment; and an optic nerve axotomy *in vivo*. **Methods:** For retinal organotypic cultures, retinas of adult C57Bl/6 mice were dissected and cultured in supplemented Neurobasal medium. For the *in vivo* model, the left optic nerve was crushed (ONC). In both models, the retinas were analyzed at intervals from 24 hours to 9 days (n= 6-8/group) and MC were immunodetected with anti-Iba1 antibody. MCs were photographed, the morphological changes were qualitatively analyzed and their mean density (MC/mm^2) in the ganglion cell layer (GCL) of the central retina was assessed (n=4 micrographs/retina/time point). **Results:** After ONC, MCs are highly branched, with long radial extensions in the direction of the optic nerve. In contrast, the MCs observed in the *in vitro* model are rounder, barely branched, thicker and with fewer extensions. Quantitatively, there is an increase in MCs density in the GCL that is progressive and follows the same trend in both models, although the density of MCs *in vitro* is, at all time points, significantly smaller than *in vivo*. **Conclusions:** The morphology of MCs differs *in vivo* and *in vitro*, suggesting a differential activation. In addition, the density of MCs *in vitro* is lower than *in vivo*, indicating that *in vivo* either MCs or circulating macrophages invade the retina to cope with RGC degeneration.

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Validation of the Recently Developed ATN Classification and Grading System for Myopic Maculopathy

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Purpose: To assess the reliability of the ATN classification in patients with pathologic myopia (PM) and its correlation with best-corrected visual acuity (BCVA). Design: Cross-sectional study. **Methods:** 100 highly myopic eyes with spherical equivalent (SE) > -6.0 D or axial length (AL) >26 mm and a total ATN score ≥3 underwent a complete ophthalmological examination, including fundus photography and swept-source optical coherence tomography (SS-OCT). Five observers graded each eye using the ATN system. Mean A, T, and N scores were calculated and correlated with age, BCVA (LogMAR), and AL. Patients were considered to present severe PM if either A or T components were ≥3 and/or N was ≥2. **Results:** 100 eyes (53 left) from 91 patients (78 females) were classified. Mean age, BCVA, and AL values were, respectively, 65.1±11.7 years (range, 36 to 97), -0.63±0.62 (-3.00 to 0.00), and 29.26±2.7 mm (26.01 to 37.66). Mean ATN grades for each component were: A=2.51±0.78 (0.6 to 4.0), T= 0.88±1.14 (0.0 to 5.0), and N=1.31±1.40 (0.0 to 3.0). Weighted interobserver agreement was 98.1%, 98.7%, and 94.6%, for A, T and N respectively. In eyes with severe PM, BCVA was significantly lower and AL significantly longer. **Conclusions:** The excellent interobserver rate in this study demonstrates that the updated ATN grading system is an accurate and reliable tool to classify patients with PM. These findings show that BCVA is more compromised in eyes with severe PM, particularly those graded ≥ A3 and/or T3.

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Morphological Analysis of the Papillae of Both Eyes in Healthy Individuals. Can We Assume Papillary Symmetry between Both Eyes of the Same Individual?

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Purpose: In clinical practice, the asymmetry of the papillary excavation between both eyes is a warning sign. However, there is little information on the anatomical variability between the optic discs of the same individual. This study provides a comparative morphologic analysis of the optic disc of both eyes in individuals without ocular pathology. **Methods**: In individuals without ocular pathology, retinographies were acquired (Topcon TRC-NW400 non-mydriatic retinograph) and the axial length of each eye was determined. The morphologic characteristics of the optic disc were studied using image processing techniques. Each optic disc was manually segmented by marking the disc and excavation boundaries by two experts. The papillary size and the differences of the CDR (cup-to-disc ratio) were analyzed considering the vertical axis, horizontal axis and area of the papillary/excavation of both eyes, as well as the RDR (rim-to-disc ratio) values establishing the degree of symmetry between the papillae of both eyes of the same individual. **Results**: We studied 163 patients (159 females and 54 males) with a mean age of 59.17±12.63 years. The mean axial length was 23.46±1.10 mm. The papillary area was classified into megapapillae (area >3 mm^2), micropapillae (area <1.9 mm^2), and normopapillae (>1.9 mm^2 and < 3 mm^2) and related to axial length. The difference between vertical CDR, horizontal CDR, and area-based CDR between both eyes was 0.057±0.05 mm, 0.069±0.05 mm, and 0.055±0.04 mm^2, respectively. The difference between the RDR of both papillae was 0.0785±0.07 mm. The CDR showed no significant differences between the different papillary sizes nor between papillary size and axial length. **Conclusions**: In healthy eyes, the differences in papillary size do not condition a greater asymmetry between both eyes. There is no relationship between papillary size and axial length.

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Phenotype Characterization of a Mice Model Retinal Degeneration Induced by Sodium Iodate Intraperitoneal Injection

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Purpose: To characterize the histological retinal degenerations, electrophysiological responses, photoresponse and visual behavioral modifications after sodium iodate ($NaIO_3$) intraperitoneal injection, as an attempt to have an animal model of complete blindness which may become useful in the studies of new ophthalmological approaches. Prior studies have assessed the degenerative capacity of $NaIO_3$, an oxidative stress agent that affects adversely the retinal pigment epithelium. **Methods:** A single dose of $NaIO_3$ (40 mg/kg) was intraperitoneal injected injection into a series of C57BL/6J mice with a mutation in the $Opn4^{-/-}$ gene. The effect of $NaIO_3$ on the retina was assessed at 7, 14, 21 and 28 days after the injection by means of electroretinography (ERG) recordings, photoresponse and behavioral tests and immunohistochemical staining. **Results:** A gradual reduction of the visual capacities of the animals was observed at different times after $NaIO_3$ injection. The pupillary reflex showed a decrease in amplitude after agent injection, reaching an absolute photoresponse abolition by 28 days testing. Similar results were observed in the optomotor test. Likewise, the components of the ERG waves showed a marked decrease in the amplitude along 28 days period after $NaIO_3$ injection. Surprisingly, the immunohistochemical studies showed no effect on retinal cellularity and just few irregularities at the outer retinal layers. **Conclusions:** The assessment of the retina structure and function in the $NaIO_3$ animal model reveals a decrease of its functional capacity after the $NaIO_3$ injection but not mayor structural changes. More detailed immunohistochemical studies will be necessary to confirm the loss cell functionality.

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Diagnosis and Classification of Diabetic Retinopathy. Artificial Intelligence Versus Medical Evaluation

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Purpose: To evaluate the agreement between the diagnosis and classification of diabetic retinopathy (DR) and diabetic macular edema (DME) by a retinal specialist with a fundoscopic examination and artificial intelligence (AI) reading of fundus images. **Methods:** A clinical evaluation of 100 diabetic patients was done by a retinal specialist with diagnosis and classification of DR and DME. In the same visit mydriatic color fundus photos of both eyes (one centered in the macula and one centered in the optic nerve head) were obtained with the DRI OCT Triton plus®. The images were then exported to the EyeArt v2.1.0, EyeArt AI Eye Screening System, Automated DR Screening software ® for analysis. The diagnosis and treatment were dictated by the retinal specialist criteria based on clinical evaluation and an informed consent was obtained from the patients. The results of the ophthalmological evaluation were compared with the diagnosis and classification of DR and DME of the AI software. The statistical analysis was performed with SPSS statistics, and the agreement between the two diagnosis methods was calculated using Kappa coefficients. **Results:** We analyzed the data of 100 diabetic patients with a mean age of 61.3± 13.2 years (from 23 to 90 years). The AI software analyzed 85% of the images and was inconclusive in cases with proliferative DR treated with retinal photocoagulation or media opacities. The agreement between the ophthalmological exam and the AI regarding the diagnosis of DR showed a Kappa coefficient of 0.49 (Standard Error (SE) 0.68), $p<0.05$. The agreement regarding the diagnosis of DME was 0.42 (SE 0.9), $p<0.05$. The interobserver agreement for the classification of the DR showed a Kappa statistic of 0.27 (SE 0.51) $p<0.05$. **Conclusions:** Our study shows that AI interpretation was not possible in cases with other ocular findings. There was moderate agreement between the AI software and the clinical diagnosis of DR and DME and fair agreement in the classification of DR.

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Vascular Study of the Optic Nerve and Macula with OCT-A in Patients with Primary Open Angle and Pseudoexfoliative Glaucoma

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Purpose: Vascular conditions are risk factors for glaucoma. Optical coherence tomography angiography (OCT-A) has been introduced in the vascular study of glaucoma. OCT-A is an opportunity to analyze vascular differences between primary open angle glaucoma (POAG) and pseudoexfoliative glaucoma (PEXG). To evaluate the reproducibility of the parameters of vascular density and blood flow index of the optic nerve and macula in healthy individuals using OCT-A. The same parameters will be evaluated in patients with POAG and PEXG. **Methods:** The Swept-Source Plex Elite 9000 OCT-A device was used. Two studies were designed. Study I: to evaluate the reproducibility: 18 healthy people (29.11 ± 4.04 years old) were included. Study II: 136 subjects (70.3 ± 11.86 years old) were included. **Results:** Study I; related to the optic nerve the peripapillary RNFL flow index (FI) (83.11% of the variability is explained with a single factor) and the capillary perfusion density (CPD) of the peripapillary RNFL (65.97% of the variability is explained with a single factor). Related to retinal macular vascular perfusion density (86.83% of variability is explained with a single factor). Study II; Peripapillary RNFL FI, peripapillary CPD, perfusion density in global retina, superficial and deep plexus were significantly higher in healthy subjects than in PEXG and POAG. But no differences were found according to the type of glaucoma. **Conclusions:** The reproducibility of the variables measured with OCT-A is good in healthy subjects. Glaucoma patients present a decrease in both macular and peripapillary vascular density measured with OCT-A compared to healthy patients. No differences were found in the OCT-A parameters studied between POAG and GPEX patients.

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Neuroprotective Effect of Immunosuppression and Minocycline Administration after Optic Nerve Crush

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Purpose: Modulation of microglial activation and inflammation is a promising treatment after traumatic and degenerative lesions in the central nervous system. We purpose to assess the effect of the selective microglial inhibition (daily intraperitoneal minocycline administration) and systemic immunosuppression (daily intraperitoneal cortisone administration and cyclosporine A supplementation in water). on retinal ganglion cell (RGC) survival after optic nerve crush (ONC)**.**

Methods: Sprague Dawley albino female rats were divided into 6 groups (n=6/group): i/ intact, ii/ intact plus microglial inhibition, iii/ intact plus systemic immunosuppression, iv/ ONC in left optic nerve, v/ONC and microglial inhibition and, vi/ ONC and systemic immunosuppression. Animal were perfused after 7 or 21 days and flatmounted retinas immunodetected against Brn3a and Iba1. Total $Brn3a^+$RGCs were quantified, and their spatial distribution analyzed with isodensity maps.

Results: Microglial morphology is altered in all treated groups compared to their control ones. Twenty-one days of immunosuppression or microglial inhibition does not affect the RGC population in intact retinas. At 7 days after ONC the number of RGCs is significantly higher in the minocycline ($p<0.005$) and immunosuppressed groups ($p<0.001$) compared to ONC alone. Neuroprotection afforded by systemic immunosuppression is significantly higher than microglial inhibition by minocycline ($p<0.01$). **Conclusions:** In rats, specific microglial inhibition and systemic immunosuppression delay axotomy induced-RGC death. Because RGC neuroprotection is significantly higher after immunosuppressing than after specific microglial inhibition, there must be a crosstalk between the central nervous system and the systemic immune system that plays an important role in RGC death after ONC in rats.

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Morphological Evaluation of Meibomian Gland in Relation to Epidemiological Factors and Ocular Surface Parameters

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Purpose: To assess the morphology of Meibomian glands quantified by non-contact infrared meibography and the relationship with epidemiological characteristics and ocular surface clinical parameters. **Methods**: A total of 116 eyes of Caucasian adults, 75 women and 41 men with a mean age of 48.87 (20-87) years were enrolled in this cross-sectional and analytical study. No-contact infrared meibography was performed to evaluate the loss of Meibomian glands (meiboscore) and the influence of epidemiological characteristics such as age, sex, type 2 diabetes mellitus, hypertension, dyslipidemia, toxic consumption and drug use such as thiazide diuretics, angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor antagonists (AIIRA), anxiolytics, antidepressants, antipsychotics, thyroxine, contraceptives and antiandrogen therapy; as well as the presence of cutaneous affections (rosacea, seborrheic dermatitis, atopic dermatitis, and psoriasis). In addition, the relationship between mebioscore with ocular surface disease index, tear break-up time, fluorescein corneal staining, Oxford scale, eyelid margin abnormalities and Schirmer's test was also studied. **Results:** The values obtained in the mebioscore were cataloged in three grades, from less to greater affectation being 47.41% grade 1 (N=55), 36.21% grade 2 (N=42) and 16.38% grade 3 (N=19). Statistically significant results were found ($p<0.05$) when relating the mebioscore with age ($p=0.0012$), type 2 diabetes mellitus ($p=0.013$), hypertension ($p=0.012$), consumption of ACEi/AIIRA ($p=0.035$) and eyelid margin abnormalities explored with slit lamp ($p=0.027$). **Conclusions:** The morphology of Meibomian glands can change in relation to different epidemiological factors, being useful for the study of these glands the exploration by slit lamp and objective methods such as Meibography.

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Corneal Ulcer Infiltrated by Epicoccum Nigrum

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Purpose: To highlight the relevance of the study of microbiological corneal samples in the treatment and typing of atypical corneal ulcers. Epicoccum Nigrum is a fungus belonging to the yeast category. It is found in cereal and fruit crops. It is used in the medical and agricultural industry as an antimicrobial. **Methods:** We present a descriptive study (case report). We have performed a clinical, imaging and microbiological follow up of a patient with an atypical corneal ulcer in the right eye. A corneal scraping was performed for microbiological analysis, resulting in the presence of a filamentous fungus. The sample was sent to a specialized laboratory in Majadahonda confirming the presence of Epicoccum Nigrum in the corneal tissue. **Results:** The patient presented with decreased visual acuity and pain in the right eye unrelated to trauma, nor is she a contact lens wearer. The patient lives in a rural area and works as a fruit picker. Examination showed an infiltrated corneal ulcer with significant epithelial defect and stromal edema. The best corrected visual acuity was 0.05. A sample was taken for culture and treatment with Voriconazole and Chlorhexidine was started. The culture confirms the existence of filamentous fungus, and treatment with topical Natamycin was started. The culture was analyzed in a laboratory outside our center and was positive for Epicoccum Nigrum. After 10 months with no activity observed, penetrating keratoplasty was performed on the right eye and the patient currently has a better corrected visual acuity of 0.3. **Conclusions:** This is the first clinical case in which the presence of Epicoccum Nigrum is detected in corneal tissue. There are no previous cases documented in scientific literature. Due to the difficulty in confirming its presence in cultures in non-specialized centers, we could suppose that perhaps its prevalence in corneal ulcers was higher than what is known.

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Cone Impairment and Microglia Activation in Human Donor Retinas with COVID-19

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Purpose: Several ocular alterations have already been observed along with the presence of SARS-CoV-2 in the eye although the possible morphological disturbances of retinal cells in COVID-19 patients are unknown. Thus, the morphology of the retinal cells and the state of microglia cells in the retinas of human donor deceased by COVID-19 were studied. **Methods:** Retinas of human donors with SARS-CoV-2 infection (n=9) and control subjects (n=5) obtained from the General University Hospital Consortium of Valencia were analyzed. Immunohistochemistry staining in cross-sections and wholemount retinas was performed to study the photoreceptors and the retinal structure (using markers as calbindin, recoverin, GFAP, CRALBP and Collagen Type IV), the presence of ACE2 in the retina and the microglia cells (using Iba-1). Confocal microscopy imaging and a quantitative analysis of Iba-1 positive cells were carried out. **Results:** COVID-19 and control patients had a mean age of 77±11 and 68±7 years, respectively. Most patients of both groups had arterial hypertension. ACE2 immunostaining was observed in Müller cells, retinal pigment epithelium and in the outer segment of photoreceptors. Some COVID-19 patients presented a weaker expression of ACE2 protein in the outer segment of photoreceptors. Swelling of the axon terminal of cone photoreceptors and alterations in the morphology of Müller cells were found in the retina of several patients. Microglial cells changed from a ramified morphology to an ameboid-shape and most of them migrated to the retinal vessels. In addition, a reduction of the total area occupied by these cells was also found which confirmed a microglia activation in the retina of COVID-19 group. **Conclusions:** Retinas of COVID-19 patients showed a microglia activation, variations in the ACE2 immunostaining and morphological alterations in the cone photoreceptors as well as Müller cells.

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Co-Encapsulation of Glial Cell-Line-Derived Neurotrophic Factor (GDNF) and Tauroursodeoxycholic Acid (TUDCA) in PLGA Microspheres for the Therapy of Retinal Diseases

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Purpose: To develop a multi-loaded PLGA-microspheres (MSs) incorporating two neuroprotectants agents (glial cell-line-derived neurotrophic factor -GDNF- and tauroursodeoxycholic acid -TUDCA-) as a potential tool for retinitis pigmentosa treatment. **Methods:** To MSs preparation, a solid-in-oil-in-water emulsion was employed. Moreover, particle size, morphology, encapsulation efficiencies and *in-vitro* release studies were evaluated. To optimise the formulation, different technological parameters were considered such as the incorporation of a water-soluble co-solvent (EtOH) to methylene chloride (MC) in the internal organic phase, the addition of NaCl and a viscosifying agent (hydroxypropyl methylcellulose -HPMC-) in the external aqueous phase. Preliminary *in-vivo* studies were carried out in P23H heterozygous mice (n=7) in which ERG recordings at P120 and P180 were studied. **Results:** Optimised MSs formulation resulted in using a MC/EtOH ratio of 75:25 and HPMC (1%w/v). In comparison with initial formulation, GDNF and TUDCA encapsulation efficiencies increased 20.33% and 32.93%, respectively, and the initial release of GDNF decreased 38.64%. A sustained *in-vitro* release of both compounds over 91 days was observed. No significant differences were found in a- and b-wave amplitudes at P120 and P180 between animals receiving a single blank-MSs injection and the GDNF/TUDCA-MSs. **Conclusions:** the GDNF/TUDCA-MSs created in this work were able to sustained release both neuroprotective compounds for at least 91 days**.** Further studies including several administrations are necessary to evaluate the efficacy of the formulation.

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Ophthalmologic Evaluation of Patients Treated with Hydroxychloroquine in Health Area Seven of the Region of Murcia (Spain)

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Purpose: An observational retrospective transversal study was performed to evaluate hydroxychloroquine therapy ophthalmologic monitoring in the health area seven of Murcia, Spain. **Methods:** Through the pharmaceutical management service of the health system of Murcia, the identification number of the patients in health area seven of Murcia who had been prescribed hydroxychloroquine between January and November 2019 was provided. Afterwards, demographic data, prescribing specialty and pathology for which hydroxychloroquine was prescribed, year of beginning of the treatment and tests performed for each patient in the ophthalmology department were recorded and analyzed. **Results:** Finally, 202 patients were studied. The services that prescribed hydroxychloroquine were rheumatology (59.9%), internal medicine (23.3%), dermatology (10.9%) and nephrology (2%). The main pathologies were systemic lupus erythematosus (29.9%), rheumatoid arthritis (25.7%), discoid lupus and subacute cutaneous lupus (8.4%). 63.9% of patients were referred for baseline testing, in which fundus examination was performed in 96.9%, optical coherence tomography (OCT) in 33.3% and no retinography was performed. Monitoring was performed in 61.8%, with autofluorescence in 5.6%, central visual field in 44%, OCT in 71%, retinography in 0.8%, electrophysiological tests in 2.4%, and fundus examination in all the patients. There are significant differences between services and whether the patients were referred for baseline testing ($p=0.03$). The year the treatment begins is related to the performance of monitoring ($p<0.001$). There also was a significant ($p=0.011$) and inverse relationship (correlation coefficient -0.226) between the year the treatment begins and the monitoring frequency, and between the former and the time period until the first revision ($p=0.000034$ and correlation coefficient -0.360). Alterations were found in 1.6% of the baseline tests and 6.5% of the revisions. **Conclusions:** We have found a deviation from the latest guidelines in the monitoring of hydroxychloroquine treatment in our health area. Our objective is to plan common protocols to implement better approaches to improve hydroxychloroquine monitoring.

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Study of Retinal and Choroidal Vascular Network through OCT and Angio-OCT in Patients with High Genetic Risk for Developing Alzheimer's Disease.

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Purpose: Alzheimer's Disease (AD) is the most common form of dementia. In AD patients and preclinical AD, thinning of the choroid and retina and therefore an enlargement of the foveal avascular zone (FAZ), has been observed, giving an opportunity to study those ocular changes as early biomarkers of AD. **Methods:** The present study evaluates choroidal thickness and retinal FAZ in 184 individuals asymptomatic at risk of developing AD, by optical coherence tomography (OCT) and OCT-angiography (OCTA). The participants were divided into individuals who have at least one first-degree relative suffering AD (n=127) or individuals without familiar history of AD (n=57), adding two more covariates: having at least one ε4 allele for the APOE gene (n=67) and having retinal hard drusen (n=54). **Results:** Choroidal thinning was statistical significate at superior and inferior sectors in ApoE ε4- controls as compared to ApoE ε4- controls with drusen ($p<0.03$); as well as at superior sector in ApoE ε4- relatives with drusen ($p<0.04$) and superior and inferior sectors in ApoE ε4+ relatives with drusen ($p<0.02$, $p<0.04$ respectively) as compared to ApoE ε4- controls with drusen. The enlargement of superficial FAZ was statistically significant in ApoE ε4- relatives ($p<0.001$) and ApoE ε4+ relatives ($p<0.01$) as compared to ApoE ε4- relatives with drusen. **Conclusions:** Oculo-vascular changes were not observed in healthy asymptomatic individuals at risk for developing AD. Monitoring of healthy subjects with drusen, choroidal thinning and enlargement of superficial FAZ, should be done to determine whether they will develop AD or age related macular degeneration.

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Development of Thermo-Responsive Hydrogel Formulations Based on PLGA-PEG-PLGA as Novel Strategy for Co-delivery of Retinal Neuroprotective Agents through Intravitreal Administration

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Purpose: Neurodegenerative diseases of the retina are one of the leading causes of blindness in the world. Most degenerative processes require the delivery of neuroprotective active substances overtime at the target site of action (retina). The present work aims to develop a thermoresponsive hydrogel (HyG) based on PLGA-PEG-PLGA for intravitreal administration able to deliver neuroprotective agents to the retina over extended periods of time. **Methods:** Hydrogels (HyG-1 and HyG-2) were developed by combining different PEG:LA:GA ratios (1:1.54:23.1 and 1:2.25:22.5 respectively). Hydrogels were physiochemically characterized and gelation was evaluated. HyG-1 was loaded with dexamethasone (0.2%) or ketorolac (0.5%) alone and combined with idebenone (1μM) or vitaminE-PEG (0.002%). *In-vitro* release studies and cell-tolerance of formulations were assessed. Oxidative-stress studies were performed in retinal epithelial cells by inducing cell death through H_2O_2 addition Furthermore, anti-inflammatory ability of formulations to reduce TNFα was evaluated. **Results:** HyG-1 (25% w/v) showed low polydispersity index (PDI=1.22) and strong gelation at 31-34ºC. Sustained release of dexamethasone and ketorolac was achieved for 70 and 50 days respectively). Tolerance of single HyG-1 was 84.49±3.20% with excellent values (100%) when including neuroprotective agents. Idebenone addition (0.2%), promoted high protection to oxidative stress (86.24±14.68%). Finally, ketorolac-based formulations were the most effective in reducing TNF-α albeit those loaded with dexamethasone also showed significant results. **Conclusion:** The hydrogel formulations developed in the present work result to be a potential biodegradable and biocompatible therapy to treat retinal diseases that develop with neurodegeneration overtime.

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Cellular-molecular-genetics Insights into the Role of p53 Gene in the Pathogenesis of Retinopathies. The Super p53 Mice Model

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Purpose: Retinal diseases cause visual impairment and blindness worldwide. Recognized pathological mechanisms underlying the retinopathies are oxidative stress (OS), angiogenesis (AG), apoptosis (AP), and neurodegeneration (ND). New clues on identifying cells, molecules and genes regulating these processes are highly required. Biological p53 gene actions involve mainly cell-cycle regulation, AP induction, cell differentiation, development, AG inhibition, OS regulation, neuroprotection (NP), telomere protection, cell senescence-ageing and age-related diseases. We evaluated the role of p53 gene in the normal/pathologic retina by cellular-molecular-genetics assessments in the transgenic super p53 mice model (sp53-tg/tg). **Methods:** 72 eyes from sp53-tg/tg mice (carrying 2 extra p53 gene copies; n=18) and wild-type mice (wt; n=18) were enucleated and 72 retinas were processed to histochemistry-immunohistochemistry, enzyme-like immunosorbent assays and western blot-immunoblotting. Data were analyzed by the SPSS-24.0 program. **Results:** Whole-mount retinas showed striking immunohistochemical changes in sp53-tg/tg retinal astroglia. The p53 signaling pathway was activated in the sp53-tg/tg mice, by p53 gene exerting a set of retinal activities by regulating outstanding OS, AG, AP, and ND/NP processes. Expression of malondialdehyde, vascular endothelial growth factor, poly(ADP-ribose) polymerase-1, and brain derived growth factor was significantly higher in the sp53-tg/tg versus wt retinal homogenates (p=0.043, p=0.002, p=0.0016, p=0.00003 respectively). **Conclusions:** Within the realm of the genetic regulation of retinopathies and the ability of p53 gene to trigger proliferation arrest/cell death upon the occurrence of a variety of stresses, including those affecting the eyes, data support an interesting set of p53 gene retinal regulations that may help to design alternative diagnostic-therapeutic strategies for the most prevalent and challenging sight-threatening retinal diseases.

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Silicone Oil-Related Complications as Intraocular Tamponade

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Purpose: Silicone oil (SO) still represents the main choice for long-term intraocular tamponade in complicated vitreoretinal surgery. This review compared the complications associated with the use of SO and other vitreous substitutes after pars plana vitrectomy in patients with different diseases. **Methods:** This meta-analysis was conducted in accordance with the PRISMA guidelines. We retrieved retrospective case-control studies and randomized clinical trials (RCTs) evaluating the risk of SO published between 1994-2020, conducting a computer-based search of the databases. The primary outcome was the rate of complications such as intraocular hypertension, retinal re-detachment, unexpected vision loss, or hypotony. The secondary outcome was to compare the rate of adverse events of different SO viscosities, including particularly emulsification. **Results:** Forty-two articles were included. There were significant differences in intraocular hypertension (P=0.0002, OR=1.66; 95% CI=1.27-2.18) and retinal re-detachment (P=0.0009, OR=0.65; 95% CI=0.50-0.64) between SO and other agents, including placebo. However, there were no differences in other complication rates. SO-emulsification is non-significantly higher in low than high SO viscosity and results from other complications were comparable in both groups. **Conclusions:** The high quality of most of the studies included in this study is noteworthy, which provides some certainty to the conclusions. Among them, the high variability of the residence time of the SO. The fact that ocular hypertension and not hypotension are related to SO use. That a clear relationship is not found for the so-called unexplained vision loss, which affects a significant percentage of eyes. The cases of re-detachment are less if SO is used and that surprisingly there does not seem to be a relationship in the percentage of emulsification between the low and high viscosity silicones.

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Systemic Administration of Lipopolysaccharide (LPS) Endotoxin Causes Retinal Ganglion Cell Death in Mice

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Purpose: To ascertain whether systemic LPS causes retinal inflammation and neuronal death, and to investigate if this effect is the same in males and females. **Methods:** Male and female C57BL/6J mice were used in this work. To assess the right LPS dose male mice were administered one single intraperitoneal injection of increasing doses of LPS (2, 3, 4, 5, 7 and 10 mg/kg) and the total number of Retinal Ganglion Cells (RGCs) and microglial activation in the ganglion cell layer was studied at 3 days. Once the right dose was established, males and female were treated with LPS (5mg/kg) and their retinas evaluated at 3 and 7 days. **Results:** Anatomical microglial activation was observed from 3mg/kg onwards. However, with the lowest dose (2mg/kg) there was already a significant loss of RGCs, that significantly increased at higher concentrations (5 and 7 mg/kg). Because there was no difference in RGC death between 5 or 7 mg/kg, we chose 5mg/kg for subsequent studies. The effect of LPS on microglial activation and on RGC death is similar between sexes, but somehow more attenuated in females: RGC loss at 3 days was 13% in females and 20% in males (percent of death compared to intact retinas) and advanced with time, being significantly higher at 7 days (30% females, 34% males). **Conclusions:** Systemic administration of LPS causes retinal inflammation and neuronal death that progresses with time and is softer in female than male mice.

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In vitro, *In vivo*, and *Ex vivo* Sterilization Studies of Dexamethasone PLGA Microspheres for Sub-Tenon Administration

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Purpose: Despite being the preferred route for delivering drugs to the back of the eye in chronic treatments, intravitreal injection can cause unpleasant side effects. Periocular administration of drug delivery systems can represent a route to overcome the limitations associated with intravitreal injections and achieve sustained drug concentrations. **Methods:** Biodegradable dexamethasone-loaded poly(lactic-*co*-glycolic)acid microspheres (DX-MSs) were elaborated using the oil-in-water emulsion solvent evaporation technique, sterilized by gamma radiation (25 kGy) and characterized *in-vitro* (morphology, particle size, production yield, encapsulation efficiency, and *in-vitro* release profile). *In-vivo* evaluation was performed in 22 rabbits. Eyes received 5 mg of DX-MSs (dose: 828µg DX) via sub-Tenon injection. Anterior pole, posterior one and intraocular pressure (IOP) were respectively physically examined and measured from the time of injection until 42 days. Inflammation was then evaluated *ex-vivo* on rabbits' eyes. **Results:** The selected 53-20µm granulometric fraction of DX-MSs exhibited smooth and spherical shape and high drug loading (165.6±3.6µg DX/mg MSs) which remained unaltered after sterilization. *In-vitro* release profile sensibly increased over time after sterilization due to PLGA molecular weight reduction and polymeric matrix changes. For both sterilized and non-sterilized DX-MSs, DX was released for nine weeks. Minimal signs of conjunctival congestion were observed at 1 day and 7 days after DX-MSs sub-Tenon administration in rabbits. Moreover, IOP remained unchanged (18.8±2.8mmHg on experimental eyes) during the follow-up days. Histological analysis showed presence of occasional foamy macrophages and sporadic microspheres residues close to injection site. **Conclusions:** Sub-Tenon administration of microspheres loaded with anti-inflammatory drug was well tolerated, thus it could represent an interesting clinical need for retinal diseases treatment.

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Functional Evaluation in Zebrafish of the Glaucoma Candidate Gene GUCA1C

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Purpose: Primary congenital glaucoma (PCG) is a heterogeneous, inherited, and severe optical neuropathy caused by apoptotic degeneration of the retinal ganglion cell layer. Homozygous nonsense GUCA1C variant (c.52G > T) has been detected by our group in a PCG family. This gene encodes GCAP3, a guanylate cyclase activating protein involved in phototransduction and with a potential role in intraocular pressure regulation. The main objective of this work was to evaluate the ocular effect of GUCA1C loss-of-function (LoF). **Methods:** CRISPR/Cas9 genome editing was employed to obtain a zebrafish line with guca1c LoF. Immunohistochemistry and confocal microscopy on eye cryosections of adult fishes was employed to detect guca1c expression and to characterize phenotypical alterations. **Results:** Sanger sequencing revealed the introduction of an indel mutation predicted to produce a non-functional protein p. (Thr47Argfs*63) in the guca1c knockout zebrafish line. Immunohistochemistry demonstrated the presence of GCAP3 in the non-pigmented ciliary epithelium and retina of adult wild-type fishes. KO animals presented up-regulation of the glial fibrillary acidic protein in Müller cells and evidence of retinal ganglion cell apoptosis, indicating the existence of gliosis and glaucoma-like retinal damage. **Conclusions:** Our data provide evidence for the role of GUCA1C as a candidate gene in PCG and offer new insights into the function of this gene in the ocular anterior segment and the retina.

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Efficacy of XEN Implant in Primary Open-Angle Glaucoma: One-year Follow-up

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Purpose: To assess the hypotensive effect of XEN implant for the treatment of primary open-angle glaucoma (POAG) in the short term. **Methods:** Twenty-six eyes (16 right and 10 left ones) from 26 POAG patients (14 men and 12 women) aged 68.50±9.96 years had XEN implantation. Intraocular pressure (IOP) and the number of topical hypotensive eyedrops used (NHEU) were measured during one year. Comparisons between baseline and follow-up results were calculated using Wilcoxon signed rank test. **Results:** Preoperatively, IOP was 21.53±4.96 mmHg and NHEU was 2.80±0.49. IOP at 1st day (12.52±7.16 mmHg), 1st week (13.80±6.76 mmHg), 1st month (16.92±4.55 mmHg), and 12th month (17.20±2.83 mmHg) were lower than preoperatively ($p<0.05$). In contrast, 3rd month (19.36±6.63 mmHg) and 6th month IOP (20.40±6.58 mmHg) results were similar than preoperative IOP ($p>0.05$). NHEU were found lower at 1st month (0.11±0.43), 3rd month (0.48±0.87), 6th month (0.86±0.88) and 12th month (0.87±0.88) than preoperatively ($p\leq 0.001$ in all comparisons). **Conclusions:** XEN implantation seems to decrease IOP but, mainly, the need of NHEU for the treatment of POAG in the short term.

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Effect of 24-month Nutraceutical Supplementation on Redox Status and Visual Function of Patients with Retinitis Pigmentosa: A Pilot Randomized, Double-blind, Placebo-controlled Trial

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Purpose: It has been widely suggested that oxidative stress plays a major role in the pathogenesis of retinitis pigmentosa (RP). The purpose of this study was to evaluate the effect of 24-month nutritional intervention with antioxidant nutraceuticals on visual function and redox status of RP patients. **Methods**: Thirty-one patients with RP and 35 subjects without systemic or ocular oxidative-stress related disease as controls were enrolled in this randomized, double-blind, placebo-controlled study. RP patients randomly received either a mixture of nutraceuticals containing antioxidant nutrients or placebo daily for 2 years. At baseline and two-year follow-up, dietetic-nutritional evaluations, plasma and aqueous humor concentration of several markers of redox status, and visual function were assessed. Retinal function and structure were assessed by multifocal electroretinogram, optic coherence tomography and visual field test. Bayesian approach was performed to determine the probability of an effect. Region of practical equivalence (ROPE) was used. **Results**: Twenty-five patients completed the follow-up at 24 months. At baseline, we confirmed an imbalanced ocular redox status and, to a lesser extent, altered systemic redox status in RP patients. The Bayesian analysis suggested that 24-month nutraceutical supplementation increased the probability to restore some parameters of ocular redox status and ameliorated visual function in RP patients compared with RP patients who received placebo. **Conclusions**: This study suggest that long-term nutraceutical supplementation could, with high probability, ameliorate ocular oxidative stress and improve visual function in RP.

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Effect of Optical Zone Diameter Modification in Orthokeratology on Peripheral Refraction and Topography

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Purpose: Nowadays, there are few studies that relate the change in diameter of the optical zone of orthokeratology contact lens with the visual function, so this work intended to evaluate the effect of the change in diameter of the optical zone on peripheral refraction, corneal topography and aberrations. **Methods:** A total of 20 myopic subjects between -1.00 and -6.00 D with astigmatism less than or equal to -1.75D, with average age of 21.56 ± 2.6 years, wore three orthokeratology lenses of different optical zone diameter (5.0, 5.5 and 6.0 mm), obtained by a composite topography from the corneal topographer. Peripheral refraction was measured with an open field autorefractometer, and topographic profile was measured with a corneal topographer. High order aberrations were measured with an aberrometer. All measurements were made before the treatment (PRE), after the first night (1N), after a week (7N) and after two weeks (14N). **Results:** there are no statistically significant differences for none of the three designs for peripheral refraction profile ($p>0.05$). Statistically significant differences were found between the initial evaluation and after 15 days of orthokeratology lens wear, however, no statistically significant differences were found between the three lens designs. However, statistically significant differences ($p<0.05$) were found in the corneal curvature profile, with the smaller optical zone diameter flattening the central. No statistical differences in aberrations were found between the three lens designs, but differences were found between the pre-visit and after 15 days of treatment ($p<0.05$). **Conclusions:** Changes in the optical zone diameter of the orthokeratology lens cause changes in the topographic profile, being flatter in the central zone and thicker in the mid-periphery with a smaller optical zone diameter design. While in peripheral refraction a myopic peripheral defocus is obtained, although no differences are found between the three lens designs.

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Brain Inflammatory Response after an Unilateral Optic Nerve Damage in Mice

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Purpose: Unilateral optic nerve crush induces a bilateral response that causes an inflammatory response in the contralateral un-injured retinas. Our aim was to analyze the micro- and macroglial response in other regions of the central nervous system (CNS), either in the visual pathway (superior colliculus, SC) or outside it (hippocampus, H). **Methods:** C57Bl/6 male mice were divided into 3 groups: Optic nerve crush (ONC) of the left optic nerve, SHAM, and intact. Brain cryosections were prepared and microglia was analyzed through Iba1+ cell density and activation (CD68). Macroglial response was studied measuring the area occupied by GFAP signal (considering 100% the surface of GFAP signal in intact brains) and MHCII expression in both SCs and H, i.e. ipsi (i)- and contralateral (c) to the ONC. **Results:** In the ONC group, the cSC showed a gradual and significant increase of Iba1+ and CD68+ cell density over time compared to intact (3d, 146±9%; 9d, 161±10%; 30d, 192±12%) while the elevation in iSC was significant at 30 days (115±5.6%). ONC group presented a similar increase of GFAP+ area in cSC (3d, 138±22%; 9d, 152±35%; 30d, 314±30%) and iSC (3d, 138±29%; 9d, 162±39%; 30d, 264±65%). In the cH, Iba1+ cell density was significantly higher than intact in Sham 30d group, and there was a significant elevation of Iba1+ cell density (144±10%) at 30 days in iH. We did not observe a consistent response of macroglial cells in the hippocampus. **Conclusions:** A local injury in CNS as ONC induces a microglial and macroglial response along the visual pathway while impact in non-visual areas may be long-term dependent.

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Evaluation of Apoptosis during the Neuroprotective Effect of Mesenchymal Stem Cells Secretome over *In vitro* Neuroretinal Degeneration

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Purpose: Retinal degeneration is a common characteristic of many retinal pathologies. These pathologies have a significant social and economic impact and represent one of the main causes of blindness worldwide. Apoptosis or programmed cell death is a normal process that plays a fundamental role in the development of tissue homeostasis. The aim of this study was to evaluate the apoptosis during the neuroprotective effect of mesenchymal stem cells (MSC) secretome over in vitro retinal neurodegeneration. **Methods:** Four experimental conditions were analysed: Fresh porcine neuroretina (NR), NR cultured for 72 hours, NR cocultured with MSC for 72 hours and NR cocultured only with MSC secretome for 72 hours (previously obtained through the coculture of porcine NR explants and MSC under standard conditions for 72 hours). mRNA expression of *BCL2, BAX, CASP-3, CASP-8* and *CASP-9* genes were analysed by qPCR Morphological characteristics and immunoreactivity of AIF protein was analyzed by immunohistochemistry. **Results:** mRNA expression of *CASP-3* gene increased in all experimental condition compared to fresh NR ($p<0.001$). *CASP-8*, *BAX*, *BCL2* and *CASP-9* genes mRNA expression were similar between fresh NR and NR cocultured with MSC for 72 hours ($p>0.05$). In addition, mRNA expression of *BCL2* was also similar between fresh NR and NR cocultured with MSC secretome ($p>0.05$). Immunohistochemical evaluation of AIF protein showed that the immunoreactivity increased in the NR cultured and cocultured for 72 hours and it was located mostly in the outer segment of the photoreceptor layer. The AIF protein immunoreactivity in fresh NR was low and restricted to the ganglion cells layer. **Conclusion:** The MSC secretome decreases apoptosis in vitro retinal neurodegeneration.

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Autophagy Analysis during the Neuroprotective Effect of Mesenchymal Stem Cells Secretome over *In Vitro* Retinal Degeneration

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Purpose: Autophagy is a degradation process mediated by the lysosomal system, that is associated with retinal pathology. The aim of this study was to evaluate the autophagy during the neuroprotective effect of mesenchymal stem cells (MSC) secretome over *in vitro* retinal neurodegeneration. **Methods:** Four experimental conditions were analysed: Fresh porcine neuroretina (NR), NR cultured for 72 hours, NR cocultured with MSC for 72 hours and NR cocultured only with MSC secretome for 72 hours (previously obtained through the coculture of porcine NR explants and MSC for 72 hours). Autophagy process was evaluated by qPCR to measure the mRNA expression of the *mTOR, SQSTM1, LC3B, BCLIN1* and *ATG7 genes*. Additionally, the localization of p62 protein was analyzed by immunohistochemistry. **Results:** mRNA expression of *mTOR, ATG7* and *BCLIN1* genes increased in all experimental conditions compared to fresh NR ($p<0.001$). mRNA expression of *SQSTM1* and *LC3B* genes decreased in all experimental conditions in comparison to fresh NR ($p<0.001$). mRNA expression of *mTOR*, *BCLIN1, SQSTM1* and *LC3B* were similar in NR cocultured with MSC for 72 hours and NR cocultured only with MSC secretome ($p>0.05$). In addition, MSC secretome modified the mRNA expression of *ATG7, BCLIN1*, *SQSTM1 and LC3B* genes in comparison to NR cultured for 72 hours ($p<0.01$). The immunohistochemistry analysis of the p62 protein showed that the immunoreactivity was restricted mostly to the outer segments of the photoreceptors. NR cocultured with MSC and cocultured only with MSC secretome showed similar p62 protein immunoreactivity. **Conclusions:** The MSC secretome has the capacity to modulate the autophagy during the *in vitro* retinal neurodegeneration.

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Axial Length Cut-off Values to Objectively Define Pathologic Myopia and Severe Pathologic Myopia: Clinical Characteristics and ATN Grading System Correlation

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Purpose: This study had two aims: 1) determine AL cut-off values to distinguish between pathologic myopia (PM) and severe PM; and 2) identify clinical differences between PM and severe PM according to ATN grading system. **Methods:** Cross-sectional, non-interventional study. A series of 656 eyes from 352 highly myopic patients were included. All patients underwent complete ophthalmologic examination, ATN grading and multimodal imaging. Results: The eyes were graded on the ATN system and classified as PM (≥A2) or severe PM (≥A3, ≥T3, and/or N2). Significant between-group (PM vs. severe PM) differences ($p<0.05$) were observed on the individual ATN components (atrophic [A], tractional [T] and neovascular [N]). Patients classified with severe PM were older, had longer AL, and worse BCVA ($p<0.05$). ROC curve analysis showed the optimal AL cut-off value to distinguish between PM and severe PM at 28 mm (AUC ROC curve: 0.813, specificity: 75%, sensitivity: 75%) and 29.50 mm (AUC ROC curve: 0.760, specificity: 75%, sensitivity: 70%), respectively. **Conclusions:** The optimal cut-off points for axial length to detect PM and severe PM are 28 mm and 29.5 mm, respectively. These AL cut-off values would help to establish myopic conditions numerically by relying on the clinical features defined previously; and should be taken into account for closer follow-up, ophthalmic management and treatment.

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Mutation Spectrum of Inherited Retinal Dystrophies in a Cohort from the Basque Country (Spain)

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Purpose: Inherited retinal dystrophies (IRD) are a heterogeneous group of diseases that mainly affect the retina, with more than 250 genes involved in its pathogenesis. The clinical and genetic heterogeneity complicate the identification of causative mutations. Here we present the results of a genetic-molecular characterization in a cohort of Basque patients. **Methods:** A retrospective study was carried out on 744 IRD affected individuals (from 266 unrelated families) using different molecular techniques, including gene panel, whole exome sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA) and hybridation arrays. **Results:** Overall, 50% (133/266) of the studied families were genetically characterized. Regarding the inheritance pattern, we found 67% (89/133) of the studied families with recessive inheritance, within which 34% (45/133) were compound heterozygous and 33% (44/133) were homozygous, 28% (38/133) with dominant inheritance, 4% (5/133) with X-linked inheritance, and one case of digenism. 126 different likely causative variants were identified. Most variants, 81, were missense/nosense; 28 were small insertion/deletions, 12 variants affected splice regions, 4 involved copy number variations and 1 was a complex rearrangement. These variants were identified in 42 different genes. The most recurrently mutated genes were USH2A, CERKL and RHO, found in 21, 10 and 10 families respectively. Most frequent pathogenic variants were c.2276G > T (p.Cys759Phe) in USH2A, c.847C > T (p.Arg283Ter) in CERKL and c.3260C > T (p.Ser1087Leu) in SNRNP200, identified in 12, 10 and 8 families respectively. **Conclusions:** Our study allowed us to characterize 50% of the families in our cohort, having important implications for genetic diagnosis and counselling to the Basque population.

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Study of RPE Functions, Proliferation and Apoptosis under Physiological and Pathological Conditions

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Purpose: The retinal pigment epithelium (RPE) is a monolayer of cuboidal and polarized cells important for the blood/retina barrier interface. The aim of this work is to analyze the levels of various proteins involved in the functional roles of the RPE when this epithelium is subjected to the physiological and pathological conditions happening during age-related macular degeneration (AMD). **Methods:** Human RPE cells cultures (hRPE) were exposed to different concentrations of serum from the blood of ophthalmic patients diagnosed with dry and wet AMD or control patients. To quantify the modifications in the levels of diverse hRPE functional proteins, such as MerTK, RPE65 and Pmel17, western blots were performed in lysates of cells that were cultured under the different experimental conditions. In addition, the levels of proliferation and apoptosis-related proteins such as Histone 3 and Caspase 3, respectively, were also analyzed. Additionally, the expression of the basal polarity protein Scribble, was investigated during the polarization and differentiation processes of hRPE cells at 7, 14, and 21 days in culture. **Results:** A significant increase in the levels of Pmel17 and RPE65 was observed in cells exposed to the serum of control and AMD patients in comparison with those of the control conditions (without serum), demonstrating that these cells are activated at the early stages of the disease in an attempt to counteract the damage. In addition, an increase in procaspase 3 is observed in cells exposed to the serum of patients, also indicating that the cells react by down-regulating apoptosis after exposure to a stressful environment. Scribble protein is increased in the developing epithelium as cell polarity and polarity complexes are established. **Conclusions:** When hRPE cells are subjected to different conditions recreating the environment of this epithelium during the development of AMD, they react by becoming more active and reducing apoptotic cell death. Scribble expression increases as hRPE cells in culture become polarized and differentiated.

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Influence of Age in Retinal Thickness Measurements Using Spectral Domain Optical Coherence Tomography in Children

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Purpose: To evaluate the influence of age in the repeatability of retinal thickness measurements in a healthy pediatric population with spectral domain Optical Coherence Tomography (SD-OCT). Knowledge of retinal nerve fiber layer (RNFL) parameters and macular characteristics in a normal population and differences between ages and sexes is useful for evaluating normal development of the eyes. Repeatability is important for distinguishing normal subjects from patients with disease as well as for following ocular modifications over time for follow-up after treatment, especially in young population. **Methods:** 76 eyes from 76 and 81 eyes from 81 healthy pediatric subjects underwent three fast macula scans and three RNFL measurements using a Spectralis OCT device respectively. Mean thicknesses in the nine Early Treatment Diabetic Retinopathy Study (ETDRS) and in the seven optic nerve RNFL areas were performed. Mean values were evaluated by age and by sex. Children were divided in two groups, Group 1: 6-year-old or younger and group 2: children older than 6 years. We calculated the repeatability comparing the two age groups, looking for intraclass correlation coefficient (ICC) and coefficient of variation (COV) values. **Results:** In the younger group central macular thickness value was 262.52±18.28, and the central RNFL 104.03±7.99. In the group of children older than 6, central macular thickness was 270.57±18.49 and the central RNFL 100.27±8.58. No statically significant differences were found. Central retinal thickness COVs were 7.00% in group 1 and 6.83% in Group 2. Optic disc RNFL thickness COVs were 7.72% in group 1 and 8.69% in group 2. All ICCs were higher than 0.8 showing excellent correlation among the three measurements performed by the same operator. **Conclusions:** Repeatability of Spectralis OCTs was high in healthy macula measurements, and significantly higher with Spectralis. Highly limited differences were found between repeatability of macular thickness measurements obtained by both devices analyzed by age.

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Intravitreal Injections of Humanized Anti-VEGF Drugs Cause Inflammation and RGC Loss in the Rat Retina in a Dose-dependent Manner

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Purpose: Intravitreal injections (IVI) of drugs directed against vascular endothelial growth factor (VEGF) are an essential therapeutic strategy for various retinal diseases like neovascular age-related macular degeneration (AMD) or diabetic retinopathy, but may have also ocular adverse effects. In this work, we study the effects of the IVI of a polyclonal antibody against rat VEGF and two humanized anti-human VEGF drugs on the adult rat retina. **Methods:** Albino Sprague-Dawley rats were divided into a control and 6 experimental groups that received each a 5 µl IVI of one of the following substances: phosphate buffered saline (PBS), ranibizumab (Lucentis®, 10 µg/µl or 0.38 µg/µl), aflibercept (Eylea®, 40 µg/µl or 1.5 µg/µl) or of a goat anti-rat polyclonal VEGF antibody (0.015 µg/µl). Animals were processed 7 or 30 days after the IVI and retinas were dissected as whole-mounts and immunodetected with antibodies against: Brn3a to label retinal ganglion cells (RGCs), melanopsin to label intrinsically photosensitive RGCs (ipRGCs), Iba1 to label retinal microglia and GFAP to label retinal macroglia. **Results:** The IVI of the high concentration of the humanized anti-VEGF drugs: ranibizumab and aflibercept caused a strong macro and microglial cell response and a significant decrease of the numbers of Brn3a+RGCs (approximately 10%) but not of ipRGCs. When these agents were injected at lower concentrations, calculated for the rat vitreous volume, the macro- and microglial response was milder and there were no decreases of the numbers of RGCs or ipRGCs. The IVI of PBS or the polyclonal anti-rat VEGF caused also a mild macro- and microglial reaction and did not cause a decrease of the numbers of RGCs and ipRGCs. **Conclusions:** In the rat, IVI of anti-VEGF drugs cause macro- and microglial responses (immunogenicity) that depend on the structure and concentration of the injected substance. The IVI of humanized anti-VEGF drugs, especially at high concentrations cause also RGC loss.

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Long-Term Outcomes of Internal Limiting Membrane Peeling, Autologous Fibrin and Gas Tamponade for High Myopic Macular Hole Retinal Detachment

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Purpose: To investigate the long-term safety and efficacy of microincisional pars plana vitrectomy with internal limiting membrane (ILM) peeling, autologous plasma rich in growth factor (PRGF) and gas tamponade in the treatment of the myopic macular hole retinal detachment (MMHRD). **Methods:** Five high myopic patients with macular hole and posterior detachment were included in this prospective observational nonrandomized study between November 2011 and September 2019. All patients underwent microincisional (23-25 G) pars plana vitrectomy with ILM peeling, PRGF and C2F6 gas tamponade. All patients were prospectively evaluated. The main outcomes were anatomic closure of the macular hole, macular attachment status and the best-corrected visual acuity (BCVA). **Results:** Retinal detachment was resolved in all patients (100%) included. Mean follow -up was 24±4 months (range, 110-14). At final visit, all of them had the macula attached. Postoperatively, macular hole resolved in four cases, but a patient suffered a reopening one year after the surgery. The final BCVA improved significantly compared with baseline. **Conclusions:** Microincisional pars plana vitrectomy with ILM peeling, PRGF and gas tamponade could be an effective treatment in the surgical management of myopic macular hole retinal detachment. The use of autologous fibrin as adjuvant to vitrectomy showed to be reproducible, safe and efficient in these cases.

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Saffron Exerts Protective Effect on Retinal Ganglion Cells in a Glaucoma Model

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Purpose: To analyze whether saffron extract oral supplementation, with neuroprotective, anti-inflammatory and anti-oxidant properties reduces microglial activation and protects retinal ganglion cells (RGC), in a unilateral laser-induced ocular hypertension (OHT) model. **Methods:** Six groups of Albino Swiss mice were done: naïve (NG), saffron naïve (SNG), laser group 3 days (LG3d), laser group 7 days (LG7d), saffron laser group 3 days (SLG3d) and saffron laser group 7 days (SLG7d). Unilateral OHT was induced by laser photocoagulation. OHT and contralateral eyes were analyzed. In retinal whole-mounts we analyzed: Iba-1$^+$cells number; Area of retina occupied by Iba-1$^+$cells (Iba-1-RA) in the nerve fiber layer-ganglion cell layer; Cell body area of Iba-1$^+$cells (CbIbac); and Arbor area of Iba-1$^+$cells (AAIbac); P2RY12$^+$resident microglia, and Brn3a$^+$RGCs. **Results:** No differences were found between naïve groups. LG3d-OHT retinas showed a significant increase of Ibacn, Iba-1 RA and CbIbac and a significant decrease of AAIbac compared to NG. SLG3d-OHT, and their contralateral retinas showed a significant reduction of all signs of microglial activation compared to LG3d-OHT. In NG all Iba-1$^+$cells were P2RY12$^+$. Compared to NG, in LG3d-OHT retinas P2RY12 expression was down-regulated, while in SLG3d-OHT, the saffron extract led to a smaller decrease. In contralateral eyes from the LG3d and SLG3d groups, P2RY12 expression was similar to NG. In LG7d-OHT there was a significant reduction in the number of Brn3a$^+$RGCs compared to NG. Saffron extract counteracted this reduction and a significant difference was found between SLG7d and LG7d retinas. No differences were found between LG7d and SLG7d contralateral retinas. **Conclusions:** Saffron extract decreases the number of microglial cells and the morphological signs of microglial activation, diminishes down-regulation of P2RY12 and counteracts the RGCs loss. Thus, saffron extract reduces neuroinflammation associated with IOP increase and protects RGCs.

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Early Anatomical and Functional Changes in Patients with Pachychoroidal Diseases after Photodynamic Therapy: A Pilot Study.

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Purpose: To analyze the response after one month of photodynamic therapy (PDT) in patients with pachychoroidal diseases using Macular Integrity Assessment Microperimetry (MAIA) and swept-source optical coherence tomography (SS-OCT). **Methods:** Prospective study during one month of 11 patients with pachychoroidal diseases treated with half-fluece and full-dose PDT for the standard duration of treatment after fluorescein angiography (FA) and indocyanine green angiography (ICG). 22 eyes (11 cases and 11 controls) were evaluated before and one month after PDT with SS-OCT, measuring retinal thickness and the choroid thickness in addition to the retinal sensitivity, measured by. Furthermore, the correlation of both parameters was analysed. **Results:** Pachychoroid spectrum was diagnosed by FA and ICG (four central serous chorioretinopathy (n=4), four pachychoroid pigment epitheliopathy (n=4), one pachychoroid neovasculopathy (n=1) and two polypoidal choroidal vasculopathy (n=2). Before PDT, cases showed a lower retinal sensitivity in all 14 regions analyzed by MAIA compared to the control group ($p<0.05$), as well as one month after treatment (except superoexternal area). Regarding OCT parameters, no significant changes were observed between both groups. Cases showed an increase in retinal sensitivity in the foveal region C ($p<0.05$) and in the rest of the sectors ($p>0.05$) after PDT. A decrease in retinal and choroid thickness were found by SS-OCT ($p>0.05$). When correlating the upper, nasal, lower and temporal internal quadrants of the retinal and choroidal parafoveal ring, using SS-OCT and the thresholds of the 3- and 5-degree radii (MAIA), a medium-high negative correlation was observed ($p> 0.05$) in the retinal quadrants besides a mean negative correlation ($p> 0.05$) in the choroidal quadrants after PDT. **Conclusions:** It was observed an early increase in retinal sensitivity at the foveal level after one month of PDT in patients with pachychoroid diseases.

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Associations between Age Related Macular Degeneration, Alzheimer's Disease and ApoE

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Purpose: Age-Related Macular Degeneration (AMD) and Alzheimer's disease (AD) are neurodegenerative diseases, which share some risk factors and have common elements in their pathogenesis. The goal of this review is to present the relationship between AMD, (AD) and ApoE alleles. **Methods:** A literature search was carried out using the "MESH" terms in PubMed and Google Scholar with the following keywords and word combination: "AMD", "AD", "ApoE" and others. We selected articles from the last 20 years. **Results:** AMD and AD share environmental risk factors, and there are some similarities in their pathogenesis. The main one is the deposit of amyloid β. One of the principal causes of neurotoxicity in AD is the extracellular accumulation of amyloid β (Aβ). In AMD, it has been shown that Aβ-peptide is present in drusen, and it may be associated with complement activation during drusen formation. Regarding the genetic factors, in AD, the ε4 allele of the APOE gene is a risk factor for its presence, and while ε2 is a protective factor. Conversely, ApoE ε4 allele is a protective factor for the development of AMD, and ApoE ε2 is associated with an increased risk of AMD. A recent study has shown that AMD patients have a higher risk for AD, in comparison to non-AMD participants. However, most of the studies do not show an increased risk of AD in subjects with AMD. **Conclusions:** AMD and AD share some risk factors, as well as the presence of Aβ. The ApoE genotype acts in the opposite way in both pathologies. There is no consensus on whether having one disease increases the risk of developing the other, so more studies are needed.

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Retinal Neuroinflammatory Response to Peripheral Inflammation

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Purpose: To study the participation of the mononuclear phagocytic system in the neuroinflammatory response at the retinal level in an experimental model of peripheral inflammatory process induced by Freund's complete adjuvant (FCA). **Methods:** In adult C57BL/6J lysozyme M-eGFP-knock-in (LysM-eGFP) mice, that express LysM-eGFP selectively in myelomonocytic cells, a local unilateral arthritic inflammatory process was induced by intra-plantar FCA administration in the hind limb. At different survival intervals (ranging 3-10 days post-lesion; dpl), FCA treated, sham or naïve mice were processed. Both retinas were prepared as whole-mounts and analyzed histologically for the expression of microglia markers and LysM-eGFP. Qualitative and quantitative changes in the appearance and distribution pattern of microglia and monocyte-derived macrophages were analyzed. **Results:** Morphological signs compatibles with microglia activation were observed in the eGFP+ cell population in the innermost retina layer at 3 days after FCA challenge and persisted along the time-course of study. The three sub-population of eGFP+ cells identified, amoeboid, ramified and peripheral, showed significant and relevant changes in the distribution pattern respect to that observed in untreated retinas from sham or naïve mice. Along the time-course analyzed the number of eGFP+ and Iba-1 immunonegative amoeboid cells was constant and similar to those of sham or control retinas, but at early stages (3 dpl) there is a significant depletion of eGFP+ and Iba-1+ amoeboid cells to reach normal values at later time points. However, the number of eGFP+ and Iba-1+ ramified cells peaked at 7 dpl to decreasing thereafter to normal values. The number of eGFP+ and Iba-1+ perivascular cells was constant and similar for all study groups and along the time-course. **Conclusions:** Induction of a peripheral inflammatory process by administration of CFA elicits a response from the peripheral bone marrow-derived monocyte-macrophage population in the retina that is recruited and differentiated into microglia.

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Trpm8-Dependent Activity of Cold-Sensitive Trigeminal Ganglion Neurons Encode Ocular Surface Temperature Changes and Shape the Reflex Blink

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Purpose: Ocular surface temperature (OST) changes are detected by cold thermosensitive trigeminal ganglion (TG) neurons expressing TRPM8 ion channels. Their sensory input evokes ocular sensations and is also used by the CNS to integrate protective reflex mechanisms as tearing and blinking. The aim of this work was to describe the ability of cold-sensitive TG neurons to encode OST changes and to establish the correlation between the magnitude of this sensory input and the magnitude of the reflex blink, evidenced as the amplitude of *orbicularis oculi* EMG (OOemg) signal. **Methods:** Spontaneous and stimulus-evoked impulse activity of cold-sensitive TG neurons was recorded extracellularly in anesthetized rats with tungsten electrodes. OOemg was recorded with intramuscular silver electrodes. OST changes (-1 to -15°C) were induced by instilling saline drops at different temperatures. TG-neuron impulse activity and the amplitude (area under the curve-AUC) of OOemg signals evoked by OST changes in control conditions and 15 min after topical treatment with TRPM8-blocker AMTB (1mM) were compared. **Results:** Low background (LB) and high background (HB) cold-sensitive neurons were identified. In control conditions, HB responded to all applied cold stimuli, reaching maximum firing values for OST drops of -1°C and over. On the contrary, firing of LB was proportional to the OST reduction (r^2:0.607, $p<0.001$), as well as the AUC of OOemg activity ($r^2=0.354$, $p=0.001$). AMTB decreased the ability of LB and HB to detect cold stimuli ($p<0.001$), reduced impulse response of LB and HB to OST drops, and almost abolished reflex OOemg activity ($p<0.001$). **Conclusions:** Low background cold-sensitive neurons innervating the ocular surface encode temperature changes between -1 and -15°C, while high background cold-sensitive neurons signal the presence of cold stimuli without encoding their intensity. Blockade of TRPM8 channels reduces the impulse response of cold-sensitive neurons (probably depending on the density of TRPM8 channels constitutively present in LB and HB neurons) as well as the reflex activation of the *orbicularis oculi* muscle in response to OST decreases.

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Adult-onset Vitelliform Macular Dystrophy: Tomography Evolution of a Case Series

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Purpose: To report a case series diagnosed of adult-onset vitelliform macular dystrophy (AVMD), evolution of macular injury using optical coherence tomography (OCT) and change in visual acuity (VA) during a monitoring period of time between 5-8 years. **Methods:** Retrospective observational study of a consecutive cases series diagnosed with AVMD at the Hospital General Universitario Reina Sofía. Revision of digitised medical history and analysis of multimodal image of spectral domain OCT. **Results:** Five eyes belonging to 4 patients between 63-74 years old diagnosed with AVMD were monitored during 70-106 months (92.8 average). After monitoring period, OCT showed complete reabsorption of vitelliform material in 4 eyes, and the time required was between 66-92 months (76.4 average). The best corrected VA at diagnosis was 0.5-1 and decreased after monitoring to 0.1-0.8; In 2 eyes improved or remained stable, and it decreased in 3 eyes. Out of the 3 eyes in which the VA decreased, in 2 of them the vitelliform material had been reabsorbed and in all 3 cases there was a destructuring of the ellipsoid layer with atrophy of retinal pigment epithelium and photoreceptors in OCT. **Conclusions:** The AVMD is a disease described by Gass less than 50 years ago, characterized by the presence of yellowish subfoveal deposits visualized like an hyperreflective cupuliform material of subretinal location in OCT. There are few studies with a sufficient monitoring period, but it has been reported that the reabsorption of the vitelliform material occurs over a period of several years, as we can see in our patients. The long-term visual prognosis is variable, in some cases vision may improve after reabsorption of the material, but it usually leads to a slow loss of central vision due to progression to atrophy or choroidal neovascularization.

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Morphometric Analysis of Corneal Innervation in Dry Eye Disease Patients. An *In Vivo* Confocal Microscopy Study

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Purpose: To measure the innervation of the corneal subbasal nerve plexus in Dry Eye Disease patients and compare the results with normal values of healthy patients. **Methods:** A prospective and observational study was conducted analyzing 31 eyes of 23 women and 8 men aged 54.3±9.4 years (range 37 to 70 years) which underwent in vivo confocal microscopy microscopy (IVMC) with the Rodstock cornea Module attached to Heildelberg's HRT3**.** Two hundred thirty-one images were analyzed and only one eye of each patient was included in the study. Nineteen eyes of healthy volunteers were included as a control group. At least 3 images of each eye were selected. Following subbasal central nervous plexus parameters were measured with ACC Metrics© software: Corneal Nerve Fiber Density (CNFD), Corneal Nerve Branch Density (CNBD), Corneal Nerve Fiber Length (CNFL), Corneal Nerve Fiber Total Branch Density (CTBD), Corneal Nerve Fiber Area (CNFA), Corneal Nerve Fiber Width (CNFW), Corneal Nerve Fractal Dimension (CNFrD). Data analysis was performed with SPSS® software for Windows 22.0 (SPSS® Inc, Chicago, IL.). The normality of the sample was checked with the Shapiro-Wilk test and the results were compared with the T test or the Man-Whitney U test based on the distribution of the data. The differences were considered statistically significant for $P<0.05$. **Results:** The mean of the analyzed variables and their standard deviations were CNFD: 17.85±1.31 fibers/mm^2, CNBD: 21.85±2.46 branches/mm^2, CNFL: 12.32±0.55 mm/mm^2, CNFA: 0.0059±0.0002 mm^2/mm^2, CNFW: 0.022±0.0003 mm/mm^2, CNFrD: 1.47±0.005. The values were decreased comparing healthy eyes data, and the differences were statistically significant in CNFD and CNFW. **Conclusions:** According to the data obtained, corneal subbasal nerve plexus is decreased in dry eye disease, compared to the healthy control group. However, studies with larger samples and divided by age groups are needed for more reliable results.

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Bioaccesibility of Antioxidants Provided by an Oral Supplement Containing Vitamins and Omega 3 Fatty Acids in the Diabetic Retina

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Purpose: Diabetic retinopathy (DR) is the leading cause of blindness in young adults. Oxidative stress (OS) is a critical DR pathogenic mechanism, once the overproduction of reactive oxygen species (ROS) and activation of ROS signaling pathways damage the eyes. We evaluated OS markers and the effects of a nutritional supplement regimen containing antioxidants and omega 3 fatty acids (A/ω3) in type 2 diabetic (T2DM) eyes. **Methods:** 480 participants were divided into 2 groups: 1) T2DM patients (n=287) with (+)/without (-) non-proliferative diabetic retinopathy (NPDR) and 2) controls (CG; n=194). Participants were randomly assigned (or not) to a daily pill of A/ω3 trough 38-months follow-up. Clinical and blood biochemical data were recorded. Statistical analysis was made using the SPSS 24.0 program. **Results:** Statistically higher circulating pro-oxidants (p=0.001) and lower antioxidants (p=0.0001) were detected in T2DM patient vs the CG. At the end-of-study the above parameters changed to a significantly higher antioxidant capacity (p=0.042) in the T2DM+NPDR as compared to the T2DM-NPDR with A/ω3 supplementation. **Conclusions:** This long-term follow up study, reinforces the need for strategies to increase the antioxidants supply as achieved with appropriated nutritional supplements. This intervention regimen can be useful for counteracting the OS of the diabetic eyes.

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Retinal Changes by Optical Coherence Tomography in Subject at High Genetic Risk for Developing Alzheimer´s Disease.

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Purpose: To assess the retinal changes by optical coherence tomography (OCT) in asymptomatic subject with two genetic risk factors to develop Alzheimer's disease (AD): First-degree family history of AD and being carrier of ε4 allele for the ApoE gene. **Methods:** A case-control study was conducted in 35 subjects with family history of AD (FH+) and ApoE ε4 carriers and 29 age-matched control subjects without a family history of AD (FH-) and ApoE ε4 non carriers. All subjects, free of ocular pathology, underwent a complete eye examination and OCT. **Results:** We found statistical significance decreases ($p<0.05$) in FH+ ApoE ε4 carriers compared to FH- ApoE ε4 non carriers in: i) the foveal area of the macular RNFL (11.89 ± 1.95,FH+ vs 12.86±1.62,FH-); ii) the inferior and nasal sectors in the outer (26.77±2.74,FH+ vs 28.17±2.82, FH) (29.49±2.89,FH+ vs 30.93±3.85,FH-) and inner (40.49±2.51,FH+ vs 42.10±3.48,FH-) (41.94±2.74, FH+ vs 43.52±3.57, FH-) macular ring in the IPL; iii)the foveal area (18.82±3.61, FH+ vs 21.21±3.88, FH-) and the inferior sector in the outer macular ring (30.91±2.72,FH+ vs 32.10±2.81,FH-) in INL; iv)the inferior sector of the outer macular ring (27.60±2.75,FH+ vs 29.97±3.82, FH-) in OPL. In pRNFL, no statistically significant differences were found. **Conclusions:** In subjects at high genetic risk of developing AD, the initial changes appear in the macular area. These slight changes detected by OCT could be used as an early biomarker of AD.

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Long-Term Effectiveness and Safety of Early Lensectomy in Patients with Pseudoexfoliation

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Purpose: To assess and compare the effectiveness, predictability and safety (in the early and long-term follow-up) of cataract surgery with intraocular lens (IOL) implantation in patients presenting advanced pseudoexfoliation (PEX) (symmetric stage) and mild PEX (asymmetric presentation). **Methods:** Retrospective single-center study that included PEX patients who underwent phacoemulsification in both eyes. A postoperative follow-up ≥5 years, ≥3 preoperative reliable visual field (VF) tests and VF tests until the analysis visit were required. One hundred sixty-one patients (322 eyes) were included (age: 71.4±5.7 years), and they were classified as "symmetric PEX" (both eyes had PEX; 204 eyes of 102 patients), and "asymmetric PEX" (only one eye presented clinically apparent PEX; 118 eyes of 59 patients). Preoperative and postoperative visual acuity, IOP, number of hypotensive medications, and VF mean deviation (MD) were registered, as well as the appearance of intraoperative and postoperative complications. Wilcoxon test was used for statistical analysis. **Results:** The mean follow-up time was 8.5±2.8 years. A hydrophobic acrylic IOL was implanted in all the cases. Six months after cataract surgery, 95% and 96% of eyes were within ±1.00D in symmetric and asymmetric groups, respectively. At the final follow-up, IOP decreased only in the asymmetric group ($p=0.004$), with a reduction in the number of medications in both ($p<0.001$). MD changed from -8.8 dB to -11.6 dB in the symmetric group ($p<0.001$). Intraoperative complications were only registered in the symmetric group: 7 (3.4%; $p=0.04$). Ten cases (4.9%) of late IOL dislocation were found, all from the symmetric group ($p=0.03$). **Conclusions:** Early lensectomy in patients with PEX before its symmetric presentation resulted effective, safe and predictable in the long term. In addition, a lower rate of glaucoma progression and a better control of IOP were found when surgery was performed in the initial stages of the disease.

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Morphometric Analysis of Corneal Innervation in Patients Diagnosed with Dry Eye Disease Treated with Plasma Rich in Growth Factor

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Purpose: This study examined the effect of plasma rich in growth factor treatment (PRGF), on subbasal nervous plexus innervation of patients diagnosed with dry eye disease (DED). **Methods:** A prospective, observational study was conducted analyzing 31eyes images of 31 patients with DED (22 women and 9 males) aged 53.4±8.5 years (range 36 to 71 years). Subjects underwent microscopy confocal in vivo (IVMC) before (bf) and after (af) 3 months of treatment with PRFG. At least 3 images of each eye were selected and the following subbasal central nervous plexus parameters were measured with ACC Metrics software: Corneal Nerve Fiber Density (CNFD), Corneal Nerve Branch Density (CNBD), Corneal Nerve Fiber Length (CNFL), Corneal Nerve Fiber Total Branch Density (CTBD), Corneal Nerve Fiber Area (CNFA), Corneal Nerve Fiber Width (CNFW) and Corneal Nerve Fractal Dimension (CNFrD). Data analysis was performed with SPSS® software for Windows 22.0 (SPSS® Inc, Chicago, IL.). The normality of the sample was checked with the Shapiro-Wilk test and the results were compared to the *t* test or the Wilcoxon test based on the distribution of the data. The differences were considered statistically significant for $P<0.05$. **Results:** The mean and their standard deviations obtained for the variables analyzed were: CNFDbf: 17,854±1,311 and CNFDaf: 18,590±1,699 fibers/mm^2, CNBDbf: 21,847±2,465 and CNBDaf: 24,591±3,310 branches/mm^2, CNFLbf: 12,324±0,548 and CNFLaf: 12,828±0,789mm/mm^2, CTBDbf: 37,728±3,428 and CTBDaf: 41,187±5,124 branches/mm^2, CNFAbf: 0.00598±0.00277 and CNFAaf: 0.00596 ± 0.00332 mm^2 by mm^2, CNFWbf: 0.0223±0.0030 and CNFWaf: 0.0224±0.0029 mm^2 by mm^2, CNFrDaf: 1,471±0,006 and CNFrDbf: 1,463±0,02. Differences versus before and after treatment values were not statistically significant in all values analyzed. **Conclusions:** nervous plexus parameters improved after treatment with PRGF, although without being statistically significant. Further studies should include a larger sample.

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Autosomal Dominant Optic Atrophy Due to OPA1 Gene Mutation: A Family Case Series

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Purpose: Autosomal dominant optic atrophy (ADOA) is a pathology from the group of hereditary optic neuropathies. 75% of cases are caused by mutations in the OPA1 gene, which codes for the synthesis of inner mitochondrial membrane proteins. This gene is responsible for regulating several mitochondrial processes, such as the control of apoptosis and metabolism. This disease affects retinal ganglion cells and their axons. **Methods:** Ophthalmological and neurophysiological diagnostic tests were performed on three members of the same family (maternal aunt, mother and son) affected by ADOA, who are followed up at Reina Sofía University Hospital in Murcia. A genetic study was also performed in two of them. **Results:** In all three patients, an ophthalmologic examination showed a visual acuity below 20/100 and dyschromatopsia in blue-yellow axis (tritanopia). A fundus examination was performed, where a bilateral and predominantly temporal pallor of the optic papilla was detected. Optical coherence tomography showed an optic nerve (ON) fiber layer thickness defect bilaterally, greater in the temporal half. The visual field of two patients showed a paracentral scotoma in both eyes. In the third patient, the visual field showed a bilateral central scotoma. Visual evoked potentials test showed reduced wave amplitudes correlating with delayed conduction of ON fibers. An electroretinogram was normal in two patients, showing a low b-wave amplitude value in the third one. In the genetic study the pathogenic variant NM_015560c.112C>T (p.Arg38*NS) was found in the OPA1 gene, associated with Autosomal Dominant Optic Atrophy (ADOA-OMIM # 165500). **Conclusions:** ADOA is an inherited ophthalmologic disease that commonly presents in the first decades of life. The involvement is usually bilateral and symmetrical and it can result in moderate to severe visual loss. Unfortunately, there is no effective treatment for this pathology, so it is necessary to continue investigating on this topic.

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The Role of RUNX1 in Experimental Choroidal Neovascularization

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Purpose: Choroidal neovascularization (CNV) is a prevalent cause of vision loss in various ocular diseases. Current treatment involves repeated intravitreal injection of drugs that target *vascular endothelial growth factor* (VEGF). *Runt-related transcription factor* 1 (RUNX1) has been recently identified as a key mediator of retinal neovascularization. In this study, we wanted to evaluate the preclinical efficacy of RUNX1 inhibition with small molecule Ro5-3335 as a new therapeutic approach for CNV. **Methods:** Laser-induced CNV model was used. A single intravitreal injection of either phosphate-buffered saline (vehicle), Ro5-3335, aflibercept (an approved anti-VEGF) or combination of Ro5-3335+aflibercept was performed immediately after laser. RUNX1 expression was measured by immunofluorescence and polymerase chain reaction. CNV size was quantified from choroidal flatmounts stained with isolectin B4. Vascular leakage was assessed by fluorescein angiography. **Results:** RUNX1 expression was found in the main cell types involved in CNV: endothelial cells (CD31+), mononuclear phagocytes (macrophages/microglia) (CD11b+), retinal pigment epithelial cells (RPE65+), vascular smooth muscle cells/myofibroblasts (alpha-smooth muscle actin+) and Müller cells (glial fibrillary acidic protein+). Importantly, there was no RUNX1 expression in uninjured retina. Peak of RUNX1 levels were found at day 3. Ro5-3335 significantly decreased CNV area, and when combined with aflibercept, reduced vascular leakage more effectively than aflibercept alone. **Conclusions:** We demonstrate RUNX1 expression in the main cell types involved in CNV and report the preclinical efficacy of RUNX1 inhibition in experimental CNV. These data suggest that RUNX1 inhibition alone or in combination with anti-VEGF drugs should be further investigated as a novel treatment for CNV.

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A2B Retrobulbar Shunt in Tight Orbit Syndrome

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Purpose: Tight orbit syndrome is characterized by the eyelids pressing firmly against the globe, limiting globe exposure during tonometry, gonioscopy and surgery, and high-pressure open angle glaucoma that is extremely difficult to manage. **Methods:** Clinical case. 88-year-old patient with advanced glaucoma BE mainly on his RE. Visual acuity was PL and HM, IOP of 26/ 20 mmHg under maximum topical treatment plus edemox (with a great variability up to 40mmHg). Ophthalmological antecedents on his RE were extracapsular extraction trabeculectomy, Ahmed Valve explanted for implant plate exposure and endothelial decompensation keratoplasty and DSAEK. The patient's palpebral cleft was narrow and made it very difficult for the upper conjunctival exposure. The presumptive diagnosis of Tight Orbit Syndrome was reached, for which reason Shunt A2B of retrobulbar shunt given the conjunctiva state. **Results:** The surgery was performed without complications with an IOP of 4 mmHg and a wide camera 24 hours after the surgery. Three months later, VA remains at LP, IOP was 12mmHg without antiglaucoma treatment and any sign of graft failure. **Conclusions:** The Tight Orbit Syndrome show significant variability in IOP and have a history of multiple failed surgeries that cause highly altered conjunctivae. The retrobulbar shunt A2B shows efficacy in challenging cases, being an alternative in these patients as its implantation is easier and safer than Ahmed Valve. The retrobulbar space has a better cicatrization profile because of the fat tissue nature and fewer fibrosis elements. The size and shape of the shunt could reduce the device exposure in this type of eyes.

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Evaluation of the Morphogeometric Differences in Down Syndrome Patients with Keratoconus Using 3D Virtual Models

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Purpose: This study investigates and characterizes a previously developed 3D corneal model in patients with Down Syndrome (DS), aiming to stablish new clinical diagnosis criteria using volumetric and superficial changes as evidences. **Methods:** This retrospective case series study evaluated a sample of 108 eyes of 108 patients (34.72±11.12 years) divided into two main groups: 46 DS patients (29.1±9.2 years) and 62 age-sex-matched non-DS patients (37.2±8.6 years). The DS group was divided into two subgroups, designated as "DS with normal topography" and "DS with abnormal topography", depending on the topographical classification. Morphogeometric characteristics were established using an in vivo, non-finite global 3D analysis of the cornea. Data engagement scores were confirmed by the Kolmogorov–Smirnov test. Kruskal-Wallis test with post-hoc correction was performed to compare quantitative variables among the control, DS with normal topography and DS with abnormal topography groups. **Results:** Anterior apex deviation was significantly higher in both DS groups (with normal and abnormal topography) than in the control group ($p<0.05$). Moreover, the DS group with abnormal topography had higher Anterior/Posterior minimum thickness point deviation values than in the control group. Regarding volumetric parameters, again both DS groups had decreased Total Volume and Anterior/Posterior Apex Volume for each 0.05 mm step of the radius value (between 0.1 to 1.5mm diameter), when compared with the control group ($p<0.0166$). **Conclusions:** This study allowed differentiating between healthy non-DS and DS groups, with both normal and abnormal tomographies, by studying the irregularity of the cornea in patients that present this condition. These conclusions may open a new perspective in the diagnosis of keratoconus in DS patients.

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Minocycline Reduces Caspase-3 Activation but not Retinal Ganglion Cells Loss after Ocular Hypertension in Mice

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Purpose: To analyse the effect of minocycline administration on the evolution of retinal ganglion cell (RGC) loss and the activation of the apoptotic signal Caspase-3 after ocular hypertension (OHT) in mice. **Methods:** Ocular hypertension was induced by diode laser photocoagulation of limbal and episcleral veins in the left eyes of Swiss albino mice and intraocular pressure (IOP) was monitored with a TonoLab®. Mice received intraperitoneal injections of minocycline hydrochloride (45 mg/kg) diluted in saline (n=7 per group/time point), administered twice the first day of OHT induction and daily for the remaining days. Control groups received saline injections administered with the same routine (n=5-6 per group/time point). Mice were analyzed at 4 or 15 days after OHT. Retinas were whole-mounted and double immunodetection against Brn3a and active-Caspase-3 was performed to identify surviving and apoptotic RGCs, respectively. Total Brn3a$^+$RGCs and a-Casp3$^+$RGCs were quantified automatically and manually, respectively. The distribution of both populations was analyzed by topographic maps. **Results:** The evolution of IOP in the groups treated with minocycline or vehicle was similar. At 24 hours there was a significant elevation (34.8±5.6 and 33.2±6.1 mmHg, respectively) which decreased to normal values at one week (18.3±4.4 mmHg and 17.3±5.3 mmHg, respectively). The number of Brn3a$^+$RGCs in the minocycline-treated group did not differ from the vehicle group neither at 4 (40,878.7±4860, 43,526.8±2714, respectively) nor at 15 (16,161.4±12650, 12,919±6085, respectively) days after OHT. However, the number of a-Casp3$^+$RGCs was significantly lower in the minocycline-treated groups at both 4 (58.6%) and 15 (33.6%) days compared to the saline-treated groups (100%). **Conclusions:** Treatment with minocycline decreases activation of caspase 3 after OHT but does not rescue RGCs from OHT-induced death.

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Response of the Retinal Glia to Syngeneic, Allogeneic, or Xenogeneic Intravitreal Transplants of BM-MSCs

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Purpose: Cell therapy as neuroprotective approach in the central nervous system (CNS) is being widely researched. In patients cell transplants are mostly allogeneic or autologous (syngeneic), and preclinical studies are carried out with human cells tested in animals, i.e. xenotransplant. Little is known of the effect of these transplants in the healthy CNS. Here, we purpose to analyze the response of retinal glia to intravitreal administration of Bone Marrow Mesenchymal Stromal Cells (BM-MSCs) in syngeneic, allogeneic and xenogeneic transplants. **Methods:** Human BM-MSCs were obtained by direct percutaneous aspiration from the iliac crest of healthy volunteers, and mouse BM-MSCs from the tibias and femurs of the C57BL/6-Tg(CAG-EGFP) strain. 20,000 cells were intravitreally injected into the left eye of C57BL/6 (syngeneic and xenogeneic transplants) or BALB/c (allogeneic transplant) mice strains. Retinas were analyzed 5 or 21 days after the transplants (n=4/time point and transplant) and Iba1, CD45, GFAP, AQP4, Vimentin and GLUT1 immunoidentified in cross-sections. mBM-MSCs were identified by their GFP expression and human ones by anti-human mitochondrial immunodetection. **Results:** Transplanted cells were observed in the vitreous at 5 and 21 days in the three models although at 21 days very few cells remained in the allotransplant. All transplants caused Müller cell hypertrophy, microglial activation, and a decrease of AQP4 and GLUT1 signal. All these changes were more evident at 21 than at 5 days and stronger in the xenotransplant. Retinal folding and infiltration of $CD45^{+}$cells from the choroid was observed in half of the syngeneic and all xenotransplanted retinas. **Conclusions:** Intravitreal administration of BM-MSCs is not innocuous, causing macro and microglial activation as well as retinal folding and cell infiltration.

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Resident Dendritic Cells in the Cornea: Important Players in Ocular Surface Homeostasis

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Purpose: To determine the role of resident dendritic cells (DC) in modulating corneal sensory nerve activity, basal tearing and pain. **Methods:** Anesthetized C57BL/6 CD11c-DTR mice (5-6 months old, both sexes) received bilateral subconjunctival injections of diphtheria toxin (DT) to induce short-term (48h, STDT) and long-term (8 days, LTDT) DC depletion. PBS injections were used as sham and naïve mice (no DT-injections) as control. Background and stimulus-evoked (cooling and heating ramps) activity of cold thermoreceptor nerve terminals was recorded ex vivo. Basal tearing rate was measured using commercial phenol red threads during 30s under isoflurane anesthesia. To quantify eye pain, eye closure ratio was calculated dividing the height by the width of mice palpebral fissure. **Results:** STDT produced a significant decrease in heating threshold (37.7±0.7ºC, n=10 vs. naïve, 41.6±1.8ºC, n=4 and LTDT, 42.3±1ºC, n=8; p=0.003, ANOVA) and a slight reduction in basal tearing (2,8± 0,6mm before DT vs. 1,2±0,38mm at 48h, n=6; p=0.119, paired t-test). In LTDT lower temperature values were needed to reach both cooling threshold and peak frequency during cooling ramps (30.7±0.5ºC, n=17 vs. naïve, 32.3±0.3ºC, n=20, and STDT, 32.2±0.4ºC, n=17; p=0.005, ANOVA). Spontaneous pain was present in both STDT and LTDT, being eye closure ratio lower than before DT-injections (STDT: 0,92±0,01 before DT vs. 0,18±0,02 at 48h, n=23, p≤0.001, paired t-test; LTDT: 0.9±0.03 before DT vs. 0.17±0.02 at 48h vs. 0.46±0.07 at day 8, n=3, p≤0.001, RM ANOVA). Sham injections significantly increased cooling response (ST-PBS 23±4.2 imp/s, n=7 vs. naïve, 13.4±2.4 imp/s, n=20 and LTPBS, 21.6±3.9 imp/s, n=8; p=0.022, ANOVA) without inducing changes in tearing rate or pain behavior. **Conclusions:** Differences observed in nerve activity and the subsequent changes in basal tearing and signs of pain after dendritic cell depletion suggest a crucial role of these cells in corneal nerve activity and ocular surface homeostasis.

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Cell Death by Necroptosis during Neuroprotection by Mesenchymal Stem Cells Secretome over *In vitro* Retinal Degeneration

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Purpose: The pathophysiological response to retinal injury is similar in many retinal neurodegenerative diseases, in that process cell death mechanisms has major implication. Necroptosis is a cell death mechanism that resembles a programmed necrosis in which cells show disruption of their cell membranes. The aim of the study was to evaluate the necroptosis during the neuroprotective effect of mesenchymal stem cells (MSC) secretome over in vitro neuroretina (NR) degeneration. **Methods:** Four experimental conditions were analysed: Fresh porcine NR, NR cultured alone for 72 hours, NR cocultured with MSC for 72 hours and NR cocultured with MSC secretome for 72 hours (previously obtained through the coculture of porcine NR explants and MSC under standard conditions for 72 hours). The mRNA expression of *MKLL, RIPK1* and *RIPK3* genes were analysed by qPCR. The morphological characteristics and immunoreactivity of MLKL was also analyzed by immunohistochemistry. **Results:** mRNA expression of *RIPK3* and *MLKL* genes were similar in fresh NR and NR cocultured with MSC ($p>0.05$). *RIPK1* mRNA expression was increased in all experimental conditions compared to Fresh NR ($p<0.001$). NR cultured alone for 72 hours showed the highest levels of mRNA expression of *RIPK3*, *RIPK1* and *MLKL* genes. On the other hand, Immunoreactivity distribution of the MLKL over the neuroretinal tissue was similar in all the experimental conditions; however, its intensity was considerably higher in the NR cultured alone. **Conclusions:** MSC secretome causes the decline of cell death by necroptosis in the in vitro retinal neurodegeneration.

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A Specific Decrease of Fatty Acids in the Retina is Associated with Neurodegeneration in a Retinitis Pigmentosa Mouse Model.

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Purpose: Retinal degenerative diseases are characterized by a complex interaction among apoptotic, inflammatory, and oxidative pathways. Fatty acids are highly enriched in the retina, having relevant roles in its physiology. The specific fatty acid composition of the membrane of photoreceptors impacts the proper functioning of the phototransduction cascade. The omega-3 DHA is probably the most important fatty acid for photoreceptor survival. The analysis of fatty acids in the rd10 mouse model can apport new information about the molecular pathways that contribute to retinal degeneration. **Methods:** C57BL/6J (n=4) and rd10 (n=9) mice were kept at a photoperiod of 12:12 (L:D), and a light intensity of 50 lux. At P25, animals were euthanized, and cryostat sections were obtained for morphological studies of the retina using immunohistochemistry. Fatty acid composition was assessed by lipid extraction, and the profile was quantified by GC/MSD. **Results:** We found a significant decrease of rows of photoreceptor cells in the retina of rd10 compared to the retina of C57BL/6J mice. Also, rhodopsin was mislocalized in the dystrophic retinas. Cone arrestin immunolabeling revealed a shortened of the outer segments and axons of cones. Additionally, dendrites of bipolar cells were diminished. The fatty acid profile of the retina of rd10 mice showed a decrease of specific fatty acids. DHA decreased markedly, originating an unbalance between omega-3 and omega-6 fatty acids. Furthermore, we found a positive correlation between these fatty acids and the number of photoreceptor rows. **Conclusions:** Specific fatty acids are altered in retinitis pigmentosa, which can be contributing to the disruption of phototransduction and the exacerbation of apoptosis, inflammation, and oxidative stress in these cells. The lipidomics analysis of fatty acids in the retina of the rd10 mouse model can apport new molecular targets to improve the therapeutical strategies.

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Effect of Single or Combined Treatment with bFGF and Minocycline on the Cone Population Following a Model of Focal Light-Emitting Diode (LED)-Induced Phototoxicity (LIP) in Pigmented Mice

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Purpose: To study the effects of single or combined administration of basic fibroblast growth factor (bFGF) and minocycline on the cone population after a focal blue light-emitting diode (LED)-induced phototoxicity (LIP). **Methods:** In dark-adapted anesthetized adult pigmented C57BL/6 mice, left eyes with previously dilated pupils were exposed to LIP (400 nm, 500 lux for 45 seconds) placed perpendicular to the cornea. Mice treated with bFGF (0.5μg) and/or minocycline (45mg/kg) were longitudinally monitored *in vivo* with a SD-OCT at 1, 3, 5 and 7 days (d) after LIP. Cone outer segments (OS) immunodetected with arrestin were analyzed *ex vivo* at 3 or 7d after LIP within a predetermined fixed-size area (PFA) centred on the lesion in flattened retinas (n=10-13). As control groups, mice were treated with vehicle following the same administration routine (n=10 each group). **Results:** Optical OCT sections showed a focal lesion in the supero-temporal quadrant with progressive thinning of the outer retina. In mice treated with bFGF or bFGF+minocycline retinal thickness was better preserved at 5d after LIP than in the other groups. However, at 7d all groups had thinned similarly. Whole-mounted retinas showed within the focal lesion a progressive decrease in arrestin^{+}OS within PFA at 3 and 7d (5162±307 and 3892±318, respectively). By 3d there were no significant differences in the arrestin^{+}OS population between groups. However, 7d after LIP, the groups treated with bFGF alone (4519±320) or in combination with minocycline (4882±446) had significantly higher numbers of arrestin^{+}OS than the other groups (p=0,0063). **Conclusions:** LIP results in a progressive outer retinal damage affecting the OS population. Administration of bFGF had an effect on arrestin^{+}OS survival and its combination with minocycline did not increase this effect.

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Simultaneous Recording of Electroretinographic Responses and Visual Evoked Potential for the Study of Retinal Function

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Purpose: To assess the mechanisms of synaptic transmission in the retina by analyzing the combined electrophysiological responses of the flash electroretinogram (fERG) and visual evoked potential (VEP). **Methods:** The study was carried out in healthy adult mice of the C57BL6 / J strain. The fERG and VEP signals were simultaneously recorded in response to full-field light stimulation of increasing intensities, both in scotopic and photopic conditions. The right eye of each animal was injected with 1 μL of a PBS solution containing one of the following agents, GABA (100 mM), Glutamate (100 mM), Bicuculline (10 mM), DNQX (30 mM), APB (25mM), HEPES (25mM), TPMPA (5mM), or a combination of some of them. Eye and cortical responses of the injected animals (n = 4) were analyzed comparatively with the corresponding control animals (n = 8). **Results:** The analysis of the rod b wave of the fERG showed a decrease in the amplitude to all the injected agents. A delay in the latency of the P2 component of the VEP was observed under scotopic conditions. Analysis of the mixed b-wave showed an amplitude decrease induced by Glutamate, HEPES, TPMPA, DNQX/Bicuculline and wave cancellation by APB. Analysis of VEP responses to stimuli of high intensity under scotopic and photopic conditions showed a delay in N1 and P2 latency in animals injected with APB, GABA, Glutamate, APB/GABA, GABA/Glutamate, HEPES. The responses dominated by cone showed a decrease in the b wave after DNQX, HEPES, DNQX/Bicuculline injection. **Conclusions:** The amplitude of the b wave of the fERG as well as the latency of the N1 and P2 components of the VEP allow to analyze the participation of retinal synaptic mechanisms in visual processing. The participation of synaptic transmission between rods and bipolar cells in the visual cortical response is demonstrated. The analysis of the effect of the different neurotransmitters demonstrates the role of the first synapse of the visual pathway on the cortical response.

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Teleophtalmology with Smartphones. Role of a New Medical Device: The Open Retinoscope

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Purpose: The use of smartphones to provide specialist ophthalmology services is becoming a more commonly used method to support patients with eye pathologies. During the COVID-19 pandemic, demand for telehealth services such as tele-ophthalmology, is increasing rapidly. **Methods:** In 2019, the agreement between diagnostic tests was investigated by comparing the diagnostic performance for eye posterior pole pathologies of the images obtained by a smartphone coupled to a medical device known as open retinoscope (OR), handled by a nurse and subsequently assessed by an ophthalmologist versus the images obtained by an ophthalmologist using a slit lamp associated to a 76 diopter indirect ophthalmic lens (Volk Super FieldVR) (SL-IOL) at the outpatient department of a hospital. The OR used in this study worked with a 28-diopter indirect lens. **Results:** An examination of 151 dilated eyes (79 adult patients, mean age of 66.7 years, 59.5% women) was conducted. Sensitivity was 98.9%, specificity was 89.8%, the positive predictive value was 93.8% and the negative predictive value was 98.2%. The kappa index between both tests was 0.90 (95% CI: 0.830.97) in basic diagnosis, 0.81 (95% CI: 0.74-0.89) in syndromic diagnosis (13 categories) and 0.70 (95% CI: 0.62-0.77) in advanced diagnosis (23 categories). **Conclusions:** Images obtained by a nurse using a smartphone coupled to the OR and subsequently assessed by an ophthalmologist showed a high diagnostic performance for eye posterior pole pathologies, which could pave the way for remote ophthalmology systems for this patient group.

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Role of the *ADAMTSL4* Gene in Juvenile Glaucoma: Functional Analysis inZebrafish

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Purpose: Early-onset glaucoma (EOG) is a heterogeneous, inherited and severe optical neuropathy that originates from maldevelopment of the anterior segment of the eye. It encompasses different diseases, ranging from primary congenital glaucoma (CG) to juvenil glaucoma (JG), which are usually diagnosed before three years or between 5 to 18 years of age, respectively. Using exome sequencing we have identified *ADAMTSL4* as a candidate gene in JG. The main objective of this study was to evaluate the loss-of-function (LoF) effect of this candidate gene in zebrafish. **Methods:** Evolutionary conservation of human and zebrafish orthologue genes was evaluated bioinformatically. A *knock out* zebrafish line was generated by CRISPR/Cas9. Disruption of the gene was corroborated in zebrafish embryos (144 hpf) by RT-qPCR. Morphological characterization of zebrafish embryos (144 hpf) was done under a SMZ18 (Nikon) steromicroscope. **Results:** Bioinformatic analysis also revealed a high evolutionary conservation between human and zebrafish genes. RT-qPCR showed a significant in *adamtsl4* mRNA reduction in the established knockout zebrafish line (c.234_351del) at 144 hpf. Preliminary, complete *adamtsl4* disruption in zebrafish embryos (144 hpf) results in a lethal phenotype in 40% of homozygous embryos, characterized by varying degrees of gross developmental craniofacial abnormalities, including microphthalmia, and pericardial and periocular edemas. **Conclusions:** Overall, our data indicate that *ADAMTSL4* loss-of-function may affect ocular development. Because complete *adamtsl4* LoF is lethal, partial functional inactivation of this gene would be required to investigate its possible role in glaucoma.

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Wild olive (acebuche) oil-enriched diet as an anti-inflammatory and anti-fibrotic nutraceutical tool in hypertensive retinas

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Purpose: Inflammation and fibrosis play a pivotal role in the development/progression of ocular diseases, including vascular retinopathies. Despite the well-known beneficial effects of extra virgin olive oil (EVOO) on inflammatory diseases, very little is known about the potential therapeutic effects of the cultivated olive tree's counterpart, the wild olive tree (also known in Spain as acebuche, ACE). Here we aimed to characterize the anti-inflammatory and anti-fibrotic effects of ACE oil in the retina of hypertensive mice. **Methods:** C57B/6J mice became hypertensive following administration of NG-nitro-L-arginine-methyl-ester (L-NAME) and were simultaneously subjected to dietary supplementation with either ACE oil or EVOO for comparison purposes. Retinal function was evaluated by electroretinography (ERG), and determinations of inflammation- and fibrosis-related biomarkers (PPARα/γ, IL-1β/6/10, TNF-α, COX-2, collagen and TGF-β isoforms, CTGF, and MMP/TIMP ratio) were performed in retinal homogenates or quantified in retinal layers. **Results:** Our ACE oil-enriched diet prevented the characteristic retinal dysfunction recorded in ERGs from hypertensive mice. Furthermore, the diet supplemented with ACE oil increased the expression of anti-inflammatory biomarkers PPARα, PPARγ and IL-10, while downregulating COX-2 and proinflammatory cytokines IL-1β, IL-6, and TNF-α. Collagen deposition and metabolism also improved via ACE oil-dependent regulation of MMP/TIMP/CTGF/TGFβ expression. Interestingly, although EVOO-enriched diet also proved beneficial against retinal inflammation and fibrosis, the administration of ACE oil appears to be even more effective. **Conclusions:** This is the first study reporting comparative anti-inflammatory and anti-fibrotic effects of diets enriched with either ACE oil or a reference EVOO, with better outcomes in favor of the former, in the setting of hypertension-related retinal damage.

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An Optic Role of Mitochondria in the Cone Photoreceptor of a Mammalian Retina

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Purpose: Mitochondria are cellular organelles chiefly intended for energy production. Mammalian photoceptors aggregate numerous mitochondria in the ellipsoid region immediately adjacent to their light-sensitive outer segments (OS). While these mitochondria are required to support the high metabolic demands of phototransduction, they may potentially impair light delivery to the OS due to either premature absorption or scattering by their numerous membrane structures. Conversely, they might enhance the delivery of light to the OS, taking up a potential optic role, which is not unprecedented for retinal structures (e.g. rod nuclei, Müller glia, and cone oil droplets). We thus set out to investigate the potential optic role of mitochondria in photoreceptor ellipsoid region. **Methods:** Using a horizontal slice preparation from the ground squirrel retina, in which plentiful cones contain mitochondria in a bundled arrangement closely resembling those in primate, we directly imaged light transmitted through the mitochondria bundle (MtB). In addition, we performed electromagnetic simulations of light transmission based on the MtB structures translated from reconstructions of Blockface Scanning EM images. **Results:** We directly demonstrated that such MtB concentrates light several fold onto the OS for detection. In addition, this "microlens"-like feature of cone mitochondria produces an angular dependence of light intensity quantitively consistent with the Stiles-Crawford effect, a psychophysical phenomenon believed to improve visual resolution. **Conclusions:** We thus establish an unconventional optical function for mitochondria, energy-producing organelles in cone photoreceptors, offering insight into their role in the interpretation of noninvasive optical tools for vision research and clinics.

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The Bandwidth Ground Squirrel Retina as a Model for Neural Injury and Neuroprotection

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Purpose: Retinal ganglion cell (RGC) death occurs after optic nerve damage due either to acute trauma or chronic degenerative conditions. RGC loss leads to permanent vision lost without an effective therapeutic approach, calling for more laboratory investigations. Modeling human RGC/optic nerve diseases with primate model is ideal but with practical limitations, high cost and ethical considerations. Commonly used rodents, mice and rats, are nocturnal with retinal structures that differs significantly from that of the primates, often possessing less than one tenth of the RGCs. To establish the diurnal thirteen-lined ground squirrel (TLGS) as a model for RGC pathology in injury/neurodegeneration, we aim to characterize the features of RGCs and other cells in the ganglion cell layer (GCL) of the TLGS retina in normal and injury conditions. **Methods:** Cell-type specific antibodies were used to quantify RGCs, glia, and microglia in the GCL. The optic nerves were injured by totally (tONC) or partially crush (nasal half, pONC). The number and topography of RGCs were automatically quantified by an algorithm. After ONC, the retina, ganglion cell complex (GCC) and nerve fiber (RNFL) thinning were examined in vivo by the spectral-domain optical coherence tomography and structural changes were investigated with ex vivo imaging. **Results:** The TLGS retina possesses ~600,000 RGCs with high density along the equatorial retina matching the high cone density area (visual streak). The number and distribution of RGCs are much closer to primate retina than other rodent models. TLGS and primate retinas also share a similar interlocking pattern between RGC axons and astrocyte processes in the RNFL. At 14d after both types of ONC, the average RGC survival rate is about 12% in the injured area, compatible to other models. In vivo OCT and ex vivo microscopic examinations confirm RGC loss precedes proximal axon degeneration. **Conclusions:** TLGS retina is an excellent model, between commonly used rodent species and primate, for translational research in neurodegeneration and neuroprotection. This study provides a foundation for such future research endeavors.

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Structural and functional findings in patients with moderate diabetic retinopathy

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Purpose: To evaluate structural and functional ocular changes in patients with type 2 diabetes mellitus (DM2) and moderate diabetic retinopathy (DR) without apparent diabetic macular edema (DME) assessed by optical coherence tomography (OCT) and microperimetry. **Methods:** This was a single-center cross-sectional descriptive study for which 75 healthy controls and 66 DM2 patients with moderate DR were recruited (one eye per patient was included). All eyes underwent a complete ophthalmic examination (axial length, macular imaging with swept-source OCT, and MAIA microperimetry). Macular thicknesses, ganglion cell complex (GCC) thicknesses, and central retinal sensitivity were compared between groups, and the relationships between the OCT and microperimetry parameters were evaluated. **Results:** Macular thickness was similar in both groups. There was a diminution in the parafoveal areas thickness in the DM2 group in the GCC complex. Retinal sensitivity was reduced in all sectors in the DM2 group. Moderate correlations were detected between the central sector of MAIA microperimetry and retina total central thickness (-0.347; p=0.0035). Age, visual acuity, and hemoglobin A1c levels also correlated with retinal sensitivity. **Conclusion:** Macular GCC thickness and central retinal sensitivity were reduced in patients with moderate DR without DME, suggesting the presence of macular neurodegeneration prior to the appearance of DME.

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Systemic Administration of 7, 8-Dihydroxyflavone Protects Adult Rat Retinal Ganglion Cells from Axotomy-induced Loss

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Purpose: To examine the responses of different types of retinal ganglion cells (RGCs) to intraorbital optic nerve transection (IONT) and systemic treatment with 7,8-Dihydroxyflavone (DHF), a selective TrkB agonist. **Methods:** Adult rats had a left IONT and were treated with daily i.p. injections of 5 mg/kg DHF or vehicle (saline). Animals were analyzed at survival intervals ranging 7 to 60 days and the retinas were prepared as wholemounts and immunolabelled for Brn3a and melanopsin to identify surviving RGCs. Additional animals were analyzed with western-blot at 1, 3 or 7 days to examine TrkB activation in the retina (n=4 each). **Results:** Following IONT and treatment with saline or with DHF caused loss of 41% or 7%, 72% or 10%, 83% or 14%, 87% or 83%, 90% or 90% and 92% or 92% of the original Brn3a$^+$RGC population at 7, 10, 14, 21, 30 or 60 days, respectively (n=8 each). Thus, DHF neuroprotection was maximal at 7 days and remained for up to 21 days. When quantifying the m$^+$RGC population, treatment with saline or with DHF caused loss of approximately 86% or 85%, 76% or 75%, 59% or 48%, 56% or 49%, 57% or 50% and 63% or 52% of the original m$^+$RGC population at 7, 10, 14, 21, 30 or 60 days, respectively (n=6 each). At 7 days, optimal neuroprotection resulted in significant retinal TrkB phosphorylation. **Conclusions:** DHF produces TrkB activation and protects Brn3a$^+$RGCs and m$^+$RGCs. DHF neuroprotection for Brn3a$^+$RGCs is maximal at 7 days but remains for up to 21 days, whereas neuroprotection for m$^+$RGCs is observed at 14 days and is permanent.

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Potential of Broccoli and/or Tigernut for Ocular Health

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Purpose: Diet influences vision. The lack of essential nutrients can damage visual health by increasing the risk of developing ocular diseases. We address the benefits of natural food, broccoli (BC) or tigernut (TN) on the eyes. **Methods:** The role of a daily, core nutritional regimen containing BC in macular health was analysed in 14 voluntaries divided into two groups: daily BC consumers of 375g for 4 consecutive weeks (BCG; n=7), and non-BC consumers (CG; n=7). Plasma total antioxidant activity (TAC) and measurement of macular pigment optical density (MPOD) by the Visucam 500® retinographies were performed. To evaluate the effects of a daily TN intake in the eyes, two studies were run: A) dry eye disorder (DED) patients were examined by the Schirmer and break-up-time (BUT) tests (n=20), and B) healthy volunteers (n=30) were evaluated for analysing plasmatic TAC and measuring MPOD. Statistics was done by the SPSS program. **Results:** The regular BC regimen induced amelioration of visual subjective sensations, significantly increasing the MPOD (30% more than CG; $p<0.05$). Also, significantly higher plasmatic TAC levels were found in the BCG (baseline: 1.231 ± 0.120mM; end-of-study: 1.858±0.393mM; $p<0.001$). The nutritional TN intervention resulted in significantly higher ($p<0.05$) BUT (right-eye: 7.4±0.7sec. vs. 9.8 ± 0.4 sec; left-eye: 7.5±0.7sec vs. 9.7±0.4 sec) and Schirmer (right-eye: 7.1±0.7mm vs. 10.5±0.9 351mm; 7.0±0.6mm vs. 12.9±1.8 mm) tests respect to baseline. About the redox status, an increase in plasma TAC levels as well as 50% more of MPOD in both eyes were detected after a 30g daily TN intake for 3-months. **Conclusions:** Natural food have the potential to increase antioxidant status, to provide carotenoids to the macula as well as to promote tear film stability by counteracting the oxidative and inflammatory load in people at risk of eye diseases.

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Choroidal Thickness Analysis in Keratoconus Patients

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Purpose: Inflammatory alterations recently identified in keratoconus (KC) patients could be related to an enhanced vascular flow in adjacent tissues such as the choroid, one of the most vascularized of our anatomy. Our purpose is to determine and compare choroidal thickness (CT) in KC patients using Enhanced Depth Imaging (EDI) Spectral-Domain OCT. Our secondary goal was to compare topographic and keratometric indexes with the mean CT in KC patients **Methods:** A comparative cross-sectional study including 26 patients with KC and 26 healthy controls assessed with Pentacam and EDI-OCT was performed. CT was measured manually with the Caliper function at thirteen locations at 500 µm regular intervals by the same technician. **Results:** The average CT in T6 was 253.96 ± 88.95 µm in the KC group, and of 309.39 ± 94.11 µm in the control group ($p < 0.041$). No significant differences in mean CT were observed in the rest of the points, including the subfoveal CT (M), 351.48 ± 106.3 vs 365.35 ± 114.6 µm, ($p = 0.66$). No correlation was observed between the mean subfoveal CT (M) in the KC group, and the values of K1 ($p = 0.977$ and $p = 0.498$ respectively), K2 ($p = 0.450$ and $p = 0.656$), corneal asphericity (Q) ($p = 0.986$ and $p = 0.902$), minimal pachymetry (Pachy) ($p = 0.408$ and $p = 0.688$), keratoconus index (KI) ($p = 0.601$ and $p = 0.217$), vertical asymmetry index (VAT) ($p = 0.296$ and $p = 0.523$), staging of KC (TKC) ($p = 0.549$ and $p = 0.08$) and corneal apex morphology. **Conclusions:** No significant difference of CT values between KC patients and healthy subjects was found. We found no association between the refractive and keratometric indexes obtained by corneal topography and subfoveal CT

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Extracellular Vesicles in Ophthalmology: Biomarkers and Therapeutic Opportunities

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Purpose: Extracellular vesicles (EVs) are lipid bilayered nanovesicles (30-500nm) produced by all cell types. EVs contains lipids, protein and RNA cargo resembling their cells of origin and the cellular state. Cells release vesicles in physiologically in response to external factors as mediators of intercellular communication. Different types of EVs have been shown to be involved in many pathological processes. They may also play an important role in the treatment of eye diseases such as glaucoma, diabetic retinopathy and keratitis. Our group is interested in finding new EV-based biomarkers of eye diseases, and to this end we compared different sources and methods for obtaining eye-fluid derived EVs. **Methods:** We analysed EVs from human tears (collected using capillary tube or Schirmer strips) and aqueous humor (AH, collected during cataract surgery). EVs were isolated by SEC. This method allows to recover EVs with a very low level of non-EV related proteins, thus permitting the definition of EV-related biomarkers. Isolated EVs were analyzed by bead-based flow cytometry and protein content (microBCA). **Results:** Interestingly, we have found increased levels of EVs markers (CD9, CD63, and CD81) in tears than in AH. This, together with the minimal invasiveness of the collection method, point to tears as a preferential source of EVs for biomarker discovery. Also, both capillary tubes and Schirmer strips are a suitable method for obtaining EV, with some technical advantages for the strip. The possibility to perform patient-based proteomic analyses of these samples (instead of pooling samples) is currently being evaluated. **Conclusions:** Eye fluid derived-EVs may be isolated from tears and AH by SEC, thus permitting the identification of EV-related biomarkers for eye-related pathologies. The future definition of such biomarkers may open new opportunities for the treatment of several eye diseases.

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Design and Test of New Drugs with Therapeutic Potential for the Treatment of Retinal Degenerative Diseases

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Purpose: Oxidative stress and inflammation are common pathogenic features of retinal degenerative diseases. Drugs with anti-oxidant and/or anti-inflammatory properties have a therapeutic potential in slowing-down the degenerative process. In a collaborative work between our groups of the University of Alicante (UA), Instituto Teófilo Hernando of the Universidad Autónoma de Madrid (ITH-UAM) and IQM-CSIC, we design, synthetized and test new drugs with therapeutic potential for the treatment of retinal degenerative diseases. As a first collaboration, we established the *in vivo* proof of concept of ITH12674, a new Nrf2 inducer, as a neuroprotectant. In an ongoing work, we test the potential therapeutical effect of ITH15004, a P2X7R blocker, in retinal degeneration. **Methods:** First, ITH12674 was intraperitoneally treated at a dose of 1 or 10 mg/Kg in rd10 mice from P16 to P30. At P30, retinal function was evaluated by electroretinography and optomotor test. Retinal morphology was evaluated by immunohistochemistry. Oxidative stress and inflammatory state were evaluated by Western-blot, and flow cytometry. In ongoing experiments, the ability of ITH15004 to prevent ATP-induced cell death is being tested in cultures of both, the photoreceptor-derived 661W cell line and in the Müller-derived MU-PH1 cell line. **Results:** ITH12674 (10 mg/kg) preserved retinal functionality and morphology and reduced the inflammatory state, showing neuroprotective capabilities. ITH15004 (30 μM) shows protection to ATP (10 mM)-treated 661W and MU-PH1 cell lines. **Conclusions:** The collaboration of our groups, with experience in SNC degenerative diseases and chemical design (ITH-UAM; IQM-CSIC) and in retinal degeneration (UA) provides a synergistic effect in the search of new drugs with therapeutic potential for the treatment of retinal degenerative diseases.

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In vivo systemic administration of ITH-IB6 prevents NDMA-induced but not Axotomy-induced Retinal Ganglion Cell death

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Purpose: To study the neuroprotective effects of ITH-IB6 on the survival of adult rat retinal ganglion cells (RGCs) in two models of retinal injury, axotomy and NMDA-induced excitotoxicity. This compound has been described to protect against several toxic stimuli related to neurodegeneration, such as Tau hyperphosphorylation or glutamate excitotoxicy. **Methods:** Adult Sprague Dawley rats received a daily subcutaneous (sc) injection of ITH-IB6 (30 m g/kg, in saline with 1%DMSO) or vehicle (saline with 1% DMSO) starting just before retinal injury. In one group (n=12), the left optic nerve was intraorbitally transected (IONT) and in another group (n=10) the left eye received an intravitreal injection of 100nM NMDA. Rats were sacrificed one week later, perfused through the heart with saline and 4% paraformaldehyde, both eyes were enucleated, the retinas were prepared as flattened wholemounts with four radial cuts, the deepest one signaling the superior pole, and immunolabelled with Brn3a to identify surviving RGCs. Retinas were photographed, total numbers of Brn3a+RGCs were automatically counted and topographic maps were constructed for each retina to investigate their retinal distribution. **Results:** Following IONT and daily sc treatment with ITH-IB6 or vehicle caused the loss of approximately 36.5% (n=6) or 40.6% (n=6), respectively, of the original Brn3a+RGC population at 7 days. Following NMDA injection and sc treatment with ITH-IB6 or vehicle, resulted in the loss of approximately 39.2% (n=4) or 72.3%, respectively, of the original Brn3a+RGC population at 7 days. **Conclusions:** While ITH-IB6 does not prevent axotomy-induced loss of RGCs, it has a potent neuroprotective effect against NMDA-induced RGC loss, with prevention by 7 days of the loss of approximately 32% of the original Brn3a+RGC population. Thus, ITH-IB6 affords neuroprotection against excitotoxicity-induced RGC loss.