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# MD Research News

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## Drug treatment

**Br J Ophthalmol. 2015 Mar 16. [Epub ahead of print]**

**Significance of the hyperautofluorescent ring associated with choroidal neovascularisation in eyes undergoing anti-VEGF therapy for wet age-related macular degeneration.**

Camacho N, Barteselli G, Nezgoda JT, El-Emam S, Cheng L, Bartsch DU, Freeman WR.

AIM: To characterise the presence of a hyperautofluorescent (HAF) ring associated with choroidal neovascularisation (CNV) complex in patients with wet age-related macular degeneration (AMD).

METHODS: Fundus autofluorescence images and spectral-domain optical coherence tomography (OCT) scans from 362 eyes with wet AMD were reviewed. The presence and size of an HAF ring associated with the CNV complex was evaluated. A subgroup of 64 treatment-naive eyes with new-onset CNV was studied to analyse the relationship between pretreatment OCT characteristics and the presence of the HAF ring.

RESULTS: An HAF ring was present in 38% of the entire cohort of eyes and in 39% of treatment-naive eyes. The presence of the HAF ring was significantly correlated with the extent of baseline subretinal fluid (SRF) on OCT (p=0.0113), the number of antivascular endothelial growth factor (VEGF) injections (p=0.0439) and the number of treatment cycles (p=0.0154). Eyes with an HAF ring were more likely to have disruption of the ellipsoid zone line once the SRF was resolved compared with eyes without an HAF ring (p=0.0002). In multivariate analysis, the best predictors for HAF ring were the baseline area of SRF (p=0.0449) and the number of anti-VEGF treatments received (p=0.0568).

CONCLUSIONS: Nearly 40% of wet AMD eyes had an HAF ring. In treatment-naive eyes, the HAF ring had a significant association with SRF and was found as early as the baseline measurement and as long as 18 months after beginning treatment, persisting for up to 6 years after the initial diagnosis. Its association with baseline SRF and disruption of the ellipsoid zone line of the photoreceptors on OCT could indicate continuous stress on the outer retinal structures after exposure to prolonged SRF and/or transmitted autofluorescence from loss of the photoreceptors overlying the retinal pigment epithelium.

PMID: 25777818 [PubMed - as supplied by publisher]

**Br J Ophthalmol. 2015 Mar 16. [Epub ahead of print]**

**Aflibercept therapy for polypoidal choroidal vasculopathy: short-term results of a multicentre study.**

Koizumi H, Kano M, Yamamoto A, Saito M, Maruko I, Sekiryu T, Okada AA, Iida T.

BACKGROUND/AIMS: To investigate short-term outcomes of intravitreal aflibercept injections (IAIs) for polypoidal choroidal vasculopathy (PCV).

METHODS: 91 eyes of 88 consecutive patients with treatment-naive PCV examined at three university hospitals received IAI monthly for 3 months. One month after the third IAI, changes in best-corrected visual acuity (BCVA) and macular morphology were retrospectively evaluated. Additionally, possible baseline characteristics predictive of persistent retinal fluid were analysed.

RESULTS: The mean BCVA (logarithm of the minimum angle of resolution units) of the 91 eyes improved from 0.31 at baseline to 0.21 at 3 months ($p<0.0001$). The mean central retinal thickness and mean subfoveal choroidal thickness decreased from 323 μm and 270 μm at baseline to 185 μm and 232 μm at 3 months, respectively ($p<0.0001$ for both). Seventy-three eyes (80.2%) achieved a dry macula defined as absence of retinal fluid. Presence of the baseline characteristics of subretinal haemorrhage and greater size of the largest polyp were significantly associated with inability to achieve a dry macula ($p=0.008$ and 0.03, respectively). However, this association was not found on multivariate logistic regression. Of the 90 eyes that underwent indocyanine green angiography at 3 months, 43 eyes (47.8%) showed complete and 28 eyes (31.1%) showed partial resolution of polyps. Twenty-four eyes (24.4%) also showed partial regression of branching choroidal vascular networks.

CONCLUSIONS: IAIs for the treatment of a large number of PCV eyes were found to improve both visual acuity and macular morphology over the short term.

PMID: 25777816 [PubMed - as supplied by publisher]

**Eur J Pharm Biopharm. 2015 Mar 14. [Epub ahead of print]**

**Injectable formulations for an intravitreal sustained-release application of a novel single-chain VEGF antibody fragment.**

Asmus LR, Grimshaw JP, Richle P, Eicher B, Urech DM, Gurny R, Möller M.

Abstract: Sustained-release formulations of a single-chain anti-VEGF-A antibody fragment were investigated in vitro toward their potential use for intravitreal applications. The hydrophobic polyester hexylsubstituted poly(lactic acid) (hexPLA) was selected as the sustained-release excipient for its biodegradability and semi-solid aggregate state, allowing an easy and mild formulation procedure. The lyophilized antibody fragment ESBA903 was micronized and incorporated into the liquid polymer matrix by cryo-milling, forming homogeneous and injectable suspensions. The protein showed excellent compatibility with the hexPLA polymer and storage stability at 4°C for 10weeks. Additionally, hexPLA shielded the incorporated active substance from the surrounding medium, resulting in a better stability of ESBA903 inside the polymer than after its release in the buffer solution. Formulations of ESBA903 with hexPLA having drug loadings between 1.25% and 5.0% and polymer molecular weights of 1500g/mol, 2500g/mol, 3500g/mol and 5000g/mol were investigated regarding their in vitro release. All formulations except with the highest molecular weight formed spherical depots in aqueous buffer solutions and released the antibody fragment for at least 6-14weeks. The polymer viscosity derived from the molecular weight strongly influenced the release rate, while the drug loading had minor influence, allowing customization of the release profile and the daily drug release. Size exclusion chromatography and SDS-PAGE revealed that the antibody fragment structure was kept intact during incorporation and release from the liquid matrix. Furthermore, the released protein monomer maintained its high affinity to human VEGF-A, as measured by surface plasmon resonance analysis. Formulations of ESBA903 in hexPLA meet the basic needs to be used for intravitreal sustained-release applications in age-related macular degeneration treatment.

PMID: 25779352 [PubMed - as supplied by publisher]

**Trials. 2015 Dec;16(1):608. Epub 2015 Mar 10.**

**Comparing different dosing regimens of bevacizumab in the treatment of neovascular macular degeneration: study protocol for a randomised controlled trial.**

Foss A, Childs M, Reeves B, Empeslidis T, Tesha P, Dhar-Munshi S, Mughal S, Culliford L, Rogers C, Tan W, Montgomery A.

BACKGROUND: Bevacizumab (Avastin®) is as effective as ranibizumab (Lucentis®) in the treatment of neovascular age-related macular degeneration (nAMD). However it has two important structural differences. First, it has two active sites instead of one; second, it retains the Fc portion of the antibody which would be expected to confer a significantly longer half-life. These agents have been associated with systemic complications including strokes, so it is desirable to use the smallest effective dose. Furthermore, the standard dosing regimen requires monthly hospital visits, which present a significant challenge both to the hospital services and to the patients (who are elderly).

METHODS/DESIGN: Patients ≥50 years who are eligible for anti-vascular endothelial growth factor (VEGF) treatment of nAMD in the NHS, who are either newly referred for treatment or have reactivation of nAMD and who have not received treatment to either eye for the previous six months. We have designed a factorial multi-centre masked randomised controlled trial using bevacizumab as the intervention, with patients randomised to one of four arms: to standard or low dose and to monthly or two-monthly patient review. The aim is to recruit sufficient patients (around 1,000) to obtain 304 patients meeting the endpoint over a four-year period. The primary endpoint is time to treatment failure to be analysed using Cox regression.

DISCUSSION: This randomised control trial will show if half dose and two monthly as required is as effective as full dose and monthly regimes. A two monthly as required regimen of Bevacizumab would significantly reduce both the cost and the service delivery burden for the treatment of nAMD while a reduced dose would be expected to enhance the safety profile of this treatment regime.

PMID: 25778604 [PubMed - in process]

**Retina. 2015 Mar 16. [Epub ahead of print]**

**TREATMENT OF EXUDATIVE AGE-RELATED MACULAR DEGENERATION WITH RANIBIZUMAB COMBINED WITH KETOROLAC EYEDROPS OR PHOTODYNAMIC THERAPY.**

Semeraro F, Russo A, Delcassi L, Romano MR, Rinaldi M, Chiosi F, Costagliola C.

PURPOSE: To evaluate whether ketorolac eyedrops plus intravitreal ranibizumab (IVR) or verteporfin photodynamic therapy plus IVR provides additional benefit over IVR monotherapy for treatment of choroidal neovascularization in age-related macular degeneration.

METHODS: This was a prospective, randomized, pilot study in 75 patients with naive choroidal neovascularization. Patients were randomized 1:1:1 into 3 groups: ranibizumab monotherapy (RM), ranibizumab plus ketorolac, or ranibizumab plus loading-phase reduced-fluence verteporfin photodynamic therapy (RV) groups.

RESULTS: At 12 months, all groups showed significant improvement in both best-corrected visual acuity and central retinal thickness. The mean best-corrected visual acuity change from baseline to 12 months was -0.14 ± 0.52 logMAR (20/73 ± 20/29), -0.25 ± 0.60 logMAR (20/46 ± 20/27), and -0.10 ± 0.30 (20/97 ± 20/40) logMAR in RM, ranibizumab plus ketorolac, and RV groups, respectively. The mean central retinal thickness change from baseline to 12 months was -125 ± 15 μm, -141 ± 21 μm, and -130 ± 15 μm in RM, ranibizumab plus ketorolac, and RV groups, respectively. Both ranibizumab plus ketorolac and RV groups required fewer IVR treatments than RM.

CONCLUSION: Compared with RM and ranibizumab plus verteporfin photodynamic therapy, the

combination of 0.45% ketorolac eyedrops 3 times a day and ranibizumab in patients with choroidal neovascularization provided superior best-corrected visual acuity and central retinal thickness outcomes. Both combination regimens required fewer IVR injections than RM during the 12-month follow-up period.

PMID: 25784358 [PubMed - as supplied by publisher]

**Wien Klin Wochenschr. 2015 Mar 19. [Epub ahead of print]**

**Predictors of 1-year visual outcome in OCT analysis comparing ranibizumab monotherapy versus combination therapy with PDT in exudative age-related macular degeneration.**

Weingessel B, Mihaltz K, Vécsei-Marlovits PV.

AIM: The aim of this study was to find predictive factors of 1-year visual outcome, analyzing novel optical coherence tomography (OCT) biomarkers in exsudative age-related macular degeneration (choroidal neovascularization (CNV)) in two groups of different treatment modalities.

METHODS: In all, 34 consecutive patients with new-onset CNV were randomized 1:1 to receive either ranibizumab monotherapy or ranibizumab combined with photodynamic therapy (PDT) with verteporfin. After three initial injections with ranibizumab, re-treatment was performed according to an as-needed scheme; PDT was performed once at baseline. Best-corrected visual acuity (BCVA) and OCT parameters like central macular volume (CMV), central macular thickness (or central retinal thickness (CRT)), subretinal and intraretinal fluid, fibrovascular lesion thickness, or inner segment/outer segment (IS/OS) junction were analyzed.

RESULTS: After 12 months, a visual gain of 6.1 letters was found in the monotherapy group, whereas patients in the combination therapy group lost - 4.8 letters from baseline to the 12-month visit. CMV and CRT decreased considerably between baseline and month 2-3 in both groups, with a following slight increase until month 12. Additional application of PDT had negative effect to 12-month BCVA, whereas higher baseline BCVA and integrity of the IS/OS junction at month 12 had positive effect to 12-month BCVA.

CONCLUSIONS: Better baseline BCVA and the integrity of IS/OS junction at 12-month visit were the most important predictive factors for final BCVA. Combination therapy caused worse final BCVA and a higher degree of IS/OS disruption.

PMID: 25787216 [PubMed - as supplied by publisher]

**Curr Opin Ophthalmol. 2015 Mar 13. [Epub ahead of print]**

**Current state of therapeutic strategies with ranibizumab in neovascular age-related macular degeneration.**

Gallego-Pinazo R, Figueroa MS, García-Layana A.

PURPOSE OF REVIEW: To summarize the current dosing strategies in the management of neovascular age-related macular degeneration with intravitreal injections of ranibizumab.

RECENT FINDINGS: A variety of therapeutic strategies has been recently described as an alternative to the monthly fixed treatment. The efficacy and local and systemic safety results of each approach is relevant in order to make a clinical decision and to provide patients an accurate information.

SUMMARY: The proposed therapeutic strategies achieve positive visual outcomes when compared with monthly fixed regimen in the clinical trials. However, the real-life practice does not reflect these results. The main cause of this difference is the incapability to adopt any of the different strategies as the clinics are completely booked and this turns into a delay in the diagnostic and treatment visits.

PMID: 25774961 [PubMed - as supplied by publisher]

**Doc Ophthalmol. 2015 Mar 15. [Epub ahead of print]**

**Peripheral retinal function assessed with 30-Hz flicker seems to improve after treatment with Lucentis in patients with diabetic macular oedema.**

Holm K, Schroeder M, Lövestam Adrian M.

PURPOSE: To evaluate the influence of ranibizumab on the multifocal electroretinogram (Mf-ERG), full-field electroretinogram (Ff-ERG) and optical coherence tomography (OCT) in diabetic eyes (n = 20) with macular oedema.

METHODS: In 20 eyes (20 diabetic subjects) with no or background diabetic retinopathy and macular oedema (age 65.7 ± 9.8 years, duration 16.5 ± 10.0 years), the change in ETDRS letters, Mf-ERG, Ff-ERG and OCT was analysed, at baseline, 4 weeks after the first injection, (just before the second injection), and 4 weeks after the last injection with ranibizumab.

RESULTS: From baseline, mean BCVA improved from 64.0 ± 10.0 ETDRS letters to 75.0 ± 7.3 ETDRS letters (p = 0.005) 1 month after the last injection. Mean OCT thickness reduced after the first injection from 418 ± 117 to 311 ± 126 µm; (p = 0.001) and to 302 ± 74 µm after the third injection. Mf-ERG demonstrated in the innermost three rings a shorter implicit time after the first injection with p values of 0.002, 0.005 and 0.017, respectively. After the third injection, implicit time was prolonged to almost the original levels. Cone implicit time with 30-Hz flicker improved significantly between baseline (35.5 ± 3.6 ms) and final follow-up (34.6 ± 3.1 ms) (p = 0.04).

DISCUSSION: Though the central retinal thickness was reduced after three injections of ranibizumab and the subjects gained a mean of 11 ETDRS letters, there was no significant change in amplitude or implicit time in Mf-ERG. The shortened 30-Hz flicker implicit time might imply that ranibizumab has no negative impact on the entire peripheral cone function, but can improve it instead.

PMID: 25773362 [PubMed - as supplied by publisher]

**Ophthalmol Ther. 2015 Mar 14. [Epub ahead of print]**

**Missed Hospital Appointments of Patients Receiving Ranibizumab Therapy for Neovascular Age-Related Macular Degeneration.**

Karampelas M, Pefkianaki M, Rees A, Gill N, Kotecha A, Hamilton R, Nikita E, Patel PJ.

INTRODUCTION: The aim of this study was to investigate the frequency and duration of missed hospital appointments (MHAs) in a consecutive cohort of patients treated with ranibizumab for neovascular age-related macular degeneration (nAMD) and to assess their impact on outcomes of therapy in a real-world clinical setting.

METHODS: Retrospective, cross-sectional study of consecutive patients attending medical retina clinics for nAMD treatment with ranibizumab.

RESULTS: Seventy-eight eyes of 78 patients met the inclusion criteria for data analysis. Mean age was 78 years with mean follow-up of 27 months. Mean visual acuity (VA) was 52 ± 16 letters at baseline, 56 ± 17 letters at year 1 and 58 ± 16 letters at year 2. At the end of the second year, 90% of the patients had lost <15 letters, 26% had gained ≥15 letters and 10% had lost ≥15 letters. Nineteen patients had at least one MHA (24%) over 2 years. There were 26 MHA episodes in total leading to a median duration of 79 days (range 35-159) between attended hospital visits. None of these MHAs occurred during the first 3 months after treatment initiation. Mean VA and central retinal thickness difference between 2 years and baseline for the MHA group was not statistically different compared with the non-MHA group.

CONCLUSIONS: Our data suggest that MHA may be a relatively common occurrence in AMD treatment clinics, but good outcomes of treatment can be achieved over 2 years despite missed hospital visits if

patients are reviewed on average six times in the first year after an initial loading phase of three injections and nine times in the second year of treatment.

PMID: 25769782 [PubMed - as supplied by publisher]

**Am J Ophthalmol. 2015 Mar 10. [Epub ahead of print]**

**Progression of retinal pigment epithelial atrophy in antiangiogenic therapy of neovascular age-related macular degeneration.**

Schütze C, Wedl M, Baumann B, Pircher M, Hitzenberger CK, Schmidt-Erfurth U.

PURPOSE: To monitor retinal pigment epithelial (RPE) atrophy progression during antiangiogenic therapy of neovascular age-related macular degeneration (AMD) over two years using polarization-sensitive optical coherence tomography (OCT).

DESIGN: Prospective interventional case series.

METHODS: Setting: Clinical practice Study Population: 30 patients (31 eyes) with treatment-naïve neovascular AMD.

OBSERVATION PROCEDURES: Standard intravitreal therapy (0.5mg ranibizumab) was administered monthly during the first year and pro re nata (PRN) as-needed during the second year. Spectral domain (SD) OCT and polarization-sensitive OCT (selectively imaging the RPE) examinations were performed at baseline and 1, 3, 6, 12 and 24 months using a standardized protocol. RPE-related changes were evaluated using a semi-automated polarization-sensitive OCT segmentation algorithm and correlated with SD-OCT and fundus autofluorescence (FAF) findings.

MAIN OUTCOME MEASURES: RPE response, geographic atrophy (GA) progression RESULTS: Atrophic RPE changes included RPE thinning, RPE porosity, focal RPE atrophy and development of GA. Early RPE loss (i.e. RPE porosity, focal atrophy) increased progressively during initial monthly treatment and remained stable during subsequent PRN-based therapy. GA developed in 61% of eyes at month 24. Mean GA area increased from 0.77mm2 at 12 months to 1.10mm2 (standard deviation=1.09mm2) at 24 months. Reactive accumulation of RPE-related material at the lesion borders increased until month 3 and subsequently decreased.

CONCLUSIONS: Progressive RPE atrophy and GA developed in the majority of eyes. RPE migration signifies certain RPE plasticity. Polarization-sensitive OCT specifically images RPE-related changes in neovascular AMD contrary to conventional imaging methods. Polarization-sensitive OCT allows to precisely monitor the sequence of RPE-related morphologic changes.

PMID: 25769245 [PubMed - as supplied by publisher]

## Other treatment & diagnosis

**Curr Opin Ophthalmol. 2015 Mar 16. [Epub ahead of print]**

**Introduction to microperimetry and its use in analysis of geographic atrophy in age-related macular degeneration.**

Hanout M, Horan N, Do DV.

PURPOSE OF REVIEW: This article discusses recent advances in the fundus-guided perimetry (microperimetry) and its utilization in evaluation and monitoring of patients with geographic atrophy.

RECENT FINDINGS: Although best-corrected visual acuity has been gold standard in clinical practice for

decades, it does not provide an entire assessment of visual function that determines daily activity and quality of life of a patient. Furthermore, psychophysical tests, including low-luminance visual acuity, reading speed, and contrast sensitivity, cannot be used to quantify retinal sensitivity or detect pattern of retinal dysfunction. Microperimetry provides a true evaluation of visual function by offering fundus-controlled testing through eye-tracking technology that allows for structural and functional correlation and test-retest reliability for the same test point. Furthermore, it enables precise assessment of location and stability of fixation. Recent research has shown microperimetry to be more representative of the macular function in macular diseases.

SUMMARY: Microperimetry is currently the clinical investigation of choice to assess residual visual functions and functional vision in macular degenerative diseases, especially geographic atrophy. There is an increasing popularity to employ microperimetry in clinical trials investigating new treatments for geographic atrophy, as well as other macular degenerative diseases, as a reliable functional outcome measure.

PMID: 25784112 [PubMed - as supplied by publisher]

**Reprod Biol Endocrinol. 2015 Dec;13(1):5. Epub 2015 Feb 22.**

**Human embryonic stem cell cultivation: historical perspective and evolution of xeno-free culture systems.**

Desai N, Rambhia P, Gishto A.

Abstract: Human embryonic stem cells (hESC) have emerged as attractive candidates for cell-based therapies that are capable of restoring lost cell and tissue function. These unique cells are able to self-renew indefinitely and have the capacity to differentiate in to all three germ layers (ectoderm, endoderm and mesoderm). Harnessing the power of these pluripotent stem cells could potentially offer new therapeutic treatment options for a variety of medical conditions. Since the initial derivation of hESC lines in 1998, tremendous headway has been made in better understanding stem cell biology and culture requirements for maintenance of pluripotency. The approval of the first clinical trials of hESC cells for treatment of spinal cord injury and macular degeneration in 2010 marked the beginning of a new era in regenerative medicine. Yet it was clearly recognized that the clinical utility of hESC transplantation was still limited by several challenges. One of the most immediate issues has been the exposure of stem cells to animal pathogens, during hESC derivation and during in vitro propagation. Initial culture protocols used co-culture with inactivated mouse fibroblast feeder (MEF) or human feeder layers with fetal bovine serum or alternatively serum replacement proteins to support stem cell proliferation. Most hESC lines currently in use have been exposed to animal products, thus carrying the risk of xeno-transmitted infections and immune reaction. This mini review provides a historic perspective on human embryonic stem cell culture and the evolution of new culture models. We highlight the challenges and advances being made towards the development of xeno-free culture systems suitable for therapeutic applications.

PMID: 25778481 [PubMed - in process]

**IEEE Trans Med Imaging. 2015 Mar 6. [Epub ahead of print]**

**Stratified Sampling Voxel Classification for Segmentation of Intraretinal and Subretinal Fluid in Longitudinal Clinical OCT Data.**

Xu X, Lee K, Zhang L, Sonka M, Abramoff M.

Abstract: Automated three-dimensional retinal fluid (named symptomatic exudate-associated derangements, SEAD) segmentation in 3D OCT volumes is of high interest in the improved management of neovascular Age Related Macular Degeneration (AMD). SEAD segmentation plays an important role in the treatment of neovascular AMD, but accurate segmentation is challenging because of the large diversity of

SEAD size, location, and shape. Here a novel voxel classification based approach using a layer-dependent stratified sampling strategy was developed to address the class imbalance problem in SEAD detection. The method was validated on a set of 30 longitudinal 3D OCT scans from 10 patients who underwent anti-VEGF treatment. Two retinal specialists manually delineated all intraretinal and subretinal fluid. Leave-one-patient-out evaluation resulted in a true positive rate and true negative rate of 96% and 0.16% respectively. This method showed promise for image guided therapy of neovascular AMD treatment.

PMID: 25769146 [PubMed - as supplied by publisher]

**Retina. 2015 Mar 12. [Epub ahead of print]**

**REFRACTILE DRUSEN: Clinical Imaging and Candidate Histology.**

Suzuki M1, Curcio CA, Mullins RF, Spaide RF.

PURPOSE: To evaluate eyes with refractile drusen using clinical imaging and to identify candidate histologic correlates of refractile drusen.

METHODS: Refractile drusen were defined as drusenoid material containing small refractile spherules. Retrospective analysis of color, autofluorescence, and spectral domain optical coherence tomography images of eyes with refractile drusen was performed to characterize the morphology and topography of these lesions. Macular sections from donor eyes were processed with a von Kossa stain for calcium phosphate and viewed by light microscopy. Punches of retinal pigment epithelium-choroid from donors with geographic atrophy were prepared for transmission electron microscopy.

RESULTS: Fundus findings of 14 eyes of 10 patients with age-related macular degeneration (age, 82.9 ± 5.6 years) were evaluated. A generalized loss of autofluorescence signal over refractile drusen appeared to spread over a larger area than each druse, for drusen located centrally. By color fundus photography, refractile drusen showed corresponding depigmentation around drusen that were located in the center of the macula. Optical coherence tomography imaging of refractile drusen showed hyperreflective dots. In the histologic specimens, drusen contained many small spherules rich in calcium phosphate. Ultrastructural examination of the spherules showed complex assemblies consisting of concentric shells containing thin layers of calcium.

CONCLUSION: Refractile drusen appear to be a stage of drusen regression marked by loss of retinal pigment epithelium, thus contributing to the development of geographic atrophy. Calcium-containing spherules appear to account for the glistening appearance.

PMID: 25768253 [PubMed - as supplied by publisher]

**Retina. 2015 Mar 12. [Epub ahead of print]**

**EPIRETINAL MEMBRANES IN NEOVASCULAR AGE-RELATED MACULAR DEGENERATION: Effect on Outcomes of Anti-vascular Endothelial Growth Factor Therapy.**

Karaca EE, Kepez Yldz B, Çubuk MÖ, Özdek Ş.

PURPOSE: To investigate the role of epiretinal membrane (ERM) on outcomes of anti-vascular endothelial growth factor therapy in patients with neovascular age-related macular degeneration (nAMD).

METHODS: This study is a retrospective observational case series and was conducted at the Gazi University School of Medicine, Ankara, Turkey. The reports of the patients with a diagnosis of new-onset nAMD, who were aged at least 50 years and treated with intravitreal anti-vascular endothelial growth factors (ranibizumab or bevacuzimab) between October 2010 and September 2013 in our retina clinic, were reviewed for the vitreomacular interface changes.

RESULTS: The study included 90 eyes of 90 patients with nAMD. The mean age of the patients was 70 ± 7.5 years, with 35 (38.9%) being male and 55 (61.1%) being female. According to the examinations with optical coherence tomography and B-mode ultrasonography, 43 patients had "concurrent" vitreomacular adhesion (30 focal, 13 broad; Group 1). Twenty-nine patients had complete posterior vitreous detachment (Group 2) and 18 patients (Group 3) had ERM. The number of injections was highest for the patients with ERM (Group 3), and this difference was statistically significant ($P < 0.001$). The mean interval between injections and the mean longest interval were shorter in Group 3 ($P < 0.05$).

CONCLUSION: The presence of ERM in association with nAMD seems to increase the number of anti-vascular endothelial growth factor injections and decrease the injection intervals for the treatment of nAMD. Although the anatomical and functional results are similar in eyes with or without ERM, the increased need for anti-vascular endothelial growth factors may mean that these membranes may decrease the penetration of the drugs through these membranes, which may act as a physical barrier. Additionally, increased inflammation in patients with ERM probably requires more frequent injections.

PMID: 25768251 [PubMed - as supplied by publisher]

**Drug Deliv Transl Res. 2015 Apr;5(2):168-86. [eCollection 2015]**

**Integration of drug, protein, and gene delivery systems with regenerative medicine.**

Lorden ER, Levinson HM, Leong KW.

Abstract: Regenerative medicine has the potential to drastically change the field of health care from reactive to preventative and restorative. Exciting advances in stem cell biology and cellular reprogramming have fueled the progress of this field. Biochemical cues in the form of small molecule drugs, growth factors, zinc finger protein transcription factors and nucleases, transcription activator-like effector nucleases, monoclonal antibodies, plasmid DNA, aptamers, or RNA interference agents can play an important role to influence stem cell differentiation and the outcome of tissue regeneration. Many of these biochemical factors are fragile and must act intracellularly at the molecular level. They require an effective delivery system, which can take the form of a scaffold (e.g., hydrogels and electrospun fibers), carrier (viral and nonviral), nano- and microparticle, or genetically modified cell. In this review, we will discuss the history and current technologies of drug, protein, and gene delivery in the context of regenerative medicine. Next, we will present case examples of how delivery technologies are being applied to promote angiogenesis in nonhealing wounds or prevent angiogenesis in age related macular degeneration. Finally, we will conclude with a brief discussion of the regulatory pathway from bench to bedside for the clinical translation of these novel therapeutics.

PMID: 25787742 [PubMed - in process]

## Pathogenesis

**J Mol Med (Berl). 2015 Mar 18. [Epub ahead of print]**

**Light induces NLRP3 inflammasome activation in retinal pigment epithelial cells via lipofuscin-mediated photooxidative damage.**

Brandstetter C, Mohr LK, Latz E, Holz FG, Krohne TU.

Abstract: Photooxidative damage and chronic innate immune activation have been implicated in retinal pigment epithelium (RPE) dysfunction, a process that underlies blinding diseases such as age-related macular degeneration (AMD). To identify a potential molecular link between these mechanisms, we investigated whether lipofuscin-mediated phototoxicity activates the NLRP3 inflammasome in RPE cells in vitro. We found that blue light irradiation (dominant wavelength 448 nm, irradiance 0.8 mW/cm2, duration 6 h) of lipofuscin-loaded primary human RPE cells and ARPE-19 cells induced photooxidative damage,

lysosomal membrane permeabilization (79.5 % of cells vs. 3.8 % in nonirradiated controls), and cytosolic leakage of lysosomal enzymes. This resulted in activation of the inflammasome with activation of caspase-1 and secretion of interleukin-1β (14.6 vs. 0.9 pg/ml in nonirradiated controls) and interleukin-18 (87.7 vs. 0.2 pg/ml in nonirradiated controls). Interleukin secretion was dependent on the activity of NLRP3, caspase-1, and lysosomal proteases cathepsin B and L. These results demonstrate that accumulation of lipofuscin-like material in vitro renders RPE cells susceptible to phototoxic destabilization of lysosomes, resulting in NLRP3 inflammasome activation and secretion of inflammatory cytokines. This new mechanism of inflammasome activation links photooxidative damage and innate immune activation in RPE pathology and may provide novel targets for therapeutic intervention in retinal diseases such as AMD.

KEY MESSAGE: Visible light irradiation of lipofuscin-loaded RPE cells activates inflammasome. • Inflammasome activation results from lysosomal permeabilization and enzyme leakage. • Inflammasome activation induces secretion of inflammatory cytokines by RPE cells. • Photooxidative damage by visible light as new mechanism of inflammasome activation. • Novel link between hallmark pathogenetic features of retinal degenerative diseases.

PMID: 25783493 [PubMed - as supplied by publisher]

**Free Radic Biol Med. 2015 Mar 12. [Epub ahead of print]**

**Light-induced disulfide dimerization of recoverin under ex vivo and in vivo Conditions.**

Zernii EY, Nazipova AA, Gancharova OS, Kazakov AS, Serebryakova MV, Zinchenko DV, Tikhomirova NK, Senin II, Philippov PP, Permyakov EA, Permyakov SE.

Abstract: Despite vast knowledge of molecular mechanisms underlying photochemical damage of photoreceptors, linked to progression of age-related macular degeneration, the information on specific protein targets of the light-induced oxidative stress is scarce. Here, we demonstrate that prolonged intense illumination (halogen bulb, 1,500lx, 1-5h) of mammalian eyes under ex vivo (cow) or in vivo (rabbit) conditions induces disulfide dimerization of recoverin, a Ca2+-dependent inhibitor of rhodopsin kinase. Western blotting and mass spectrometry analysis of retinal extracts reveals illumination time-dependent accumulation of disulfide homodimer of recoverin and its higher order disulfide cross-linked species, including a minor fraction of mixed disulfides with intracellular proteins (tubulins, etc.). Meanwhile, monomeric bovine recoverin remains mostly reduced. These effects are accompanied by accumulation of disulfide homodimer of visual arrestin. Histological studies demonstrate that the light-induced oxidation of recoverin and arrestin occurs in intact retina (illumination for 2h), while illumination for 5h is associated with damage of photoreceptor layer. A comparison of ex vivo levels of disulfide homodimer of bovine recoverin with redox dependence of its in vitro thiol-disulfide equilibrium (glutathione redox pair) gives the lowest estimate of redox potential in rod outer segments under the illumination from -160mV to -155mV. Chemical crosslinking and dynamic light scattering data demonstrate an increased propensity of disulfide dimer of bovine recoverin to multimerization/aggregation. Overall, the oxidative stress caused by the prolonged intense illumination of retina might affect rhodopsin desensitization via concerted disulfide dimerization of recoverin and arrestin. The developed herein models of eye illumination are useful for studies of the light-induced thiol oxidation of visual proteins.

PMID: 25772009 [PubMed - as supplied by publisher]

**Transl Vis Sci Technol. 2015 Mar 10;4(2):6. eCollection 2015.**

**Suppression of Laser-Induced Choroidal Neovascularization by the Oral Medicine Targeting Histamine Receptor H4 in Mice.**

Ijima R, Kaneko H, Ye F, Takayama K, Nagasaka Y, Kataoka K, Funahashi Y, Iwase T, Kachi S, Kato S, Terasaki H.

PURPOSE: This study aimed to examine relationship of histamine receptor H4 (HRH4) and the pathogenesis of laser-induced choroidal neovascularization (laser-CNV) and to determine whether oral administration of HRH4 antagonists suppressed laser-CNV in mice.

METHODS: Laser photocoagulation was performed in mice to induce the laser-CNV. Histamine was administered intravitreously, and CNV volume was measured. Laser photocoagulation and intravitreous injection of HRH4 antagonist JNJ7777120 were performed after intraperitoneal injection of clodronate liposome, which depletes circulating monocyte-derived macrophages; CNV volume was compared with that in mice injected with control (dimethyl sulfoxide [DMSO]/PBS). Three days after laser-CNV, the F4/80+CD11b+ macrophage population in retinal pigment epithelium (RPE)/choroid complex was quantified with flow cytometry in wild-type and Hrh4-/- mice. The long-acting HRH4 antagonist JNJ28307474 was then administrated periorally, and the laser-CNV volume was compared with controls.

RESULTS: Intravitreous injection of histamine did not affect laser-CNV volume. The laser-CNV from the eye injected with JNJ7777120 was equivalent to that injected with the DMSO/PBS in mice that had intraperitoneally received clodronate liposome. Flow cytometry after laser-CNV induction revealed a smaller F4/80+CD11b+ macrophage population in the RPE/choroid complex of Hrh4-/- mice than in wild-type mice. Oral administration of JNJ28307474 significantly reduced laser-CNV volume in wild-type mice.

CONCLUSIONS: Our results suggested that HRH4-positive macrophages played an important role in the pathogenesis of laser-CNV and that they require a different ligand from that of histamine. The oral administration of an HRH4 antagonist successfully reduced laser-CNV.

PMID: 25774332 [PubMed] PMCID: PMC4356033

**Exp Eye Res. 2015 Mar 13. [Epub ahead of print]**

**Molecular mechanisms of subretinal fibrosis in age-related macular degeneration.**

Ishikawa K, Kannan R, Hinton DR.

Abstract: Subretinal fibrosis is a result of a wound healing response that follows choroidal neovascularization in neovascular age-related macular degeneration (nAMD). Although anti-vascular endothelial growth factor therapy has become a standard treatment that improves visual acuity in many nAMD patients, unsuccessful treatment outcomes have often been attributed to the progression of subretinal fibrosis. In this review, we summarize the cellular and extracellular components of subretinal fibrous membranes and also discuss the possible molecular mechanisms including the functional involvement of growth factors and the inflammatory response in the process. Moreover, we present an murine animal model of subretinal fibrosis that might facilitate greater understanding of the pathophysiology and the development of novel therapeutic strategies for the inhibition of subretinal fibrosis in nAMD.

PMID: 25773985 [PubMed - as supplied by publisher]

**Sci Rep. 2015 Mar 16;5:9144.**

**7-Ketocholesterol Increases Retinal Microglial Migration, Activation, and Angiogenicity: A Potential Pathogenic Mechanism Underlying Age-related Macular Degeneration.**

Indaram M, Ma W, Zhao L, Fariss RN, Rodriguez IR, Wong WT.

Abstract: Age-related macular degeneration (AMD) has been associated with both accumulation of lipid and lipid oxidative products, as well as increased neuroinflammatory changes and microglial activation in the outer retina. However, the relationships between these factors are incompletely understood. 7-Ketocholesterol (7KCh) is a cholesterol oxidation product localized to the outer retina with prominent pro-inflammatory effects. To explore the potential relationship between 7KCh and microglial activation, we localized 7KCh and microglia to the outer retina of aged mice and investigated 7KCh effects on retinal

microglia in both in vitro and in vivo systems. We found that retinal microglia demonstrated a prominent chemotropism to 7KCh and readily internalized 7KCh. Sublethal concentrations of 7KCh resulted in microglial activation and polarization to a pro-inflammatory M1 state via NLRP3 inflammasome activation. Microglia exposed to 7KCh reduced expression of neurotrophic growth factors but increased expression of angiogenic factors, transitioning to a more neurotoxic and pro-angiogenic phenotype. Finally, subretinal transplantation of 7KCh-exposed microglia promoted choroidal neovascularization (CNV) relative to control microglia in a Matrigel-CNV model. The interaction of retinal microglia with 7KCh in the aged retina may thus underlie how outer retinal lipid accumulation in intermediate AMD results in neuroinflammation that ultimately drives progression towards advanced AMD.

PMID: 25775051 [PubMed - in process] PMCID: PMC4360733

**Photochem Photobiol Sci. 2015 Mar 20. [Epub ahead of print]**

**Liposomal hypocrellin B as a potential photosensitizer for age-related macular degeneration: pharmacokinetics, photodynamic efficacy, and skin phototoxicity in vivo.**

Li T, Hou X, Deng H, Zhao J, Huang N, Zeng J, Chen H, Gu Y.

Abstract: Photodynamic therapy (PDT) has been successfully implemented as a treatment for wet age-related macular degeneration (AMD), but very few photosensitizers have been developed for clinical use. Herein, we describe a novel formulation of liposomal hypocrellin B (LHB) that was prepared by high-pressure homogenization. The encapsulation efficiency and PDT efficacy in vitro of this new preparation were found to remain nearly constant over 1 year. Moreover, LHB is rapidly cleared from the blood, with a half-life of 2.319 ± 0.462 h and a very low serum concentration at 24 h after injection. Testing in a rat model of choroidal neovascularization (CNV) showed that leakage of blood vessels in CNV lesions was significantly reduced when LHB PDT was given at a dose of 1 mg kg-1 along with yellow laser irradiation; the damage to the collateral retina and the retinal pigment epithelium was minimal. Skin phototoxicity assays showed that only two of the 200 mice given a 4 mg per kg dose of LHB experienced an inflammatory reaction in the auricle irradiated at 24 h after dosing. These data collectively indicate that LHB may be a safe and effective photosensitizer for vascular-targeted PDT of AMD.

PMID: 25793654 [PubMed - as supplied by publisher]

**Invest Ophthalmol Vis Sci. 2015 Feb 24;56(3):1820-9.**

**Identification of miRNAs in a Model of Retinal Degenerations.**

Saxena K, Rutar MV, Provis JM, Natoli RC.

PURPOSE: We investigated the expression profile of and identify all microRNAs (miRNAs) that potentially regulate inflammation in a light-induced model of focal retinal degeneration.

METHODS: Sprague Dawley (SD) rats aged 90 to 140 postnatal days were exposed to 1000 lux white fluorescent light for 24 hours. At 24 hours, and 3 and 7 days after exposure, the animals were euthanized and retinas processed for RNA. Expression of 750 miRNAs at 24 hours of exposure was assessed using low density array analysis. Significantly modulated miRNAs and their target mRNAs were used to assess the potential biological effects. Expression of seven miRNAs, potentially modulating inflammation, was investigated across a protracted time course after light exposure using quantitative PCR. Photoreceptor cell death was analyzed using TUNEL.

RESULTS: Intense light exposure for 24 hours led to differential expression of a number of miRNAs, 37 of which were significantly modulated by 2-fold or more. Of those, 19 may potentially regulate the inflammatory immune response observed in the model. MicroRNAs -125-3p, -155, -207, -347, -449a, -351, and -542-3p are all upregulated at 24 hours of exposure along with peak photoreceptor cell death. The

MiRNAs -542-3p and -351 reached maximum expression at 7 days after exposure, while -125-3p, -155, -207, -347, and -449 reached a peak expression at 3 days.

CONCLUSIONS: The results of the study show that miRNAs are modulated in response to light damage (LD). These miRNAs potentially regulate the inflammatory immune response, triggered as a result of the acute retinal damage, which is a key mediator of retinal degeneration in this model and age-related macular degeneration.

PMID: 25711632 [PubMed - in process]

## Epidemiology

**Ophthalmic Epidemiol. 2015 Apr;22(2):75-84.**

**Age-related macular degeneration in ethnically diverse Australia: Melbourne Collaborative Cohort Study.**

Robman LD, Islam FM, Chong EW, Adams MK, Simpson JA, Aung KZ, Makeyeva GA, Hopper JL, English DR, Giles GG, Baird PN, Guymer RH.

PURPOSE: To determine and compare the prevalence of age-related macular degeneration (AMD) in older Australians of Anglo-Celtic and Southern European origin.

METHODS: A total of 21,132 participants of the Melbourne Collaborative Cohort Study, aged 47-86 years, were assessed for AMD in 2003-2007 with non-mydriatic fundus photography. Of these, 14% were born in Southern Europe (Greece, Italy or Malta), with the remaining 86% of Anglo-Celtic origin, born in Australia, the United Kingdom or New Zealand.

RESULTS: Overall, 2694 participants (12.7%) had early stages of AMD, defined as either one or more drusen ≥125 μm (with or without pigmentary abnormalities) or one or more drusen 63-124 μm with pigmentary abnormalities in a 6000-μm diameter grading grid, in the absence of late AMD in either eye. A total of 122 participants (0.6%) had late AMD, defined as either geographic atrophy or neovascular AMD. In logistic regression analysis, adjusted for age, sex, smoking, education and physical activity, Southern Europeans compared to Anglo-Celts had a higher prevalence of the early stages of AMD (odds ratio, OR, 1.15, 95% confidence interval, CI, 1.00-1.34), and lower prevalence of late AMD (OR 0.36, 95% CI 0.17-0.78).

CONCLUSIONS: Australians of Southern European origin have a higher prevalence of the early stages of AMD and lower prevalence of late AMD compared to those of Anglo-Celtic origin. Although AMD prevalence in the older age group(s) of Southern Europeans could be underestimated due to disparity in participation rates, it is likely that both lifestyle and genetic factors play their parts in differential AMD prevalence in these ethnic groups.

PMID: 25777306 [PubMed - in process]

Sci Rep. 2015 Mar 20;5:9345.

Calcium, ARMS2 Genotype, and Chlamydia Pneumoniae Infection in Early Age-Related Macular Degeneration: a Multivariate Analysis from the Nagahama Study.

Nakata I, Yamashiro K, Kawaguchi T, Nakanishi H, Akagi-Kurashige Y, Miyake M, Tsujikawa A, Yamada R, Matsuda F, Yoshimura N; Nagahama Study Group.

Abstract: Although various risk factors have been identified for the development of age-related macular

degeneration (AMD), risk factors of early AMD have been relatively under studied. We aimed to investigate AMD risk factors by evaluating multiple factors in association with large drusen, an important component of AMD, simultaneously. In a community-based cross-sectional survey in Japan, 971 large drusen cases and 3,209 controls were compared for 65 variables, including systemic, environmental, and genetic factors. The association and the effect size of each factor were evaluated with logistic regression analysis using a backward-elimination approach. Multivariate analyses identified a significant association in serum calcium level (odds ratio [OR] = 0.932, P = 1.05 × 10(-3)), ARMS2 A69S (rs10490924) genotype (OR = 1.046, P < 0.001), Chlamydia pneumoniae IgG (OR = 1.020, P = 0.0440), and age (OR = 1.013, P < 0.001) for large drusen. Hypocalcemia was observed in 7.2% of large drusen cases and in 5.5% of controls (P = 0.0490). C. pneumoniae infections was more frequent in large drusen cases (56.4%) than in controls (51.7%, P = 0.00956). These results suggest that calcium, ARMS2 genotype, C. pneumonia infection, and age are significant factors in the development of the early stages of AMD.

PMID: 25792034 [PubMed - in process]

Am J Ophthalmol. 2015 Mar 10. [Epub ahead of print]

Prevalence of Intermediate-Stage Age-Related Macular Degeneration in Patients with the Acquired Immunodeficiency Syndrome.

Jabs DA, Van Natta ML, Sezgin E, Pak JW, Danis R; Studies of the Ocular Complications of AIDS Research Group.

PURPOSE: To evaluate the prevalence of intermediate-stage age-related macular degeneration (AMD) in patients with the acquired immunodeficiency syndrome (AIDS).

DESIGN: Cross sectional study of patients with AIDS enrolled in the Longitudinal Study of the Ocular Complications of AIDS METHODS: Intermediate-stage AMD was determined from enrollment retinal photographs by graders at a centralized Reading Center, using the Age-Related Eye Disease Study grading system. Graders were masked as to clinical data.

RESULTS: Of 1825 participants with AIDS and no ocular opportunistic infections, 9.9% had intermediate-stage AMD. Risk factors included age, with an odds ratio (OR) of 1.9 (95% confidence interval [CI] 1.6, 2.3, P<0.001) for every decade of age; the prevalence of AMD ranged from 4.0% for participants 30-39 years old to 24.3% for participants >60 years old. Other risk factors included the HIV risk groups of injection drug use (OR= 2.4, 95% CI 1.5, 3.9, P<0.001) or heterosexual contact (OR=1.9, 95% CI 1.3, 2.8, P=0.001). Compared with the HIV-uninfected population in the Beaver Dam Offspring Study, there was an approximate 4-fold increased age-adjusted prevalence of intermediate-stage AMD.

CONCLUSIONS: Patients with AIDS have an increased age-adjusted prevalence of intermediate-stage AMD compared with that found in a non-Human Immunodeficiency Virus (HIV)-infected cohort evaluated with similar methods. This increased prevalence is consistent with the increased prevalence of other age-related diseases in antiretroviral-treated, immune-restored, HIV-infected persons when compared to non-HIV-infected persons.

PMID: 25769246 [PubMed - as supplied by publisher]

**J Ophthalmol. 2015;2015:605814. [Epub ahead of print]**

**The Prevalence of Age-Related Eye Diseases and Cataract Surgery among Older Adults in the City of Lodz, Poland.**

Nowak MS, Smigielski J.

Purpose: To determine the prevalence of age-related eye diseases and cataract surgery among older adults in the city of Lodz, in central Poland.

Material and Methods: The study design was cross-sectional and observational study. A total of 1107

women and men of predominantly Caucasian origin were successfully enumerated and recruited for the study. All selected subjects were interviewed and underwent detailed ophthalmic examinations.

Results: Overall 8.04% (95% CI 6.44-9.64) subjects had cataract surgery in either eye. After excluding subjects with bilateral cataract surgery, the prevalence of cataract was 12.10% (95% CI 10.18-14.03). AMD was found in 4.33% (95% CI 3.14-5.54 ) of all subjects. Of them 3.25% (95% CI 2.21-4.30 ) had early AMD and 1.08% (95% CI 0.47-1.69) had late AMD. Various types of glaucoma were diagnosed in 5.51% (95% CI 4.17-6.85) of subjects and 2.62% (95% CI 1.68-3.56) had OHT. The prevalence rates of DR and myopic macular degeneration were 1.72% (95% CI 0.95-2.48) and 0.45% (95% CI 0.06-0.85), respectively. All multiple logistic regression models were only significantly associated with older age. The highest rate of visual impairment was observed among subjects with retinal diseases.

Conclusions: The study revealed high prevalence of age-related eye diseases in this older population.

PMID: 25789169 [PubMed] PMCID: PMC4350620

**Invest Ophthalmol Vis Sci. 2015 Mar 18. [Epub ahead of print]**

**Risk Factors for Age-Related Macular Degeneration in an Elderly Japanese Population: The Hatoyama Study.**

Aoki A, Tan X, Yamagishi R, Shinkai S, Obata R, Miyaji T, Yamaguchi T, Numaga J, Ito H, Yanagi Y.

PURPOSE: To estimate the risk factors of age-related macular degeneration (AMD) in an elderly Japanese population from a suburban area north of metropolitan Tokyo.

METHODS: The Hatoyama Cohort Study was launched in 2010, and 742 persons participated in the baseline study. Among these participants, 596 persons who attended the 2-year follow-up examinations in 2012 were evaluated, and the presence of early and late AMD was determined via grading of their fundus photographs. Based on the cohorts' data, logistic regression analyses were performed to identify the risk factors for AMD. The possible risk factors that we examined were age, sex, medical history of systemic disorders, smoking, inflammatory markers at baseline, and the complement factor H (CFH) I62V and age-related maculopathy susceptibility 2 (ARMS2) A69S variants.

RESULTS: We assessed 480 participants (40.0% women) who had a gradable fundus photographs. The prevalence of early AMD was 37.9% and the prevalence of late AMD was 0.6%. Mantel-Haenszel analysis revealed that the CFH I62V and ARMS2 A69S variants were significantly associated with the prevalence of AMD (P = 0.029 and 0.025, respectively).

CONCLUSIONS: The CFH I62V and ARMS2 A69S variants were significantly associated with the prevalence of AMD.

PMID: 25788651 [PubMed - as supplied by publisher]

## Genetics

**PLoS One. 2015 Mar 18;10(3):e0119570.**

**Lens Status Influences the Association between CFH Polymorphisms and Age-Related Macular Degeneration: Findings from Two Population-Based Studies in Singapore.**

Wong CW, Liao J, Cheung GC, Khor CC, Vithana EN, Wang JJ, Mitchell P, Aung T, Wong TY, Cheng CY.

AIMS: To determine the differential effects of genetic polymorphism in CFH and ARMS2 on risk of age-related macular degeneration (AMD) between phakic vs. pseudophakic/aphakic eyes.

METHODS: 9,529 eyes of 4,918 participants from the Singapore Malay Eye Study and Singapore Indian

Eye Study were analyzed. Participants had detailed eye examinations, including slit-lamp examinations and dilated fundus photography. AMD grading was performed according to the Wisconsin age-related maculopathy grading system. Lens status was defined. Single nucleotide polymorphisms (SNPs) rs10801555 (Y402H) within CFH and rs3750847 in ARMS2 were assessed. The main outcome measure was early AMD or any AMD.

RESULTS: No significant associations between the CFH Y402H genotypes and early AMD were found in phakic individuals. In contrast, among pseudophakic/aphakic individuals, the CFH Y402H risk genotypes were significantly associated with higher odds of early AMD, with an OR of 1.57 (95% CI: 1.07-2.29) for GA genotype and 2.40 (95% CI: 1.25-4.61) for AA genotype, compared to those with GG genotype. There was significant interaction between pseudophakic/aphakic status and CFH Y402H variant on risk of early AMD (p = 0.037), adjusting for age, gender, and the first 5 genetic principal components. No significant interaction was found between lens status and ARMS2 rs3750847.

CONCLUSIONS: CFH genetic polymorphism and pseudophakic/aphakic status may have a potential synergistic effect on early AMD, suggesting roles for the complement system and related pathways in the pathogenesis of AMD in eyes after cataract surgery.

PMID: 25786237 [PubMed - in process]

**Eye (Lond). 2015 Mar 13. [Epub ahead of print]**

**Gene-gene interaction of CFH, ARMS2, and ARMS2/HTRA1 on the risk of neovascular age-related macular degeneration and polypoidal choroidal vasculopathy in Chinese population.**

Huang L, Meng Q, Zhang C, Sun Y, Bai Y, Li S, Deng X, Wang B, Yu W, Zhao M, Li X.

Purpose: To evaluate the association and interaction of five single-nucleotide polymorphisms (SNPs) in three genes (CFH, ARMS2, and ARMS2/HTRA1) with neovascular age-related macular degeneration (nAMD) and polypoidal choroidal vasculopathy (PCV) in Chinese population.

Methods: A total of 300 nAMD and 300 PCV patients and 301 normal subjects participated in the present study. The allelic variants of rs800292, rs2274700, rs3750847, rs3793917, and rs1065489 were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Gene-gene interactions were evaluated by the data mining approach multifactor-dimensionality reduction (MDR) method.

Results: The risk alleles of CFH rs800292, rs2274700, ARMS2 rs3057847, and ARMS2/HTRA1 rs3793917 showed significant difference between nAMD or PCV patients and controls (all P<0.01). The homozygosity of risk alleles for rs800292, rs2274700, rs3750847, and rs3793917 were significantly different between nAMD patients and controls (all P<0.01), and predisposed to PCV patients (all P<0.01). After cross-validation consistency (CVC) and permutation tests, the two-locus model rs2274700_rs3750847 has a balanced accuracy of 64.37% in predicting nAMD disease risk. The one-marker model, rs3750847, and two -locus model rs2274700_rs3750847 has a balanced accuracy of 66.07% and 65.89% in predicting PCV disease risk, respectively. Furthermore, CFH rs1065489 did not show significant association with nAMD (P>0.01), but was strongly associated with PCV in Chinese patients (P<0.001).

Conclusions: In this study, we found that the interaction of ARMS2 and ARMS2/HTRA1 is significantly associated with nAMD, and the interaction of CFH and ARMS2 is pronounced in PCV development in Chinese population.

PMID: 25771815 [PubMed - as supplied by publisher]

**Hum Mol Genet. 2015 Mar 18. [Epub ahead of print]**

**Rare Genetic Variants in the CFI Gene are Associated with Advanced Age-Related Macular**

**Degeneration and Commonly Result in Reduced Serum Factor I Levels.**

Kavanagh D, Yu Y, Schramm EC, Triebwasser M, Wagner E, Raychaudhuri S, Daly MJ, Atkinson JP, Seddon JM.

Abstract: To assess a potential diagnostic and therapeutic biomarker for age-related macular degeneration (AMD), we sequenced the complement factor I gene (CFI) in 2266 individuals with AMD and 1400 without, identifying 231 individuals with rare genetic variants. We evaluated the functional impact by measuring circulating serum factor I (FI) protein levels in individuals with and without rare CFI variants. The burden of very rare (frequency<1/1000) variants in CFI was strongly associated with disease (P=1.1 x 10-8). In addition, we examined eight coding variants with counts≥5 and saw evidence for association with AMD in three variants. Individuals with advanced AMD carrying a rare CFI variant had lower mean FI compared to non-AMD subjects carrying a variant (P<0.001). Further new evidence that FI levels drive AMD risk comes from analyses showing individuals with a CFI rare variant and low FI were more likely to have advanced AMD (P=5.6 x 10-5). Controlling for covariates, low FI increased the risk of advanced AMD among those with a variant compared to individuals without advanced AMD with a rare CFI variant (OR 13.6, P=1.6 x 10-4), and also compared to control individuals without a rare CFI variant (OR 19.0, P=1.1 x 10-5). Thus, low FI levels are strongly associated with rare CFI variants and AMD. Enhancing FI activity may be therapeutic and measuring FI provides a screening tool for identifying patients who are most likely to benefit from complement inhibitory therapy.

PMID: 25788521 [PubMed - as supplied by publisher]

**JAMA Ophthalmol. 2015 Mar 19. [Epub ahead of print]**

**Genetic Testing for Age-Related Macular Degeneration: Not Indicated Now.**

Stone EM.

Abstract: Age-related macular degeneration is a very common condition that is caused by a complex interplay of genetic and environmental factors. It is likely that, in the future, genetic testing will allow physicians to achieve better clinical outcomes by administering specific treatments to patients based on their genotypes. However, improved outcomes for genotyped patients have not yet been demonstrated in a prospective clinical trial, and as a result, the costs and risks of routine genetic testing currently outweigh the benefits for patients with age-related macular degeneration.

PMID: 25789813 [PubMed - as supplied by publisher]

**BMC Ophthalmol. 2015 Dec;15(1):8. [Epub ahead of print]**

**Common synonymous variants in ABCA4 are protective for chloroquine induced maculopathy (toxic maculopathy).**

Grassmann F, Bergholz R, Mändl J, Jägle H, Ruether K, Weber BH.

BACKGROUND: Chloroquine (CQ) and hydroxychloroquine (HCQ) are used to treat auto-immune related diseases such as rheumatoid arthritis (RA) or systemic lupus erythematosus. Both drugs however can cause retinal toxicity eventually leading to irreversible maculopathy and retinopathy. Established risk factors are duration and dosage of treatment while the involvement of genetic factors contributing to toxic maculopathy is largely unclear. To address the latter issue, this study aimed to expand on earlier efforts by (1) evaluating risk-altering variants known to be associated with age-related macular degeneration (AMD), a frequent maculopathy in individuals over 55 years of age, and (2) determining the contribution of genetic variants in the coding sequence of the ABCA4 gene.

METHODS: The ABCA4 gene was analyzed by deep sequencing technology using a personal genome machine (Ion Torrent) with 200 bp read length. Assessment of AMD variants was done by restriction enzyme digestion of PCR products and TaqMan SNP genotyping. Effect sizes, p-values and confidence

intervals of common variants were evaluated by logistic regression (Firth's bias corrected). To account for multiple testing, p-values were adjusted according to the false discovery rate.

RESULTS: We found no effects of known AMD-associated variants on the risk of toxic maculopathy. In contrast, we report a statistically significant association of common variants in the ABCA4 gene with retinal disease, assessed by a score-based variance-component test (PSKAT = 0.0055). This association remained significant after adjustment for environmental factors like age and duration of medication and was driven by three common variants in ABCA4 (c.5682G > C, c.5814A > G, c.5844A > G), all conferring a reduced risk for toxic maculopathy.

CONCLUSIONS: Our findings demonstrate that minor alleles of common genetic variants in ABCA4 significantly reduce susceptibility to develop toxic maculopathy under CQ treatment. A refined risk profile based on genetic and environmental factors may have implications for revised recommendations in CQ as well as HCQ treatment.

PMID: 25784260 [PubMed - in process] PMCID: PMC4352241

## Diet, lifestyle and low vision

**Ophthalmic Epidemiol. 2015 Apr;22(2):85-93.**

**Prospective Study of Plasma Homocysteine Level and Risk of Age-related Macular Degeneration in Women.**

Christen WG, Cook NR, Ridker PM, Buring JE.

PURPOSE: Prospective data to examine the association of homocysteine with age-related macular degeneration (AMD) are limited. We examined the prospective relation of plasma homocysteine level and AMD in a large cohort of apparently healthy women.

METHODS: We evaluated the relationship between baseline levels of plasma homocysteine and incident AMD among 27,479 female health professionals aged 40 years or older. Main outcome measures were total AMD, defined as self-report documented by medical record evidence of an initial diagnosis after randomization, and visually significant AMD, defined as confirmed incident AMD with visual acuity 20/30 or worse attributable to this condition.

RESULTS: During an average 10 years of follow-up, a total of 452 cases of AMD, including 182 cases of visually significant AMD, were documented. Women in the highest versus lowest quartile of plasma homocysteine had modestly, but statistically non-significant, increased risks of total AMD (hazard ratio, HR, 1.24, 95% confidence interval, CI, 0.95-1.63; p for trend 0.07) and visually significant AMD (HR 1.41, 95% CI 0.92-2.17; p for trend 0.052) in age- and treatment-adjusted analyses.

CONCLUSIONS: These prospective data from a large cohort of apparently healthy women do not support a strong role for homocysteine in AMD occurrence.

PMID: 25777307 [PubMed - in process] PMCID: PMC4363940

**Invest Ophthalmol Vis Sci. 2015 Mar 17. [Epub ahead of print]**

**The Influence of Coping on Vision-Related Quality of Life (VRQoL) in Patients with Low Vision: A Prospective Longitudinal Study.**

Sturrock BA, Xie J, Holloway EE, Lamoureux EL, Keeffe JE, Fenwick E, Rees G.

PURPOSE: To determine the longitudinal impact of specific coping strategies on vision-related quality of life (VRQoL) in patients with low vision.

METHODS: A single group, longitudinal study, utilising telephone-administered interviews conducted at baseline, 3-and 6-months with patients (visual acuity <6/12 in the better eye) recruited from low vision services. The Coping Strategy Indicator (CSI) assessed three strategies used specifically in relation to vision related problems: avoidant coping, problem-solving coping, and seeking social support. VRQoL was assessed using the Impact of Vision Impairment (IVI) questionnaire, which comprises two domains: vision-related functioning and vision-related emotional well-being. We used multivariable mixed linear regression, including time as an independent variable to assess change in VRQoL.

RESULTS: The study comprised 162 patients (Mean age = 69.8 years, 66% female), most with age-related macular degeneration (42%) and moderate vision impairment (41%; <6/18-6/60). Multivariable mixed linear regression showed that avoidant coping was a significant determinant of decline in vision-related functioning (β = -0.11, 95% confidence interval [CI] -0.22 to -0.01, p = 0.036) and emotional well-being (β = -0.29, 95% CI -0.45 to -0.13, p < 0.001) over a 6 month period.

CONCLUSIONS: Our findings showed that avoidant coping has a detrimental impact on VRQoL over time. Low vision specialists should be aware of their patients' coping strategies and encourage them to engage in active rather than avoidant coping to deal with the impact of their vision impairment.

PMID: 25783600 [PubMed - as supplied by publisher]

**RBMC Ophthalmol. 2015 Dec;15(1):13. [Epub ahead of print]**

**Baseline traits of patients presenting at a low vision clinic in Shanghai, China.**

Gao G, Ouyang C, Dai J, Xue F, Wang X, Zou L, Chen M, Ma F, Yu M.

BACKGROUND: Low vision, along with cataract, trachoma, onchocerciasis, childhood blindness and refractive error, is one of the priorities in the global initiative, VISION 2020-The Right to Sight. The purpose of this study was to characterize the traits of patients presenting at a low vision clinic in China.

METHODS: A retrospective study was conducted of the records of 299 patients who visited the Low Vision Clinic of Eye and ENT Hospital Affiliated to Fudan University from January 2009 to May 2014. Reviewed parameters included age, gender, education, occupation, cause of visual impairment and types of low vision aids (LVAs) dispensed.

RESULTS: Of all the patients (193 male; aged from 3 to 96 years, with a mean of 29.74 ± 25.23 years), 43.48% experienced moderate visual impairment, 25.42% had severe visual impairment and 21.07% were blind. The four major causes of visual impairment were congenital cataract (14.38%), degenerative myopia (13.71%), juvenile macular degeneration (9.36%) and retinitis pigmentosa (9.36%). The most common causes of visual impairment were congenital cataract (22.67%) in 0-19-year-olds, retinitis pigmentosa (20.62%) in 20-59-year-olds, and age-related macular degeneration (36.54%) in the 60+ group. With the help of LVAs, a significant improvement of distance and/or near vision or visual field was observed in 243 patients, of whom 185 accepted LVAs and 58 patients refused due to high price, inconvenience, young age (≤6 y), clumsy appearance and ignorance. The most commonly dispensed LVAs were stand magnifiers (21.57%) followed by spectacle-type LVAs (19.21%).

CONCLUSIONS: The majority of the patients in our low vision clinic were young, the main causes of visual impairment were congenital and hereditary diseases. Stand magnifiers were the most commonly dispensed LVAs. High price was the major reason for refusing LVAs.

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4-Hydroxy-7-oxo-5-heptenoic Acid (HOHA) Lactone is a Biologically Active Precursor for the Generation of 2-(ω-Carboxyethyl)pyrrole (CEP) Derivatives of Proteins and Ethanolamine Phospholipids.

Wang H, Linetsky MD, Guo J, Choi J, Hong L, Chamberlain AS, Howell SJ, Howes AM, Salomon RG.

Abstract: 2-(ω-Carboxyethyl)pyrrole (CEP) derivatives of proteins were previously shown to have significant pathological and physiological relevance to age-related macular degeneration, cancer and wound healing. Previously, we showed that CEPs are generated in the reaction of ε-amino groups of protein lysyl residues with 1-palmityl-2-(4-hydroxy-7-oxo-5-heptenoyl)-sn-glycero-3-phosphatidylcholine (HOHA-PC), a lipid oxidation product uniquely generated by oxidative truncation of docosahexanenate-containing phosphatidylcholine. More recently, we found that HOHA-PC rapidly releases HOHA-lactone and 2-lyso-PC (t1/2 = 30 min at 37 °C) by non-enzymatic transesterification/deacylation. Now we report that HOHA-lactone reacts with Ac-Gly-Lys-OMe or human serum albumin to form CEP derivatives in vitro. Incubation of human red blood cell ghosts with HOHA-lactone generates CEP derivatives of membrane proteins and ethanolamine phospholipids. Quantitative analysis of the products generated in the reaction HOHA-PC with Ac-Gly-Lys-OMe showed that HOHA-PC mainly forms CEP-dipeptide that is not esterified to 2-lysophosphatidycholine. Thus, the HOHA-lactone pathway predominates over the direct reaction of HOHA-PC to produce the CEP-PC-dipeptide derivative. Myleoperoxidase/H2O2/NO2- promoted in vitro oxidation of either 1-palmityl-2-docosahexaneoyl-sn-glycero-3-phosphatidylcholine (DHA-PC) or docosahexaenoic acid (DHA) generates HOHA-lactone in yields of 0.45% and 0.78%, respectively. Lipid oxidation in human red blood cell ghosts also releases HOHA-lactone. Oxidative injury of ARPE-19 human retinal pigmented epithelial cells by exposure to H2O2 generated CEP derivatives. Treatment of ARPE-19 cells with HOHA-lactone generated CEP-modified proteins. Low (submicromolar), but not high, concentra¬tions of HOHA-lactone promote increased vascular endothelial growth factor (VEGF) secretion by ARPE-19 cells. Therefore, HOHA-lactone not only serves as an intermediate for the generation of CEPs but also is a biologically active oxidative truncation product from docosahexaenoate lipids.

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**Protective Effects of Resveratrol against UVA-Induced Damage in ARPE19 Cells.**

Chan CM, Huang CH, Li HJ, Hsiao CY, Su CC, Lee PL, Hung CF.

Abstract: Ultraviolet radiation, especially UVA, can penetrate the lens, reach the retina, and induce oxidative stress to retinal pigment epithelial (RPE) cells. Even though it is weakly absorbed by protein and DNA, it may trigger the production of reactive oxygen species (ROS) and generate oxidative injury; oxidative injury to the retinal pigment epithelium has been implicated to play a contributory role in age-related macular degeneration (AMD). Studies showed that resveratrol, an abundant and active component of red grapes, can protect several cell types from oxidative stress. In this study, adult RPE cells being treated with different concentrations of resveratrol were used to evaluate the protective effect of resveratrol on RPE cells against UVA-induced damage. Cell viability assay showed that resveratrol reduced the UVA-induced decrease in RPE cell viability. Through flow cytometry analysis, we found that the generation of intracellular H2O2 induced by UVA irradiation in RPE cells could be suppressed by resveratrol in a concentration-dependent manner. Results of Western blot analysis demonstrated that resveratrol lowered the activation of UVA-induced extracellular signal-regulated kinase, c-jun-NH2 terminal kinase and p38 kinase in RPE cells. In addition, there was also a reduction in UVA-induced cyclooxygenase-2 (COX-2) expression in RPE cells pretreated with resveratrol. Our observations suggest that resveratrol is effective in preventing RPE cells from being damaged by UVA radiation, and is worth considering for further development as a chemoprotective agent for the prevention of early AMD.

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