

Issue 293

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This free weekly bulletin lists the latest published research articles on macular degeneration (MD) and some other macular diseases as indexed in the NCBI, PubMed (Medline) and Entrez (GenBank) databases.

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## Drug Treatment

**Acta Ophthalmol. 2016 Aug 18. [Epub ahead of print]**

**Neovascular age-related macular degeneration treated with ranibizumab or aflibercept in the same large clinical setting: visual outcome and number of injections.**

Rasmussen A, Sander B, Larsen M, Brandt S, Fuchs J, Hansen LH, Lund-Andersen H.

**PURPOSE:** To study visual outcome and number of annual injections in treatment-naïve patients with neovascular age-related macular degeneration (nAMD) before and after a change in first-line therapy from ranibizumab to aflibercept in a high-volume clinical practice.

**METHODS:** This was a retrospective chart review of routine clinical practice. The study included 1027 treatment-naïve patients, 559 of whom started intravitreal ranibizumab therapy in 2011-2012 and 468 of whom started intravitreal aflibercept therapy in 2013-2014, a fixed loading dose of three injections followed by a pro re nata treatment regimen used in both periods.

**RESULTS:** Snellen best-corrected visual acuity (BCVA) at baseline and after one year was 0.23 and 0.31 ( $p < 0.0001$ ), respectively, for patients treated with ranibizumab and 0.25 and 0.33 ( $p < 0.0001$ ) for patients treated with aflibercept, last observation carried forward. The share of patients (73%) still in treatment with ranibizumab at year 1 had a baseline BCVA of 0.26 but 0.40 at year 1 ( $p < 0.0001$ ), and the patients (75%) still in treatment with aflibercept at year 1 had a baseline BCVA of 0.28 but 0.42 at year 1 ( $p < 0.0001$ ). Proportional visual gains for both cohorts were comparable for one year ( $p = 0.14$ ). The number of injections given within year 1 including first injection was 6.9 for ranibizumab and 5.9 for aflibercept ( $p < 0.0001$ ). In patients continuing treatment through year 1, the number of injections was 8.0 for ranibizumab and 6.6 for aflibercept ( $p < 0.0001$ ). The two cohorts had similar cause-of-discontinuation profiles.

**CONCLUSION:** Treatment of nAMD at a single centre in two sequential cohorts yielded comparable BCVA outcomes with 15% fewer injections of aflibercept compared to ranibizumab.

PMID: 27535819

**Retina. 2016 Aug 16. [Epub ahead of print]**

**VISUAL ACUITY OUTCOMES OF RANIBIZUMAB TREATMENT IN PATHOLOGIC MYOPIC EYES WITH MACULAR RETINOSCHISIS AND CHOROIDAL NEOVASCULARIZATION.**

Cekic L, Munk MR, Wolf-Schnurbusch U, Gekkieva M, Wolf S.

**PURPOSE:** To investigate visual and morphological outcome in eyes with MRS and choroidal neovascularization (CNV) secondary to pathologic myopia treated with intravitreal (IVT) ranibizumab.

**METHODS:** Post hoc analysis of the patients included in the RADIANCE trial (n = 277) was performed to evaluate the impact of MRS on the functional outcome in patients with myopic choroidal neovascularization (mCNV) undergoing intravitreal ranibizumab injections.

**RESULTS:** Prevalence of MRS in pathologic myopia population is 6%. Respective patients were generally older than patients without MRS. Study eyes with MRS at baseline (BL) showed an initially poor treatment response after 3 months (mean change in best corrected visual acuity (BCVA) was  $2.8 \pm 12.4$  letters,  $P = 0.009$ ). After 12 months of treatment however, the mean change in BCVA was  $7.1 \pm 14.5$  early treatment diabetic retinopathy study (ETDRS) letters ( $P = 0.025$ ). Patients with MRS at baseline received more intravitreal injections than the other RADIANCE patients without MRS (MRS, n = 15 eyes:  $5.8 \pm 2.1$  vs. RADIANCE non-MRS [n = 207 eyes]:  $4.0 \pm 2.9$ ;  $P = 0.0001$ ).

**CONCLUSION:** Improvement of visual acuity is delayed and reduced after 3 months intravitreal ranibizumab in eyes with MRS and myopic choroidal neovascularization compared to eyes without MRS. More ranibizumab injections are needed in eyes with MRS to gain comparable BCVA at Month 12. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

PMID: 27533774

**Graefes Arch Clin Exp Ophthalmol. 2016 Aug 18. [Epub ahead of print]**

**Short-term focal macular electroretinogram of eyes treated by aflibercept & photodynamic therapy for polypoidal choroidal vasculopathy.**

Takayama K, Kaneko H, Kataoka K, Ueno S, Chang-Hua P, Ito Y, Terasaki H.

**BACKGROUND:** To compare short-term outcomes of intravitreal aflibercept injection (IAI) with or without initial photodynamic therapy (PDT) for polypoidal choroidal vasculopathy (PCV) using focal macular electroretinography (FMERG).

**DESIGN:** Observation case series.

**METHODS:** Twelve patients (6 males, 6 females; 12 eyes) with naïve PCV received 3 initial IAIs and a single session of PDT 3 days after the first IAI (combination group), and 13 patients (7 males, 6 females; 13 eyes) with naïve PCV received 3 initial IAIs only (IAI group) were retrospectively observed. Changes in visual acuity, central retinal thickness (CRT), central choroidal thickness (CCT), and FMERG parameters (FMERGs) were compared.

**RESULTS:** The combination group showed improved visual acuity after the second and third IAI ( $P = 0.040$ ,  $0.019$ , respectively); both groups showed reduced CRT after the first IAI ( $P < 0.01$ , each). Only the combination group showed reduced CCT after the third IAI ( $P = 0.031$ ). The FMERGs of the IAI group showed improved amplitudes of a-waves after the third IAI ( $P = 0.026$ ) and of b-waves after the first and third IAI ( $P = 0.034$ ,  $< 0.01$ , respectively); the combination group did not show improvement. The implicit times of the a- and b-waves were not changed in either group.

**CONCLUSIONS:** Combination therapy and IAI monotherapy each improved visual acuity and retinal structure to a similar degree; combination therapy reduced choroidal thickness but did not improve FMERGs in the short term.

PMID: 27538907

**Ophthalmol Eye Dis. 2016 Aug 7;8:31-2. eCollection 2016.**

# **Association of Anti-VEGF Injections with Progression of Geographic Atrophy.**

Enslow R, Bhuvanagiri S, Vegunta S, Cutler B, Neff M, Stagg B.

**ABSTRACT:** Age-related macular degeneration (AMD) is one of the leading causes of blindness in developed countries in people over the age of 60 years. One of the forms of advanced AMD is wet AMD. Wet AMD is a result of leakage and bleeding from abnormal neovascularization. The principal treatment for wet AMD is intravitreal anti-VEGF injections. A second form of advanced AMD is geographic atrophy (GA). GA refers to large areas of retinal pigment epithelium loss. In the literature, there is some concern that anti-VEGF injections administered to treat wet AMD may be associated with progression of GA. This review discusses evidence suggesting the association of anti-VEGF injections with progression of GA.

PMID: 27528805

**Ophthalmology. 2016 Aug 11. [Epub ahead of print]**

# **Introducing Anti-Vascular Endothelial Growth Factor Therapies for AMD Did Not Raise Risk of Myocardial Infarction, Stroke, and Death.**

Yashkin AP, Hahn P, Sloan FA.

**PURPOSE:** To assess the effect of availability of anti-vascular endothelial growth factor (VEGF) therapy on mortality and hospitalizations for acute myocardial infarction (AMI) and stroke over a 5-year follow-up period in United States Medicare beneficiaries newly diagnosed with exudative age-related macular degeneration (AMD) in 2006 compared with control groups consisting of beneficiaries (1) newly diagnosed with exudative AMD at a time when anti-VEGF therapy was not possible and (2) newly diagnosed with nonexudative AMD.

**DESIGN:** Retrospective cohort study.

**PARTICIPANTS:** Beneficiaries newly diagnosed with exudative and nonexudative AMD in 2000 and 2006 selected from a random longitudinal sample of Medicare 5% claims and enrollment files.

**METHODS:** Beneficiaries with a first diagnosis of exudative AMD in 2006 were the treatment group; beneficiaries newly diagnosed with exudative AMD in 2000 or nonexudative AMD in 2000 or 2006 were control groups. To deal with potential selection bias, we designed an intent-to-treat study, which controlled for nonadherence to prescribed regimens. The treatment group consisted of patients with clinically appropriate characteristics to receive anti-VEGF injections given that the therapy is available, bypassing the need to monitor whether treatment was actually received. Control groups consisted of patients with clinically appropriate characteristics but first diagnosed at a time when the therapy was unavailable (2000) and similar patients but for whom the therapy was not clinically indicated (2000, 2006). We used a Cox proportional hazard model.

**MAIN OUTCOME MEASURES:** All-cause mortality and hospitalization for AMI and stroke during follow-up.

**RESULTS:** No statistically significant changes in probabilities of death and hospitalizations for AMI and stroke within a 5-year follow-up period were identified in exudative AMD beneficiaries newly diagnosed in 2006, the beginning of widespread anti-VEGF use, compared with 2000. As an alternative to our main analysis, which excluded beneficiaries from nonexudative AMD group who received anti-VEGF therapies during follow-up, we performed a sensitivity analysis with this group of individuals reincluded (11% of beneficiaries newly diagnosed with nonexudative AMD in 2006). Results were similar.

**CONCLUSIONS:** Introduction of anti-VEGF agents in 2006 for treating exudative AMD has not posed a threat of increased risk of AMI, stroke, or all-cause mortality.

PMID: 27523614

**Can J Ophthalmol. 2016 Aug;51(4):302-5. Epub 2016 Jun 30.**

### **Ozurdex in age-related macular degeneration as adjunct to ranibizumab (The OARA Study).**

Chaudhary V, Barbosa J, Lam WC, Mak M, Mavrikakis E, Mohaghegh P SM.

**OBJECTIVE:** To evaluate the utility of dexamethasone intravitreal implant (DXI; Ozurdex; Allergan, Irvine, Calif.) in combination with ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland) versus ranibizumab monotherapy on visual acuity (VA) and anatomical outcomes in a neovascular age-related macular degeneration (nAMD) cohort.

**DESIGN:** Multicentred, single-blinded, pilot randomized control trial.

**PARTICIPANTS:** Ten patients 50 years or older with subfoveal choroidal neovascularization secondary to AMD were randomized to receive DXI in combination with ranibizumab (group 1) or ranibizumab alone (group 2) after a 3-month ranibizumab loading period.

**METHODS:** Group 1 patients received 1 DXI after the loading phase with the option of retreatment at months 4 to 6. Ranibizumab was administered pro re nata for 6 months in both study arms. Mean VA and central macular thickness (CMT) reductions from baseline to study endpoint (9 months) were reported in addition to adverse event frequency across study cohorts.

**RESULTS:** From baseline to the study endpoint, VA improved by  $10.8 \pm 13.2$  Early Treatment of Diabetic Retinopathy Study letters in the control arm and  $3.0 \pm 10.5$  letters in the intervention arm ( $p = 0.331$ ). CMT decreased by  $31.7\% \pm 17.5\%$  and  $13.3\% \pm 27.0\%$  ( $p = 0.236$ ) for the control and intervention cohorts, respectively. One patient developed intraocular pressure in excess of 30 mm Hg 3 months after DXI administration.

**CONCLUSIONS:** For this nAMD population, no visual or anatomical benefits were observed when treating with DXI in adjunct to ranibizumab relative to ranibizumab monotherapy. DXI-related adverse events were consistent with those previously documented for dexamethasone.

PMID: 27521672

## **Other Treatment and Diagnosis**

Retina. 2016 Aug 16. [Epub ahead of print]

### **OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY OF THE FOVEAL MICROVASCULATURE IN GEOGRAPHIC ATROPHY.**

Kvanta A, Casselholm de Salles M, Amrén U, Bartuma H.

**PURPOSE:** To examine the retinal and choroidal foveal and parafoveal vasculature in patients with bilateral geographic atrophy (GA) secondary to age-related macular degeneration using optical coherence tomography angiography (OCTA).

**METHODS:** Fourteen eyes from 7 patients with and without fovea-sparing bilateral GA at St. Erik Eye Hospital. All patients were examined by optical coherence tomography angiography, en face OCT and fundus autofluorescence (FAF). Segmented optical coherence tomography angiography flow scans were obtained from the superficial retinal vascular layer (SL) and the choriocapillaris (CC) and correlated with areas of retinal pigment epithelial (RPE) loss on fundus autofluorescence. The foveal avascular zone (FAZ) was measured on superficial retinal vascular layer scans and compared to the GA area of each patient.

**RESULTS:** No significant correlation ( $r = -0.17$ ,  $P = 0.58$ ) was found between superficial retinal vascular layer foveal avascular zone ( $0.49 \text{ mm} \pm 0.23 \text{ mm}$ ) and GA area ( $7.36 \text{ mm} \pm 4.36 \text{ mm}$ ). Absent or severely

impaired CC flow was observed inside all GA lesions and to varied extent outside the GA margins including areas of fovea sparing. A high level of symmetry was observed in CC flow between fellow eyes.

**CONCLUSION:** In this cross-sectional study, no relation was found between superficial retinal vascular layer foveal avascular zone and GA area. CC flow inside the GA was severely impaired, whereas CC flow outside the GA correlated poorly with both RPE integrity and visual acuity. Fellow eye symmetry suggests that CC monitoring may be a relevant clinical end point in interventional GA studies.

PMID: 27533772

## **Br J Ophthalmol. 2016 Aug 16. [Epub ahead of print]**

### **Classification of diabetic macular oedema using ultra-widefield angiography and implications for response to anti-VEGF therapy.**

Xue K, Yang E, Chong NV.

**AIMS:** To characterise differential pathogeneses of diabetic macular oedema (DMO) using ultra-widefield fluorescein angiography (UWFA) and evaluate responses to anti-vascular endothelial growth factor (anti-VEGF) therapy.

**METHODS:** Ninety-nine eyes (73 consecutive patients) with anti-VEGF naïve DMO underwent UWFA and optical coherence tomography, of which 60 with central retinal thickness (CRT) >400 µm received monthly intravitreal ranibizumab injections. Best-corrected visual acuity (BCVA) and CRT were measured at baseline and after three injections.

**RESULTS:** After excluding tractional factors, DMO was categorised into three types based on UWFA: (A) microaneurysm driven (49%), (B) peripheral ischaemia (37%) and (C) neovascularisation (15%). While all three types showed similar mean CRT ( $p=0.257$ ), types B and C were associated with more diffuse oedema, which extended beyond the 6.0 mm central macula ( $p=0.0034$ ). Following anti-VEGF treatment, all three types showed improvement in CRT and BCVA, which reached statistical significance for types A and B. A positive correlation was found between the Peripheral Ischaemia Index and improvement in CRT (slope=2.09,  $R^2=0.1169$ ,  $p=0.0151$ ) but not BCVA (slope=-0.00037,  $R^2=0.001149$ ,  $p=0.8152$ ).

**CONCLUSIONS:** UWFA facilitates the detection of peripheral ischaemia, which is associated with a significant proportion of DMO. While this group of DMO responded well to anti-VEGF therapy, it remains to be determined whether addressing the peripheral ischaemia may reduce recurrence.

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PMID: 27531355

## **Ophthalmology. 2016 Aug 9. [Epub ahead of print]**

### **Clinical and Genetic Characteristics of Japanese Patients with Age-Related Macular Degeneration and Pseudodrusen.**

Elfandi S, Ooto S, Ueda-Arakawa N, Takahashi A, Yoshikawa M, Nakanishi H, Tamura H, Oishi A, Yamashiro K, Yoshimura N.

**PURPOSE:** To investigate differences in clinical characteristics and genotype distribution in Japanese patients with age-related macular degeneration (AMD) and pseudodrusen using multimodal imaging.

**DESIGN:** Retrospective, observational case series.

**PARTICIPANTS:** A total of 101 patients (101 eyes) with AMD and pseudodrusen.



**METHODS:** Patients underwent complete ophthalmologic examination, including color fundus photography, infrared reflectance (IR) imaging, fundus autofluorescence, confocal blue reflectance, fluorescein and indocyanine green (ICG) angiography, and spectral-domain optical coherence tomography (SD OCT). Pseudodrusen subtype was identified with multiple imaging techniques. Patients were genotyped to identify major single nucleotide polymorphisms associated with AMD (CFH Y402, CFH I62V, and ARMS2 A69S).

**MAIN OUTCOME MEASURES:** Clinical characteristics and genetic distributions of patients with pseudodrusen.

**RESULTS:** At least 1 imaging technique identified dot pseudodrusen in all 101 eyes and ribbon pseudodrusen in 53 eyes (52.5%). Forty-eight eyes (47.5%) had only dot pseudodrusen, but no eyes had only ribbon pseudodrusen or midperipheral drusen. Forty-five of 49 bilateral cases (91.8%) had the same pseudodrusen subtype in both eyes. Pseudodrusen subtype did not change during the observation period in 100 eyes (99.0%), but dot-dominant type changed to dot-ribbon type in 1 eye (1.0%). The dot and ribbon subtypes were detected in 84 (83.1%) and 51 (96.2%) eyes, respectively, using color fundus photographs. Detection sensitivity of dot pseudodrusen was high for IR (97.0%), confocal blue reflectance (95.1%), fundus autofluorescence (93.1%), and ICG (100%) imaging. Detection sensitivity for ribbon pseudodrusen was high for color fundus photography (96.2%), confocal blue reflectance (94.3%), and fundus autofluorescence (90.6%), but not for IR imaging and ICG angiography. Risk allele frequency of the CFH I62V polymorphism was 79.8% and 67.0% in patients with dot-dominant and dot-ribbon pseudodrusen, respectively ( $P = 0.053$ ). The genotype frequency of CFH Y402H and ARMS2 A69S polymorphisms was not significantly different between the patients with dot-dominant type and dot-ribbon type ( $P = 0.647$  and  $P = 0.354$ , respectively).

**CONCLUSIONS:** Patients with pseudodrusen can be classified with dot-dominant or dot-ribbon type, and these subtypes usually are the same in both eyes. The distribution of CFH I62V polymorphisms may have an association with pseudodrusen subtypes.

PMID: 27521170

**Ann Neurosci. 2016 Mar;23(1):62.Epub 2016 Mar 11.**

**Imaging of Wet Age-Related Macular Degeneration.**

Kenan D.

PMID: 27536025

## Pathogenesis

**J Fr Ophtalmol. 2016 Aug 18. [Epub ahead of print]**

**Relationships between macular pigment optical density and lacquer cracks in high myopia.**

Benoudis L, Ingrand P, Jeau J, Lichtwitz O, Boissonnot M, Leveziel N.

**PURPOSE:** A low concentration of macular carotenoid pigment (lutein and zeaxanthin) is a significant risk factor for macular degeneration. The goal of this paper is to investigate the relationship between macular pigment optical density (MPOD) and lacquer cracks (LC) in high myopia.

**METHODS:** This is a prospective comparative observational study (NCT02205632) including high myopic patients with or without LC. High myopia was defined as a refractive error greater than 6 diopters of myopia or axial length greater than 26mm. All patients underwent best-corrected visual acuity in logMAR, MPOD measurement, multicolor imaging, SD-OCT, autofluorescence and axial length measurement. MPOD was

calculated using heterochromatic flicker photometry. Group 1 was defined as eyes without LC and group 2 as eyes with LC.

**RESULTS:** Forty-five eyes of 32 patients with a mean age of 51.3 years were included in group 1, and 15 eyes of 13 patients aged 54.1 in group 2 ( $P=0.56$ ). Mean spherical equivalent was -10.11 diopters in group 1 and -15.11 in group 2 ( $P=0.0004$ ). Mean visual acuity was +0.08 logMAR (0.8 in decimal notation) in group 1 and +0.11 logMAR (0.8 in decimal notation) in group 2 ( $P=0.061$ ). Axial length was 27.8mm in group 1 and 29.2 in group 2 ( $P=0.0052$ ). Central macular thickness was lower in group 1 (295 $\mu$ m) than in group 2 (305 $\mu$ m) ( $P<0.0001$ ), and macular choroidal thickness did not differ between the two groups ( $P=0.094$ ). Mean MPOD in group 2 was 0.52 and 0.63 in group 1 ( $P=0.042$ ). Differences in axial length were not related to MPOD measurements ( $P=0.74$ ).

**CONCLUSION:** A lower rate of MPOD was observed in cases of LC in high myopia. Further studies are needed to investigate if dietary carotenoids could have a protective effect in reducing the risk of LC.

PMID: 27544327

**Invest Ophthalmol Vis Sci. 2016 Aug 1;57(10):4144-50.**

### **The Intraocular Cytokine Profile and Therapeutic Response in Persistent Neovascular Age-Related Macular Degeneration.**

Rezar-Dreindl S, Sacu S, Eibenberger K, Pollreis A, Bühl W, Georgopoulos M, Krall C, Weigert G, Schmidt-Erfurth U.

**PURPOSE:** To investigate the course of inflammatory and angiogenic cytokines in the aqueous humor of patients with persistent/recurrent neovascular age-related macular degeneration (nAMD) under ranibizumab monotherapy (IVM) or ranibizumab plus dexamethasone combination treatment.

**METHODS:** In this 12-month prospective study, 40 eyes with nAMD were treated with either IVM or combined treatment with ranibizumab plus intravitreal dexamethasone implant (IVC). Patients in the IVM group were treated following an "as needed" treatment regimen; patients in the IVC group received ranibizumab and a dexamethasone implant at baseline and were re-treated with ranibizumab. At baseline and at each time of retreatment aqueous humor samples were taken.

**RESULTS:** Before treatment, levels of macrophage chemoattractant protein (MCP)-1, monokine induced by  $\gamma$  interferon (MIG), and lipocalin-2/ neutrophil gelatinase-associated lipocalin (NGAL) were elevated in nAMD patients compared to healthy controls ( $P = 0.024$ ;  $P = 0.04$ ;  $P = 0.01$ ). In contrast, tumor necrosis factor  $\alpha$ , IL-12p70, and secreted protein acidic and rich in cysteine (SPARC) concentrations were lower ( $P = 0.001$ ;  $P = 0.008$ ;  $P = 0.03$ ), while vascular endothelial growth factor (VEGF) was not altered ( $45 \pm 6/51 \pm 12$  pg/mL nAMD/control group;  $P = 0.6$ ). During IVC, levels of VEGF, MIG, platelet-derived growth factor (PDGF)-AA, and transforming growth factor  $\beta 1$  ( $P = 0.005$ ;  $P = 0.011$ ;  $P = 0.008$ ;  $P = 0.013$ ) were reduced. Ranibizumab monotherapy did not influence the course of any inflammatory/angiogenic cytokine. Interleukin 6 and PDGF-AA levels correlated with central retinal thickness changes ( $P = 0.007$ ;  $P = 0.022$ ). Over the 12-month period visual function was maintained with no significant differences during or between both treatment groups.

**CONCLUSIONS:** Inflammatory proteins are involved in the pathogenesis of chronic macular edema due to AMD and are associated with disease activity. During combined treatment, levels of inflammatory and angiogenic cytokines decreased over a 12-month period with no superiority in functional outcome.

PMID: 27537264

**Graefes Arch Clin Exp Ophthalmol. 2016 Aug 12. [Epub ahead of print]**

### **Stress responses of human retinal pigment epithelial cells to glyoxal.**

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Roehlecke C, Valtink M, Frenzel A, Goetze D, Knels L, Morawietz H, Funk RH.

**PURPOSE:** Intracellular formation of advanced glycation end products (AGEs) is a crucial pathological process in retinal diseases such as age-related macular degeneration (AMD) or diabetic retinopathy (DR). Glyoxal is a physiological metabolite produced during formation of AGEs and has also been shown to derive from photodegraded bisretinoid fluorophores in aging retinal pigment epithelial (RPE) cells.

**METHODS:** Flow cytometry was combined with either: 1) immunocytochemical staining to detect glyoxal induced formation of Nε-carboxymethyllysine (CML)-modifications of intracellular proteins (AGEs) and changes in the production of stress response proteins; or 2) vital staining to determine apoptosis rates (annexin V binding), formation of intracellular reactive oxygen species (ROS), mitochondrial membrane potential (MMP), and changes in intracellular pH upon treatment of cells with glyoxal. The percentage of apoptotic cells was further quantified by flow cytometry after staining of fixed cells with propidium iodide to determine cells with a subdiploid (fragmented) DNA content. Apoptosis related activation of caspase 3 was determined by Western blotting. Glyoxal induced changes in VEGF-A165a mRNA expression and protein production were determined by real-time PCR and by flow cytometry after immunocytochemical staining.

**RESULTS:** Increasing glyoxal concentrations resulted in enhanced formation of AGEs, such as CML modifications of proteins. This was associated with elevated levels of intracellular reactive oxygen species, a depolarized MMP, and a decreased intracellular pH, resulting in an increased number of apoptotic cells. Apoptosis related caspase 3 activation increased in a dose dependent manner after glyoxal incubation. In consequence, the cells activated compensatory mechanisms and increased the levels of the anti-oxidative and stress-related proteins heme oxygenase-1, osteopontin, heat shock protein 27, copper/zinc superoxide dismutase, manganese superoxide dismutase, and cathepsin D. Furthermore, VEGF-A165a mRNA expression and VEGF-A protein production were significantly increased after incubation with glyoxal in ARPE-19 cells.

**CONCLUSIONS:** The glyoxal-induced oxidative stress and apoptosis in ARPE-19 cells may provide a suitable in vitro model for studying RPE cellular reactions to AGEs that occur in AMD or in DR.

PMID: 27520463

**J Biol Chem. 2016 Aug 11. [Epub ahead of print]**

**Retinal hypercholesterolemia triggers cholesterol accumulation and esterification in photoreceptor cells.**

Saadane A, Mast N, Dao T, Ahmad B, Pikuleva IA.

**ABSTRACT:** The process of vision is impossible without the photoreceptor cells that have a unique structure and specific maintenance of cholesterol. Herein we report on the previously-unrecognized cholesterol-related pathway in the retina discovered during follow up characterizations of Cyp27a1<sup>-/-</sup>-Cyp46a1<sup>-/-</sup> mice. These animals have retinal hypercholesterolemia and convert retinal cholesterol excess into cholesterol esters, present normally in the retina in very small amounts. We established that in the Cyp27a1<sup>-/-</sup>-Cyp46a1<sup>-/-</sup> retina, cholesterol esters are mainly generated by and accumulate in the photoreceptor outer segments (OS), the retinal layer with the lowest cholesterol content. Mouse OS were also found to express the cholesterol-esterifying enzyme ACAT1, but not LCAT, and differ from humans in retinal expression of ACAT1. Nevertheless, cholesterol esters were discovered to be abundant in human OS. We suggest a mechanism for cholesterol ester accumulation in the OS and that activity impairment of ACAT1 in humans may underlie the development of subretinal drusenoid deposits, a hallmark of age-related macular degeneration, which is a common blinding disease. We generated Cyp27a1<sup>-/-</sup>-Cyp46a1<sup>-/-</sup>-Acat1<sup>-/-</sup> mice, characterized their retina by different imaging modalities, confirmed that unesterified cholesterol does accumulate in their OS and that there is photoreceptor apoptosis and OS degeneration in this line. Our results provide insights into the retinal response to local hypercholesterolemia and retinal significance of cholesterol esterification, which could be cell-specific and be both beneficial and detrimental for the retina structure and function.

PMID: 27514747



**J Med Assoc Thai. 2016 Jan;99(1):65-70.**

**Keratoconus in Patients with Macular Stromal Dystrophy.**

Kosrirukvongs P, Ngowyutagon P, Booranapong W.

**OBJECTIVE:** To show the association between keratoconus and macular dystrophy.

**MATERIAL AND METHOD:** All patients with macular dystrophy and associated clinical findings leading to a diagnosis of keratoconus by corneal topography were retrospectively reviewed during a 10-year period. Uncorrected and best-corrected visual acuity, automated refraction, manifest refraction, corneal thickness, and corneal curvature by corneal topography were evaluated

**RESULTS:** Three patients with macular dystrophy exhibiting decreased vision, multifocal white dense deposits, and haze surrounding the deposits in the corneal stroma were evaluated. All had a steep corneal curvature of >47 diopters and a thin cornea consistent with keratoconus. Penetrating keratoplasty was performed in one patient with severely decreased vision. Macular dystrophy was diagnosed based on an Alcian blue-stained pathological specimen.

**CONCLUSION:** Keratoconus may develop as a result of changes associated with macular dystrophy. Therefore, patients with severely decreased vision should be evaluated for keratoconus to ensure proper management.

PMID: 27455826

**Oncotarget. 2016 Aug 10. [Epub ahead of print]**

**BMP9/ALK1 inhibits neovascularization in mouse models of age-related macular degeneration.**

Ntumba K, Akla N, Oh SP, Eichmann A, Larrivée B.

**ABSTRACT:** Age-related macular degeneration (AMD) is the leading cause of blindness in aging populations of industrialized countries. The drawbacks of inhibitors of vascular endothelial growth factor (VEGFs) currently used for the treatment of AMD, which include resistance and potential serious side-effects, require the identification of new therapeutic targets to modulate angiogenesis. BMP9 signaling through the endothelial Alk1 serine-threonine kinase receptor modulates the response of endothelial cells to VEGF and promotes vessel quiescence and maturation during development. Here, we show that BMP9/Alk1 signaling inhibits neovessel formation in mouse models of pathological ocular angiogenesis relevant to AMD. Activating Alk1 signaling in laser-induced choroidal neovascularization (CNV) and oxygen-induced retinopathy (OIR) inhibited neovascularization and reduced the volume of vascular lesions. Alk1 signaling was also found to interfere with VEGF signaling in endothelial cells whereas BMP9 potentiated the inhibitory effects of VEGFR2 signaling blockade, both in OIR and laser-induced CNV. Together, our data show that targeting BMP9/Alk1 efficiently prevents the growth of neovessels in AMD models and introduce a new approach to improve conventional anti-VEGF therapies.

PMID: 27517154

**Hum Mol Genet. 2016 Aug 11. [Epub ahead of print]**

**Reprogramming toward anabolism impedes degeneration in a preclinical model of retinitis pigmentosa.**

Zhang L, Justus S, Xu Y, Pluchenik T, Hsu CW, Yang J, Duong JK, Lin CS, Jia Y, Bassuk AG, Mahajan VB, Tsang SH.

**ABSTRACT:** Retinitis pigmentosa (RP) is an incurable neurodegenerative condition featuring photoreceptor death that leads to blindness. Currently, there is no approved therapeutic for photoreceptor degenerative conditions like RP and atrophic age-related macular degeneration (AMD). Although there are promising results in human gene therapy, RP is a genetically diverse disorder, such that gene-specific therapies would be practical in a small fraction of RP patients. Here, we explore a non-gene-specific strategy that entails reprogramming photoreceptors

towards anabolism by upregulating the mechanistic target of rapamycin (mTOR) pathway. We conditionally ablated Tsc1, an mTOR inhibitor, in the rods of the Pde6bH620Q/H620Q preclinical RP mouse model and observed, functionally and morphologically, an improvement in the survival of rods and cones at early and late disease stages. These results elucidate the ability of reprogramming the metabolome to slow photoreceptor degeneration. This strategy may also be applicable to a wider range of neurodegenerative diseases, as enhancement of nutrient uptake is not gene-specific and is implicated in multiple pathologies. Enhancing anabolism promoted neuronal survival and function and could potentially benefit a number of photoreceptor and other degenerative conditions.

PMID: 27516389

## Epidemiology

**Ophthalmologe. 2016 Aug 19. [Epub ahead of print] [Article in German]**

**Brandl C, Stark KJ, Wintergerst M, Heinemann M, Heid IM, Finger RP.**

Epidemiology of age-related macular degeneration

**ABSTRACT:** Age-related macular degeneration (AMD) is the main cause of blindness in industrialized societies. Population-based epidemiological investigations generate important data on prevalence, incidence, risk factors, and future trends. This review summarizes the most important epidemiological studies on AMD with a focus on their transferability to Germany including existing evidence for the main risk factors for AMD development and progression. Future tasks, such as the standardization of grading systems and the use of recent retinal imaging technology in epidemiological studies are discussed. In Germany, epidemiological data on AMD are scarce. However, the need for epidemiological research in ophthalmology is currently being addressed by several recently started population-based studies.

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**Acta Ophthalmol. 2016 Aug 20. [Epub ahead of print]**

**Neovascular age-related macular degeneration is not associated with coronary heart disease in a Chinese Population: a population-based study.**

Hu CC, Lin HC, Sheu JJ, Kao LT.

**PURPOSE:** This case-control study aimed to explore the association between prior coronary heart disease (CHD) and neovascular age-related macular degeneration (AMD) using a population-based data set in Taiwan.

**METHODS:** We analysed data sourced from the Taiwan Longitudinal Health Insurance Database 2005. The study consisted of 1970 patients with neovascular AMD as cases and 5910 age- and sex-matched controls. We performed a conditional logistic regression to examine the odds ratio (OR) and its corresponding 95% confidence interval (CI) for previously diagnosed CHD between cases and controls.

**RESULTS:** Of the 7880 sampled patients, 24.5% had a prior history of CHD; CHD was found in 25.7% of cases and in 22.7% of controls ( $p = 0.008$ ). The conditional logistic regression analysis indicated that the OR for prior CHD for cases was 1.17 [95% confidence interval (CI): 1.04-1.32] compared to the controls. However, after adjusting for patient's monthly income, geographic location, urbanization level, age, hyperlipidaemia, diabetes and hypertension, we failed to observe an association between prior CHD and AMD (OR = 1.03, 95% CI = 0.91-1.17). Additionally, the medical comorbidities of hyperlipidaemia (adjusted OR = 1.29, 95% CI = 1.15-1.45), hypertension (adjusted OR = 1.20, 95% CI = 1.05-1.37) and diabetes (adjusted OR = 1.47, 95% CI = 1.32-1.65) were significantly associated with AMD.

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**CONCLUSIONS:** This study presented no significant difference in the odds of prior CHD between patients with AMD and those without AMD after adjusting for comorbidities and sociodemographic characteristics in a Chinese population.

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## Diet, Lifestyle and Low Vision

**J Lipid Res. 2016 Aug 18. [Epub ahead of print]**

### **Mechanisms of Selective Delivery of Xanthophylls to Retinal Pigment Epithelial Cells by Human Lipoproteins.**

Thomas SE, Harrison EH.

**ABSTRACT:** The xanthophylls, lutein and zeaxanthin, are dietary carotenoids that selectively accumulate in the macula of the eye providing protection against age-related macular degeneration (AMD). To reach the macula, carotenoids cross the retinal pigment epithelium (RPE). Xanthophylls and  $\beta$ -carotene mostly associate with HDL and LDL, respectively. HDL binds to cells via a scavenger receptor class B1 (SR-B1)-dependent mechanism while LDL binds via the LDL receptor (LDLR). Using an in-vitro, human RPE cell model (ARPE-19), we studied the mechanisms of carotenoid uptake into the RPE by evaluating kinetics of cell uptake when delivered in serum or isolated LDL or HDL. For lutein and  $\beta$ -carotene, LDL delivery resulted in the highest rates and extents of uptake. In contrast, HDL was more effective in delivering zeaxanthin and meso-zeaxanthin leading to the highest rates and extents of uptake of all four carotenoids. Inhibitors of SR-B1 suppressed zeaxanthin delivery via HDL. Results show a selective HDL-mediated uptake of zeaxanthin and meso-zeaxanthin via SRB1 and a LDL-mediated uptake of lutein. This demonstrates a plausible mechanism for the selective accumulation of zeaxanthin > lutein and xanthophylls over  $\beta$ -carotene in the retina. We found no evidence of xanthophyll metabolism to apocarotenoids or lutein conversion to meso-zeaxanthin.

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**Appl Microbiol Biotechnol. 2016 Aug 16. [Epub ahead of print]**

### **Engineering of the carotenoid pathway in Xanthophyllomyces dendrorhous leading to the synthesis of zeaxanthin.**

Pollmann H, Breitenbach J, Sandmann G.

**ABSTRACT:** Zeaxanthin is an essential nutrient for prevention of macular degeneration. However, it is limited in our diet. For the production of zeaxanthin, we have engineered zeaxanthin synthesis into a carotenoid mutant of Xanthophyllomyces dendrorhous which is blocked in astaxanthin synthesis and accumulates  $\beta$ -carotene instead. Two strategies were followed to reach high-yield zeaxanthin synthesis. Total carotenoid synthesis was increased by over-expression of genes HMGR, crtE, and crtYB encoding for limiting enzymes in the pathway leading to and into carotenoid biosynthesis. Then bacterial genes crtZ were used to extend the pathway from  $\beta$ -carotene to zeaxanthin in this mutant. The increase of total carotenoids and the formation of zeaxanthin is dependent on the number of gene copies of crtYB and crtZ integrated into the X. dendrorhous upon transformation. The highest zeaxanthin content around 500  $\mu$ g/g dw was reached by shaking flask cultures after codon optimization of crtZ for Xanthophyllomyces. Stabilization of carotenoid and zeaxanthin formation in the final transformant in the absence of selection agents was achieved after passing through a sexual cycle and germination of basidiospores. The values for the transformant before and after stabilization were very similar resembling about 70 % of total carotenoids and corresponding to a conversion rate of 80 % for hydroxylation of  $\beta$ -carotene to zeaxanthin. The

stabilized transformant allowed experimental small-scale fermentation yielding X. dendrorhous cells with a zeaxanthin content similar to the shaking flask cultures. Our result demonstrates the potential of X. dendrorhous for its development as a zeaxanthin producer and its suitability for large-scale fermentation.

PMID: 27527661

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