

The Role Of Vascular Interfaces In Age-Related Macular Degeneration

PhD Thesis Synopsis

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INTRODUCTION

Age-Related Macular Degeneration (AMD) is the leading cause of severe irreversible central visual loss among the elderly in western industrialized countries. The aetiology and pathogenesis of AMD are poorly understood. AMD affects primarily the outer retinal vascular interface; the retinal pigment epithelium (RPE), Bruch's Membrane (BrM) and the choriocapillaris. Early AMD is characterized by the accumulation of lipid-rich deposits under the RPE and within BrM. These deposits may act as a barrier to transport via BrM and thus play a significant role in the pathogenesis of AMD. Sub-RPE deposits may be diffuse (basal laminar or linear deposits) or focal (drusen). Of these, drusen alone are readily detectable clinically. Soft macular drusen are hallmarks of AMD, their presence has been clearly identified by epidemiologic studies as a risk factor of late AMD and vision loss. Their immediate significance in relation to photoreceptor health and function however, are still unclear. The natural history of soft drusen may involve an increase in size, area and confluence with subsequent neovascularisation, or fading and disappearance which is generally believed to be followed by atrophy. Spontaneous regression of soft drusen without subsequent atrophy has also been reported, although the functional implications of true drusen regression as reflected by retinal function or autofluorescence have not been demonstrated. The mechanism of sub-RPE lipid deposition in AMD is unknown. The accumulation of extracellular material is a feature common to both atherosclerosis and AMD. According to the current hypothesis of atherogenesis, intimal extracellular matrix (ECM) proteoglycans may trap serum lipids by binding LDL apolipoproteins. Likely candidates for this interaction are apoB-100 and Biglycan. This pathway is also conceivable within BrM in AMD.

AIMS

1. The aim of the clinical part of our investigations was to probe the functional implications of macular soft drusen regression in AMD eyes, with the following questions in mind:
 - 1.1. Is the regression of macular soft drusen accompanied by signs of progression of the underlying AMD process, detectable in colour fundus images?
 - 1.2. Is the regression of macular soft drusen associated with consequent abnormal fundus autofluorescence?
 - 1.3. Is the regression of macular soft drusen associated with subsequent function loss?

2. In the experimental morphological part of our investigations, we aimed to characterize ultrastructural changes in Bruch's Membrane with respect to serum lipid characteristics of transgenic mice overexpressing the human apoB-100, biglycan or both genes in combination with a high-cholesterol diet (HCD), with a view to the following questions:
 - 2.1. Does a high-cholesterol diet and/or the overexpression of the apoB-100 or biglycan proteins affect serum lipid levels?
 - 2.2. How does BrM thickness correlate with the above factors?
 - 2.3. How is the ultrastructure of BrM affected by the above factors?
 - 2.4. Can morphological indications of consequences for photoreceptor cells be detected?
 - 2.5. Are there indications for an apo-B100-biglycan interaction as a triggering event for lipid entrapment within BrM?

METHODS

1. Functional aspects of drusen regression in AMD.

1.1. Patients

Patients were selected from a large ongoing collection of clinical data at the Reading Centre of Moorfields Eye Hospital. Inclusion criteria were: a clinical diagnosis of AMD, both manifest and resolved soft macular drusen, a level of fixation and general fitness sufficient to perform the full test sequence. Patients with exudative AMD in the eye studied were excluded. Sequential colour fundus and FAF images of 960 patients were screened for disappearing drusen. Soft drusen regression was detected in 34 cases, 19 met all inclusion criteria, 14 (10 female, 4 male) agreed to participate in the study, ranging in age from 52 to 84 years (median 72 years). The mean follow-up period was 5.9 years (range 2.8-14.4 years). In ten cases the drusen regressed spontaneously, four patients received prophylactic laser treatment prior to drusen regression.

1.2. Imaging

ETDRS Standard 30° stereo field 2 colour images (SCI) of the fundus were captured digitally using a fundus camera. Fundus autofluorescence (FAF) imaging was performed using a cSLO (Heidelberg Retina Angiograph 2, Heidelberg Engineering GmbH), with a 488nm solid-state laser for illumination, and a filter with a short-wavelength cut-off at 500nm for recording autofluorescence. A 30°x30° retinal area around the fovea was captured at 1536x1536 pixel resolution. To reduce noise, for each eye in the study mean images were produced by averaging 16 individually recorded images.

1.3. Phenotyping

Detailed phenotyping was performed by grading based on digital field 2 colour and FAF images, according to the system defined in the International Classification (IC) for ARM and AMD. Gradings were compared for inter- and intra-observer reliability as well as for reliability over time. In cases where the simultaneous presence of AMD, disappearance of drusen and absence of exudative disease were confirmed, the patient was invited for functional testing.

1.4. Psychophysical testing

For psychophysical testing Fine Matrix Mapping (FMM) was selected due to its superior spatial resolution and its ability to measure wide ranges of sensitivity changes. FMM was performed using a modified Humphrey Field Analyzer. Test flashes were positioned over the retinal area of interest, including manifest drusen, areas where drusen regressed and normal retinal areas. A $9^\circ \times 9^\circ$ matrix of 100 locations with 1° intervals was tested using a Goldmann size III stimulus under photopic and scotopic conditions. Detection threshold sensitivity was expressed in decibels and thresholds as log units. FMM thresholds were processed by normal filtering to improve repeatability. For each patient, sensitivity data were analyzed intra-individually as well as compared with age-matched data from normal individuals (n=8). Filtered data were used to calculate mean and maximum threshold elevations from baseline as well as elevation relative to mean threshold levels of the normal control group. Fixation stability was monitored through an infrared camera and expressed as bivariate contour ellipse area (BCEA), defined as the area of an ellipse on the retinal surface within which the centre of the fixation target was imaged 68% of the time.

1.5. Image processing and analysis

For each patient, fundus images taken at baseline and at subsequent visits as well as FAF images were imported into individual layers within a composite image in Adobe PhotoShop and adjusted to obtain exact correspondence between fundus features within individual layers. FMM sensitivity threshold contour plots were superimposed over the fundus images. Composite images were analysed for correlations at identical retinal locations between fundus appearance at different points in time, FAF and retinal sensitivity. Total area covered by drusen within the macula were measured using area markup layers. Changes in drusen area were identified by binary logical operations between markup layers. Areas with regressed drusen, persistent drusen and drusen-free areas were identified, delineated and associated levels of sensitivity loss were compared using ANOVA.

2. Characterisation of Bruch's membrane ultrastructure in apoB-100 and biglycan transgenic mice.

2.1. Mice

Transgenic mice were produced at the Biological Research Center of the Hungarian Academy of Sciences in Szeged, Hungary. For the production of apoB-100 mice, fertilized oocytes from wild-type female mice (C57BL/6xCBAF1) were collected and injected with purified P1-phagemid DNA containing the entire 43 kb human apoB-100 gene, the 19 kb of the 5' and the 14 kb of the 3' flanking genomic sequences, according to a standard technique. The apoB^{+/+} transgenic mice were homozygous for the human apoB transgene. Biglycan transgenic mice were generated using a GeneStorm[®] Expression-Ready clone expressing the human biglycan gene construct (Invitrogen, Carlsbad, CA). This involves human biglycan cDNA

fused to a CMV promoter, a V5 epitope and a 6X His Tag sequence at the 3' end of the cDNA. A 3440bp fragment including the transcription unit was separated from the vector backbone, and the purified DNA was microinjected at 2ng/ml into the fertilized oocytes of female C57BL/6xCBA F1 mice, using the standard pronucleus microinjection technique. Microinjected eggs were implanted into the oviduct of pseudopregnant Swiss female mice. In all cases transgenic founders were identified by PCR analysis on tail DNA samples. The clone expressing the human biglycan mRNA at the highest level, was selected for further study. Homozygous apoB-100 and biglycan transgenic mice were crossed to produce hemizygous double transgenic littermates. Increased gene expression levels were confirmed by QRT-PCR. Wild-type C57BL/6 mice were used as controls. All animals (n=5 per group) were initially raised on a standard diet (CRLT/N, EU registration code HU13100039). Experimental groups were switched to a high-cholesterol diet (HCD) with 2% cholesterol added, at 6 weeks of age, for 17 weeks. All mice had free access to food and water and were maintained on 12-hour light-dark cycles with standard day ambient light maintained at 500 lux.

2.2. Determination of serum cholesterol levels

Serum total and LDL cholesterol levels were measured in triplicate from blood samples obtained by cardiac puncture following anaesthesia, using colorimetric assays adapted to 96-well plates (Diagnosticum Ltd., Budapest, Hungary) calibrated using Standard Lipid Controls (Multiparametric HDL/LDL Calibrator, Sentinel Diagnostics SpA, Milano, Italy).

2.3. Transmission Electron Microscopy (TEM)

Animals were sacrificed through anesthesia by ether and the eyes were promptly fixed in a solution of 1% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M PBS at pH 7.2, post-fixed with 1% OsO₄, dehydrated and embedded in resin. Ultra-thin sections were cut and washed in 1% uranyl acetate and Reynolds' lead citrate. TEM images were collected digitally at 4008x2762 pixels resolution. Measurements of Bruch's membrane thickness were performed at progressive and wherever possible, equal intervals, on sections where the optic nerve head was clearly identified, the choriocapillary lumen was open and lined by a single layer of endothelium. Inter-capillary pillars and regions far peripheral to the optic nerve head were avoided. Three eyes per group were processed and at least 10 images per eye were taken. In each image, the boundaries of BrM were marked manually and BrM thickness was measured with single-pixel resolution. A mean thickness value per image was calculated. Focal sub-RPE nodule severity was estimated according to the system devised by Sarks and van der Schaft.

2.4. Statistical analysis

Bruch's membrane thickness and serum lipid values of the groups were compared by one-way analysis of variance (ANOVA). Serum lipid levels were expressed as mean±SEM, BrM thickness as mean±SD. Correlations were determined by calculating the Pearson Correlation Coefficient. Results were considered to be significantly different at a probability level of $p < 0.05$. Software used in this study include: Adobe Photoshop (Adobe Systems Inc.), 'FoveaPro' v4 (Reindeer Graphics Inc), ImageJ v1.38x (USNIH), Ms Excel (Microsoft Co., USA), SPSS v11 (SPSS Inc., USA) and GraphPad Prism 4 (GraphPad Software Inc., USA)

RESULTS

1. Functional aspects of drusen regression in AMD.

1.1. Phenotype

The predominant phenotype in the study eye at baseline was “soft drusen” in all cases. Disappearance of drusen was followed by CNV in one, by GA in 3 cases, while in 10 cases no indications of end-stage disease were detectable in the colour fundus images. In most cases, parallel to fading drusen, new drusen in other locations appeared and grew in size and confluence. New drusen tended to form at increasingly peripheral locations relative to the fovea. Repeated appearance of drusen in the same retinal location was not seen.

1.2. Autofluorescence

FAF associated with drusen varied from decreased to increased, no good correspondence was detectable. FAF corresponding to areas with disappearing drusen in the absence of pigmentary changes was normal in 7 cases. In two patients increased FAF was seen, in one case in an area adjacent to the junctional zone of a GA, in the other adjacent to a large crystalline druse. One other patient showed widely varying levels of FAF in connection with regressed drusen. GA was associated with decreased FAF centrally and increased FAF along the boundaries. Crystalline drusen showed decreased, coarse granular hyperpigmentation increased FAF.

1.3. Functional characteristics

Best corrected visual acuities in the study eye assessed at the time of FMM testing ranged from 6/12 to 6/5 (median=6/6). All patients had less

than two lines loss in BCVA compared to the baseline value. Fixation Stability was good in all cases. Two patients, both with end-stage disease, showed significant (more than 2 lines) deterioration in BCVA at the endpoint as compared to the baseline value. FMM showed generalised threshold elevation relative to normal controls both under photopic and scotopic conditions. Scotopic sensitivity loss exceeded photopic loss in all cases. Scotopic loss over areas with drusen or regressed drusen was analysed using ANOVA and did not differ significantly from that over non-drusen areas ($p=0.289$ and $p=0.989$ respectively). Elevated scotopic thresholds were seen associated with GA, crystalline drusen and coarse granular hyperpigmentation, all in connection with abnormal FAF. Photopic thresholds showed little topographic variation except in areas with GA.

2. Characterisation of Bruch's membrane changes in apoB-100 and biglycan mice.

2.1. Serum Lipid Levels

In cholesterol-fed apoB-100 transgenic mice both total and LDL cholesterol levels, in apoB-100/biglycan double transgenic mice, total cholesterol levels were significantly elevated ($5.0\pm 0.4\text{mmol/l}$, $2.7\pm 0.5\text{mmol/l}$ and $4.9\pm 2.1\text{mmol/l}$ respectively).

2.2. Bruch's Membrane Thickness

Relative to wild-type mice on normal diet, apoB-100 transgenic and double transgenic mice showed a significant diet-dependent increase in BrM thickness ($475\pm 116\text{nm}$ and $476\pm 138\text{nm}$ respectively, $p<0.05$ in all cases). A significant but diet-independent thickening of BrM was seen in biglycan transgenic animals ($519\pm 120\text{nm}$ on normal chow and $545\pm 195\text{nm}$ on high-

cholesterol diet $p < 0.05$ in both cases). With the biglycan-only transgenic animals excluded, there was a strong correlation between BrM thickness and cholesterol levels in the remaining samples ($r = 0.98$, $p < 0.006$).

2.3. Bruch's Membrane Ultrastructure

2.3.1. Electron-lucent profiles

Two distinct types of electron-lucent profiles were observed. One type was circular, 30-50nm in diameter with indistinct margins appearing mostly in confluent clusters. These were seen in all transgenic animals, most densely in cholesterol-fed apoB-100 and double transgenics. Profiles of the second type were larger and more variable in size (100-350nm), usually oval in shape, with sharply demarcated outlines, delimited by a double-layered electron-dense membrane and were mostly as solitary or in small clusters, often surrounded by large numbers of the smaller, slightly more electron-lucent vacuoles. This type was present in all transgenic groups, most frequently in cholesterol-fed apoB-100 transgenics, but only occasionally in 3/6 of wild-type mice on HCD and not at all on normal chow. Both types of electron-lucent profiles occurred scattered throughout the inner and outer collagenous layers of BrM, the smaller type occurring with higher density in and near the inter-capillary pillars.

2.3.2. Focal sub-RPE nodules

In transgenic animals, focal nodules of an amorphous material of intermediate-electron density were present between the plasma and basement membranes of the RPE, amid misaligned and disorganised or atrophied RPE basal processes. These nodules were most frequent and extensive in apoB-100 transgenics on HCD.

Focal thickening of BrM

In biglycan transgenic animals, a significant thickening of BrM was noted. This was in all cases associated with a continuous layer of varying thickness of a basement membrane-like material in outer BrM. Frequently, this layer became focally massive, resulting in an up to fourfold thickening of BrM. This phenomenon was also present with similar frequency but more attenuated amplitude in double transgenics and was not seen in animals not carrying the human biglycan gene. Measurement of the thickness of this layer was not possible with reasonable precision due to a lack of a clear boundary towards the inner layers of BrM.

2.3.3. Fragmentation of the elastic lamina

Longer continuous segments of the elastic lamina were only visible in wild-type and biglycan transgenic animals. In all other groups, only fragments were apparent. No differences in the degree of fragmentation could be determined.

2.4. Photoreceptor Outer Segments, RPE and Choriocapillaris

Classical signs of photoreceptor damage or atrophy were not evident. Some photoreceptor outer segments showed a cytoplasmic cap at the apex. The RPE cytoplasm contained occasional electron-lucent vacuoles of a size and structure similar to those found within BrM. Endothelial fenestrae of the choriocapillaris were of uniform size (50-70nm) and density, no differences between groups were detectable.

DISCUSSION

1. Functional aspects of drusen regression in AMD.

Regression of macular soft drusen is generally considered to presage degeneration and atrophy of the RPE and photoreceptors. It was however noted in population-based studies (Melton Mowbray study, Chesapeake Bay Waterman Study, Beaver Dam Eye Study) that in significant numbers of patients, drusen may also regress without residual signs. The prognostic implications of this phenomenon are unknown. From the clinical aspect, it raises the possibility that arrested progression or even regression of the disease process may exist naturally. In our study, 10 out of 14 patients showed no ophthalmoscopic indications of manifest or incipient end-stage disease in the fundus following drusen regression. In nine however, parallel to regression, new drusen in other, usually more peripheral locations appeared and grew in size and confluence, signifying the continued activity of the disease.

In diseases, where the RPE is primarily affected, the presence of abnormal FAF may be an early sign of progression. In our study, FAF of retinal areas with normal appearance in the colour image following drusen regression was in most cases normal and none of the patterns described by earlier studies as associated with impending atrophy was detectable.

Mean retinal sensitivity relative to normal controls was reduced in all patients tested in our study, both in the light and dark adapted states, with significantly higher loss under scotopic conditions. This observation confirms data from previous histopathological and psychophysical studies that in AMD, rods are at increased risk for degeneration and function loss occurs before progression to the late clinical stage. The retinoid deficiency

hypothesis proposed by Curcio and coworkers offers one possible interpretation of this preferential rod vulnerability. In AMD, diffuse sub-RPE deposits may act as a diffusion barrier between the choriocapillaris and the RPE, thereby disrupting transport across BrM and leading to a local scarcity of 11-cis-retinal, a compound required to regenerate the photoreceptor pigment after bleaching by light as well as for photoreceptor survival. Early defects in rod sensitivity may be signs of a local depletion of nutrients, including 11-cis-retinal. Cones have an additional retinoid delivery pathway involving Müller cells, rendering them less vulnerable to reduced transport across BrM. A clinical consequence of these findings is that tests of visual acuity, currently the standard clinical functional assessment, may underestimate the degree of visual dysfunction in the elderly and AMD patients.

Although generalised sensitivity loss was measured in all our patients, topographic variation in sensitivity over drusen relative to areas with normal appearance was not significant. This confirms earlier observations that the presence of macular soft drusen seems to have little effect on the local sensitivity of affected retinal areas, with the exception of large, soft foveal drusen. These may be regarded as small RPE detachments and showed mildly reduced photopic and considerably reduced scotopic sensitivity as well as abnormal FAF. Photopic and scotopic sensitivity over areas with regressed drusen was not significantly different from that over unaffected retinal areas equidistant from the fovea. Thus, we did not find functional evidence for manifest or incipient photoreceptor atrophy and although the generalised disease persists, retinal sensitivity and FAF, two direct, sensitive and complementary measures of retinal health do not indicate that disappearance of drusen is necessarily followed by local function loss and atrophy.

2. Characterisation of Bruch's membrane changes in apoB-100 and biglycan transgenic mice.

Several previous studies using murine models have reported degenerative changes and basal deposit formation in BrM in association with diet-induced high serum lipid levels in C57BL/6 mice as well as in mice with genetically-determined hyperlipidemia (including apoE2, apoE3, apo*E3-Leiden, apoE4 transgenics and LDL receptor knockout mice), in combination with advanced age and blue-light-induced damage.

In our animal model, the factors age and light damage were eliminated by the exclusive use of untreated mice of a single age-group. Transgenics were bred from the same C57BL/6 stock as controls. Also, the apoB-100 transgenic mouse model has a significant inherent advantage. The proteoglycan-binding sequence of apoB-100 in LDL is different from that in apoB-48, the apolipoprotein normally present in mice; and apoB-48-containing lipoprotein can also be cleared from the plasma by receptors other than LDL-R. Mice expressing the human apoB-100 gene have a lipoprotein profile similar to humans. BrM deposits seen mainly in our apoB-100 transgenics were of two main types. Located between the RPE plasma and basement membranes, we found focal deposits of an amorphous material similar to early-type nodular BlamD. A more pronounced feature was the presence of electron-lucent particles similar to membranous debris and small electron-lucent droplets described previously in basal linear deposits. The mechanism of deposit formation and the source of lipids within the deposits found in AMD remain controversial. Our data support the hypothesis that serum LDL may be a source of lipids, via a mechanism directly analogous to that established in atherosclerosis.

Biglycan transgenics exhibited a significant BrM thickening,

irrespective of the diet, while the severity of EL particles was consistently less than in corresponding apoB-100 or double transgenic animals. This thickening of BrM was attributable to a layer of an amorphous or fibrillar material in the outermost part of BrM. The most likely source of this layer is the basement membrane of the choriocapillaris. Recent reports have linked biglycan with ECM remodelling and collagen matrix formation in pulmonary fibrosis and keloid scarring. Thus, BrM thickening seen in our biglycan transgenic mice appears to be attributable mainly to increased production of a fibrous material, most likely collagen IV.

Our apoB-100/biglycan double transgenic mouse model was aimed at modeling lipoprotein entrapment, a key step in the current paradigm of atherogenesis. Relative to wild-type mice, these animals did show increased serum lipid levels, BrM thickness and EL particle severity when fed a HCD, but not on normal diet and the degree of abnormalities was not significantly different from that seen in apoB-100 transgenics. Based on previous in vitro data indicating a likely central role of the apoB-100-biglycan interaction in lipid retention and the overexpression of both factors in these mice, we expected a significantly higher level of abnormalities. One possible explanation for this may be that although biglycan is the proteoglycan that most closely and consistently co-localizes with apoB in humans, there are other candidates for lipid entrapment, including decorin. The fact that on normal diet, biglycan transgenic mice show a significant thickening of BrM while double transgenic animals do not may indicate that the apoB-100-biglycan interaction does take place, however without the expected increase in lipid deposition, while biglycan is no longer available to exert its fibrosis-inducing effect via other pathways.

CONCLUSIONS

1. In our clinical investigations we demonstrated that:

- 1.1. Macular soft drusen may disappear without detectable signs in stereoscopic colour fundus images of their earlier existence.
- 1.2. Drusen regression may occur without subsequent FAF or psychophysical signs of local dysfunction or incipient atrophy.

Thus, normal-appearing eyes at the present time may have had some features of AMD in the past. This is a potential source of misclassification and needs to be considered in epidemiologic studies as well as clinical trials.

2. In our morphological investigations, we demonstrated that:

- 2.1. ApoB-100 and double transgenic mice show elevated serum lipid levels when fed a high-cholesterol diet.
- 2.2. BrM thickness was significantly increased in apoB-100 and double transgenic mice on a HCD and there was a strong correlation between BrM thickness and serum cholesterol level.
- 2.3. ApoB-100 and double transgenic animals show EL profiles similar to BlinD in BrM as well as a BlamD-like material between the plasma and basement membranes of the RPE, Biglycan transgenic mice show a marked, diet-independent increase in BrM thickness, due to a layer of basement-membrane like material in outer BrM
- 2.4. Signs of photoreceptors damage were not detectable.
- 2.5. Our observations further implicate apoB-100 in sub-RPE lipid deposition in AMD. The role of biglycan may more likely be the preservation of the integrity of collagen structures, including basement membranes.

LIST OF PUBLICATIONS

Publications and presentations related to this thesis

1) Peer-reviewed publications

Sallo FB, Rechtman E, Peto T, Stanescu-Segall D, Vogt G, Bird AC, Fitzke FW. [Functional aspects of drusen regression in Age-Related Macular Degeneration](#). Br J Ophthalmol. 2009 Jun 18. [Epub ahead of print] PMID: 19535356 [PubMed - as supplied by publisher] (IF 2.859)

Sallo FB, Bereczki E, Csont T, Luthert PJ, Munro P, Ferdinandy P, Sántha M, Lengyel I. [Bruch's membrane changes in transgenic mice overexpressing the human biglycan and apolipoprotein b-100 genes](#). Exp Eye Res. 2009 Aug;89(2):178-86. Epub 2009 Mar 24. PMID: 19324038 (IF 2.579)

2) Presentations at national congresses

Sallo FB, Rechtman E, Peto T, Luong V, Bird AC, Fitzke FW. Functional testing of the macula in age-related macular disease. Poster presentation at the Annual Congress of the Hungarian Ophthalmological Society, Szeged, Hungary, June 9-11 2005.

3) Presentations at international congresses

Sallo FB, Luthert PJ, Munro P, Santha M, Bereczki E, Lengyel I. Changes in Bruch's Membrane of transgenic mice overexpressing ApoB100 and Biglycan proteins - implications for Age-Related Macular Degeneration. Annual Meeting of ARVO Ft. Lauderdale, April 27 - May 1, 2008 (Poster presentation)

Sallo FB, Rechtman E, Peto T, Luong V, Bird AC, Fitzke FW (1399/B168) Fine Matrix Mapping of Drusen and Non-Drusen Retinal Areas in Age-Related Maculopathy. Poster presentation at the Annual Meeting of ARVO in Ft. Lauderdale, May 1-5, 2005

4) Citable abstracts

F. B. Sallo, P. J. Luthert, P. Munro, M. Santha, E. Bereczki, and I. Lengyel. Changes in Bruch's Membrane of Transgenic Mice Overexpressing ApoB100 and Biglycan Proteins - Implications for Age-Related Macular

Degeneration. Invest. Ophthalmol. Vis. Sci. 2008 49: E-Abstract 1754.

Sallo FB, Peto T, Rechtman E, Bird AC, Fitzke FW: A macula lutea funkcionális vizsgálata idoskori maculadegenerációban Szemészet, 142. Évfolyam (2005) I. Supplementum 1-140.

F.B. Sallo, E. Rechtman, T. Peto, V. Luong, A.C. Bird, and F.W. Fitzke. Fine Matrix Mapping of Drusen and Non-Drusen Retinal Areas in Age-Related Maculopathy. Invest. Ophthalmol. Vis. Sci. 2005 46: E-Abstract 1399.

Other publications and presentations

1) Peer-reviewed publications

Sallo FB, Peto T, Leung I, Xing W, Bunce C, Bird AC. [The International Classification system and the progression of age-related macular degeneration.](#) Curr Eye Res. 2009 Mar;34(3):238-40. PMID: 19274532 (IF 1.519)

2) Presentations at national congresses

Hatvani I, **Sallo FB**. Ophthalmological aspects of dirofilarioses. Lecture at the CME conference of St Istvan University, Budapest, Hungary, November 6, 2004.

Hatvani I, **Sallo FB**: Living filarioid in the human vitreous. 2002 Congress of the Hungarian Ophthalmological Society, Miskolc, Hungary.

Bausz M, Sényi K, **Sallo FB**, Süveges I: Our conclusions from 15 years follow up of children operated with bilateral congenital cataract, SHIOL Keszthely, Hungary 2002

Sallo FB, Hatvani I. Pigment epithelial cysts of the pupillary rim of the iris causing transient blindness. SHIOL (Societas Hungarica ad Implantandam Oculi Lenticulam), 2002, Keszthely, Hungary

Hatvani I, **Sallo FB**, Axmann Á, Fok É, Albert M, Baska F: A case of intravitreal filarioidosis in Hungary. Joint Congress of the Hungarian Society of Parasitologists and the Hungarian Zoological Society, Bp 2002

Hatvani I, Czvikovszky Gy, **Sallo FB**, Refractive surgical procedures following penetrating corneal grafts. SHIOL 2001 Kaposvár, Hungary

Hatvani I., **Sallo FB.**, Current place of glaucoma implants in our practice The 2nd National Congress of Ophthalmology of the Romanian Society of Ophthalmology, Sinaia, Romania 2001

Sallo FB, Czvikovszky Gy, Hatvani I Intraoperative examination of the retina using a rigid endoscope following removal of a luxated lens due to blunt trauma – „in vivo Miyake view” The Millennium Session of the Retina Section of the Hungarian Ophthalmological Society, 2000 Tihany, Hungary

3) Presentations at international congresses

Stanescu DM, Rechtman E, **Sallo FB**, O'Toole L, Luong V, Fitzke FW (4077/B435) The Photopic and Scotopic Visual Thresholds in Eyes With Solar Retinopathy: A Comparison With the Anatomical Damage. Poster presentation at the Annual Meeting of ARVO in Ft. Lauderdale, May 1-5, 2005

Stanescu D, Bonnel S, **Sallo FB**, Sahel J: CMV retinitis in Good Syndrome, EVER 2004. (poster presentation)

Sallo FB, Peto T, Dandekar S, Leung I, Bird AC: The International Classification System and progression of AMD, Annual Meeting of ARVO, Ft. Lauderdale, USA, May 4-8, 2003 (poster presentation)

4) Citable abstracts

D.M. Stanescu, E. Rechtman, **F. Sallo**, L. O'Toole, V. Luong, and F. Fitzke. The Photopic and Scotopic Visual Thresholds in Eyes With Solar Retinopathy: A Comparison With the Anatomical Damage. Invest. Ophthalmol. Vis. Sci. 2005 46: E-Abstract 4077.

F.B. Sallo, T. Peto, S. Dandekar, I. Leung, and A.C. Bird. The International Classification System and Progression of AMD. Invest. Ophthalmol. Vis. Sci. 2003 44: E-Abstract 1811.