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Understanding Pathogenetic Mechanisms of Disease

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Friday, 20th September 2019

08:30 **Opening remarks and welcome**
Daniel Pauleikhoff (Münster)
Frank G. Holz (Bonn)

08:45 **1st session**
▼ **AMD basics**
10:35 **Moderators: SriniVas Sadda** (Los Angeles/USA)
Steffen Schmitz-Valkenberg (Bonn)

08:45
01 L **Alan C. Bird** (London/GB)

Why the macula – learnings from the MacTel project?

08:57
02 L **Philip J. Luthert** (London/GB)

Cellular structures – computational biology of the macula

The macula has evolved to offer detection of light over a very wide range of intensities and at exquisitely graded resolution whilst presumably optimising energy consumption as photoreceptors are intrinsically energy demanding and the system is 'always on'. This is achieved through the intricate interplay of choroidal circulation, retinal pigment epithelium, photoreceptors and Muller cells.
It is well known that there are complex, co-independent interactions between choriocapillaris, retinal pigment epithelium and photoreceptors and in aging there are changes in each of these elements making it challenging to understand aging and age-related disorders such as AMD. These changes are likely to impact on the complex metabolic ecosystem operating in the outer retina eloquently described in recent years by Hurley and colleagues but direct measurements of metabolism at the back of the human eye currently presents significant challenges.
One approach to address these challenges is to use data from post-mortem tissue, 'omics and the field of cell biology to construct computational models of the interplay between different cell types at the back of the eye. This approach can also be informed by the wealth of anatomical data available that describes how photoreceptor density and other parameters change with increasing eccentricity from the fovea.
It is interesting that models with rather simple 'rules' nevertheless display quite complex behaviour in relation to changing metabolite availability and some of the key patterns of metabolic fluxes determined experimentally are replicated in the models. The hope is that understanding of network control of metabolism may generate new approaches to therapy.

09:09
03 L **Christine A. Curcio[1],** D. Kar[1], D.M. Dacey[2], D.M.G. Anderson[3], A. Kotnala[3], K.L. Schey[3] ([1]Birmingham/USA., [2]Seattle/USA, [3]Nashville/USA)

Molecular microarchitecture of macula in health and AMD

Background: In normal human macula, we investigated with comprehensive molecular and ultrastructural technologies an ecosystem that contributes to drusen lipids (PMID 30357337). Two features of high-risk drusen and their precursors in Bruch's membrane implicate macular biology: enrichment in the fatty acid linoleate signifying a dietary source; epidemiologic localization within the central subfield and inner ring of the ETDRS fundus grading grid, suggesting a relationship with foveal cone photoreceptors and supporting Müller glia.
Methods: To map mass/ charge (m/z) signals from tissue lipids without using labels or stains, eyes from donors older than 80 years of age and recovered within 6 hours of death were cryosectioned for imaging mass spectrometry. To determine the proportion of retinal layers occupied by Müller glia, one eye from a 21-year-old organ donor was preserved at the withdrawal of life support and serially thin-sectioned through the fovea for volume electron microscopy.
Results: Hundreds of m/z were detected. Many lipids were identifiable via bioinformatics. Among 6 m/z patterns specific to single or grouped retinal layers, a bowtie pattern was unique to the fovea and adjacent parafovea. High signal intensity in the foveal center, HFL, IPL, and NFL of the bowtie resembled the distribution of xanthophyll carotenoids described in macaque retina (PMID 6724837). The volume fraction occupied by Müller glia is 80-90 % in the foveal floor and HFL and less than 50 % in cellular layers.
Conclusion: The bowtie m/z pattern was associated with a population of human macular cells resembling Müller glia, suggesting a distinctive lipid profile for these cells. Müller glia processes are abundant in retinal layers known to have high xanthophyll content. Data are consistent with (but do not prove) a hypothesis that soft druse lipids may be remainders from the dietary delivery of xanthophyll carotenoids used by specialized Müller glia to sustain foveal cones.

09:21
04 L **Robert Mullins** (Iowa City/USA)

Addressing choriocapillaris degeneration in AMD: Cause, consequences, and potential therapies

Both histological approaches and new imaging modalities reveal vascular loss in the choroid in AMD. Quantification of choroidal vascular lumen areas using a marker for viable endothelial cells shows loss of the choriocapillaris under intact RPE in early dry AMD, with more severe vascular degeneration in geographic atrophy. The observations that (a) choriocapillaris degeneration may precede RPE loss, (b) the membrane attack complex (MAC) of complement is predominantly localized to the choriocapillaris, (c) MAC is more abundant in eyes with AMD and in eyes with increased genetic risk for AMD, and (d) choroidal endothelial cells are susceptible to MAC-mediated lysis suggests a model for AMD pathogenesis in which injury to the choriocapillaris is an early event. This model for AMD pathogenesis will be discussed along with possible treatments for AMD by protecting the choriocapillaris in early AMD and rebuilding the choriocapillaris in late AMD, respectively.

09:33
05 L **Sandra Liakopoulos** (Cologne/D)

Macular Imaging – Morphology and changes in AMD and aging?

Imaging technologies have developed rapidly in recent years. New generation SDOCT as well as Swept-Source OCT instruments provide high quality imaging not only of the retina but also of the choroid, OCT angiography (OCTA) allows non-invasive visualization of retinal and choroidal blood flow, and en face imaging of selected retinal slabs is possible with dense OCT or OCTA volume scans. Various morphological changes are better appreciated using confocal scanning laser ophthalmoscopy compared to conventional color fundus photography. Modern multimodal imaging not only helps clinicians to better visualize morphological changes, but also allows to detect new imaging findings and a better understanding of the pathogenesis of AMD. There is an ongoing effort to describe known and new imaging findings, correlate them with morphological and histological changes and investigate their potential prognostic relevance. While some features are regarded as normal aging changes, others are first signs of the development of AMD. Both the qualitative evaluation and the quantitative measurements allow detailed follow-up of the morphological changes that ultimately lead to atrophy, currently the greatest unmet need in AMD.

09:45
67►5a L **Usha Chakravarthy[1],** B. Reeves[2], R. Evans[2], C. Rogers[2] ([1]Belfast/GB, [2]Bristol/GB)

Extended follow up from the IVAN trial

Background: To understand visual outcomes in study eyes after release from protocol in participants enrolled in the IVAN clinical trial.
Methods: 532 participants eligible for inclusion after IVAN exit. 124 died before data collection. Approval was available for passive data collection on all survivors and those deceased. 199 of the survivors attended a research visit. Data from every visit was extracted along with VA and treatments administered to each eye. BCVA, LLA and macular imaging captured in attenders and in non attenders images from the most recent visit were obtained. The mean, median of DVA after IVAN exit by type of follow up in survivors and deceased patients and change in DVA, visit and injection rates for each year of follow up by BCVA category at IVAN exit was computed. Duration of follow up was length of

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time between IVAN exit and cessation of active management and used for rate of change in DVA/year, injection and visit rates A multivariable linear random effects model was used to test effects of covariates.
Results: The median DVA was 58.0 letters (IQR 34.0, 73.0), 14 letters worse than at IVAN exit with a median change -10 letters, [IQR -22.0 to -2.0]. One third had a VA better than 68 letters. One fifth (had a VA of worse than 33 letters. The multivariable model estimated the reduction in DVA during study eye monitoring to be -4.3 letters per year (95 % CI 3.7 to 4.9). The only significant interaction was between age and time (p<0.001) with DVA deteriorating faster in older participants and in the group who died after IVAN exit but prior to data collection. Injection rate did not influence DVA decline.
Conclusion: Despite intensive active management of nAMDwith anti VEGF the VA in study eyes deteriorated with an average loss of around 4 letters per year

09:57
65►5b L **Eric Souied** (Paris/F)

Pathways from CNV to fibrosis

10:09
06 L **Marius Ueffing[1],** A. Armento[1], S. Honisch[1], M.A. Meester-Smoor[2], C. Delcourt[3], C.C.W. Klaver[4], J. Monés[5], L. Serrano[6], C. Kiel[5], A.I. den Hollander[2], I. Lengyel[7], T. Peto[7], B. de la Cerda[5], F. Pol[9], P.J. Luthert[8] for the EYE-RISK Consortium ([1]Tübingen/D, [2]Nijmegen/NL, [3]Bordeaux/F, [4]Rotterdam/NL, [5]Barcelona/E, [6]Sevilla/E, [7]Belfast UK, [8]London, UK, [9]Dublin, IRE)

Using cell lines - identify, model and validate AMD risk factors and disease drivers

Age-related macular degeneration (AMD) is a chronic and progressive disease of the retina and is the most frequent cause for legal blindness in the EU. Current research aims to prevent, predict and treat AMD. So far, genetic and epidemiologic studies have been able to pinpoint more than 35 genetic variants as well as environmental and lifestyle factors that define the individual risk for AMD. Turning this knowledge into prevention, prediction and treatment has been the goal of EYE-RISK (www.eye-risk.eu) funded by the EU programme Horizon2020 in its public health challenge PHC-01 "Understanding health, ageing and disease: determinants, risk factors and pathways". The approach of EYE-RISK integrates clinical phenotyping and diagnosis, genotyping, next-generation targeted re-sequencing, bioinformatics and statis- tics, clinical data analysis, computational biology, systems-biology oriented pathway analysis and modelling. Five major goals have been pursued: 1) robust algorithms to identify personalized risks of development of advanced AMD, and progression of dry AMD; 2) novel biomarkers for further stratification of disease risk; 3) molecular drivers/biological pathways relevant for onset and progression of advanced AMD; 4) clinical guidelines for individuals at risk of developing AMD; 5) criteria of inclusion and stratification for patients entering clinical trials. Specific emphasis has been on the question, how a combination of risks jointly shifts the regulation of homeostasis in the choriocapillaris/Bruchs-membrane/RPE interphase towards a diseased state. Recent results on the interconnection of discrete genetic risks, the interaction with lifestyle factors, and the impact of local dysregulation of complement on the development and the progression of AMD will be presented.

09:21
7 P **Anita de Breuk[1],** I.E. Acar[1], E. Kersten[1], M.M.V.A.P. Schijvenaars[1], M.A. Meester-Smoor[2], C. Delcourt[3], C.C.W. Klaver[2], J. Monés[4], D. Pauleikhoff[5], R. Silva[6], S. Fauser[7], C.B. Hoyng[1], M.J.H. Coenen[1], A.I. den Hollander[1], EYE-RISK Consortium ([1]Nijmegen/NL, [2]Rotterdam/NL, [3]Bordeaux/F, [4]Barcelona/E, [5]Münster/D, [6]Coimbra/P, [7]Cologne/D)

Development of a Genotyping Assay for Age-Related Macular Degeneration: The EYE-RISK Consortium

Background: Current genetic tests for age-related macular degeneration (AMD) are limited to a low number of genetic variants and vary widely in their risk assessment capacity. We aimed to develop an AMD genotyping assay that covers all currently known genetic variants and that can detect novel rare coding variants.
Methods: We developed a genotyping assay based on single molecule molecular inversion probes and next generation sequencing covering 87 single nucleotide polymorphisms (SNPs), and the coding and flanking regions of 13 genes (ABCA4, ARMS2, C3, C9, CD46, CFB, CFH, CFI, CTNNA1, HTRA1, PRPH2, TIMP3, SLC16A8). We included DNA samples from five European cohorts. Quality control steps were applied to ensure high quality data. Rare variants were analyzed with gene-based burden tests. Logistic regression analysis was used to assess associations of rare damaging and loss of function variants with AMD.
Results: We genotyped 2,720 AMD patients and 1,299 controls. Seventy SNPs were successfully genotyped, while seventeen SNPs were discarded due to coverage problems, deviation from Hardy-Weinberg expectations (P<10-4), low genotype concordance (<95 %) or a high proportion of missing genotypes per SNP (>10 %). We observed a high concordance rate between our platform and other genotyping platforms (96.02 %-99.96 %). Allele frequencies of the majority of the SNPs were similar to previously reported data. We observed a significant burden of rare variants in the C3 gene (P=1.00 x 10-3) and the CFH gene (P=4.17 x 10-5) in AMD cases. The CFH and CFI genes carried more damaging and loss of function variants in cases compared to controls (CFH gene OR 2.1, P=0.09; CFI gene OR 8.4, P=0.04).
Conclusion: We developed a comprehensive AMD genotyping assay which successfully genotyped 70 SNPs and the coding regions of 13 genes. After further optimization the assay can be used for risk assessment of AMD development and AMD progression.

09:28
8 P **Thomas J. Heesterbeek[1],** E.K. de Jong[1], I.E. Acar[1], J.M.M. Groenewoud[1], B. Liefers[1], C.I. Sánchez[1], T. Peto[2], C.B. Hoyng[1], D. Pauleikhoff[3], H.W. Hense[3], A.I. den Hollander[1] ([1]Nijmegen/NL, [2]Belfast/GB, [3]Münster/D)

Genetic Risk Score has added value over initial clinical grading stage in predicting disease progression in patients with non-advanced age-related macular degeneration – The Muenster Aging and Retina Study (MARS)

Background: Several prediction models for progression of age-related macular degeneration (AMD) have been developed, but the added value of using genetic information in those models in addition to clinical characteristics is ambiguous. In this study, we investigated the association between the genetic risk score (GRS) and disease progression in patients with non-advanced AMD, and explored the added value of the GRS in addition to the drusen coverage at baseline.
Methods: A total of 177 patients with non-advanced (early or intermediate) AMD in the worst eye at baseline and a follow-up visit after 6.5 years were selected from the Muenster Aging and Retina Study (MARS). After DNA genotyping, 52 AMD-associated variants were extracted to generate a GRS. Fundus images were graded according to the Age-Related Eye Disease Study (AREDS) basic clinical classification scale, and the drusen coverage was quantified using a computer-aided detection system.
Results: The GRS was strongly associated with the drusen coverage at baseline (P<0.001) and both the GRS and drusen coverage were associated with disease progression. When the GRS was added as predictor in addition to the drusen coverage, R2 increased from 0.46 to 0.56. This improvement by the GRS was predominantly seen in patients with a drusen coverage <15 %. In patients with a larger drusen coverage, the GRS had less added value to predict disease progression.
Conclusion: Genetic information has added value over clinical characteristics in predicting disease progression in AMD, but only in patients with a less severe disease stage. Patients with a high GRS should be made aware of their risk and could be selected for clinical trials for arresting progression.

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11:05 **2nd session**
▼ **Genetics and risk factors of AMD**
12:45 **Moderators: Carel B. Hoyng** (Nimwegen/NL)
Bernhard H.F. Weber (Regensburg/D)

11:05
09 L **Bernhard H.F. Weber** (Regensburg/D)

Age-related macular degeneration – Searching for targeted therapies

Background: Age-related macular degeneration (AMD) is a severe condition in elderly people and is the leading cause of vision impairment in industrialized countries. Little is known, however, about the molecular biology underlying this disease and thus about targeted approaches to develop innovative treatment options. Deciphering the genetic architecture underlying AMD development but also progression of the disease once manifestations have already developed may shed light on pathways and target molecules to treat this devastating condition.
Methods: Common single nucleotide polymorphism (SNP)-based genetic association studies (GWAS) are suited to correlate genetic variation to any trait or disease of interest. Generally, 800.000 to 1.000.000 SNPs are directly genotyped throughout the genome in a disease/trait group and compared to a matched control population. Statistical analysis of allele frequencies between the two groups eventually leads to genome-wide significant data which allow pinpointing pathways and specific target genes suitable to address in future clinical studies.
Results: To date, the genetic risk to develop AMD has essentially been worked out. There are more than 34 genetic loci accounting for at least 52 independent genetic risk variants associated with late stage AMD and with major effects at the CFH and the ARMS2 / HTRA1 loci, respectively. Progression of AMD may be a more crucial medical trait to be addressed and may underlie its own genetic architecture. Progression can be understood in several ways and currently is focused on progression from early to late stages of AMD, progression from early to a specific late stage subtypes of AMD and progression of geographic atrophy lesion growth. Here, we are at the beginning to understand the genetic background of the various progression traits.
Conclusions: Genetic studies have the potential to guide the development of therapeutic treatment by pointing to disease-associated pathways and genes. So far, we have become aware that the various aspects of disease development and progression will likely lead to different target genes which can now be addressed in novel therapeutic approaches and first clinical trials.

11:17
10 L **Carel B. Hoyng** (Nimwegen/NL)

Fenotype-genotype correlation in AMD

Fenotype-genotype correlation in AMD is of increasing importance.
1. Certain fenotypes correlate with a higher incidence of so-called 'rare variants'. These are mutations in risk genes (especially CFH and CFI). With emerging gene therapy trials it is important to recognize these fenotypes. Furthermore, these fenotypes have a higher incidence within families.
2. Certain fenotypes are correlated with a faster progression of AMD.
3. Still a reasonable number of monogenetic diseases, especially caused by mutations in the PRPH2 or ABCA4 gene are diagnosed as dry AMD (up to 5 % of dry AMD cohorts). Their progression and familial involvement is different from AMD and with several upcoming therapies for dry AMD, but alsof or monogenetic diseases they should be recognized seperated from each other.r.

11:29
11 L **Magda A. Meester-Smoor,** C.C.W. Klaver (Rotterdam/NL)

EyeRisk Study – risk factors for AMD phenotypes and progression?

The H2020 funded EYE-RISK project aimed to identify frequency and morbidity of AMD in Europe, and to specify who is at risk of developing AMD, who is at risk for progression, why and how risks combine to advance progression, and what we can do to lower the risk.
Existing epidemiologic data from >45,000 individuals aged 40+ from ten countries in Europe were harmonized and entered into a common database. Prevalence, visual decline, genetic and non-genetic risk factors were investigated and analysed for geographic differences and time trends.
We found that AMD will become an even more significant health problem in Europe in the near future and that long term visual function of AMD continues to be poor. Furthermore, we showed that genetic testing combined with environmental factors for AMD can be very predictive and that lipids are implicated in AMD with a surprising direction of effect.

11:41
12 L **Giovanni Staurenghi** (Milan/I)

How to differentiate GA phenotypes – the CAM initiative

11:53
13 L **Frank G. Holz,** S. Schmitz-Valckenberg, M. Schmid, G.S. Rubin, H. Dunbar, A. Tufail, D.P. Crabb, A. Binns, C.I. Sánchez, P. Margaron, G. Normand, M.K. Durbin, U.F.O. Luhmann, P. Zamiri, J. Cunha-Vaz, F. Asmus F, R.P. Finger, on behalf of the MACUSTAR consortium (Bonn/D)

MACUSTAR: Development and Clinical Validation of Functional, Structural, and Patient-Reported Endpoints in Intermediate Age-Related Macular Degeneration

Purpose: Currently, no outcome measures are clinically validated and accepted as clinical endpoints by regulatory agencies for drug development in intermediate age-related macular degeneration (iAMD). The MACUSTAR Consortium, a public-private research group funded by the European Innovative Medicines Initiative intends to close this gap.
Procedures: Development of study protocol and statistical analysis plan including predictive modelling of multimodal endpoints based on a review of the literature and expert consensus.
Results: This observational study consists of a cross-sectional and a longitudinal part. Functional outcome measures assessed under low contrast and low luminance have the potential to detect progression of visual deficit within iAMD and to late AMD. Structural outcome measures will be multimodal and investigate topographical relationships with function. Current patient-reported outcome measures (PROMs) are not acceptable to regulators and may not capture the functional deficit specific to iAMD with needed precision, justifying development of novel PROMs for iAMD. The total sample size will be n = 750, consisting mainly of subjects with iAMD (n = 600).
Conclusions: As clinical endpoints currently accepted by regulators cannot detect functional loss or patient-relevant impact in iAMD, we will clinically validate novel candidate endpoints for iAMD.

12:05
14 L **Steffen Schmitz-Valkenberg** (Bonn/D)

Challenges in phenotyping geographic atrophy

Retinal atrophy is the end-stage manifestation of various retinal and choroidal diseases and thus not exclusive for AMD. The manifestation and lesion size progression of well-defined areas of atrophy- commonly called geographic atrophy – is also not limited to AMD. Multimodal retinal imaging allows for a better detection of retinal and choroidal changes and thus a refined phenotyping. This includes the differentiation between AMD and other macular diseases, causing outer retinal degeneration and atrophy in the same age group, but associated with a distinct genetic background, such as late-onset Stargardt disease and central areolar choroidal dystrophy (CACD). Further, within AMD cohorts, distinct patterns outside atrophy can be detected, one of the most obvious phenotype being the diffuse-trickling pattern, showing several peculiar multimodal imaging findings, suspicious demographic observations with regards to general medical history and exhibiting a non-expected AMD genotype. Still in other situations, the differential diagnosis is challenging and not so clear. We are still in the process to further advance the AMD definition which requires a multidisciplinary approach. Today, it is quite evident that the term "AMD" is currently used too frequently rather than to further classify the degeneration in question, both in clinical management and also and explicitly in clinical research.

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12:17
15 L **Andrew Lotery[1],** J.R. Sutton[1], S. Khandhadia[1], G. Menon[2], C. Bailey[3], S. Sivasprasad[4], Q. Mohamed[5], P. Bishop[6], P. Charbel Issa[7], D. Steel[8], T. Peto[3], W. Amoaku[9], R. Arora[10], P. Prakash[11], W. Innes[12], R. Maclaren[7], D. Kavanagh[12] ([1] Southampton/GB, [2]Camberley/GB, [3]Bristol/GB, [4]London/GB, [5]Cheltenham/GB, [6]Manchester/GB, [7]Oxford/GB, [8]Sunderland/GB, [9]Nottingham/GB, [10]Salisbury/GB, [11]Harlow/GB, [12]Newcastle/GB)

Rare genetic variants in geographic atrophy

Background: Rare variants in complement factor I (CFI) are strongly associated with risk of developing AMD (Age-related Macular Degeneration). Furthermore low systemic CFI levels have been found in patients with AMD. We aimed to identify the prevalence of patients with advanced dry AMD (geographic atrophy) who also had low systemic CFI protein levels and CFI genetics mutations. Our hypothesis is these patients may benefit from Complement Factor I supplementation.
Methods: 13 hospitals in the United Kingdom invited patients with geographic atrophy to participate in this ethically approved study. Patients had serum Complement Factor I and C-reactive protein measured. If serum CFI levels were low, sequencing of CFI was undertaken to identify rare variants. CFI levels were measured by ELISA and genetic analysis was undertaken using Sanger sequencing and performed by Multiplex Ligation-dependent Probe Amplification.
Results: Currently 409 patients have been recruited. Of these 66 patients have a low serum CFI level defined as below 15.6 ug/mL ie 16 %. Range of CFI proteins (6.2 - 36.0 ug/mL). Of those patients with a low CFI serum level, 9 had an identified rare variant in CFI and 57 did not (2 % of the total population). In patients with a low CFI level C-RP range and average was <0.3 – 14.2mg/L (2.09) and for those with a normal CFI level, 0.4 – 59 mg/L (4.62) The range and average age of GA patients with CFI mutations was 61-84 with (77) and of those without 46 – 101 (79).
Conclusions: In the UK rare variants in CFI with low serum levels are found in 2 % of patients with geographic atrophy. These patients might be particularly suitable for CFI supplementation in future therapeutic trials.

12:29
16 L **Sobha Sivaprasad** (London/GB)

Early Loss of Color Vision on Drusen and Reticular Pseudodrusen

14:00 ▼ **15:30** **3rd session**
Complement, inflammation and cellular energetics in AMD
Moderators: Philip J. Luthert (London/GB)
Marius Ueffing (Tübingen/D)

14:00
18 L **Bradley D. Gelfand,** J. Ambati (Charlottesville/USA)

Inflammasome in AMD

Activation of the NLRP3 inflammasome, an innate immune signaling platform involved in sensing foreign and endogenous danger signals, induces RPE death in geographic atrophy. One agonist of NLRP3 inflammasome is retro-element derived Alu RNAs, which accumulate in RPE due to deficiency of DICER1 enzymatic processing. However, the consequences of Alu RNA accumulation and NLRP3 inflammasome activation in choroidal neovascularization (CNV), another hallmark of advanced AMD, is unknown. Here, we report that multiple, complementary mouse models of DICER1 deficiency develop spontaneous CNV, which is abrogated in mice lacking NLRP3 inflammasome constituents. Alu RNA aggravates experimental choroidal CNV. This angiostimulatory activity is diminished or abrogated in mice lacking constituents of the inflammasome including P2X7, NLRP3, Caspase-1, and MyD88 and in mice with pharmacologic inflammasome inhibitors. Alu RNA induces NLRP3 inflammasome-dependent activation of canonical hypoxia signaling, including degradation of VHL, HIF-2α accumulation, and VEGF production. Collectively, these observations suggest that NLRP3 inflammasome activation due to Alu RNA promotes aberrant angiogenesis, and thereby may contribute to diseases of aberrant angiogenesis including neovascular AMD.

14:12
19 L **Tim U. Krohne[1],** L. Wang[1], S. Schmidt[1], J. Rossa[1], P.P. Larsen[1], J.H. Meyer[1], W.R. Roush[2], E. Latz[1,2], F.G. Holz[1] ([1]Bonn/D, [2]Boston/USA)

NLRP3 inflammasome as a therapeutic target in AMD

Background: NLRP3 inflammasome activation in the retinal pigment epithelium (RPE) is observed in atrophic age-related macular degeneration (AMD). Pharmacological NLRP3 inhibition may provide a therapeutic strategy to halt disease progression. We tested novel selective NLRP3 inhibitors (IFM-514, IFM-632, and CRID3) for their efficacy in human and murine RPE cells.
Methods: Inflammasome activation was induced in primary human RPE cells, ARPE-19 cells, and murine RPE tissue cultures by different stimuli. For this, cells were priming with IL-1a and subsequently subjected to either lysosomal membrane permeabilisation by Leu-Leu-OMe, oxidative damage induced by hydrogen peroxide, lipofuscin-mediated photooxidative damage induced by incubation with 4-hydroxynonenal-modified photoreceptor outer segments and subsequent blue light irradiation, or P2X7 activation by BzATP. RPE/choroid/sclera eye cups from Abca4-/-, Abca4-/-/Nlrp3-/-, and wildtype mice were exposed to blue light irradiation or Leu-Leu-OMe. Inflammasome activation was assessed by means of IL-1b release, lactate dehydrogenase release, and ZO-1 staining.
Results: Independent of activation mechanism, treatment with the NLRP3 inhibitors IFM-514, IFM-632, and CRID3 resulted in a significant suppression of inflammasome activation as assessed by IL-1b and LDH release. E.g., 0.01 µM IFM-632 reduced IL-1b release induced by lysosomal permeabilisation, oxidative damage, photooxidative damage, and P2X7 activation in ARPE-19 cells to 16.6 % (p=0.005), 6.9 % (p=0.003), 39.4 % (p=0.010), and 12.7 % (p<0.001), respectively. Likewise, inflammasome activation in blue light-irradiated Abca4-/- mouse and Leu-Leu-OMe-treated wildtype mouse RPE/choroid/sclera eye cups was significantly reduced by treatment with the NLRP3 inhibitors.
Conclusion: The investigated selective NLRP3 inhibitors demonstrated efficacy in human and murine RPE cells and thus represent promising agents for the future evaluation of inflammasome inhibition as therapeutic strategy for atrophic AMD.

14:24
20 L **Glen Jeffery** (London/GB)

Mitochondrial decline precedes phenotype development in the complement factor H mouse model of retinal degeneration but can be corrected by near infrared light

Mitochondrial function declines with age and is marked in the metabolically demanding retina. In aged murine AMD models (CFH-/-, CFH+/-), ATP decline is significant over matched controls by 25 % with abnormal respiration patterns. But earlier retinal development is also abnormal with enlarged mitochondria and delayed retinal formation. Complement regulates retinal development and its absence (CFH-/-) or compromise (CFH-/+) may establish weaknesses exploited by age. Evidence for this is present in human complement polymorphisms decades before expected disease establishment.
However, reduced mitochondrial function can be corrected optically with specific wavelengths that increase ATP, but only when delivered within specific temporal windows matching points in the mitochondrial circadian cycle when there is spare capacity. These need to be at specific energies. The importance of retinal mitochondria in ageing and potentially AMD is highlighted by the >70 % decline in retinal ATP in healthy aged primate and the move towards potentially toxic glycolysis.

Friday, 20th September 2019

14:36
21 L **Imre Lengyel** (Belfast/IRL)

Is mineralization a major factor in drusen pathogenesis?

Purpose: Drusen are a defining feature of the ageing eye and it is associated with age-related macular degeneration (AMD), a common sight-threatening disease of older adults. With an odds ratio of > 7 large drusen is a significant risk factor for progression to advanced AMD, still, the molecular mechanism behind this increased risk was largely unknown. We recently proposed that calcium and phosphate containing minerals are key in this process and identified the mineral components of Bruch's membrane (BrM) calcification as well large "calcified" nodules and compare these to the previously reported hydroxyapatite spherules.
Methods: Human cadaveric eyes were dissected and embedded in epoxy resin, sectioned at 0.5 to 2 µm and mounted on to glass slides or on to ultralene when appropriate. The elemental composition of BrM plaques and large calcified nodules was investigated using density dependent-scanning electron microscopy (DDC-SEM), energy dispersive x-ray spectroscopy (EDX), secondary ion mass spectroscopy (SIMS), and synchrotron microfocus x-ray fluorescence (µXRF). The mineral components were determined using transmission electron microscopy-selected area electron diffraction (TEM-SAED).
Results: Using DDC-SEM, spherules, BrM plaques and large calcified nodules were shown to contain dense material, later show to contain Ca and P by EDX and SIMS: spherules (spherules, n=100), BrM plaques (eyes, n=3) and calcified nodules (eyes, n=3). TEM-SAED confirmed that all 3 calcifications were composed of apatite.
Conclusions: We found calcium and phosphate in all types of calcific lesion: spherules, Bruch's membrane plaques and large calcified nodules. However, differences in crystal structure and composition of the developing form, shape and size of apatite can be affected by the environment they are deposited in. These results highlight the need for further research on calcium and phosphate homeostasis at the RPE-Choroid complex. Understanding these processes may aid the development of novel therapeutic interventions that target the progression of early-stage lesions to advanced stages of AMD.

14:48
22 P **Martin Zinkernagel,** D. Zysset, I. Keller, L. Berger, B. Yilmaz, S. Wolf (Bern/CH)

Associations of the intestinal microbiome with age-related macular degeneration

Background: Recent evidence has shown that the gut microbiom, directly or indirectly through its components, is associated with age related macular degeneration.
Methods: The gut microbiome of 57 patients with age related macular degeneration and 58 healthc age and sex matched controls was sequenced using the Illumina HiSeq 3000 platform. All participants were subjected to an ophthalmic examination including optical coherence tomography and standard fundus color photography.
Results: Several taxonomic features of the intestinal microbiome that differ between patients and healthy controls were identified: The genus Oscillibacter and Bacteroides species were found to be enriched in healthy controls relative to AMD patients. In contrast, the phyla Firmicutes was enriched in AMD patients compared to controls.
Conclusion: This data confirms earlier findings that the composition of the gut microbiome is associated with age related macular degeneration. Whether this effect is mediated via alterations in metabolite uptake or via microbiota-cytokine interactions remains to be investigated.

14:55
23 P **Ilhan Erkin Acar[1],** L. Lores de Motta[1], M.A. Meester[2], S. Ajana[3], D. Pauleikhoff[4], S. Fauser[5], C.B. Hoyng[1], C. Delcourt[3], C.C.W. Klaver[2], T.E. Galesloot[1], A.I. den Hollander[1] ([1]Nijmegen/NL, [2]Rotterdam/NL, [3]Bordeaux/F, [4]Münster/D, [5]Cologne/D)

Metabolomics identifies lipoprotein subclasses and dietary metabolites that are associated with age-related macular degeneration: The EYE-RISK Consortium

Background: Metabolomics, the high-throughput analysis of a wide range of metabolites in body fluids, has great potential to uncover biomarkers and pathways that contribute to disease pathophysiology. Studies into metabolomics in age-related macular degeneration (AMD) are scarce and limited by relatively small sample sizes. We therefore performed the largest metabolomics study in AMD to date, to identify associations of amino acids, glycolysis measures, ketone bodies, inflammation-related metabolites, fatty acids and lipoprotein subclasses with AMD.
Methods: A total of 7,753 samples from five cohorts (EUGENDA, Rotterdam Study, ALIENOR, MARS, CORRBI) were analyzed by NMR-based metabolomics at Nightingale Health to quantify 146 individual and 79 derivative metabolite measurements. Data was quality controlled, followed by cohort-specific logistic regression analysis and random effects meta-analysis to determine the association of each measurement with AMD, adjusting for age, gender, and cohort-specific confounders. The significance threshold was determined to be 0.05 after Benjamini-Hochberg correction (false discovery rate correction, FDR) for multiple testing.
Results: Meta-analysis identified 70 individual and 12 derivative measurements significantly associated with AMD. Increasing levels of extra-large (XL-) and large (L-) HDL subclasses were associated with increased risk for AMD (e.g. phospholipids in XL-HDL OR=1.12, 95 % CI=1.06-1.20, pFDR=0.01), while small (S-) and medium (M-) HDL subclasses and all VLDL subclasses were associated with decreased risk of AMD (e.g. cholesterol esters in L-VLDL OR=0.90, 95 % CI=0.85-0.96, pFDR=0.01). Six dietary amino acids were associated with decreased risk of AMD (e.g. phenylalanine OR=0.86, 95 % CI=0.81-0.91, pFDR=1.69x10-4).
Conclusion: HDL and VLDL lipoprotein subclasses showed strong associations with AMD, underscoring an important role for lipid metabolism in AMD pathogenesis. Interestingly, directions of effect differed among HDL subclasses, indicating that HDL particle size and composition are relevant in AMD. Associations with dietary fatty acids and amino acids highlight the role of nutrition in AMD.

15:02
24 P **Yara Lechanteur[1],** T. Heesterbeek[1], L. Lorés-Motta[1], T. Schick[2], M. Daha[3], L. Altay[2], S. Liakopoulos[2], D. Smailhodzic[1], A. den Hollander[1], C. Hoyng[1], E. de Jong[1], B. Klevering[1] (Nijmegen/NL, [2]Cologne/D, [3]Leiden/NL)

Complement activation levels are related to disease stage in age-related macular degeneration

Background: To study the levels of complement activation in different disease stages of age-related macular degeneration (AMD) and the influence of genetic polymorphisms in complement genes.
Methods: We included 797 AMD patients and 945 controls from the European Genetic Database. Patients were grouped into five AMD stages: early AMD, intermediate AMD, geographic atrophy (GA), active choroidal neovascularization (CNV) or inactive CNV. Differences in complement activation, as defined by the systemic C3d/C3 ratio, between AMD stages were evaluated using general linear modeling. In addition, we evaluated the influence of eighteen genetic AMD polymorphisms in complement genes and their effect on complement activation. Differences in complement activation were evaluated stratifying by complement associated haplotypes.
Results: Complement activation levels differed significantly between AMD disease stages. As compared to controls, the C3d/C3 ratio was higher in patients with intermediate AMD($P<0.001$) and highest in GA($P=0.002$). Two polymorphisms in CFH (rs10922109 and rs570618) and one in CFB (rs116503776) were significantly associated with complement activation. The association between AMD disease stage and complement activation was more pronounced in patients with a high genetic risk for complement activation.
Conclusion: Systemic complement activation levels differ between AMD disease stages, especially in individuals with high risk variants in complement genes. In general, consecutive AMD disease stages showed increasing levels of complement activation, except for active and inactive CNV. These findings contribute to the discussion on the pathogenesis of AMD in relation to complement activation and suggest refinement in the treatment with complement inhibitors with regard to patient selection and the optimum window of treatment.

Friday, 20th September 2019

15:09
25 P **Laura Lorés de Motta[1],** V. Cipriani[2], F. He[3], D. Fathalla[4], S. McHarg[3], N. Bayatti[3], I.E. Acar[1], C.C.B. Hoyng[1], S. Fauser[5,6], A. Moore[2,7], J.R.W. Yates[2,8], P. Morgan[4], E.K. de Jong[1], A.I. den Hollander[1], P.N. Bishop[3], S.J. Clark[3] ([1]Nijmegen/NL, [2]London/GB, [3]Manchester/GB, [4]Cardiff/GB, [5]Cologne/D, [6]Basel/CH, [7]San Francisco/USA, [8]Cambridge/GB,)

Factor H-Related Protein 4 (FHR-4) drives complement dysregulation in age-related macular degeneration

Background: Age-related macular degeneration (AMD) is a leading cause of blindness. Genetic studies reported strong associations at the CFH locus, with 8 independent signals across KCNT2, CFH, and CFHR1-5. How these variants impact protein expression and function remains to be disentangled. Recently, a variant in CFHR4 which associates with increased systemic complement activation and AMD risk was identified (Lorés-Motta et al., Ophthalmology 2018). Here we investigated whether FHR-4 directly impacts AMD pathogenesis.
Methods: Blood levels of FHR-4 and FH were measured in 484 late AMD cases and 522 controls from two independent cohorts (Cambridge and EUGENDA). Phenotyped human macular sections were stained for FHR-4 and C3b. Affinity measurements of FHR-4 binding to C3b and C3b breakdown functional assays were performed. Association of FHR-4 and FH levels with the 8 AMD-associated variants at the CFH locus (and corresponding haplotypes) was assessed; GWAS meta-analyses of FHR-4 and FH levels were additionally performed.
Results: Systemic FHR-4 levels were elevated in late AMD (P=5.3x10-6), whereas no significant difference was observed for FH levels. CFHR4 was not expressed in the eye, but FHR-4 protein accumulated in the choriocapillaris, Bruch's membrane and drusen and, by competing with FH and FHL-1, causes complement over-activation at these sites. Critically, the protective allele of the strongest AMD-associated CFH locus variant rs10922109 had the highest association with reduced FHR-4 levels (P=2.2x10-56). No locus other than CFH showed genome-wide significant association in our meta-analysis of FHR-4 levels. Haplotype analysis revealed that the effect of rs10922109 is independent of the AMD-protective CFHR1-3 deletion; furthermore, FHR-4 confers protection even in those individuals that carry the high-risk allele of rs1061170 (Y402H).
Conclusions: We dissected the GWAS results for AMD at the CFH locus and identified FHR-4 as a novel key molecular player causing complement dysregulation in AMD. FHR-4 may represent a potential therapeutic target.

15:16
26 P **Anja Schlecht[1],** P. Wieghofer[1,2], P. Zhang[1], S. Bonvea[1], M. Gruber[1], R. Sankowski[1], G. Schlunck[1], H. Agostini[1], M. Prinz[1], C. Lange[1] ([1]Freiburg i.Br./D, [2]Leipzig/D)

Identification of myeloid cell populations at sites of laser-induced choroidal neovascularization by single-cell profiling

Background: Myeloid cells (MC), such as resident microglia (MG) and infiltrating blood-derived monocytes (MO), are key players in the formation of choroidal neovascularization (CNV). However, the specific discrimination between MG and MO is challenging and the precise function of MG during CNV development remains unclear. In this study, we used MG-specific reporter mice to perform single-cell RNA-Sequencing (scRNA-Seq) of MG cells with and without laser-induced CNV to analyze their transcriptional profile.
Methods: Adult Cx3cr1CreER/+:Rosa26-Tomfl/+ mice were used for RNA-Seq analysis. Six laser spots were applied to each eye by an Argon laser (532nm). Laser setting were 150 mW, 100 ms with a spot size of 100 µm to induce CNV formation, while untreated littermates served as controls. Retinal MG cells were sorted by FACS on the third day after laser injury to perform scRNA-Seq. Protein expression of most outstanding factors was investigated by ELISA and immunohistochemistry. As a therapeutic approach, antibodies against SPP1 were injected intravitreally one day after laser injury and CNV lesion size was quantified.
Results: ScRNA-Seq analysis demonstrates, that MG strongly change their expression profile following laser-induced CNV formation and that different MG cell populations are present at sites of laser-induced CNV. In the CNV-associated MG cluster GO terms involved in migration, immune response and DNA synthesis were enriched and genes associated with proliferation, hypoxia-sensing and angiogenesis were significantly upregulated. SPP1, also known as Osteopontin, was highly expressed in CNV-associated MG. Immunohistochemistry and ELISA protein analysis reveals that SPP1 accumulates in the choroid and intravitreal injection of anti-SPP1 antibodies lead to an increased lesion size when compared to control treated eyes.
Conclusion: Our study demonstrates that retinal MG cells strongly change their expression profile following laser-induced CNV formation. CNV-associated MG cells secrete a plethora of molecules among which SPP1 may have anti-angiogenic and thus neuroprotective properties.

15:23
27 P **Alexa Klettner,** K. Winkelmann, A. Brinkmann, T. Käckenmeister, J. Roider (Kiel/D)

Effect of long-term inflammation on viability and function of RPE cells

Background: Degenerative ocular disorders like age-related macular degeneration (AMD) may induce long-term pro-inflammatory signals on retinal pigment epithelial (RPE) cells. In this study, we investigated the effect of long term treatment of RPE cells with agonists of toll-like receptor (TLR)3 (Poly I:C), TLR4 (LPS) and the pro-inflammatory cytokine TNFα.
Methods: All tests were conducted with primary porcine RPE. Cells were stimulated with Poly I:C (1, 10, 100 µg/ml), LPS (0.1, 1, 10 µg/ml) or TNFα (12.5, 25 or 50 ng/ml) for 1 day, 7 days or 4 weeks. Cytokine secretion (IL-6, IL-1ß, IL-8, TNFα, TGFß) was tested in ELISA, phagocytosis in an assay, and expression of RPE65 in Western blot. Barrier function was tested in transwell-cultured cells by measuring transepithelial resistance for up to 3 days.
Results: LPS and TNFα significantly reduce cell viability after 1 day and 7 days, Poly I:C after 7 days and 4 weeks. LPS, Poly I:C and TNFα significantly induce the secretion of IL-6 and IL-8 at all tested time points. IL-1ß is increased by LPS after 1 day, but not by Poly I:C or TNFα. TNFα secretion is increased by Poly I:C and LPS after 1 day but not at later time points. TGFß secretion is not influenced by any stimulus. Concerning RPE function, LPS decreased phagocytosis after 7 days, and Poly I:C and TNFα after 1 day. Poly I:C reduced RPE65 expression after 1 day and 7 days, while incubation for 4 weeks with LPS or TNFα significantly reduced RPE65 expression. Barrier function was not affected by Poly I:C, while LPS and TNFα reduced barrier function after 1 h, 4 h and 3 days.
Conclusion: Long term pro-inflammatory stimuli reduce RPE viability, barrier properties and cellular function and therefore may contribute directly to atrophic changes in AMD.

16:15 **4th session**
▼ **Neurobiology of the outer retina I**
17:40 **Moderators: Deborah A. Ferrington** (Minneapolis/USA) **John G. Flannery** (Berkley/USA)

16:15
28 L **Thomas Langmann,** A. Wolf (Cologne/D)

Microglia as important part of the retina: Its role in AMD

This talk highlights the role of key immune pathways in the pathophysiology of major retinal degenerative diseases including age-related macular degeneration. We will first discuss the mechanisms how loss of retinal homeostasis evokes an unbalanced immune reaction involving responses of local microglia and recruited macrophages. Presenting these innate immune cells as targets, we specifically emphasize the concept of immunomodulation as potential treatment strategy to prevent or delay vision loss. Promising molecules are ligands for phagocyte receptors, intracellular regulators involved in the activation of microglia and more general modifiers of their inflammatory response and migration. We briefly summarize the scientific evidence for this strategy from preclinical animal models, human ocular tissue analyses, and clinical trials evolving in the last few years.

Friday, 20th September 2019

16:27

29 P **Clemens Lange[1],** A. Schlecht[1], S. Boneva[1], J. Wolf[1], P. Zhang[1], G. Prinz[1], R. Horres[2], F. Bucher[1], C. Auw-Haedrich[1], L. Hansen[1], A. Stahl[1,3], I. Hilgendorf[1], H. Agostini[1], P. Wieghofer[4], G. Schlunck[1] ([1]Freiburg i.Br./D, [2]Frankfurt/D, [3]Greifswald/D, [4]Leipzig/D)

Transcriptomic Characterization of Human Choroidal Neovascular Membranes Identifies Calprotectin as a Novel biomarker for patients with Age-related Macular Degeneration

Purpose: Although genome-wide association studies, animal models, and cell culture systems have yielded important insights into the pathogenesis of neovascular age-related macular degeneration (nAMD), the underlying molecular pathways remain ill defined. The current study aims to explore the transcriptome of human CNV membranes via comparative RNA-sequencing-based transcriptional analysis.
Methods: Massive Analysis of cDNA Ends (MACE) RNA sequencing was performed on four formalin-fixed and paraffin-embedded (FFPE) CNV membranes extracted from patients with nAMD during vitreoretinal surgery between 1992 and 1999. Four age-matched FFPE RPE-choroidal specimens obtained from the macular region of enucleated eyes suffering from ciliary body melanoma served as controls. Transcriptome profiles were generated for CNV membranes and control tissue and statistical and bioinformatic methods were employed to identify disease-associated gene signatures. Calprotectin (S100A8/A9) protein expression was investigated by immunohistochemistry on paraffin sections and ELISA on vitreous samples of four patients with nAMD and six patients without AMD.
Results: We identified 777 DEG (log2FC > 2, FDR <0.05, mean counts in human CNV samples > 100) that were significantly increased in CNV membranes compared to control tissue. Gene ontology (GO) enrichment analysis demonstrated that most of these DEG contributed to biological processes, such as Symbiont Process (p=3.0E-9), Blood Vessel Development (p= 1.7E-7), Extracellular Structure Organization (p= 4.3E-18), Leucocyte Degranulation (p= 2.9E-12) and Response to Wounding (p= 4.9E-8). The S100 calcium-binding protein A8 (S100A8) and S100A9 emerged among the top differentially-expressed genes in human CNV membranes, confirmed by immunohistochemistry on CNV tissue samples and protein analysis of the S100A8/A9 heterodimer (Calprotectin) in vitreous and plasma samples of nAMD patients (35.86 ± 15.06) and controls (2.856 ± 0.586, p = 0.02).
Conclusions: This study provides a high-resolution RNA-sequencing-based transcriptional signature of choroidal neovascular membranes in AMD patients and reveals S100A8/A9 as a novel biomarker and promising target for AMD-directed therapeutics and diagnostics.

16:34

30 L **Rajendra S. Apte** (St. Louis/USA)

Animal model for AMD interfering lipid trafficking

Aberrant lipid metabolism and inflammation are critical regulators of the molecular pathogenesis of AMD. Disrupted homeostasis in these processes drives drusen biogenesis, neurodegeneration and choroidal neovascularization. The current understanding of how metabolism and inflammation regulate AMD pathogenesis will be discussed and novel therapeutic avenues for disease modulation will be presented.

16:46

31 P **Christian Grimm[1],** F. Storti[1], T. Hornemann[1], A. den Hollander[2], C. Maugeais[3] ([1]Schlieren/CH, [2]Nijmegen/NL, [3]Basel/CH)

Impaired function of the lipid transporters ABCA1 and ABCG1 in the RPE leads to an AMD-like phenotype in mice

Since the RPE has to internalize large amounts of shed tips of photoreceptor outer segments every day, it requires an efficient intracellular system to handle, metabolize and/or dispose lipids. Several genes involved in lipid metabolism including the ATP-binding cassette transporter A1 (ABCA1) have been linked to AMD suggesting that an impaired lipid handling contributes to disease development. Using an in vitro system, we show that ABCA1 localizes to both sides of polarized RPE cells and is required for efflux of photoreceptor outer segment-derived cholesterol to extracellular acceptor proteins as a first step in high-density lipoprotein (HDL) biosynthesis. Mice with an RPE-specific deletion of Abca1 and its partner Abcg1 showed strong accumulation of lipids, especially of cholesteryl esters, in the RPE, reduced function of the RPE and the retina, inflammation, as well as age-dependent RPE and photoreceptor degeneration. Inactivation of Abca1 but not of Abcg1 alone was sufficient to increase the lipid load in the RPE. Expression of ABCA1 in cell lines from human patients carrying the ABCA1 AMD risk-conferring allele was reduced potentially identifying the molecular mechanism that might explain the genetic risk for AMD in these patients. Our data strengthen the hypothesis that efficient lipid efflux from the RPE is required to maintain tissue homeostasis and suggest a pathogenic contribution of locally reduced ABCA1 function to AMD.

16:53

32 L **Nicolas Bazan[1,2]** ([1]New Orleans/USA, [2]Stockholm/S)

Elovanoids are Novel Photoreceptor Survival Signals Relevant to AMD

Background: The RPE and PRC relationship restores adversities to homeostasis. DHA is a membrane component necessary for vision that is decreased in AMD. The genetic ablation of Adiponectin Receptor 1 (AdipoR1) blocks DHA uptake, reduces phosphatidylcholine (PC)-containing Very Long Chain Polyunsaturated Fatty Acids (VLC-PUFA), and leads to PRC death. Genetic ablation of the protein leads to "flecked" retinal degeneration. AdipoR1 polymorphisms are in AMD. DHA is the precursor of docosanoids (neuroprotectin D1; NPD1) and of novel mediators called elovanoids, RPE and PRC survival promoters.
Methods: AMD and control retinas were analyzed by OCT, by MALDI molecular imaging, and by LC-MS/MS precursor/mediator lipidomic analysis.
Results: Transmembrane proteins (AdipoR1 and MFRP) and cell-selective gene transcription comprise a key molecular switch in DHA retention, VLC-PUFAs formation, storage in PCs and elovanoids/NPD1 synthesis. We uncovered unique differential PC abundance in the macula and periphery, highlighting VLC-PUFA loss in AMD. MALDI imaging of early and advanced AMD retinas showed decreased PC-VLC-PUFAs in macula PRC. LC-MS/MS of peripheral and macular punches showed higher peripheral n-3 PCs (e.g., PC44:12n-3); normal periphery had more VLC-PUFAs than macula or AMD retinas. Total 54C and 56C PCs, as well as peripheral 32C and 34C PC-VLC-PUFAs, were reduced in AMD.
Conclusion: VLC-PUFAs, precursors of elovanoids, are abundant in rods (periphery); reduction in AMD may affect rod survival. Conversely, AMD-triggering events impair rod VLC-PUFA synthesis. If DHA regulation is decreased in AMD, causing VLC-PUFA reduction, peripheral rods may show early changes in retinal DHA/VLC-PUFA lipidome. VLC-PUFA reduction may reflect impaired synthesis of elovanoids, key protective mediators. Overall, we find decreased DHA retention, VLC-PUFA formation, storage in PCs and elovanoids/NPD1 synthesis in AMD. Thus, when disrupted, these uncovered mechanisms lead to onset and progression of dry-form AMD and may represent a new therapeutic target. (Supported by NIH EY005121 and by EENT Foundation)

17:05

33 L **Clare Futter,** M. Hall, H. Cardosa, M.C. Seabra (London/GB)

Lysosome dysfunction in the RPE and its role in early AMD

The lysosomes of retinal pigment epithelial (RPE) cells have an unparalleled degradative burden due to the daily phagocytosis of spent photoreceptor outer segments. Our studies of lysosomes within cultured primary porcine RPE cells have identified different subpopulations of lysosomes that can be distinguished by their morphology, content and accessibility to newly internalized cargo. We are developing models of lysosome dysfunction in cultured RPE in order to model changes that occur with age and that may contribute to the development of early AMD. Challenging RPE cells with indigestible cargo or targeting lysosomes with lysosomotropic agents causes a shift in the balance of lysosome subpopulations and induces changes resembling some of the hallmarks of early AMD. Understanding the response to lysosome dysfunction in the RPE may identify new avenues for therapeutic intervention in early AMD.

Friday, 20th September 2019

17:17
34 L **Gerard A. Lutty,** S. Ogura, R. Baldeosingh, D.S. McLeod, M.M. Edwards, I.A. Bhutto (Baltimore/USA)

Mast Cell Involvement in Geographic Atrophy

Background: Mast cells (MCs) are prominent inflammatory cells in choroid. MCs are effector cells of innate immunity that we have previously demonstrated are elevated in number and in degranulation in geographic atrophy (GA) (Bhutto et al, BJO 2016). Furthermore, MC tryptase released during degranulation is present in Bruchs membrane in GA subjects (McLeod et al, IOVS 2018). This enzyme could lead to degradation of Bruchs membrane and thinning of choroid. The goal of this study was to develop an animal model to evaluate the role of MC activation and degranulation (DG) in causing RPE degeneration and retinal and choroidal thinning, hallmarks of GA.
Methods: A hydrogel with 48/80 (a snake venom-like compound) or blank hydrogel was injected subconjunctivally in Sprague-Dawley rats. MCs were stained with nonspecific esterase (NSE), retinal pigment epithelial cells (RPE) labeled with RPE65 in whole mount choroids, and retinal and choroidal area were determined in cryosections stained with picrosirius red. The generic FDA approved MC stabilizer, ketotifen fumarate (KTF), was evaluated in the model.
Results: Choroidal MC degranulation was significant at one week post implantation of 48/80. By 4-6 weeks, there was significant loss of RPE in eyes with 48/80 compared to blank hydrogel ($p<0.005$). The areas of retina and choroid were reduced at 8-10 weeks post implantation in 48/80 eyes compared to blank hydrogel ($p<0.05$). RPE loss and retinal and choroidal thinning did not occur in rats without MCs (cKit-/-). Daily KTF (10 mg/kg) orally inhibited RPE loss at 6 wks and thinning of retina and choroid at 8-10 weeks.
Conclusion: Inducing MC degranulation in rat choroid resulted in loss of RPE at 6 weeks post implantation and reduced retinal and choroidal areas by 10 weeks. Inhibiting MC degranulation with KTF prevented three phenotypic characteristics of GA: RPE loss and retinal and choroidal thinning.

17:40 ▼ 18:00 **Lecture in memory of Gerhard Zinser:**

Moderators: Frank G. Holz (Bonn/D)
Daniel Pauleikhoff (Münster/D)

35 **Josef F. Bille** (Heidelberg/D)

Past, present and new horizons in imaging analysis – The heritage of Gerhard Zinser

Since the first scanning laser ophthalmoscope (SLO) was introduced in the early 1980s, this confocal imaging modality has been adapted and optimized for various clinical imaging applications based on different contrast mechanisms. Optical coherence tomography (OCT) has emerged to the forefront of ocular imaging because of the wide variety of information it can provide, its high resolution images, and the complex 3-dimensional (3D) data it is able to gather. For ophthalmology, optical coherence tomography is of particular utility in glaucoma and retinal diseases, since it provides high-resolution objective, quantitative assessment of the retinal cellular layers affected by each disease. Especially since glaucoma is a slowly progressing disease, objective and quantitative measures could potentially provide a more accurate and precise method for the diagnosis of glaucoma and detection of its progression. Swept-source OCT technology offers inherent characteristics that are suitable for high- resolution anterior segment imaging and analysis. Such capabilities allow for non-contact imaging, detailed visualization and analytics of anterior segment structures of the human eye including the cornea, anterior chamber, iris, and lens with one device. Swept-source OCT technology can also serve as a tool to measure the axial length of the human eye. The above-mentioned structures and parameters are used in ophthalmology for corneal topography, corneal tomography, anterior segment analysis, biometry and calculation of intraocular lens power. Adaptive optics has emerged as an empowering technology for retinal imaging with cellular resolution. This technology holds potential for non-invasive detection and diagnoses of leading eye diseases such as glaucoma, diabetic retinopathy and age-related macular degeneration (AMD). Recent micro-stimulation techniques coupled with adaptive optics scanning laser ophthalmoscopy can produce stimuli as small as single photoreceptors that can be directed to precise locations on the retina. This enables direct in vivo study of cone activity and how it relates to visual perception.

Saturday, 21st September 2019

08:30 **5th session**
▼ **Neurobiology of the outer retina II**
09:55 **Moderators: Thomas Langmann** (Cologne/D)
Anneke den Hollander (Nimwegen/NL)

08:30
36 L **Janet Sparrow** (Boston/USA)

Is lipofuscin a relevant factor in the pathogenesis of AMD?

Age-related macular degeneration is a multi-factorial disease. The fluorophores that are the source of short-wavelength fundus autofluorescence (488 nm excitation) and that constitute the lipofuscin of retina are vitamin A-adducts (bisretinoids) that form from reactions of retinaldehyde with primary amine groups such as these moieties on phosphatidylethanolamine. These compounds form in photoreceptor cells and are transferred secondarily to retinal pigment epithelial (RPE) cells. Bisretinoid lipofuscin accumulates in RPE cells as a hallmark of aging, can trigger photodamage and can account for the deposition of the dicarbonyls that modify Bruch's membrane. These features together with evidence that the photooxidative processes initiated by bisretinoid lipofuscin can explain links amongst AMD, sunlight exposure and antioxidant intake, suggest a relationship to AMD. Since photooxidation of bisretinoid leads to degradation of these fluorophores, reduction of short-wavelength fundus autofluorescence (qAF) in atrophic AMD, is consistent with a role for bisretinoids in the pathogenic mechanisms of AMD.

08:42
37 P **Thomas Ach[1],** K. Bermond[1], J. A. Gambril[2], C. Wobbe[1], A. Berlin[1], R. Heintzmann[2,3], K.R. Sloan[3], C.A. Curcio[3] ([1]Würzburg/D, [2]Jena/D, [3]Birmingham/USA)

Cellular and subcellular changes in the retinal pigment epithelium (RPE): from normal aging to early signs of age-related macular degeneration (AMD)

Background: The aged RPE undergoes substantial changes at a cellular/subcellular level, even before AMD lesions are clinically visible with available imaging modalities. Some of these alterations impact RPE geometry and autofluorescence (AF) properties, which then affect clinical fundus AF imaging. Here, we report geometry and intracellular distribution of AF granules in aged RPE to distinguish early AMD changes, which particularly impact RPE integrity and AF characteristics.
Methods: From forty RPE-flatmounts from 40 human donors (15 normals (16-90 years); 25 AMD (80-90 years)), RPE-AF (488 nm excitation) and F-actin cytoskeleton (phalloidin-labeled, 640 nm excitation) were imaged in z-stacks. Using high-resolution structured illumination microscopy, in normal RPE cells, AF granules (lipofuscin, melanolipofuscin, melanosomes) were counted and sub-classified. In AMD eyes, using confocal microscopy, RPE cells were classified for AF pattern and morphology (cell area, AF intensity, number of Voronoi neighbors, and neighborhood of each cell).
Results: In normal aging, RPE cells contain hundreds (mean 300-500) of AF granules, with the lowest number at the fovea and highest at the perifovea. Lipofuscin granules are lowest and melanolipofuscin highest at the fovea. Few melanosomes are found within the RPE cell body. In AMD eyes, RPE geometry (area, number of neighbors) and AF changes. RPE cells re-pack their intracellular granules into aggregates that are expelled from cells, leading to altered and reduced AF/cell. Other cells lose AF granules one-by-one (degranulation). Worsening RPE pathology led to larger cells, reduced AF, and higher variability in number of neighbors.
Conclusion: Information on RPE cell shape/geometry, and number and AF characteristics of intracellular granules help to inform models of normal aging and disease. Foveal AF is mainly driven by melanolipofuscin, even in normal aged eyes. Hyper-AF aggregates seem to be a hallmark of AMD diseased RPE cells – a possible future biomarker for new imaging technologies with subcellular resolution. Funding: NIH 1R01EY027948 (TA, CAC), 1R01EY06109 (CAC), Dr. Werner Jackstädt Foundation (TA)

08:49
38 L **Deborah A. Ferrington** (Minneapolis/USA)

Primary RPE cultures from donors with AMD

Background: The pathologic mechanism responsible for the death of RPE, a hallmark of geographic atrophy, is currently unclear.
Methods: Our investigations utilize human donor tissue graded for the presence and severity of AMD using the Minnesota Grading System. The experimental approach is to study changes in cellular composition, mtDNA damage and mitochondrial function in human donors at progressive stages of AMD using a combination of proteomic and molecular biology techniques, as well as performing functional testing on primary RPE cultures.
Results: Results from multiple analyses led to the unique observation that the mitochondria in the RPE are negatively impacted by the disease. Thus, RPE mitochondrial dysfunction may be driving AMD pathology.
Conclusion: Treatments that that preserve or improve mitochondrial function may be an effective therapy for slowing down or stopping vision loss in patients with early AMD.

09:01
39 P **Natalie Wagner[1],** S.C. Joachim[1], M. Gammel[1], A. Greulich[1], S. Reinehr[1], J. Hurst[2], H.B. Dick[1], S. Schnichels[2] ([1]Bochum/D, [2]Tübingen/D)

Novel porcine organ culture model for AMD research

Background: The age-related macular degeneration (AMD) is a multifactorial disease, where good research models are limited. Hence, an established porcine degeneration model (Kuehn et al., 2017) was modified to enable improved photoreceptor cultivation and make it applicable for AMD research.
Methods: Two methods, namely "filter" and "tweezers", were tested to gain porcine neuroretina explants, with the ganglion cell layer facing down. Retinas were cultivated for 4 and 8 days and compared to explants obtained with the established method, photoreceptors facing down. To characterize the explants optical coherence tomography (OCT; n=6/group), H&E staining, immunohistochemistry, and qRT-PCR (n=4/group) were performed. More specifically, cones, rods, amacrine, bipolar, and retinal ganglion cells were investigated, followed by group comparisons.
Results: OCT analyses revealed a decrease of retinal thickness to a lower extent in tweezers explants compared to filter ($p<0.001$) and established method ($p=0.04$). Moreover, measurements of retinal thickness, via H&E staining, showed for both new methods a significantly improved photoreceptor structure compared to the established method ($p<0.05$). Additionally, the rhodopsin+ area was increased in the filter ($p<0.05$) and tweezers group ($p=0.048$) in contrast to the established one. On mRNA level, we revealed an upregulation of Rhodopsin and Opsin in both new methods compared to the established one. The amount of amacrine, bipolar and retinal ganglion cells was unaltered.
Conclusion: This project aimed to develop a more suitable organ culture photoreceptor degeneration model. The cultivation using the tweezer method led to a significantly improved morphology. Subsequently, to establish a reliable AMD model a co-cultivation of neuroretina and RPE-cells will follow.

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09:08
40 L **Anneke den Hollander** (Nimwegen/NL)

Understanding disease mechanisms in AMD:
From serum biomarkers to an organ-on-a-chip model for AMD

Genome-wide association studies have identified more than 50 variants at over 30 genomic loci that are associated with age-related macular degeneration (AMD). In addition, a significant burden of rare variants has been detected in four genes. The effect of the majority of the identified common and rare variants is currently unknown. We aim to understand the effect of these genetic variants on the disease mechanisms of AMD using various approaches. We are using serum measurements for components of the complement system and the lipid metabolism, and are correlating the identified biomarkers to genetic variants that have been identified in AMD. In addition, we are using in vitro cell culture systems to understand the local effect of AMD-associated genetic variants. Patient-derived induced pluripotent stem cells are used to generate ocular cell types, including retinal pigment epithelium (RPE) and endothelium. These cells can be cultured on an organ-on-a-chip to model the RPE-Bruch's membrane interface.

09:20
41 L **John G. Flannery[1],** M.H. Berry[1,2], A. Holt[1], A. Salari[1], J. Veit[1], M. Visel[1], J. Levitz[1,3], K. Aghi[1], B. Gaub[1,4], B. Sivyer[2], E. Isacoff[1], ([1]California/USA, [2]Oregon/USA, [3]New York/USA, [4]Zürich/CH)

Restoration of high-sensitivity and adapting vision with a cone opsin

Inherited and age-related retinal degenerative diseases cause progressive loss of rod and cone photoreceptors, leading to blindness, but spare downstream retinal neurons, which can be targeted for optogenetic therapy. However, optogenetic approaches have been limited by either low light sensitivity or slow kinetics, and lack adaptation to changes in ambient light, and not been shown to restore object vision. We find that the vertebrate medium wavelength cone opsin (MW-opsin) overcomes these limitations and supports vision in dim light. MWopsin enables an otherwise blind retinitis pigmentosa mouse to discriminate temporal and spatial light patterns displayed on a standard LCD computer tablet, displays adaption to changes in ambient light, and restores open-field novel object exploration under incidental room light. By contrast, rhodopsin, which is similar in sensitivity but slower in light response and has greater rundown, fails these tests. Thus, MW-opsin provides the speed, sensitivity and adaptation needed to restore patterned vision.

09:32
42 L **Volker Busskamp** (Bonn/D)

Forward programming of human photoreceptors

The replacement of photoreceptors represents a promising option to counteract retinal degenerative diseases. However, for a viable cell therapeutic intervention, one requires human photoreceptors in high quantity and quality. While it is possible to obtain photoreceptors in low quantities by direct reprogramming from fibroblasts or from human stem-cell-derived 3D retinal organoids, we aimed at establishing an efficient 2D forward programming protocol to generate cone photoreceptors in vitro from human induced pluripotent stem cells (hiPSCs). To this end, we performed a transcription factor (TF) library-on-library screen to program photoreceptors. One validated TF combination led to a significant loss of pluripotency markers and an upregulation of photoreceptor progenitor markers. We are currently characterizing these cells in-depths.
In-vitro-engineered photoreceptors might serve as donor material for cell transplantation to treat blindness or as biomedical testbeds as sufficient quantities can be generated within few days.

09:44
43 P **Giuliana Gagliardi[1],** D.M. Feitosa-Afonsso[1,2], Y.B. Arik[2], J. Heesterbeek[1], C.B. Hoyng[1], A.D. van der Meer[2], A.I. den Hollander[1] ([1]Nijmegen/NL, [2]Enschede/NL)

Using human-induced pluripotent stem cells for modelling the blood-retinal-barrier on-a-chip

One of the major hallmarks of age-related macular degeneration (AMD) is the accumulation of protein-lipid deposits, known as 'drusen', in the tissues of the outer blood-retinal barrier (BRB). The key cells within the BRB are the retinal pigment epithelium (RPE) and the endothelial cells (ECs) forming the choroidal capillaries. The recent development of human-induced pluripotent stem cell (hiPSC)-derived RPE and ECs has led to their use as in vitro models in drug development. However, such models typically rely on simplified monolayer cultures that insufficiently capture the tissue dysfunction of AMD. In order to overcome this limitation, microfluidic organ-on-chip technology represents a promising technology for the development of 3D in vitro models. These microfluidic cell culture devices have engineered microchannels that are continuously perfused and inhabited by living cells to form tissues that exhibit organ-level physiology. The aim of this project is therefore to develop an organ-on-chip model of the outer BRB, fully based on hiPSC-derived cells. We are currently generating ECs and RPE from hiPSC lines obtained from control and AMD-affected individuals. All individuals were genotyped for 52 AMD-associated variants, and 13 AMD-related genes were sequenced to detect rare coding variants. Differentiation and maturation of hiPSCs-derived cells is assessed using immunostaining of several markers specific to each cell type. Characterized cells are then incorporated into an organ-on a-chip device containing a microchannel and an open top culture chamber, separated by a polyester membrane. ECs are seeded in the microchannel in order to mimic a capillary-like structure, and RPE cells are seeded in the open top culture chamber. For both cell types survival and maturation in their respective microenvironment is assessed. This new in vitro model will provide new knowledge on how various molecular, cellular and physical aspects interact in AMD, and can be used for testing new therapeutic molecules.

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09:55 **6th session**
▼ **Rapid Fire Presentations and Posters**
10:45 **Moderators: Daniel Pauleikhoff** (Münster)
Frank G. Holz (Bonn)

09:55
P01 **Susette Lauwen,** D.J. Lefeber, E.K. de Jong, A.I. den Hollander (Nijmegen/NL)

B3GLCT-catalyzed O-fucose glucosylation is not required for secretion of TSP1 and CTGF from retinal pigment epithelial cells

Background: Variants in the B3GLCT gene have been found to be protective for age-related macular degeneration (AMD) in a genome-wide association study. B3GLCT is coding for beta1-3 glucosyltransferase, which catalyzes the second step of glycosylation on thrombospondin type I repeats (TSR), forming Glc-beta1-3Fuc-O. This modification has been reported to play a role in protein secretion via an endoplasmatic reticulum quality control mechanism, although the terminal glucose is thought to be essential for secretion of only a subset of TSR-containing proteins. Since nothing is known yet about a possible protective mechanism in AMD via B3GLCT, we aimed to further explore this.
Methods: We generated B3GLCT knockout (KO) hTERT RPE1 cells using CRISPR/cas9 genome editing, and investigated whether this KO causes secretion defects of TSR-containing proteins highly expressed in the RPE. For this purpose, we evaluated gene expression of TSR-containing proteins by qPCR and subsequently we tested the presence of thrombospondin I (TSP1) and connective tissue growth factor (CTGF) in cell lysates and conditioned medium/Opti-MEM by Western blot.
Results: Gene expression analysis showed that 3 of the in total 60 TSR-containing proteins are highly expressed in hTERT RPE1 cells, which are TSP1, CTGF and cysteine rich angiogenesis inducer 61 (CYR61). CTGF and CYR61 had similar RNA levels in WT and KO cells, whereas TSP1 expression was slightly increased in the KO. On protein level, CTGF secretion was similar from WT and KO cells and TSP1 was slightly more abundant in conditioned medium from KO cells, corresponding to RNA levels.
Conclusion: KO of B3GLCT does not result in secretion defects of TSP1 and CTGF from RPE cells. Our future studies will investigate whether B3GLCT-attached glucose is required for secretion of other proteins from RPE, or whether it has additional functional roles, which could potentially be linked to AMD pathogenesis.

09:58
P02 **Ana Sofia Falcão[1],** M.H. Cardoso[1], M. Hall[2], C. Escrevente[1], P. Antas[1], M. Chaves-Ferreira[1], S. Tenreiro[1], C.E. Futter[2], M.C. Seabra[1] ([1]Lisbon/P; [2]London/GB)

Dissecting the role of lysosome dysfunction in AMD

Age-related macular degeneration (AMD) is a neurodegenerative retinal disease and the leading cause of blindness in developed countries. AMD pathogenesis originates from the damage of a polarized non-regenerative retinal pigment epithelium (RPE) cells. The RPE performs many functions indispensable for vison, such as phagocytosing and degrading shed photoreceptor outer segments (POS). Degradation and recycling of cellular components are the main responsibilities attributed to lysosomes. Here we report the characterization of two RPE models featuring lysosomal dysfunction: a) chloroquine-induced in hPSc-RPE and b) POS-induced primary human fetal RPE (hfRPE) cells. These models are being used to explore the importance of lysosomes in intracellular cargo processing and the role of lysosomal dysfunction in AMD. Ultimately, the identification and characterization of defective pathways responsible for the regulation of lysosomal biogenesis and activity will contribute to a better understanding of AMD pathogenesis and identification of new therapeutic targets.

10:01
P03 **Angela Armento,** S. Honisch, V. Panagiotakopoulou, I. Sonntag, A. Jacob, M. Deleidi, M. Ueffing (Tübingen/D)

Complement factor H (CFH) loss alters energy metabolism and hinders the antioxidant capacity of RPE cells

Background: Age-related macular degeneration (AMD), leading cause of blindness in the elderly population, is caused by a combination of genetic predisposition, age and external environmental factors. About 50 % of the patients affected with AMD are carriers of polymorphisms in complement factor H (CFH) gene, key regulatory factor of the complement system. CFH has been recently associated with impairment in retinal development, mitochondria stability and redox balance at systemic level. Retina homeostasis and energy demand relies on RPE cells machinery. Therefore we investigated whether CFH loss alters energy metabolism and oxidative stress response of RPE cells.
Methods: Using RNA interference, we reduced CFH levels in RPE human cell lines hTERT-RPE-1. We assessed the effects of CFH depletion in RPE cells via viability and cytotoxicity assays under normal conditions or under H_2O_2-induced oxidative stress. We used the Seahorse XFp Analyzer to measure bioenergetics, in particular oxygen consumption rate (OCR) and extra cellular acidification rate (ECAR). In parallel we measured the levels of lipid peroxidation.
Results: In CFH absence, cytotoxicity in RPE cells was increased, while oxidative stress had only a mild effect. A minor, but significant decrease in cell viability was observed, amplified by H_2O_2 pre-treatment. Bioenergetics analyses revealed a reduction in cell energy metabolism in the absence of CFH. Indeed, both mitochondrial respiration and glycolysis rate were reduced after CFH depletion. At the same time CFH loss led to a significant increase in lipid peroxidation, key aspect of AMD pathogenesis.
Conclusion: Our data highlighted the involvement of CFH in RPE cells energy metabolism and oxidative stress response. Indeed we showed that CFH absence leads to a metabolic switch toward a less energetic phenotype and impairs the antioxidant capacity of RPE cells. These findings contribute to elucidate the role of CFH in RPE cells and AMD pathogenesis.

10:04
P04 **Corinne Gonzales** (Toulouse/F)

Clinical, Morphology-Structural, Biological Biomarkers for AMD and its Atrophy and Neovascular complications

Purpose: To determine clinical,biological,morphology-structural elements as biomarkers for AMD and its Atrophy and Neovascular complications.
Methods: AMD: 320 patients: groupA: 142, with AMD drusenoid deposits "L",Lipid Type and "P",Protein-cellular type; groupB: 64, with Atrophy complication; group C :114, with Neovascular complication. Complete Ophthalmologic exam: visual acuity,Fundus examination,Multimodal imaging (FAF,OCT,OCTen face). Morphology-Structural software (M-S software): analyze drusenoid deposit (volume, contours, 3D), let contents analyze, discrimination, differentiation, let grading, measurements (volume, density, structure), so "L" and "P" deposits evaluation, characterization. Cognitive evaluation: with Mini Mental State Examination, for all patients, score allow to determine various subgroups. Lipidomic Study: Blood tests and analysis, all lipids qualitative, quantitative analysis, all the same for all patients. Blood test is done during ophthalmologic exam.Plasma congelation"snap frost"after total blood centrifugation,then liquid-liquid extraction for lipids analysis:neutral lipid,fatty acid,phospholipids,as sphingolipids,Polyinsatured fatty acids too.
Results: Mild cognitive impairment (MCI) for 73 % patients in group A, 77,5 % group B,63 % group C. In MCI subgroups: progressive decrease and similar in group A and B, lower and homogeneous in group B. Cognitive impairment differ between AMD subgroups: moderate in group A, higher in group B and C, and more in group B than in group C, but early AD only present in group C. Multi modal, M-S Software well characterize, individualize drusenoid deposits "L" and "P" and their evolution which is different. Lipidomics: similar results for group A and C for Total Neutral Lipids,Fame,Free Fatty acid,Oxysterols,Phospholipids.

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Significative difference for group C with Eicosanoids,Fame free fatty acids; for group B with sphingosins,total neutral lipids,phospholipids total and Phosphatidyl choline; for group A with Oxysterol. Results are effective, statistically significative. So, Cognitive exam, Multi modal, M-S Software, Lipidomics become and are Biomarkers for AMD and its complications, allow better AMD follow-up and etiopathogenesis understanding.
Conclusion: Each clinical,biological,multimodal entity,all,together or not,are Biomarkers,allow AMD screening,follow-up.They also lead to better etiopathogenesis understanding and therapeutics prospects

10:07
P05 **Eric Thee[1],** M. Meester[1], D. Luttikhuizen[1], D. Rizoupolos[1], C. Klaver[1,2] ([1]Rotterdam/NL, [2]Nijmegen/NL)

Performance of classification systems for age-related macular degeneration

Background: Several classifications systems for age-related macular degeneration (AMD) have been described in the literature. Although these systems all stratify disease according to severity, it is unclear how well they predict advanced disease. We compared various well-known AMD classification systems and their capability to predict occurrence of late AMD.
Methods: Color fundus photographs of 9,990 participants from the Rotterdam Study, with up to five follow-up visits during 25 years, were graded by two experienced graders. AMD lesions were graded according to the international classification system. Baseline gradings of drusen type, size and area, hyperpigmentation and RPE-degeneration were used to score patients according to six AMD classifications systems. Late AMD was considered present when central geographic atrophy or choroidal neovascularization was observed. Incidence rates were calculated for late AMD per classification and subclass, and stratified for age groups <75 and >75 years at baseline. Overall prediction of late AMD was determined by the area under the operating characteristic curve (AUC).
Results: 183 cases of incident late AMD were observed. Incidence rates increased with a higher subclass, independent of classification system. The AREDS 9-step scale score (2005) at subclass 8 and the 3-Continent harmonization classification (2014) at subclass 4 showed the highest incidence rates for patients <75 years at 30 cases/1000py. The Rotterdam classification (1993) at subclass 3 showed the highest rate for patients >75 years at 65 cases/1000py. Overall, the AREDS 9-step scale had the highest AUC at 0.79, while the 3-Continent harmonization classification showed the best linear trend for prediction of late AMD.
Conclusion: This study compared frequently used classification systems in their prediction of late AMD. Systems differ in their advantages and disadvantages, which may drive their use. The findings will help researchers and clinicians select appropriate classification systems for applications such as clinical trials and deep learning algorithms.

10:10
P06 **Damian Jaggi,** Y. Solberg, C. Dysli, S. Wolf, M. Zinkernagel (Bern/CH)

Correlations in Mulitmodal Macular Pigment Measurements and Fluorescence Lifetime Imaging Opthalmoscopy in Age-Related Macular Degeneration

Backround: Purpose of this study was to compare two macular pigment optical density (MPOD) imaging modalities with fluorescence lifetime imaging ophthalmoscopy (FLIO) in patients with nonexsudative age-related macular degeneration (AMD).
Methods: Twenty patients with nonexsudative AMD were included in this study. Macular pigment optical density (MPOD) was measured using dual wavelength retinal autofluorescence imaging (AFI) and heterochromatic flicker photometry (HFP). Retinal autofluorescence lifetimes were measured using FLIO (Heidelberg Engineering, Germany) and analyzed in two spectral channels (SSC; 498 to 560 nm, LSC; 560 to 720 nm). Statistical analysis was performed with Pearson correlations.
Results: AFI and HFP strongly correlated at 0.5 degree of eccentricity (r_2=0.83, p<0.0001). Weaker but significant correlations were observed at 2 and 9 degree (r_2=0.68, p<0.0001 and r_2=0.46, p<0.0001). FLIO correlated with both AFI (SSC; r_2=0.57, p<0.0001) and HFP (SSC; r_2=0.39, p=0.003).
Conclusion: In addition to the well-established AFI, HFP and FLIO seem to be decent methods to investigate macular pigment in AMD. We confirm the previously described inverse correlation between FLIO and MPOD measurements.

10:13
P07 **Gereon Hüttmann[1],** P. Koch[1], H. Sudkamp[1], M. Moltmann[1], D. Theisen-Kunde[1], C. Pfäffle[1], D. Hillmann[1], C. von der Burchard[2], J. Tode[2], C. Ehlken[2], T. Kepp[1], H. Handels[1], R. Birngruber[1], J. Roider[2] ([1]Lübeck/D, [2]Kiel/D)

Full-field OCT for imaging AMD progression

Background: Optical coherence tomography (OCT) is indispensable for studying the progression of age-related macular degeneration (AMD), since it can show quantitatively morphological changes of the retina. However, current OCT devices can only be used in clinical settings and do not provide functional information. We developed a full-field (FF) technology for OCT which acquires all A-scans in parallel. This technology allows for very compact (shoe box size) low cost (5000 €) devices which can be used in point of care and home care settings. FF-OCT also allows invasive marker-free functional imaging of retinal function.
Methods: Two FF-OCT devices were evaluated. The low-cost version used the time domain (TD) principle and was tested with 39 patients with neovascular AMD for detection of relevant biomarkers. The device measured a field of 2 mm x 4 mm at 15 µm resolution within 10 seconds. The second device used swept-source OCT for ultra-fast imaging of the retina with more than 100 volumes/s. Nanometer dynamic changes of the thickness of the photoreceptor and the ganglion cell layer were evaluated after optical stimulation in healthy subjects.
Results: With the low-cost version, patients were able to do unassisted OCT imaging of their retina with high success rate after only 5 to 10 min training. The most relevant biomarkers for AMD (i.e. intraretinal fluid, subretinal fluid and pigment epithelium detachment) were visible in the OCT volumes. With the ultra-fast OCT, responses of photoreceptor and neuronal cells could be measured quantitatively.
Conclusions: FF-OCT opens new perspectives in imaging AMD progression by enabling daily self-surveillance by the patient at home. Continuous evaluation of the disease may become possible using automatic evaluation by artificial intelligence. Functional imaging may enable an even earlier detection of disease progression.

10:16
P08 **Alaadin Abdin,** S. Suffo, A. Langenbucher, B. Seitz (Homburg/D)

Intravitreal ranibizumab versus aflibercept following treat and extend protocol for neovascular age-related macular degeneration (2 years follow up)

Purpose: To assess the morphological and functional outcome and stability of the treat and extend protocol using aflibercept compared to ranibizumab for the treatment of eyes with neovascular age-related macular degeneration.
Patients and Methods: This retrospective study included 100 eyes of 94 patients with primary onset neovascular age-related macular degeneration followed for 24 months. We studied two groups of eyes: group 1: 50 eyes treated with 0.5 mg/0.05 mL ranibizumab, group 2: 50 eyes treated with 2.0 mg/0.05 mL aflibercept. During the first year, all eyes received 3 aflibercept or ranibizumab injections monthly as upload phase. Then eyes were treated with a treat and extend algorithm. Main outcome measures included: best corrected visual acuity (BCVA), central macular thickness (CMT) and the number of injections. In addition, we compared recurrence rates between the two groups.
Results: BCVA (LogMar) in group 1 vs group 2 was (0.54±0.31 vs 0.49±0.30, p=0.37) before treatment and (0.49±0.25 vs 0.47±0.32, p=0.85) after treatment. CMT in group 1 vs group 2 was (375.6±98.3µm vs 369.6±103.7µm, p=0.7) before treatment and (296.7±62.7µm vs 287.6±60.7µm, p=0.5) after treatment. The decrease in CMT was (68.7±86µm vs 75±92µm, p=0.7). Number of injections/eye after upload phase in group 1 vs group 2 was (8.6±3.2 vs 9.8±3.2, p=0.7). Finally, recurrence rates in group 1 vs group 2 were (9 % vs 14 %, p=0.06).

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Conclusions: Statistically significant differences regarding BCVA, central macular thickness and number of injections were not found between aflibercept and ranibizumab at 24months. On the other hand, there was a higher tendency of recurrence rates, statistically in the aflibercept group compared to ranibizumab group after following identical treat and extend protocol.

10:19

P09 **Martin Ziegler[1],** M. Book[1], K. Rothaus[1], G. Spital[1], A. Lommatzsch[1,2], D. Pauleikhoff[1,2] ([1]Münster/D, [2]Essen/D)

Multimodal analysis and phenotypical characterisation of CNV-transformation in exudative AMD after longterm anti-VEGF- therapy

Purpose: The natural progress of exudative AMD leads to a fibrovascular transformation of CNV. But also the anti-VEGF-therapy cannot prevent such a modification completely. In this study we analysed the clinical characteristics of the fibrous and vascular part of this CNV transformation by means of multimodal imaging.
Methods: We examined 57 eyes of 48 patients with indentifiable fibrovascular lesion in funduscopy after longterm anti VEGF-therapy (minimum 12 injections within last 24 months) with exudative AMD. For this we performed a multicolor shot (MC), fundus autofluorescence (FAF), SD-OCT (97 Scans) and OCT-A (Optovue). We considered RPE-atrophy, ellipsoid zone, external limiting membrane, intra- and subretinal fluid, choroidal thickness, outer retinal tubulations, the area/thickness of the fibrovascular lesion and the variation of vascular characteristics. After all we generate a correlation plot.
Results: We found fibrovascular lesions with and without well-preserved outer retina. In 30 eyes we observed an associated RPE-atrophy. The area of the RPE-atrophy correlated with the visual acuity, with the presence of intraretinal fluid and with the interruption oft he ellipsoid zone (p=0,007/p=0,006/p=0,01). We found outer retinal tubulations in 28 eyes (49,1 %). When outer retinal tubulations were existing, we observed an associated RPE-atrophy an a reduced visual acuity (p=0,007). Presence of intraretinal fluid correlated with outer retinal tubulations (p=0,006). In the OCT-A we found 5 phenotypes of vascular characteristics (loops 13x, immature 2x, mature 15x and hypermature 7x vessels and unremarkable vessels 8x). Most phenotypes did not correlate with other measured parameters, but mature vessels do with a larger area of fibrovascular lesion (p=0,02).
Conclusion: Multimodal Imaging allows a differentiated reflection of the fibrous and vascular part of the CNV transformation. This study shows a very broad range of fibrovascular lesions. This suggests that different transformations of CNV lead to different functional effects. This could be interesting for individual therapeutical strategy in future.

10:22

P10 **Marius Book[1],** M. Ziegler[1], K. Rothaus[1], M.L. Farecki[1], G. Spital[1], A. Lommatzsch[1,2], D. Pauleikhof[f1,2] ([1]Münster/D, [2]Essen/D)

Analysis of the vascular properties of the choroidal neovascularization (CNV) undergoing fibrovascular transformation in neovascular age-related macular degeneration (nAMD) using optical coherence tomography angiography (OCTA)

Purpose The CNV in nAMD, despite Anti-VEGF therapy, transforms into a fibrovascular lesion. The variety in vasculature and their correlation with exudative changes are unknown. As most-established angiographic procedure, the fluorescein angiography allows only few conclusions about structural changes within the CNV. The aim of this study was to describe the vascular properties of the CNV undergoing fibrovascular transformation using OCTA.
Methods: In this prospective, comparative, non-interventional study, 35 eyes with CNV undergoing fibrovascular transformation in nAMD and a history of at least 24 months of Anti-VEGF therapy were included. Spectral-domain coherence tomography (SD-OCT, Spectralis) and OCTA (OptoVue) were performed. The fibrovascular lesions were delineated in the en face SD-OCT images. For the analysis of the vasculature using OCTA we defined a slab by manually adjusting the segmentation levels to 60µm beneath Bruch's membrane and the upper edge of the hyperreflective lesion. We applied the custom slab in ten eyes and superimposed the data with the respective SD-OCT images using MATLAB (MathWorks). In addition to the CNV itself, an adjacent rim (para-rim) and another one beyond (peri-rim) both with the width of 1mm, were investigated. Skeletonized OCTA images were analyzed for vascular parameters.
Results: The average CNV area of the cohort was 3.83±0.73mm^2, the average area of the para-rim was 3.74±1.06mm^2 and of the peri-rim 3.49±1.39mm^2. The mean vessel density within the fibrovascular lesion was 0.41±0.1 %, within the adjacent rims 0.38±0.01 %. The fibrovascular lesion had both a larger total vessel length (52.90±10.27mm) and a larger proportion of large-caliber vessels (5.9±2.6 %) than the para-rim (47.38±14.50mm, 5.0±0.02 %) and peri-rim (44.31±18.14mm, 5.0±0.02 %). The mean degree of the node of the vessels was 1.42±0.02 in the CNV, higher than in the para-rim (1.35±0.03) and peri-rim (1.33±0.02). The mean length of the extracted line segments was 44.89±1.26µm in the fibrovascular lesion, 46.21±1.56µm in the para-rim and 47.23±1.34µm in the peri-rim. Other parameters showed no differences in the areas.
Conclusion: In this study the vascular properties of the CNV undergoing fibrovascular transformation have been described using OCTA. Differences between the fibrovascular lesion and the adjacent para- and peri-rim could be detected. In addition to a higher vascular density, the fibrovascular lesion also showed a higher degree of branched, short and large-caliber vessels than the adjacent rims. To what extent these descriptions of the fibrovascular lesion can help to identify different patterns of regression of the CNV must be analyzed in further studies.

10:25

P11 **Matthias Gutfleisch[1],** O. Ester[1], K. Rothaus, P. Mussinghoff[1], G. Spital[1], A. Lommatzsch[1,2], H. Kurzhals[1], D. Pauleikhoff[1,2] ([1]Münster/D, [2]Essen/D)

Text mining based data collection from unstructured, electronic patient data

Question: The collection of data from electronically managed patient files is of great interest both for internal quality analyses and for the external evaluation of large care data. In addition to the collection of (partially) structured data such as visual acuity or injection frequency, the electronic extraction of unstructured data (free text) is a particular challenge, especially the allocation to the right or left eye. The aim of the present study was to develop a method to extract data from electronic patient files that are structured, partially structured or unstructured in different databases as rule-based structured information and to evaluate the received data with regard to their quality.
Methodology: In cooperation with a collaborator (Westphalia DataLab, Muenster, Germany), a text mining modeling language was used to develop software that extracted personal and date-related information from various databases of the Eye Center in AMD patients with IVOM therapy. The evaluation of free text fields identified technical terms and side localizations. After manual labelling, the data set was divided into a training data set and a test data set. The automated identification of the eye side was performed and iteratively improved by developing rules for the automated assignment of a term to a page. The size of the test set was statistically estimated in such a way that the confidence intervals of the estimators for accuracy and sensitivity were secured with a significance level of 0.99. The test set was then used for the identification of the eye site.
Results: The evaluation of the training data set resulted in an accuracy of 0.97 and a sensitivity of 0.96 for the clinical information and side localization. The test data set evaluation showed a slightly lower accuracy (-0.03) for the same rules, while the sensitivity slightly increased (+0.01).
Conclusions: Rule-based text mining can be used to extract correctly structured information, e.g. on IVOM therapy in AMD patients, from unstructured free text fields of an electronic patient file. This creates an important basis for the automated collection of relevant data from electronic clinic/practice databases for internal quality analyses and external healthcare research projects and for the analysis of large amounts of data.

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10:28
P12 **Jan Tode[1],** E. Richert[1], C.v d. Burchard[1], R. Brinkmann[2], R. Lucius[1], A. Klettner[1], J. Roider[1] ([1]Kiel/D, [2]Lübeck/D)

Translational Evaluation of Selective Retina Therapy and Thermal Stimulation of the Retina as Therapeutic Means for AMD

Background: Despite broad research effort, early and intermediate stage AMD can neither be treated nor be prevented effectively. The multifactorial disease needs a multifactorial approach. RPE regenerative selective retina therapy (SRT), as well as thermal stimulation of the retina (TS-R) evoke a broad range of processes with therapeutic implications. We evaluated these laser modalities in AMD mouse models.
Methods: One randomized eye of nuclear factor erythroid-derived 2 -like 2 (NRF2) and apolipoprotein (Apo)E knock out mice was either treated by SRT or TS-R, the fellow eye served as control. Also, untreated knock out and wild type C57BL/6J mice were controls. Clinical (fundus, OCT, angiography), anatomical (transmission electron microscopy, histology), biochemical (expression profile of inflammatory parameters in PCR array and correlation to retinal layers by RNA in-situ hybridization) and functional (optokinetic nystagmus) properties were evaluated in both treated and untreated eyes.
Results: AMD-like clinical findings were not altered by SRT or TS-R. Concerning ultrastructural pathology, one month following SRT and TS-R Bruch's membrane (BrM) thickness was reduced. SRT also affected fellow control eyes compared to treatment naïve mice. RPE regeneration and remodeling showing increased microvilli length and less vacuole-like alterations of cytoplasm were seen 1 month after SRT and TS-R. Concerning inflammation, both SRT and TS-R led to a reduction of pro-inflammatory cell mediators (especially CCL-19 expressed by neuroretinal cells and IL-6 expressed by RPE cells). Visual acuity was neither confined by SRT nor TS-R one week after treatment.
Conclusion: SRT and TS-R reduce BrM thickness to physiological values and rejuvenate RPE cells to vital and intact morphology. Inflammatory processes are inhibited in both RPE and neuroretina. Treatments are safe concerning retinal function. Translational data about influence on fat metabolism as well as clinical trials with safe devices are needed future steps. SRT and TS-R might become AMD treatment options.

10:31
P13 **Andrii Serhiienko[1],** O. Pekaryk[2], N. Dziuba[3] ([1]Vinnitsa/UA, [2]Winnipeg/CDN, [3]Kyiv/UA)

Low-energy light treatment of wet AMD after completion of standard anti-VEGF therapy

Purpose: To evaluate the efficacy of the new approach to stabilizing deterioration of a state of the retina and visual functions in patients with wet AMD who had already completed the initial course of anti-VEGF therapy.
Material and methods: 103 patients with exudative AMD after performing the anti-VEGF therapy were included in the study. The patients were divided into two groups. In the test group (62 patients) the low-energy light therapy was performed using the Spektra Light Device (Vision Aid Inc., Winnipeg, Canada). In the control group (41 patients) the only anti-VEGF therapy was applied.
Results: In the test group a rise of the visual acuity was from 30.0 ±4.22 to 32.58±4.33 letters and in the control group a decreasing of visual acuity from 35.77±5.82 to 19.34±4.33 letters. In the test group, a diminishing of the fovea thickness from 304.42±7.39 microns to 259.29±9.12 microns were found. In patients of the control group, an increase of the retinal thickness in the fovea area was registered from 279.84±8.63 to 392.89±14.82 microns.
Conclusion: Performing of low-energy light therapy to the patients with wet AMD after injections of anti-VEGF treatment resulted in stabilizing of visual acuity and improving the retina state.

10:34
P15 **Benedikt Schworm,** J. Siedlecki, F. Hagenau, K. Kortüm, N. Luft, S. Priglinger (Munich/D)

Response of secondary choroidal neovascularization in chronic central serous chorioretinopathy to an extended upload of anti-VEGF agents

Background: To determine the anatomical and functional outcome of an extended 6-month intravitreal anti-vascular endothelial growth factor (anti-VEGF) upload in choroidal neovascularization (CNV) secondary to chronic central serous chorioretinopathy (CSC).
Methods: A retrospective analysis of patients treated with an extended upload of six consecutive injections of intravitreal anti-VEGF for secondary CNV in chronic CSC was performed. Main outcome measure was anatomical response as represented by the change of central retinal subfield thickness measured on spectral domain optical coherence tomography (Heidelberg Spectralis®). Secondary outcome measures included change in visual acuity and further imaging biomarkers of chronic CSC.
Results: In a database of 2498 patients treated with intravitreal injections between 01/2016 and 12/2018, 140 patients treated with anti-VEGF injections for secondary CNV complicating chronic CSC were identified. Of these, 21 eyes of 21 patients were found eligible for the above-mentioned criteria. Mean patient age at the first injection was 65±8.3 years and 35 % of the patients (n=8) were female. Mean disease duration before diagnosis of CNV was 48+25.3 months. Four patients (19 %) had been treated with a half-fluence photodynamic therapy prior to developing CNV. Mean central retinal thickness decreased from 346±61,0µm to 257±57.4µm ($p<0.01$) after the sixth injection while mean visual acuity improved (0.65±0.35 vs. 0.49±0.29 logMAR; $p<0.05$). Notedly, there was a significant further reduction of the mean central retinal thickness after the third injection (280±45.6µm vs. 257±57.4µm, $p<0.05$). No adverse events were recorded in the observation period.
Conclusion: Favorable anatomical results can be achieved by a strict anti-VEGF injection regimen in CNV complicating chronic CSC. Significant gains of visual acuity are feasible but, however, limited by the underlying disruption of outer retinal layers owing to the chronicity of CSC.

10:37
P16 **Satpal Ahuja** (Lund/S)

Glycan analyses of rd1 mouse retinal proteins (a model of retinitis pigmentosa) indicates a need for therapy to hyper-glycosylate mutated synaptic proteins and to improve outcome of current gene therapies

Introduction: Retinal degeneration1 (rd1) mouse, an animal model of Retinitis Pigmentosa, (RP), shows mutation in β-subunit of cGMP phosphodiesterase-6 (PDE-6) gene and is associated with modified expression of ~60 other genes affecting transcription, cell-adhesion/proliferation. Deficiency of PDE-6 elevates cGMP and Ca2+ ion levels in the rod photoreceptors with loss of ~90% rods in rd1 mice. rd1 mice retinal neurons express mutated gene protein(s) and have modified ribbon synapse connectivity due to hypo-glycosylation of proteins. Such neuronal changes lead to blindness. Current gene therapies for mutated glycoproteins are inadequate as protein hypo-glycosylation processes have not been taken into consideration. Correct post-translation glycosylation of proteins, is achieved by a balance in glycosyl -transferase/-hydrolase activities. This report reviews dynamic changes in the nature/extent of retinal protein glycans which participate in retinal- function/development/degeneration; and highlights significance of glycans and glycosyl-transferases for success of the current gene therapies.
Material & Methods: Comparative dynamic changes in the nature/extent of glycosylation of retinal proteins, from neonatal wt and rd1 mice, were profiled/quantified by lectin microarray analyses and compared for their role in retinal function/development/degeneration as reported in literature.
Results (See Ref 1, Ref 2 and Ref 3): Age/mutation dependent relative and dynamic changes in high-mannose- and GlcNAc-, Siaα2-3Galβ1-4GlcNAc-glycans associated with wt and rd1 retinal proteins suggest hypo-glycosylation of retinal proteins which adversely influence their participation in the retinal function/development/degeneration. Degree of mannosylation and sialylation with Siaα2-3Gal (not Siaα2-6Gal) possibly regulates ERG function, whereas decreased core fucosylation and increased outer bisecting GlcNAc glucosylation/galactosylation was correlated with retinal degeneration. Functional/dynamic and quantitative

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differences observed in wt and rd1 retinal protein glycans suggested that rd1 mutation(s) create imbalance in the glycosyl-transferases/-hydrolases activities which modifies glycosylation of synaptic proteins.
Conclusions: Studies on mutation in glycosyl-transferases to achieve hyper-glycosylation of retinal synaptic proteins in rd1 mouse model of RP and to improve the efficacy of current gene therapies are needed. Acknowledgement: To Karin Sandqvists Stiftelse, Stockholm (Sweden) for a travel grant. Keywords: Mice, Glycans, Retinal-Development/Degeneration/Function, Gene-Therapy
References: 1. Ahuja S, 2013, IOVS 54:3272; 2. Ahuja S, 2014, IOVS 55:654; 3. Ahuja S, 2017, Int J Ophthalmol. 10:1217

10:40
P17 **Céline Koster** (Amsterdam/NL)

Towards experimental stem cell-based therapy for Age-related Macular Degeneration – Transplantation of patient derived-tissues in the rat subretinal space

Purpose: Age-related macular degeneration (AMD) is the principle cause of severe, progressive and irreversible visual impairment among the elderly. There is currently no treatment to restore vision when the function of the macula is strongly decreased. The retinal pigment epithelium (RPE) and the choroid (Ch) play major roles in the etiology of AMD. In this study we aim to transfer human induced pluripotent stem cell-derived (hiPSC) tissue into the rat subretinal space.
Methods: iPSCs were obtained from representative AMD patients and differentiated into RPE cells in vitro on an artificial Bruch's membrane (BM; scaffolds). iPSC-RPE cells were co-cultured with hiPSC-endothelial cells, fibroblasts and pericytes to create a 3D multilayerd tissue. Tissues were implanted after in vitro maturation into the subretinal space of healthy rats and rats treated with sodium iodate. The animals were followed over time non-invasively using Electroretinography (ERG), Scanning Laser Ophthalmoscopy (SLO) and Optical Coherence Tomography (OCT) for two months. ERG responses were analyzed using their a- and b-wave amplitudes and latencies (MATLAB). At various time points, eyes were collected for histology.
Results: hiPSCs were successfully adhered and differentiated into RPE on electrospun scaffolds. These cells showed the expression of several specific RPE markers (RPE65, BEST1, MITF, MERTK, ZO-1 and others). The cells were characterized in vitro and successfully transplanted as 1 mm discs into the subretinal space of rats. SLO-OCT and Fluorescein Angiography (FA) analyses showed survival of the transplant and signs of integration. According to histology, transplants survived for at least two months in the subretinal space. ERG analyses showed reduced responses two weeks after surgery, but this was regardless of treatment.
Conclusions: We developed a pre-clinical research pipe-line, in which we are able to differentiate patient derived iPSCs to RPE and transplant these cells (with or without artificial Ch) into suitable animal models. We determined the functional and structural changes following surgery over time, non-invasively. Transplantation of (3D) RPE(-Ch) tissue into the subretinal space of an animal model opens the possibility of developing tissue therapies for retinal degenerative diseases.

11:15 ▼ 13:00 **7th session**
Phenotyping AMD: Advances in Imaging
Moderators: Richard F. Spaide (New York/USA)
Giovanni Staurenghi (Milan/I)

11:15
58►43a L **Richard F. Spaide** (New York/USA)
Imaging the Choriocapillaris with OCT-angiography

11:27
44 L **SriniVas Sadda** (Los Angeles/USA)
Choriocapillaris status as a risk factor in AMD

Advances in OCT angiography (OCTA), in particular elastic registration and averaging of multiple en face OCTA images, has now allowed the choriocapillaris (CC) to be accessed non-invasively. Through these OCTA studies, we have learned that the CC progressively attenuates with age with progressive increase in the area of CC flow deficits, which correspond to the spaces in between capillaries with blood flow. In addition, this age-associated worsening of the CC appears to be more dramatic centrally. Given that AMD, and in particular drusen, tends to impact the central macula, this age-dependent central worsening of the CC may be relevant. Supporting this contention, we observed that the CC flow deficits were more severe/extensive directly under drusen, and in particular below drusen with hyporeflective cores or drusen with overlying hyper-reflective foci --- suggesting that the CC alterations appeared to mirror the overall AMD disease severity. In addition, localized CC flow deficits appeared to predict future enlargement of drusen or the appearance of new isolated drusen, suggesting that the regional distribution of drusen was not stochastic, but rather driven by the underlying choriocapillaris. In addition, CC flow deficits have been observed to surround both CNV and GA lesions, and the severity of flow deficits in eyes with GA appears to predict the rate of progression of enlargement of these GA lesions. Taken together, these observations highlight the critical role of the CC in AMD disease progression and emphasize the role of CC imaging with OCTA as an important biomarker in the assessment of AMD.

11:39
45 L **Philip J. Rosenfeld** (Miami/USA)
Choriocapillaris imaging using SS-OCT angiography in AMD

Purpose: Imaging of the choriocapillaris (CC) and choroid were performed using swept source optical coherence tomography angiography (SS-OCTA) in eyes with age-related macular degeneration (AMD). Our initial studies involved AMD eyes with geographic atrophy (GA), also known as complete retinal pigment epithelium (RPE) and outer retinal atrophy (cRORA). The associations between enlargement rates (ERs) of GA and the measurements of CC perfusion, choroidal thicknesses, and choroidal vessel vascular densities (CVVDs) were investigated.
Methods: Patients with non-exudative AMD were imaged using a 100-kHz SS-OCT instrument (PLEX Elite 9000, Carl Zeiss Meditec). Both 6x6 mm and 12X12 mm scan patterns were performed. GA area measurements were obtained from en face SS-OCT sub-RPE slab images. Visualization of the CC and quantification of flow impairment were performed using SS-OCT angiography (SS-OCTA). The percentage of CC flow deficits (FD %), the average FD area measurements, the choroidal thickness (CT) measurements, and the CVVDs were calculated in regions surrounding the GA.
Results: In 32 eyes, the annual square root ERs for GA ranged from 0.07 to 0.75 mm/year. The highest FD values were found in the region closest to the margin of GA. However, the strongest correlation was found between the ERs and average CC FD area measurements away from the margin of GA and calculated from the total scan area minus the area of GA ($p<0.001$). While CT measurements and CVVDs were decreased in AMD eyes with GA, the CC impairment correlated best with the ER of GA.
Conclusions: Contrary to expectations, the strongest correlations between the ER of GA and CC FDs were found when the entire scan area minus the area of GA was used rather than the region immediately around the GA. The observation that CC flow impairment, a thinner choroid, and a decreased CVVD exist in AMD eyes with GA strongly suggest that choroidal perfusion plays an important role in AMD progression and growth of GA.

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11:51
46 P **Martin Hammer,** R. Schultz, D. Meller (Jena/D)

Clinicopathologic Comparison of Fluorescence Lifetimes and Spectral Characteristics in AMD

Goal: to study fluorescence lifetimes of RPE/retina and drusen in vivo and compare with post-mortem donor tissue measurements.
Methods: 43 patients (mean age: 74.1±7.9 years) with non-exudative AMD and no geographic atrophy were included. Fundus AF from a 30° retinal field was investigated with the Heidelberg Engineering fluorescence lifetime imaging ophthalmoscope (FLIO) in a long- (LSC) and a short-wavelength channel (SSC). Mean fluorescence lifetimes tm were measured. Spectral ratio (sr) of both fluorescence channels was calculated as well. Two-photon microscopy was used to study unstained histological slices from 9 post-mortem donor eyes. Fluorescence lifetimes as well as spectra were recorded.
Results: 2760 individual soft drusen were identified in vivo. There was no significant difference in tm between the drusen and the unaffected fundus (SSC: 284±54ps vs. 280±57ps; LSC: 337±34ps vs. 334±40 ps), however, the sr was significantly higher in drusen (0.555±0.077 vs. 0.539±0.081, p<0.0005). On average, hyperfluorescent drusen showed longer tm than surrounding unaffected fundus (SSC: 17.9±41.9ps, p=0.074, LSC: 16.3±29.1ps, p=0.02, N=41) although most of them were not discriminable by lifetime. This difference was significantly more pronounced in small, demarcated drusen than in diffuse ones (SSC: 22.9±27.5ps vs. -2.8±20.6ps, p=0.003, LSC: 27.1±29.3ps vs. 3.0±25.7ps, p<0.0005). In histology, 171 drusen were identified. They showed considerably longer lifetimes than the RPE: (SSC: 596±201ps vs. 179±36ps, LSC: 549±153ps vs. 286±37ps, p<0.0005) and the peak emission wavelength was green-shifted as well.
Conclusions: Whereas RPE fluorescence is dominated by Lipofuscin, drusen contain other fluorophores as is evident from their different fluorescence characteristics. This is much more obvious in histology than in the in vivo situation where drusen are hidden underneath the RPE. Nevertheless, in vivo spectral and lifetime characteristics of fluorescence by FLIO revealed differences of retinal/RPE and drusen fluorescence as well as the variability of drusen fluorescence which might have clinical impact.

11:58
47 L **Sebastian Wolf,** C. Dysli, M.S. Zinkernagel (Bern/CH)

Fluorescence lifetimes in AMD

Purpose: To investigate fluorescence lifetime characteristics in patients with age-related macular degeneration (AMD) and to correlate the measurements with clinical data and optical coherence tomography (OCT) findings.
Methods: Patients with either intermediate AMD or late AMD with geographic atrophy (GA) were imaged with a fluorescence lifetime imaging ophthalmoscope (Heidelberg Engineering, Germany). Autofluorescence lifetimes were measured in a short and a long spectral channel (498-560 nm and 560-720 nm).
Results: Fluorescence lifetime maps of 105 eyes of 105 patients (80±6 years) with GA (41) and intermediate AMD (64) were analyzed. Mean retinal autofluorescence lifetimes in patients with intermediate AMD was significantly prolonged compared with the healthy control eyes (mean±SEM: SSC, 486±18 vs. 332±11 ps, P < 0.0001; LSC: 493±9 vs. 382±17 ps, P < 0.0001). Areas of drusen featured a wide range of fluorescence lifetime values. Mean lifetimes within areas of atrophy were prolonged by 624±276 ps in the short spectral channel and 418±186 ps in the long spectral channel compared to the surrounding tissue. Autofluorescence lifetime abnormalities in GA occurred with particular patterns, similar to those seen in fundus autofluorescence intensity images.
Conclusions: This study established that autofluorescence lifetime changes in intermediate AMD and GA. Various autofluorescence lifetime pattern can be distinguished. Intraretinal deposits cause prolonged lifetimes, whereas deposits in the area of the outer photoreceptor segments lead to short fluorescence lifetimes. We hypothesize that the short lifetimes seen within the atrophy may be used to estimate damage induced by atrophy and may be useful to monitor disease progression in the context of natural history or interventional therapeutic studies.

12:10
48 P **Lydia Sauer,** A.S. Vitale, N.K. Modersitzki, E.D. Hansen, C.B. Komanski, P.S. Bernstein (Salt Lake City/USA)

Imaging of AMD with FLIO (Fluorescence lifetime imaging ophthalmoscopy)

Background: Age-related macular degeneration (AMD) is the leading cause of blindness in the Western world for people above the age of 65. In this study we investigate changes in fluorescence lifetime imaging ophthalmology (FLIO) related to AMD.
Methods: 300 eyes from 150 patients with AMD and 50 age-matched healthy eyes were investigated at the Moran Eye Center in Salt Lake City, UT. AMD stages were classified according to the Beckman classification. Both neovascular as well as non-neovascular AMD eyes were included. Fundus autofluorescence was excited at 473 nm, and FLIO lifetimes were recorded in a short (SSC, 498-560 nm) and a long (LSC; 560-720 nm) spectral channel.
Results: All eyes with AMD showed a characteristic pattern of prolonged mean FLIO lifetimes in the LSC. This prolongation occurred in a ring-like area approximately 3 mm to 6 mm from the foveal center and was found in all patients with AMD, including those with both neovascular and non-neovascular forms. The pattern was also present in very early disease stages and in one-third of the healthy controls. FLIO lifetimes were longer with more advanced stages of AMD. Different forms of drusen as well as different forms of pigment epithelial detachments (PEDs) demonstrated heterogeneous FLIO lifetimes.
Discussion: FLIO detects a pattern of prolonged FLIO lifetimes in eyes with AMD. These changes are detectable in early disease stages. By showing different signals from different types of drusen or PEDs, FLIO may give additional information to pathophysiological processes involved in AMD. FLIO may also serve as a useful tool for the early diagnosis of AMD and may help to distinguish AMD from other retinal diseases.

12:17
49 L **Adam Dubis** (London/GB)

Adaptive optics photoreceptor imaging, function and survival in AMD

Adaptive optics is an additive element to optical imaging systems that enables cellular resolution of the living human eye. To date it has been applied to most ophthalmic imaging techniques, especially in the case of AMD, to fundus cameras and scanning ophthalmoscopes. Most of the work has focused on understanding where photoreceptors exist in relation to areas of geographic atrophy and differential photoreceptor survival with regards to drusen types. New techniques of AOSLO imaging have now improved our ability to look into regions of degeneration and identify not only where waveguiding photoreceptor outer segments are presents, but also residual inner segments and RPE cells. Further developments are also looking to go beyond observations of retinal structure and towards testing retinal function in areas of health and disease. With these new AOSLO techniques, we are now able to identify RPE cell integrity, investigate Bruch's Membrane permeability as well as test photoreceptor function. These new observations are sure to add new insights into our understanding of retinal structure and function in AMD. The focus of this talk will be outlining our current understanding of photoreceptor structure and function and investigate what the future may hold.

12:29
50 P **Thomas Theelen,** P.P.A. Dhooge, T.W.F. Mulders, C.B. Hoyng (Nijmegen/NL)

In vivo photoreceptor imaging by non-adaptive optics confocal scanning laser ophthalmoscopy

Purpose: Retinal photoreceptor (PR) imaging has been proven to be possible by adaptive optics (AO) systems. Here, we aim to show the mosaic of human PRs in vivo by a non-AO system.
Methods: Healthy subjects and patients with macular disease were examined by a Heidelberg Engineering Spectralis™ device equipped with the High Magnification Module (HMM)™. Besides standard 30° near-infrared confocal scanning laser opthalmoscopy (CSLO), we performed spectral domain optical coherence tomography (OCT) and 8° square CSLO imaging with HMM™.

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Results: Two healthy subjects and four patients with macular diseases (Dominant Cystoid Macular Dystrophy, Adult Vitelliform Macular Dystrophy (AVMD), retinitis Pigmentosa (RP), macular pucker) were examined. Retinal PR mosaics could be observed and analyzed in all HMM™ images. In healthy subjects and the macular pucker case, retinal PRs appeared homogeneous and equally distributed in the macular area. In AVMD and RP, the PR mosaic was less homogeneous with a reduced number of PRs and changed optical properties. Areas of reduced PR density or optical changes on HMM™ images also showed alterations or loss of the outer retinal bands on OCT images.
Conclusion: In vivo PR imaging in healthy and diseased retinas appeared possible by a non-AO device, the Heidelberg Engineering Spectralis™ system with HMM™. A major advantage was the enlarged field of view in HMM™ as opposed to AO. Within the system, image registration of HMM™ images allowed for precise localization of observed changes and correlation with other imaging modalities like OCT.

12:36
51 P **R. Theodore Smith,** K.B. Freund (New York City/USA)
Quantitative fundus autofluorescence (qAF) of geographic atrophy secondary to AMD

Purpose: Quantitative autofluorescence (qAF) offers scaled, reproducible AF measurements between patients and over time. We studied qAF in lobules of geographic atrophy (GA) to correlate with two main pathways from intermediate age-related macular degeneration (iAMD) to two phenotypes of GA.
Methods: 23 eyes of 18 patients with GA underwent spectral-domain optical coherence tomography (SD-OCT) and qAF imaging on the Heidelberg Spectralis. We examined prior serial tracked OCT scans of 52 GA Regions-of-interest (ROIs) lobules or coalescent atrophic lobules (mean follow-up, 5.5 years) to divide them into 2 pathways by the dominant predecessor iAMD lesion type: soft drusen/ pigment epithelial detachment (PED), pathway 1, subretinal drusenoid deposits (SDD), pathway 2. Some ROIs arose from both pathways, and were assigned to a mixed group. Mean qAF values of GA ROIs were measured and compared between the 3 groups.
Results: The soft drusen/PED pathway (18/52) led to GA lesions that were "black" on AF, with lower mean qAF (35.88±12.75 qAF units), generally displaying the complete atrophy of the RPE and outer retina (cRORA) phenotype. The SDD pathway (12/52) led to GA lesions that were multilobular and "gray" on AF, with higher mean qAF (71.62±12.12 qAF units, P<0.001, t-test), generally displaying the incomplete atrophy of the RPE and outer retina (iRORA) phenotype. The mean qAF of ROIs from the mixed pathway (22/52) was intermediate (58.13±11.88 qAF units).
Conclusions: GA lesions can in most cases be divided by qAF (lower/ higher) into 2 non-exclusive groups that correlate both with the precursor pathways (from drusen/PED or SDD, resp.) and their predominant final OCT atrophy classification (cRORA or iRORA), with remaining lesions arising from both forms of iAMD , with intermediate qAF. Thus, qAF of GA lobules reflects both their pathogenesis and structure, and would be an easily implemented and useful metric for clinical GA research.

12:43
52 L **Daniel F. Martin** (Cleveland/USA)
Why did GA trials fail – how to overcome obstacles?

12:55
53 P **Victor Chong** (Ingelheim/D)
Neuroprotection as a therapeutic option for GA

Background: Geographic atrophy (GA) is the leading cause of legal blindness in the developed nations with no approved treatment. The current therapeutic focus is on the complement pathway despite several failure.
Methods: Literature review and expert opinion.
Results: Neuroprotection, including photoreceptor protection, can be a therapeutic option for GA. There are extensive pre-clinical to support multiple agents can protect photoreceptor loss in various animal models. There are Phase 2 clinical trial data to support even with photoreceptor protection can slow RPE loss as shown in AF imaging.
Conclusion: It is unclear whether a sub-group of GA might benefit most from neuroprotection.

14:15 ▼ 17:00 **8th session**
Dissecting AMD: Novel approaches
Moderators: Frank G. Holz (Bonn/D)
Daniel Pauleikhoff (Münster/D).

14:15
54 L **Adnan Tufail** (London/GB)
Application of deep learning-based automated segmentation in OCT on AMD

14:27
55 L **Aaron Lee** (Washington/USA)
Deep learning applications with OCT in AMD

Background: Deep learning applications show great promise in their ability to perform automated diagnosis and segmentation. Large databases of images are required to train and build these algorithms.
Methods: Multiple deep learning algorithms were used for automated diagnosis, feature segmentation, and prediction.
Results: Algorithms were validated and were successfully trained using large datasets.
Conclusion: Deep learning holds great promise in their ability to learn features in a data driven fashion.

14:39
56 L **Karl Csaky[1],** M. Oulette[2], C. Clark[2] ([1]Dallas/USA, [2]Plano/USA)
Developing Human Based Intuitive Deep Learning Algorithms for Analyzing Intermediate AMD OCT-Images

Background: While automated evaluation procedures exist to quantitate various anatomic aspects of optical coherence tomography (OCT), OCT analysis of eyes with intermediate age-related macular degeneration (iAMD) often requires verification or modification by a trained OCT evaluator. Newer modalities including various machine learning approaches are under investigation in an attempt to automate the analysis as well as understanding algorithmic approaches to defining anatomic characteristics that correlate with iAMD progression.
Methods: Human computing has proven to be an effective way to crowdsource a variety of scientific problems, as well as to leverage human pattern-recognition ability. Video games allow users to interact with the scientific data while also leveraging the elements game developers require to maintain engagement. To investigate whether game interactions can train players to evaluate iAMD OCT images, a web-based game, Eye in the Sky: Defender, was created featuring gameplay designed around quantification of drusen.
Results: Evaluations of accuracy using the mean user line input reflected 86 % improvement from a players' initial image evaluation. Spearman rank correlation and Procrustes analysis indicate mean line accuracy within 10 % margin of error by image 4 and improved results compared to the automatically generated line in more challenging images. The preliminary result of this approach allowed the development of a human intuition filter that, when combined with standard machine learning, improved precision and accuracy of drusen identification with fewer OCT image inputs.
Conclusions: These results suggest human computation games can be used to expedite algorithmic development of iAMD OCT analysis.

14:51
57 L **Glenn J. Jaffe** (Durham/GB)
Challenges of clinical relevant phenotyping of exudative AMD

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15:03
59 L **Giuseppe Querques[1,2],** R. Sacconi[1], V. Capuano[2], A. Carnevali[1,3], D. Colantuono[2], M. Battista[1], E. Borrelli[1], A. Miere[2], M. Parravano[4], E. Costanzo[4], L. Querques[1], E.H. Souied[2], F. Bandello[1] ([1]Milan/I, [2]Creteil/F, [3]Catanzaro/I, [4]Rome/I)

„Quiescent" CNV in AMD – characteristics and consequences

Purpose: To analyze different clinical and anatomical features in treatment-naïve non-exudative macular neovascularizations (MNVs) secondary to age-related macular disease (AMD).
Methods: In this longitudinal study with a minimum follow-up of 1 year, consecutive AMD patients with treatment-naïve non-exudative MNV were enrolled. Patients were divided in: short-term activated MNV group (exudation before 6-month) and quiescent MNV group (per definition no exudation during a minimum 6-month follow-up) showing no or late activation during follow-up (persistently quiescent and long-term activated quiescent MNV group, respectively). MNV growth rate and changes in quantitative optical coherence tomography angiography (OCT-A) features during the follow-up were analyzed between different subgroups.
Results: Thirty-one eyes (28 patients, mean age 75±9 years) were included. During the follow-up (mean duration: 22±9 months) 4 eyes (13 %) showed exudation before 6-month follow-up (short-term activated MNV group), whereas 21 eyes (68 %) did not develop signs of exudation (persistently quiescent group), and 6 eyes (19 %) developed exudation after the minimum 6-month follow-up (long-term activated quiescent MNV group). Monthly MNV growth rate was significantly higher in the short-term activated MNV group (growth rate of 13.30 %/month), vs persistently quiescent MNV group (0.64 %/month, p<0.001) and long-term activated quiescent MNV group (1.07 %/month, p<0.001). Furthermore, at the baseline, PD of short-term activated MNV group was significantly greater in comparison to persistently quiescent MNV group (p=0.001) and long-term activated quiescent MNV group (p=0.106).
Conclusions: We reported two different patterns for subclinical MNVs: subclinical MNVs characterized by short-term activation which could represent simply a pre-exudative stage in the development of an ordinary type 1 MNV, and quiescent MNVs characterized by low rate of growth and possible long-term activation. Analysis of OCT-A features may predict short-term activation for subclinical MNV.

15:15
60 P **Maximilian Pfau[1],** L. von der Emde[1], C. Dysli[2], P.T. Möller[1], S. Thiele[1], M. Lindner[3], M. Schmid[1], S. Schmitz-Valckenberg[1], F.G. Holz[1], M. Fleckenstein[1] ([1]Bonn/D, [2]Bern/CH, [3]Oxford/GB)

Type-1 choroidal neovascularization reduces the progression of atrophy in eyes with age-related macular degeneration

Background: To investigate the impact of co-existent quiescent and exudative type-1 choroidal neovascularization (CNV) on the progression of retinal pigment epithelium (RPE) atrophy in age-related macular degeneration (AMD).
Methods: A total of 114 eyes with AMD of 57 patients (40 female, 17 male) with a mean (± SD) baseline age of 76.54±6.66 years and a median [IQR] review period of 1.24 years [1.02, 1.55] from the prospective, non interventional natural history study DSGA (Directional-Spread-in-Geographic-Atrophy [NCT02051998]) were included. Eyes were subdivided in three categories: geographic atrophy (GA) without evidence of CNV (n=78), GA with quiescent CNV (n=5) and RPE-atrophy with exudative CNV (n=10). Longitudinal fundus-autofluorescence and infrared-reflectance images were semi-automatically annotated for RPE-atrophy using the RegionFinder software. CNV lesions were spatially mapped to these annotations based on optical coherence tomography angiography (OCTA). Both overall and localized RPE-atrophy progressions in topographic relation to the presence of CNV were analyzed using cross-validated mixed-effects models.
Results: Baseline GA size ([estimate ± SE] 9.06±1.06 mm2]) and age did not differ significantly among the subgroups. The model to predict overall progression rates, considering the baseline lesion size and further shape-descriptive factors, achieved a high accuracy (cross-validated R2=0.428). Hereby, the observed progression rates were significantly slower than the predicted progression rates for eyes with quiescent CNV (discrepancy of 0.35±0.16 mm^2/year) and exudative CNV (0.32±0.14 mm^2/year). The localized prediction model achieved excellent results with a cross-validated Dice coefficient [95 % CI] of 0.87 [0.85, 0.89]. The localized presence of CNV significantly (P<0.001) reduced the odds for future atrophy involvement by a factor of 0.45 [0.42, 0.48].
Conclusion: The results indicate that the presence of quiescent and exudative type 1 CNV is associated with a reduced overall and localized RPE-atrophy progression. This observation highlights the potential protective effect of CNV on the RPE and overlying neurosensory retina.

15:22
61 L **David Sarraf** (Los Angeles/USA)

Mechanisms and Pathways of PED Development in neovascular AMD

Pigment epithelial detachment (PED) can develop in neovascular age related macular degeneration (AMD) by various mechanisms. This paper will review the various pathways of PED development including PED associated with type 3 neovascularization (NV) versus type 1 NV. These pathways and mechanisms will be illustrated with the presentation of PED findings noted with advanced retinal imaging including optical coherence tomography (OCT) and OCT angiography. Type 3 NV comprises approximately one third of neovascular lesions in AMD. These lesions originate from the deep retinal capillary plexus (DCP) and descend towards the retinal pigment epithelium (RPE). Infiltration of the RPE and exudation into the subRPE compartment can lead to serous PEDs that can be even greater than 1000 microns in height. These lesions are driven by the elaboration of pro angiogenic cytokines in the RPE and are typically highly responsive to antiVEGF therapy. A pro renata dosage regimen may therefore be optimal, especially because of the greater risk of macular atrophy. The elaboration of pro angiogenic cytokines in the RPE may drive the proliferation of new blood vessels downward from the DCP or upward from the choroid. Type 1 NV originates from the choroid and represents the majority of NV lesions in AMD. PED due to type 1 NV is the most common lesion type in NV AMD and can progress to 2 morphological outcomes including persistent vascularized serous PED versus multilayered PED. Persistent vascularized serous PED may represent a more unstable anatomical presentation. Due to a greater height and smaller proportionate area of choroidal neovascularization (CNV), these lesions may be at greater risk of RPE tear as a result of traction, especially after antiVEGF therapy that can increase fibrotic traction due to an imbalance in the angiofibrotic switch. Multilayered PED represents a more favorable visual and anatomical outcome of type 1 NV in which the proportionate area of the CNV is much greater. The organization of this CNV complex is comprised of fibrovascular and fibrocellular components and these lesions may recapitulate the choriocapillaris and may protect against RPE atrophy and photoreceptor loss.

15:34
62 L **K. Bailey Freund** (New York City/USA)

Type 3 CNV – a specific phenotype?

15:46
63 L **John Marshall** (London/GB)

Can a nanosecond beam modulated laser improve transport mechanisms and delaying progression towards late AMD?

Saturday, 21st September 2019

15:58
64 L **Robyn Guymer** - LEAD study team (Melbourne/AUS)

Natural history and modulation of drusen by laser:
The LEAD Randomized Controlled Trial of Sub-Threshold Nanosecond Laser Intervention in age related macular degeneration

Background: To determine the efficacy and safety of sub-threshold nanosecond laser (SNL) treatment in intermediate age-related macular degeneration (iAMD) to slow progression to late AMD.
Methods: 292 participants with bilateral drusen >125 um within 1500 um of the fovea, monocular best corrected visual acuity (BCVA) 20/40 or better were randomized to receive SNL (twelve laser or sham spots) 6-monthly for 36-months. The primary outcome was time to late AMD as defined by multimodal imaging (a combined endpoint of choroidal neovascularization, geographic atrophy or atrophy as defined on optical coherence tomography) in the study eye using the full intent to treat (ITT) cohort. The exploratory outcome measures were the rate of change in best-corrected visual acuity (BCVA), low luminance visual acuity (LLVA), microperimetric mean sensitivity (MS), drusen volume in the study and non-study eyes.
Results: Overall, progression to late AMD was non-significantly slowed with SNL compared to sham treatment (adjusted hazard ratio [HR] 0·61, 95 % CI 0·33–1·14). Slowing was more apparent for participants without coexistent reticular pseudodrusen (RPD) at baseline (adjusted HR 0·23, 95 % CI 0·09–0·59), but participants with RPD had an increased progression rate (adjusted HR 2·56, 95 % CI 0·80–8.18; adjusted interaction p=0·002). The rate of BCVA decline was slightly higher for participants in the SNL compared to sham group in the study eye (-0.8 letters/year faster; P<0.001), but not the non-study eye (0.1 letters/year; P=0.628). There was no significant difference between study groups in the rate of change of LLVA, microperimetric MS and drusen volume in the study or non-study eyes, (all Pff10.167).
Conclusions: These findings provide evidence for the need for further trials of SNL as a potential early intervention for the early stages of AMD.

16:10
66 L **D. Pauleikhoff[1,2],** Marie-Luise Farecki[1], Henrik Faatz[1], K. Rothaus[1], G. Spital[1], A. Lommatzsch[1,2], ([1]Münster/D, [2]Essen/D)

Mathematical characterization of CNV before and under anti-VEGF-therapy

Purpose: Several clinical effects observed during and after anti-VGF therapy in exudative AMD (p.e. visual and morphological outcome, length of therapy and number of injections) can not be predicted by the current diagnostic and phenotypical differentiation oft he disease. OCT-A enables the mathematical characterisation of CNV. The aim of the present study was therefore to test, if this approach can result in an improved differention of CNV with predictive impact.
Methods: 80 eyes of 62 patients with exudative AMD and indication for anti-VEGF therapy were included in this study. OCT-A (Optovue) was performed in addition to BCVA, fundus and OCT (Spectralis) examination every 3 months. CNV were delineated on the OR-RPE-CC slab of the OCTA and exported. This image was thereafter skeletonized. As parameters flow area, flow density, total vessel length, number of vessel segments and number of vessel branching was evaluated. This was compared with BCVA and central retinal thickness (CRT in OCT).
Results: A wide variation could be seen in the area and flow of different CNV. Flow area corresponded with numder of total vessel length and number of segments characterizing the size of a CNV, while flow density correlated with number of branching characterizing the complexity of a CNV. This corresponded significantly with type 1 CNV being larger and less complex while type 2 CNV were smaller and with higher complexity. There was also a significant positive corellation of the size parameter with increased CRT (p<.01). In contrast there was a negative correlation between CRT and complexity parameters (p<.01). After anti VEGF-therapy there was a reduction in all parametrs observed with increased values as signs of reperfusion associated with renewed increase in CRT. Differentiating the differences in BCVA during therapy with the mathematical parameters, parameters define larger and less complex lesions were associated with less gain in VA compared to smaler more complex CNV.
Conclusion: OCT-A offers the possibility for mathematical characterisation of CNV. In this case series the wide variability of mathematic parameters between CNV in respect to size and complexity of a lesion could be observed. This correlated with additional clinical (CRT) and functional (VA) parameters before and aduring anti-VEGD therapy. This approach may enable a different view into CNV, which relevance has to be proven in future reading center based clinical investigations of larger cohorts.

16:27
68 P **Heinrich Gerding** (Olten/CH)

10 years anti-VEGF treatment outcomes on neovascular AMD

Background: To analyse the long-term outcome of flexible anti-VEGF treatment in patients with wet AMD.
Methods: A retrospective institutional case series analysis of 104 eyes (104 patients, mean age 77.6+/-7.3, (mean+/-1 SD)), with a median baseline best corrected logMAR visual acuity (BCLMVA) of 0.7, was performed. Only eyes meeting the inclusion criteria of the MARINA/ANCHOR studies were selected. Initial treatment consisted of 3 monthly ranibizumab injections. Patients were examined monthly during year 1 (Y1), and further on according to an individualized PRN regimen.
Results: All patients continued observation until the end of Y1, 86 (83 %) until end of Y4, 55 (53 %) until Y7, and 30 (29 %) until the end of Y10. In 24 eyes (23 % of all included) therapy was transiently or permanently switched from ranibizumab to aflibercept between Y5 and Y10. Median BCLMVA improved by +1.3 lines during the loading phase and was +0.94 lines at Y1, +0.0 lines at Y4, +0.0 lines at Y7, and -0.05 lines at Y10 visit. 12 (40 %) of 30 patients reaching Y10 evaluation had presented active disease within the last year, indicating treatment. The total number of injections was 2091 (ranibizumab: 1847 (88.3 %), aflibercept: 244 (11.7 %)).The total number of injections was 608 in Y1, 205 in Y4, 133 in Y7 and 55 in Y10. The average number of injections per enrolled eye within 10 years was 0.17/month, 2.0/eye/year, and totally 20.1/eye. The mean number of cumulative injections/observed eye was 5.8+/-2.3 in Y1, 14.2+/-8.4 at the end of Y4, 22.9+/-16.4 after Y7 and 28.6+/-20.9 towards end of Y10. Eyes with active disease received 15.0+/-5.8 injections until the end of Y4, 29.9+/-13.6 until Y7, and 37.2+/-19.6 injections until the end of Y10. The average number of injections/year for eyes with active disease was nearly identical through follow-up (5.8+/-2.3 in Y1, 4.8+/-2.4 in Y4, 4.8+/-2.2 in Y7 and 4.6+/-3.6 in Y10).
Conclusions: Results of this study demonstrate that anti-VEGF therapy of wet AMD can result in long-term functional stabilization at baseline level, well above the natural course of the disease. A stable level was achieved following year 4 with a relatively low number of injections. A considerable percentage of eyes presented persistence of active and treatable disease even in year 10 of care.

Saturday, 21st September 2019

16:34
69 L **Ricarda G. Schumann,** V. Deiters, S.R. Günther, D. Vogt, J. Siedlecki, S.G. Priglinger (Munich/D)

Long-term visual acuity outcomes and anatomic results from anti-VEGF-therapy in patients with neovascular AMD in a real-life setting

Purpose: For neovascular age-related macular degeneration (nAMD), data from real-life studies on functional and anatomic outcomes of anti-VEGF treatment is still limited. Therefore, we assessed the results of anti-VEGF therapy in our patients with a long-term follow-up.
Methods: In this retrospective monocenter study, patients were included with newly diagnosed nAMD that had received minimally three intravitreal injections between January 1, 2006 and June 30, 2014 at the University Hospital, Ludwig-Maximilians-University Munich, Germany, with a follow-up period of ffl 60 months. Primary outcome measures were the evolution of best-corrected visual acuity (BCVA) and central macular thickness (CMT) on spectral-domain optical coherence tomography (OCT). Secondary outcome measures were number of intravitreal injections (IVT) and follow-up visits, treatment strategy and switch as well as evolution of morphologic features such as macular atrophy, pigment epithelium detachment and retinal tubulations. For qualitative, quantitative and longitudinal data, Pearson's chi2 test, the Mann-Whitney U-test and Wilcoxon's signed-rank test were applied at a significance level of $p < 0.05$.
Results: Of 1034 eyes (861 patients) with nAMD treated during this period, 149 eyes (123 patients) had been newly diagnosed and were consistently followed for at least 60 months. Mean follow-up period was 8.0 years (median 7.8, range 5.0–13.2 years). Mean BCVA at baseline was 0.51±0.30 LogMAR and improved to 0.42±0.30 LogMAR with a mean number of 5.3±2.3 injections during the first year. Mean number of anti-VEGF injections was 28.8±16.1 (median 26, 3-67 injections). At year 5,19.5 % of eyes declined by 15 letters or more. Pro re nata (PRN) regimen was started in the majority of eyes at baseline (95.1 %) and changed to treat&extent (T&E) in 59.8 % over the period of treatment. Treatment switch was documented in 103/149 (69.1 %) eyes, mostly from ranibizumab to aflibercept (83.2 %). In 21.3 % of eyes, treatment was re-switched during the follow-up period. At time of last follow-up, 104 eyes (69.8 %) were still under active treatment. These are preliminary data. Data from SD-OCT analysis are pending.
ConclusionThe herein presented real-life data provide an estimate of visual function developments and injection frequencies for counselling patients undergoing long-term anti-VEGF therapy.

16:46
70 P **Jakob Siedlecki,** C. Fischer, B. Schworm, T.C. Kreutzer, K. Kortüm, R. Schumann, A. Wolf, S.G. Priglinger (Munich/D)

Long-term incidence of macular atrophy and clinical outcomes in neovascular age-related macular degeneration managed to sub-retinal fluid only

Background: To determine the role of sub-retinal fluid in age-related macular degeneration concerning the long-term incidence of macular atrophy and clinical outcomes in eyes treated with anti-vascular endothelial growth factor therapy using a treat & extend regimen.
Methods: All patients with nAMD treated with anti-VEGF inhibitors since 2014 were screened for a sub-retinal fluid only phenotype, which was defined as follows: (I) Absence of intra-retinal fluid (IRF) from baseline or after anti-VEGF upload; (II) Continuous recurrence of fluctuating subfoveal fluid responsive to anti-VEGF for a duration of ffl 3 years. Sub-retinal fluid was treated as a sign of activity and not tolerated. Treatment was monitored using multimodal imaging.
Results: Twenty-seven eyes were included. Mean age was 72 ± 6 (range: 61 – 86) years. Choroidal neovascularization (CNV) was type 1 in 12 (44 %) and type 2 in 15 (56 %) eyes. The sub-retinal fluid only phenotype was seen from baseline in 14 eyes (52 %), and in the remaining 13 eyes (48 %) after a mean 1.0 ± 1.3 (1 – 3) injections. During a mean follow-up of 4.2 ± 0.9 (3 – 5) years, 7.5, 5.9, 6.1, 6.1 and 7.0 anti-VEGF injections were given in years 1 to 5 (p=0.33). Cumulative macular atrophy incidence was 11.5 % at year 1, 15.4 % throughout years 2 to 4, and 21.0 % at year 5. Mean visual acuity improved from baseline 37.8 to 43.9 ETDRS letters at year one (p=0.006) and remained stable through years 2 to 5 (p=0.20) to a final 42.8 letters.
Conclusion: Eyes manifesting activity by sub-retinal fluid only in treat & extend anti-VEGF regimen for neovascular AMD seem to exhibit little long-term atrophy and stable visual outcomes during long-term follow-up. Recurrence of subretinal fluid without intraretinal fluid might be an indicator of more a benign form of neovascular AMD.

17:00 **Adjourn**
Frank G. Holz (Bonn/D)
Daniel Pauleikhoff (Münster/D)

Notes