Association for Research in Vision and Ophthalmology

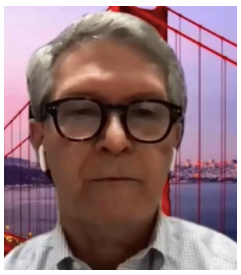
ARVO Annual Meeting In-depth Report

May 1st – 7th, 2021

In-Depth Report

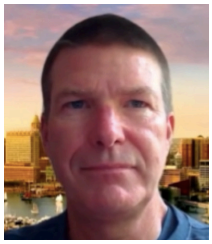
Welcome to ARVO 2021

Delegates attending ARVO 2021 were welcomed by Immediate Past President, Daniel W. Stamer, Durham, USA, and ARVO President, Stephen C. Pflugfelder, Houston, Texas. Despite the many obstacles brought on by the COVID-19 pandemic, Daniel W. Stamer and Stephen C. Pflugfleder highlighted that technology has connected researchers from around the globe, with the occurrence of virtual lectures, laboratory meetings, symposia, and international meetings such as ARVO 2021 facilitating the exchange of ideas and research.



Daniel W. Stamer thanked the ARVO staff for all their efforts in organising ARVO 2021, who were forced to adapt to the ever-dynamic conditions associated with the pandemic.

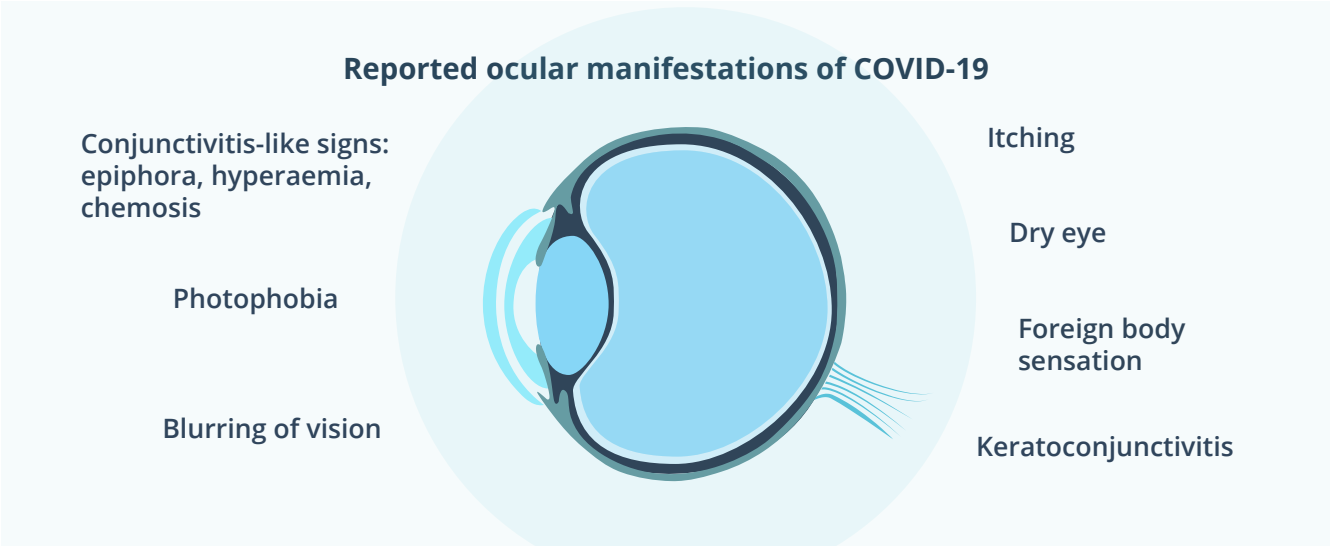
> "We have all faced unexpected challenges over the past year, but despite the hardships, our researchers have endured from the pandemic. We stand strong as a global community"
>
> **- Daniel W. Stamer, Durham, USA**



COVID-19 and the eye

Over the last year, extensive research has furthered our understanding of COVID-19, with emerging evidence indicating the potential involvement of the eye in the infection and transmission of COVID-19. Rupesh Agrawal, Novena, Singapore, presented data on the ocular surface and intraocular manifestations of COVID-19,

providing an overview of research into COVID-19 transmission via tears and conjunctival secretions. He highlighted results from a recent study which found a significant increase in choroidal thickness (p=0.0175), as well as a significant reduction in the choroidal vascularity index (p=0.0069) in patients with COVID-19, demonstrating ocular involvement of COVID-19 infection. In order to understand the mechanisms of ocular COVID-19 infection, however, further research is needed, and COVID-19 patients presenting with ocular symptoms should be thoroughly assessed by an ophthalmologist.



Elia Duh, Maryland, USA, outlined research which explored the extent to which ocular surface cells may be susceptible to COVID-19 infection, including whether these cells express key determinants for viral entry. Critical host entry factors ACE2 and TMPRSS2 were investigated using immunohistochemistry and western blotting. ACE2 was present in all specimens in the cornea, limbus, and conjunctiva, while TMPRSS2 staining was observed in all specimens and expressed throughout the entire conjunctival epithelial layer. Dr Duh concluded that the expression of *ACE2* and *TMPRSS2* demonstrates some susceptibility of the ocular surface to COVID-19 infection, indicating that the eyes may act as an additional infection gateway. These findings support the need for the use of eye protection for healthcare professionals and those at high risk of COVID-19.

"Expression of critical host entry factors supports the likelihood that the ocular surface can be infected by SARS CoV-2, although to a much lesser degree than nasal/respiratory passages"

– Elia Duh, Maryland, USA

Timothy Blenkinsop, New York, USA, presented results from a study which sought to investigate the role of productive COVID-19 replication on the ocular surface. Using whole transciptome RNA sequencing in ocular cells, host response to infection and viral replication were assessed.

Limbus cells were found to be at the highest risk for COVID-19 infection when compared with the central cornea. Furthermore, their findings indicated that COVID-19 antagonises the innate immune response while suppressing normal gene transcription.

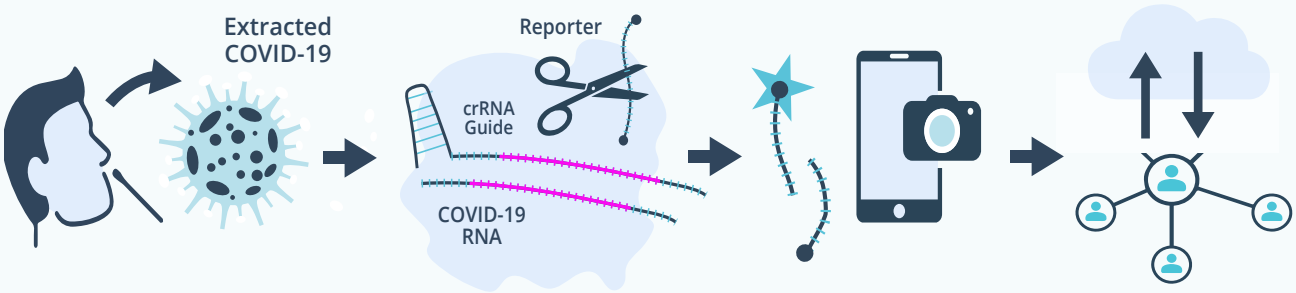
The use of mobile phone technology as an alternative to plate readers in the detection of COVID-19 was proposed by Melanie Ott, California, USA. Her team's research uncovered that when comparing the two in terms of noise level, the mobile phone outperformed the plate reader with higher consistency, and with around ten times enhanced sensitivity. The researchers subsequently demonstrated that their mobile phone assay could accurately detect 5 out of 5 of COVID-19 RNA samples within 5 minutes in a variant-insensitive manner. Dr Ott further outlined the ongoing review of a test designed to process RNA from a nasal swab sample using a mobile phone camera to detect the presence of COVID-19. If successful, this technology could be rapidly adapted to existing viruses, such as influenza, and novel viruses which may emerge in future.

A mobile phone assay accurately detected **5 out of 5** of COVID-19 RNA samples within **5 minutes** in a variant-insensitive manner

"We need to invest into novel ways to treat and detect viruses in the future in order to be prepared"

- Melanie Ott, California, USA

Simple, 15-minute, mobile phone-based COVID-19 test for all



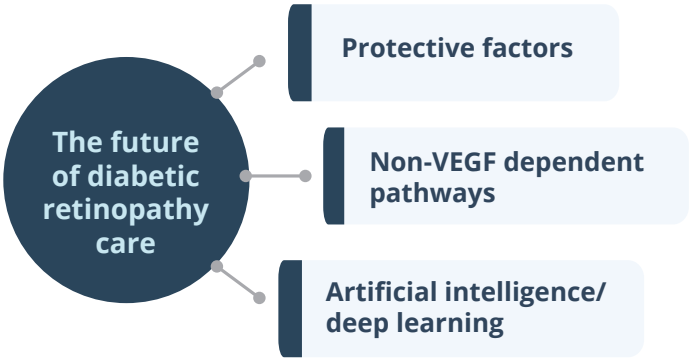
1. Simple nasal or mouth swab
2. Mix swab in one-step "programmed" CRISPR reagent-reaction occurs if viral RNA present, no amplification needed
3. Smartphone camera re-purposed as microscope detects reaction fluorescence and displays result, including viral load
4. Store and analyse quantitative results in the cloud with geo-location and symptoms to track viral spread

Can be adopted for COVID-19 variants and other viruses

Diabetic retinopathy

Diabetic retinopathy (DR) is the 5th most common cause of blindness globally, and accounted for 1 in every 39 people with blindness in 2010. In the last two decades, blindness caused by DR increased in all world regions, within the exception of Western Europe and economically developed North America, who saw a slight decrease in DR cases. Improved glycaemic control and the introduction of DR screening programs have proven effective in the avoidance of DR-related blindness.

Lloyd Aiello, Massachusetts, USA, provided an overview of diabetes and its related health complications such as DR and diabetic macular edema (DME). He explored evidence supporting anti-VEGF treatment as a future therapeutic option for non-proliferative DR. Recent studies have demonstrated that those treated with anti-VEGFs show substantially better improvement in retinopathy severity from baseline when compared to those in a sham group (nominal p<0.0001), and also show substantially lower rates of worsening (nominal p<0.001). Anti-VEGF treatment also correlated with a reduced likelihood of vision-threatening complications and centre-involved DME (nominal p<0.001). However, lack of patient awareness remains a major hurdle in DR therapy, owing to a large reported discrepancy between retinal disease state and patient-reported awareness, with 42% of 18,341 patients found to be unware of any DR even post-follow-up with a retinal specialist.



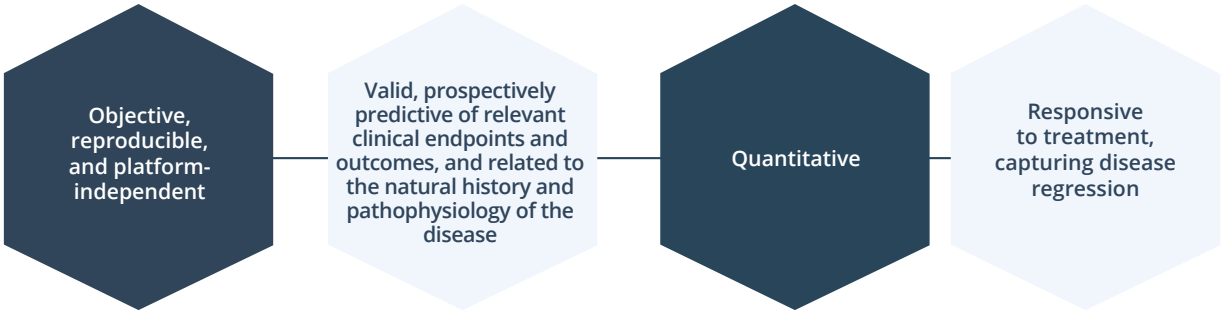
> "Lack of patient awareness is considered a major contributing factor for non-adherence to eye care guidelines and poor visual outcomes"
>
> **– Lloyd Aiello, Massachusetts, USA**

Tien Wong, Singapore, presented the history and evolution of DR and age-related macular edema (AMD) imaging and classification, from the Airlie House Classification of DR, developed in 1968, to the International Diabetic Retinopathy Severity Scale (DRSS). The International DRSS was developed in the 2000s, and is now widely used by clinicians and physicians for the assessment and diagnosis of DR.

Professor Wong further explained the present need for new classification systems for both DR and AMD due to new imaging technologies, biomarkers, treatments, and clinically-relevant outcomes.

VA, visual acuity

The ideal features of a new classification system would include a clearly defined aim of the system – whether it is intended for research or clinical use – and would be:



Tunde Peto, Belfast, Ireland, discussed the future of teleophthalmology in the treatment of DR. The COVID-19 pandemic has accelerated the implementation of virtual consultations as well as optical coherence tomography home monitoring in healthcare. Wide-field cameras now also provide the option for patients to capture their own images, or for a photographer to use a long lead in order to facilitate social distancing. Such technologies may open the doors to drive-through retinal imaging. This would allow patients to be imaged and diagnosed in their own vehicle using AI software, a similar system to that used currently for drive-through glaucoma testing, therefore protecting both patients and healthcare providers.

The roles of mitochondrial homeostasis and epigenomics in mitochondrial genomic and functional stability in DR were highlighted by Renu Kowluru, Michigan, USA. She illustrated the processes by which epigenetic modifications may cause mitochondrial DNA and structure to become more vulnerable to damage via DNA methylation. Innovative therapies such as histone deacetylase/methyltransferase inhibitors and DNA methylating agents are in the process of being trialled as preventative treatments in DR through protecting mitochondrial homeostasis.

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Age-related macular degeneration

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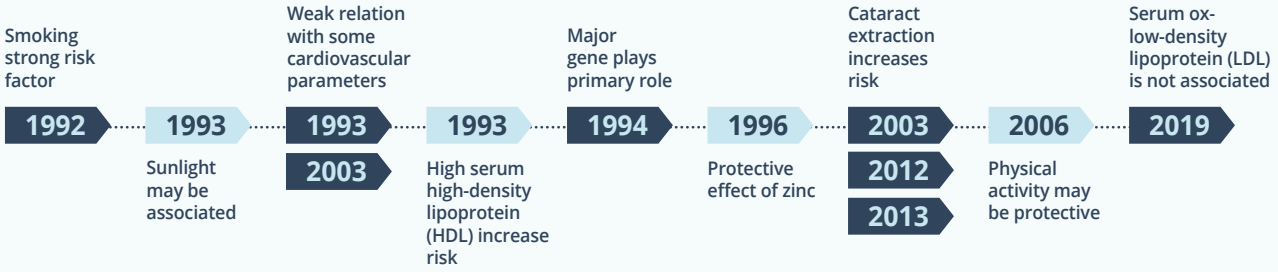
A symposium was dedicated to Professors Ron and Barbara Klein, whose combined work yielded crucial insights into the epidemiology of both AMD and DR. Professors Ron and Barbara Klein contributed to ground-breaking studies such as the Beaver Dam Eye Study (BDES) and the Wisconsin Epidemiology Study of Diabetic Retinopathy.

AMD is the primary cause of blindness in adults over 60-years-old, and is associated with loss of central vision, leading to difficulties in reading, driving, facial recognition, amongst other activities of daily living. Common risk factors include genetic background, smoking and obesity. However, the primary risk factor for AMD is ageing. From a genetic perspective, AMD is the most understood complex age-related disorder. Despite this knowledge, effective treatments for the most prevalent disease forms remain elusive.

Groundbreaking observations about AMD and BDES:

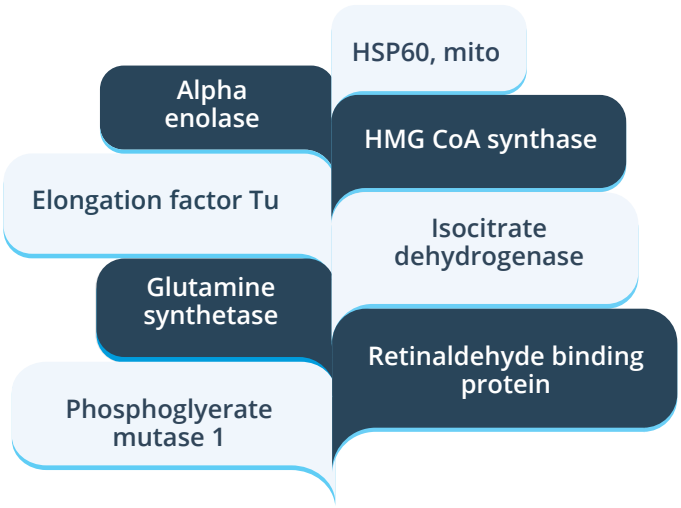


Caroline Klaver, Rotterdam, Netherlands, recounted the seminal BDES and its impact on AMD studies today. The 3 Continent Consortium built upon the BDES with a focus on epidemiology and risk factors for AMD. They confirmed an association with smoking (OR = 4.6; 95% CI 2.7–7.5), and a synergistic effect between smoking, fish, and lutein intake in AMD. Later, the EYE-RISK consortium investigated the genetic bases of AMD. When assessing ORs for AMD risk alleles as a function of allele frequency, age-related maculopathy susceptibility 2 (*ARMS2*), complement factor H (*CFH*), and complement component 3 (*C3*) harboured common but risky genetic variants. *CFH* and complement factor I also carried rare but high-risk alleles. Further research illustrated that variants in *ARMS2, CFH* and *C3* indeed determine a large proportion of late AMD cases, while variants in *C2* and other variants in *CFH* determine a large number of controls.

A positive genetic risk score was present in the majority of late AMD cases, but was also present in a significant fraction of controls. When calculating risk scores for complement, lipids, extracellular matrix and 'other' pathways, findings indicated that the average person with late AMD carries risk variants in at least three of these pathways.

Poor nutrition and smoking have also been confirmed as prominent risk factors for AMD. Unsurprisingly, a good diet and not smoking are vital to health regardless of AMD genetic risk category, however, both have the most positive influential effect in those at high genetic risk. Furthermore, Professor Klaver discussed a study of pooled data from the European Eye Epidemiology consortium which investigated serum lipids in 4600 AMD cases and 17000 controls, finding significant associations with HDLs (OR = 1.39, $p<0.0001$), LDLs (OR = 0.95, $p=0.019$), and triglycerides (OR = 0.89, $p<0.0001$). No or little physical exercise increased the risk of early and intermediate AMD.

The benefits of the Mediterranean diet in AMD were discussed by Emily Chew, Maryland, USA. Using dietary intake data from 7756 participants in the AREDS and AREDS2 studies, a dose response was identified, suggesting that a higher Mediterranean diet intake was associated with a 23%, 29%, and 16% reduction in risk of progression to late AMD, geographic atrophy, and neovascular AMD, respectively. In addition, fish intake was associated with a 31% decreased risk of progression to geographic atrophy (GA), and there was an increased beneficial effect of fish for those with protective alleles of CFH.

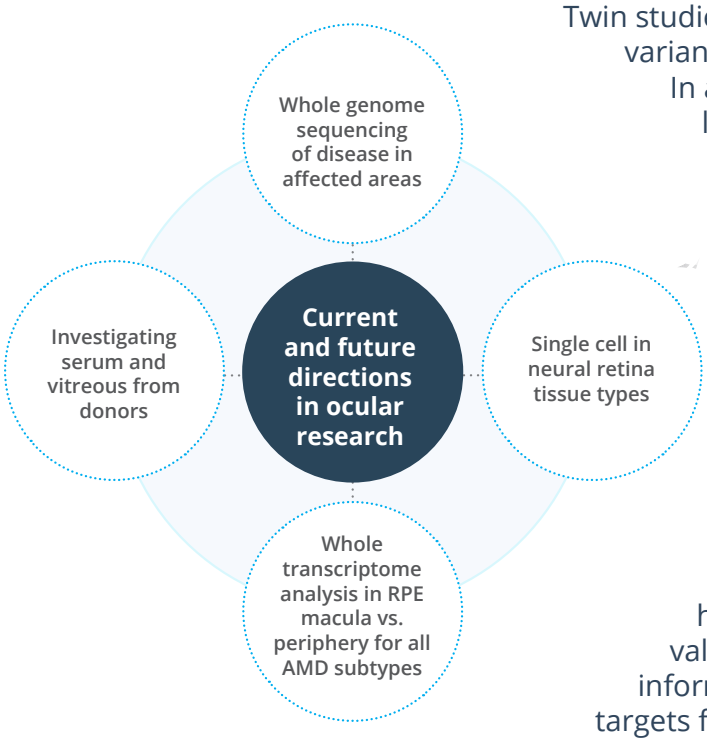


Deborah Ferrington, Minnesota, USA, presented results from proteomic analyses of retinal pigment epithelium (RPE) from human donors during normal ageing versus donors exhibiting varying severities of AMD. Using 2D gel electrophoresis and mass spectrometry, the team were able to identify eight overlapping proteins associated with normal ageing versus AMD. However, significant divergence was observed between affected pathways: proteins involved in energy metabolism and signal transduction were observed to decrease with ageing while increasing in AMD. In contrast, protein metabolism, apoptosis and stress response were increased in AMD. These results highlight that separate cellular process underlie ageing and AMD.

"Ageing and AMD involve different cellular processes"

- Deborah Ferrington, Minnesota, USA

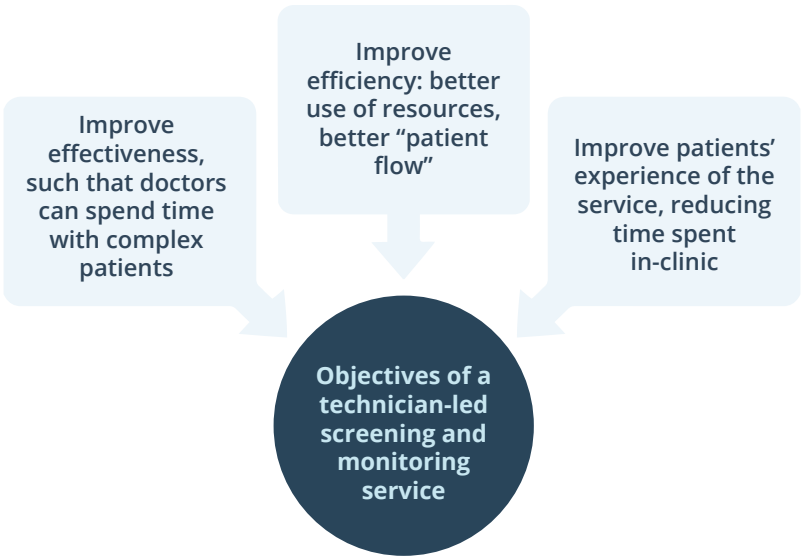
Louise Porter, Strasbourg, France, explored the epigenetic mechanisms of gene regulation in AMD, reporting results from AMD-relevant epigenetic clock analyses and age-matched control RPE samples, ingenuity pathway analysis (IPA), and IPA causal network analysis. Epigenetic clocks demonstrated a deceleration of epigenetic age in RPE, and dysfunction in ageing-related epigenetic maintenance systems was found to underlie more frequent epigenetic aberrations. Furthermore, ageing-related epigenetic maintenance system dysfunction in the RPE may cause epigenetic aberrations to occur more frequently.



Twin studies in AMD reveal that the presence of risk variants does not define disease development. In a set of identical twins with the same risk loci at chromosomes 10 and 1, matched for epidemiological factors, one exhibited a severe form of AMD though his brother showed no evidence of AMD. This confirms that genetics do not explain 100% of AMD risk and it could be a multifactorial disorder, necessitating further understanding of epigenetic mechanisms. Margaret deAngelis, New York, USA, hypothesised that disruptions in DNA methylation processes may lead to alterations in gene expression and function. She also stressed the importance of a systems biology approach in AMD to help fine-tune targets most appropriate to validate for functional analyses, using this information to design personalised therapeutic targets for AMD in future.

Renata Puertas, London, UK, proposed that due to our ageing population and low AMD clinic discharge rates, the asynchronous model of non-face-to-face clinics is the best way forward for ophthalmology services in order to increase efficiency and effectiveness of ophthalmic care. Since AMD is easily monitored by imaging and earlier treatment facilitates better AMD outcomes, she suggests that a technician-led service with remote review by consultants may lend itself well to AMD virtual or digital care. Benefits of such would include:

However, no direct contact with doctors and a possible extra appointment may pose disadvantages. Dr Puertas also reported that, when comparing face-to-face versus virtual clinics in AMD, no significant differences were identified in visual outcomes between groups (20/184.8 vs. 20/180.7, p=0.99) in those monitored for recurrence.



The process of developing a reference map of genetic regulation of gene expression quantitative trait loci (eQTLs) and splicing quantitative trait loci (sQTLs) in AMD was described by Ayellet Segre, Massachusetts, USA. Her team used eQTL and sQTL data to begin to elucidate regulatory and causal mechanisms that underlie ocular diseases. In doing so, they found that eQTLs in the hypothalamus and amygdala are most strongly associated with AMD, suggesting a proxy for neuronal tissues in the retina. QTLs in lymphocytes and the liver may also propose roles in AMD for the immune system and lipid metabolism, respectively.

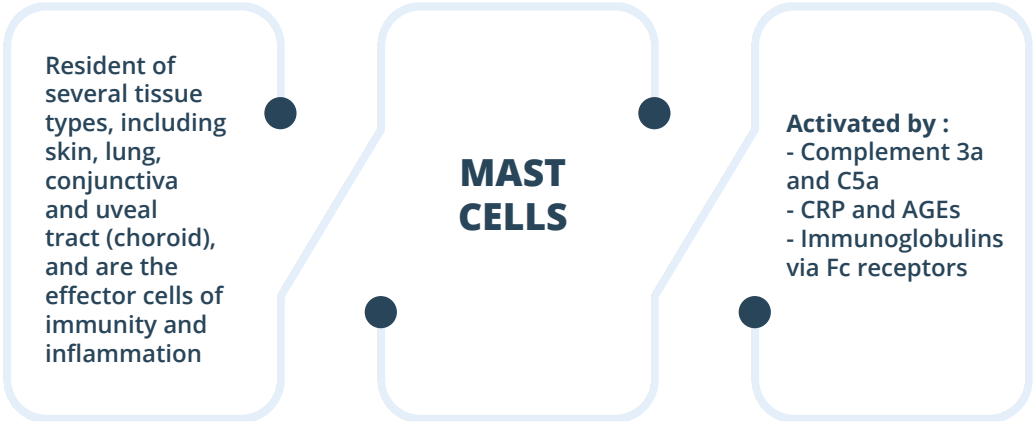
To further discern the aetiology of AMD, the team used LeafCutter to identify 98,616 splice events for 12,720 retinal genes. Around three-quarters of these splicing events were shared between peripheral and macula locations. Looking further into the tissue specificity distribution of these splicing events, around 7% of splicing events in 2605 genes were identified as retina-specific, and there was strong enrichment of tissue retina specificity amongst the rare inherited retina degeneration splicing events. Using a colocalisation method, they found AMD-associated genes proposed by retina sQTLs, including *TBC1D23* and *SNHG32*, some of which were not found with retinal eQTLs. This suggests that genetic regulation of splicing for some of these genes may represent the underlying causal mechanism for AMD.

Lastly, the researchers looked beyond genome-wide significant associations in an attempt to find additional AMD associations amongst retinal sQTLs and eQTLs. They discovered that the AMD associations were most strongly enriched among the macular sQTLs, proposing 82 sQTLs and 216 eQTLs that may be associated with AMD.

Geographic atrophy

Geographic atrophy (GA) is the most common subtype of advanced AMD, and characterised by loss of RPE and choriocapillaris, and retinal and choroidal thinning. GA can lead to irreversible blindness, however, no effective therapies are currently in use for the disorder, and functional evaluation methods remain unreliable and time-consuming.

Gerard Lutty, Maryland, USA, described a rat model for assessing the role of choroidal mast cell degranulation in GA. Mast cells were stimulated for degranulation with 48/80, a snake venom-like compound, and were placed in hydrogel, which was subsequently inserted subconjunctivally. After 4–10 weeks, loss of RPE, reduced electroretinogram amplitude and retinal and choroidal thinning – all phenotypic characteristics of GA – were observed. Prevention of this phenotype was possible through the administration of oral ketotifen and a tryptase inhibitor, demonstrating that mast cell degranulation may represent a therapeutic target for GA.



AGE, advanced glycation end product; CRP, C-reactive protein

Closing Remarks

The ARVO annual meeting ended with closing remarks by Maureen Maguire, PhD, Pennsylvania, USA, the newly-installed ARVO President. Dr Maguire thanked all presenters and speakers for their contributions of important and innovative research and stated that, over the next years, we must continue to adjust, pivot, and adapt in order to navigate the many challenges of returning to the 'new normal'.

"While this past year has provided many challenges for all of us, perseverance is the nature of our research community" **- Maureen Maguire, Pennsylvania, USA**

ARVO 2022 will be taking place May 1st–5th in Denver, Colorado, USA.