



Emerging trends and hot topics in glaucoma from the 2015 ARVO annual meeting.

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Once again, the Association for Research in Vision and Ophthalmology (ARVO) annual meeting, held this year in Denver, gave participants the opportunity to discover the latest and most innovative research being conducted in ophthalmology. We propose to provide an overview of the most relevant and promising research topics in Glaucoma presented at the meeting. This selection has been identified by all of the 19 members of the European Glaucoma Panel (EGP). The EGP and Mentoring Program is a 3-year educational and personal development program for young Glaucoma specialists from Europe with a keen interest in research. Under the mentorship of Professor David Garway-Heath (Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK) and facilitated by Dr Tuan Ho, (Moorfields Eye Hospital, London, UK), this programme, among other aspects, focuses on the selection and critical discussion of the most relevant findings from key scientific meetings. This exercise aims at providing the EGP members with in-depth insight into Glaucoma. Below is the summary of our week-long, collaborative work, which we would like to share with our colleagues outside the EGP and mentoring program.

European Glaucoma Panel participants: Sabina Anderson (**Sweden**), Laura Beltran-Agullo (**Spain**), Mehmet Cem Mocan (**Turkey**), Maurizio Digiuni (**Italy**), Cornelia Hirn (**Switzerland/Austria**), Hari Jayaram (**UK**), Themis Karmiris (**Greece**), Sergio Mahave (**Spain**), Juliane Matlach (**Germany**), Karl Mercieca (**UK**), Manuele Michelessi (**Italy**), Marcos Munoz (**Spain**), Eduardo Normando (**UK**), Luis Abegão Pinto (**Portugal/Belgium**), Muriel Poli (**France**), Verena Prokosch-Willing (**Germany**), Gokulan Ratnarajan (**UK**), Andrew Scott (**UK**), Katarzyna Skonieczna (**Poland**).

- **NEW TRENDS IN GENETICS AND EPIDEMIOLOGY**

MicroRNAs in glaucoma pathogenesis. MicroRNAs (miRNAs) are small, endogenous non-coding RNAs that modulate post-transcriptional gene expression. The work of Dr H. Jayaram et al (Portland, OR, USA), showed evidence that **changes in**



retinal microRNA expression are observed in glaucomatous rat models, demonstrating similarities to comparable mechanisms observed in central nervous system injury. Dr T. Gaasterland et al (San Diego, CA, USA) also presented preliminary data of in vitro microRNA expression in glaucomatous human optic nerve. This cutting edge approach in understanding the pathophysiology of glaucomatous optic neuropathy **may ultimately lead to novel therapeutic interventions to attenuate the mechanisms that lead to glaucomatous damage.**

Scleral and trabecular meshwork stiffness, Matrix Gla protein gene and glaucoma. Recent studies indicate that age-associated increased stiffness of trabecular meshwork (TM) and sclera could play a crucial role in the pathogenesis of glaucoma. Matrix Gla protein (MGP), an inhibitor of calcification with anti-stiffness properties, has been identified in TM and sclera. Dr T. Borrás et al (North Carolina, USA) investigated the expression and distribution of MGP in MGP-knock-in mice and found **high levels of MGP in the peri-papillary sclera as well as in the TM.** This study **contributes to stiffness theory in glaucoma pathogenesis** and may identify a potential therapeutic candidate through which stiffness may be regulated in glaucoma models using **a gene therapy approach.**

Glaucoma in Europe: Dr A. Khawaja et al (Cambridge (UK) conducted a large epidemiologic study investigating variations and determinants of intraocular pressure (IOP) across Europe. The study found new associations such as **lower IOP in taller people,** but showed **no evidence of a significant variation in IOP across Europe.** This last point is an important finding that supports collaborative pooling of data from studies examining genetic determinants of IOP. Moreover, the authors conclude that elucidating why these associations exist may help us better understand what controls IOP with potential new targets for glaucoma treatment.

- **NEW TRENDS IN PATHOPHYSIOLOGY.**

Autoimmune responses in Glaucoma. Dr O. W. Gramlich *et al.* (Iowa, USA) demonstrated in mice models that **glaucoma induced T-cell dependent adaptive**



immune processes against retinal ganglion cells (RGC), promoting IOP-independent RGC loss. They proposed that this autoimmune response may explain RGC loss in glaucoma patients despite a significant reduction in IOP. Further studies would provide useful insight into the role of this pathway in the pathophysiology of low-tension glaucoma.

Tau protein in Glaucoma. Dr M. Chiasseu *et al.* (Montreal, Canada) demonstrated that, ocular hypertension (OHT) leads to abnormal translocation of *Tau* protein from RGC axons and thus to *Tau* accumulation in the somato-dendritic compartment of rats RGC. In this animal model, selective knock-down of *Tau* expression with short-interfering RNA (siRNA) led to marked protection of RGC soma and axons from OHT-induced damage, suggesting that ***Tau* protein may play a critical role in glaucomatous neurodegeneration.**

Autophagy in Glaucoma. Recent studies suggest that autophagy, a cellular process by which cells recycle energy and nutrients, may protect cells from stressors. Abnormal autophagy has also been associated with age-related and neurodegenerative conditions. Dr P. B. Liton *et al.* (Durham, NC, USA) demonstrated that autophagy was triggered in the drainage angle, retina and TM of mice in response to elevated IOP. Conversely, in this animal model, an over-activation of autophagy *in vivo* may contribute to the RGC loss and neurodegeneration in glaucoma. Activating this cellular process **is an appealing therapeutic target** to attenuate glaucomatous damage.

Neuroinflammation in Glaucoma. Over the past decade, studies have found that inflammation play a large role in the pathogenesis of ocular diseases such as ocular surface disorders, age-related macular degeneration, etc. Dr A. Sapienza *et al.* (Paris, France) demonstrated that **neuroinflammation within the retina and cerebral structures contributed to glaucoma progression** in a rat model of chronic OHT. These findings help to provide **new insights into the central issues surrounding the pathogenesis of glaucoma, with potential innovative therapeutic issues..**



Optic nerve head and retinal blood flow in Glaucoma. Dr Y. Maiya *et al.* (Sendai, Japan) showed that a **significant reduction in optic nerve head blood flow preceded the development of glaucomatous visual field defects** in patients with pre-perimetric glaucoma and early normal-tension glaucoma, independent of ocular perfusion pressure. Moreover, Dr L. Tobe *et al.* (IN, USA) used *in vivo* human retinal flowmetry to illustrate that **reduced capillary blood flow may predict structural progression in glaucoma.**

Human peripapillary scleral stiffness in Glaucoma pathophysiology. Dr J. Palko *et al.* (Columbus, OH, USA) demonstrated **increased stiffness in the human peripapillary sclera, histologically corresponding to the arterial circle of Zinn-Haller.** During IOP elevation, these regions of increased strains could **influence blood supply to the optic nerve head and the lamina cribrosa (LC)** by the compression and distortion of peripapillary vasculature. Moreover, in such conditions, it **may influence LC biomechanics** in the same way as the translaminal pressure gradient. This finding could define a new risk factor for glaucoma with potential application for glaucoma management diagnosis and treatment.

- **NEW IDEAS IN AQUEOUS OUTFLOW AND TRABECULAR MESHWORK**

Collector channel opening in Glaucoma. Dr M. A. Johnstone *et al.* (Seattle, WA, USA) used *ex-vivo* OCT of non-human primates (NHP) and human eyes to show that **Schlemm's canal, collagen septa at collector channel ostia and collector channels lumen dimensions were rapidly modified by IOP changes.** This mechanism could determine distal outflow system resistance and may be a target for glaucoma treatment.

Influence of vascular endothelial growth factor (VEGF) on conventional outflow facility. Dr D. R. Overby *et al.* (London, UK) assessed the influence of vascular endothelial growth factor (VEGF), which regulates the permeability of vascular endothelia, on the conventional outflow facility and thus on IOP. Since VEGF is present in aqueous humour and VEGF receptors are expressed by Schlemm's canal endothelium, they measured the effects of VEGF and anti-VEGF on outflow facility in mice and humans. **Inhibition of VEGF signalling was observed to lead to a reduction in outflow facility.** Therefore anti-VEGF therapy may contribute to the

sustained IOP elevation reported in a subset of patients treated for retinal disease.

A novel 3D-culture model of trabecular meshwork (TM). Dr Francoise Brignole-Baudouin *et al.* (Paris, France) validated a **novel bioengineered 3D-culture model of TM** using extracellular matrix compound and primary TM cells (ScienceCell) in order to mimic the TM organization *in vivo*. This promising 3D-model may lead to better understanding of TM behaviour under pharmacological, chemical or physical stress conditions and further understand the pathophysiology of this important structure.

A similar study with a co-culture of human Schlemm's canal and TM cells in order to mimic the Schlemm's canal inner wall was reported by Dr K. Y. Torrejon (NY, USA). This 3D tissue provides normal and disease-relevant *in vitro* model systems for understanding the trabecular outflow physiology and its impact on glaucoma pathology.

- **NEW TRENDS IN LAMINA CRIBROSA**

Lamina cribrosa (LC) microstructural deformation is associated with Glaucoma.

Dr B. Wang *et al.* (Pittsburgh, PA, USA) demonstrated that both IOP and cerebrospinal fluid pressure (CSFP) played a key role in the pathogenesis of glaucoma by determining the translaminal pressure difference (TLPD = IOP - CSFP), itself closely correlated with LC thickness and microstructure. Acute *in-vivo* alterations of IOP and CSFP in non-human primates showed that increasing TLPD was associated with **decreased beam thickness** and **increased pore diameter**. Thus, they concluded that **TLPD was significantly associated with LC microstructural deformation**. These *in-vivo* observations emphasize the importance of considering both IOP and CSFP when evaluating the role of the LC in glaucoma pathogenesis. Moreover, Dr J. Albon (Cardiff, UK) also underlined the influence of scleral tension as well as connective tissue stiffness and microarchitecture on TLPD. These factors are modified by ageing (increasing of collagen type I, elastin and thickness of the LC by physiological age-related cross-linking), with potential impact on TLPD and particular relevance to glaucoma, where ageing has been identified as a strong risk factor of the disease .

Hereditary LC microstructural changes may predispose to glaucoma. Dr S.

Zwillinger *et al.* (Paris, France) used *in vivo* adaptive optics to demonstrate **that morphological changes of LC pores seen in glaucoma such as ovalisation and**

enlargement of average surface area were also observed in normal subjects from primary open glaucoma (POAG) families, suggesting that LC changes may precede neuronal loss and may be an additional risk factor for glaucomatous optic neuropathy.

Glaucomatous LC microstructural changes may be partially reversible. Dr R. Abumasmah *et al.* (New York, USA) demonstrated a **partial reversibility of LC changes** characterized by significant anterior repositioning of the LC in case of IOP reduction and an associated slowing of visual field (VF) progression. In this study patients with a greater IOP reduction showed slower VF progression as well as a larger anterior repositioning of the LC. It has yet to be determined whether these changes correspond to a tissue repair or hypotonic oedema, the first assumption bringing new hope for a potential partial reversibility of the glaucomatous damages.

- **NEW TRENDS IN CEREBROSPINAL FLUID PRESSURE (CSFP) MEASUREMENT**

A new formula for cerebrospinal fluid pressure estimates. Recent studies have clearly established a close relationship between CSFP and glaucoma, especially in normal-tension glaucoma (NTG). In order to avoid the invasive lumbar puncture, which is currently the only way to measure CSFP, Dr **Jonas** *et al.* proposed last year a regression formula derived from clinical data such as body mass index, diastolic blood pressure (both positively correlated) and age (negatively correlated) to estimate CSFP (Jonas *et al.*, PLoS One, 2014).

Dr A. E. Kiely *et al.* (Durham) questioned the accuracy of this formula and proposed a new one taking gender into consideration. They concluded that estimated **CSFP derived from clinical data failed to accurately predict CSFP as compared to lumbar puncture.**

- **NEW IMAGING TECHNOLOGIES FOR GLAUCOMA**



Mitochondrial flavoprotein autofluorescence assessment for early diagnosis of glaucoma. The crucial role of mitochondrial dysfunction in the pathogenesis of glaucoma and many other degenerative diseases has been clearly established. Dr A. Pinhas *et al.* (New York, USA) studied the macular **mitochondrial flavoprotein autofluorescence (FPF) in eyes with primary POAG**. They aimed to **quantitatively compare mitochondrial dysfunction** in the maculae and optic nerve of POAG patients and healthy controls, and observed that macular mitochondrial FPF, IOP and cup/disk ratio were weakly correlated. **By detecting an increased mitochondrial dysfunction in POAG, this novel approach may detect functional changes earlier than structural changes.** Nevertheless, the EGP members questioned the possible detection of mitochondrial activity in photoreceptors which could interfere with that in the RGCs.

Detection of apoptosing retinal cells (DARC) and Glaucoma. Dr M-A. Ghaffar *et al.* (London, UK) presented the DARC technology, which enables ***in vivo* real-time non-invasive imaging of apoptosing RGCs** by fluorescent labelling of retinal cells in animal models (mice and rats) of glaucoma and neurodegeneration. Subtraction of fluorescence on one retinal image compared to another is a new method **of spatially identifying newly apoptosing RGCs**, with potential application for glaucoma progression monitoring. A Phase I clinical trial of DARC in glaucoma patients is expected to start shortly.

En-face Doppler OCT in POAG. High-speed swept-source OCT imaging can resolve blood flow pulsatility, allowing accurate mean total retinal blood flow (TRBF) measurement. In addition, several studies have shown associations between the attenuation of retinal vessels and the severity of glaucomatous damage. Dr B. Lee *et al.* (Cambridge, MA, USA) demonstrated that **TRBF was significantly reduced in POAG compared to healthy controls**. This new technology may be **useful for assessing glaucoma progression**, but may **not be a sensitive diagnostic marker for detecting glaucoma because of the large variation of TRBF** in the normal population.



OCT-angiography and glaucoma. OCT angiography by high-speed 1060-nm wavelength swept-source OCT provides a non-invasive method for quantifying disc perfusion. Dr D. Zhu *et al.* from (Los Angeles, CA, USA) demonstrated that the **disc flow index**, a dimensionless parameter measuring vessel area, density and flow velocity, was **significantly reduced** in each level of the retina, choroid and LC in **patients with low or normal tension Glaucoma (NTG)**. These results provide additional insights into the pathologic role of altered perfusion in NTG.

- **NEW TRENDS IN STRUCTURE-FUNCTION CORRELATION**

Individual customised structure-function mapping enhances predictability in detecting Glaucoma. Dr F. Tanabe *et al.* (Osaka-Sayama, Japan) showed that **both optic disc and temporal raphe positions impact the Humphrey Field Analyzer 10-2 test points in the inferior and superior clusters in approximately 46% of normal subjects**. Dr A. M. McKendrick (Melbourne, Australia), demonstrated that **individual, rather than population-based, customising structure-function mapping could compensate variance in optic nerve head position, axial length and position of the temporal raphe**, thus potentially improving diagnosis and monitoring progression of glaucoma within individuals.

Sectorial analysis supplants total analysis in detecting glaucoma. Dr V.M. Danthurebandara *et al.* (Halifax, Canada) demonstrated that, irrespective of the level of visual field damage, **sectorial analysis of both RNFL thickness and Bruch's membrane opening-minimum rim width (BMO-MRW) supplemented total analysis in detecting glaucoma with the same specificity**. Thus, the authors conclude that **sectorial rather than total analysis of these parameters should be used for a better assessment of glaucoma progression**.

A new automated OCT-based 24-2 visual threshold estimation. Dr M. D. Abramoff *et al.* (Iowa, USA), demonstrated that an automated OCT-based 24-2 visual threshold estimation based upon structural information derived from both retinal nerve fibre layer and ganglion cell layer-inner plexiform layer (GCL-IPL) thicknesses had **an incomplete**



but good correlation with SAP SITA Humphrey 24-2 in patients with all stages of open-angle glaucoma { $R=0,68$ (0.47 - 0.82)}, with better intra-individual repeatability (0,98 vs 0,88). This promising approach may **complement VF testing for patients who struggle or cannot perform VF**, and may be adapted to future OCT technology to increase its correlation with subjective VF data.

Imaging improves the accuracy of visual field progression analysis: Prof D. F.

Garway-Heath *et al* (London, UK) demonstrated that imaging could be used to improve the accuracy of VF progression analysis in Glaucoma. A new, more accurate method for VF progression analysis, called '**ANSWERS**' (Analysis with Non-Stationary Weibull Error Regression and Spatial Enhancement), which takes into account increasing measurement variability as glaucoma progresses produces significantly more accurate predictions than ordinary linear regression in predicting future VFs, over a short period of time and with lower prediction errors. Incorporating the rate of structure progression with VF measurements (**sANSWERS**) further improves both prediction accuracy and predictive error. More accurate prediction of visual field will help clinicians to better define individual target pressure.

A new trend-based progression analysis algorithm for OCT. Dr C. K. Leung *et al.*

from the Chinese University of Hong Kong presented a new algorithm for visualizing the topology of progressive RNFL thinning in glaucoma, which was validated on 244 eyes of 140 glaucoma patients followed every 4 months over a mean of 5.8 years. Analysing the "false discovery rate" (FDR) (which represents the area of RNFL detected with false positives) and rates of change of RNFL thinning, the Trend-Based Progression Analysis (TPA) was found to **outperform Glaucoma Progression Analysis (GPA) in detecting more eyes with progressive RNFL thinning at similarly high specificity.**

- **NEW TRENDS IN NEUROPROTECTION**

Stem cell therapies for Glaucoma. Dr B. Mead *et al.* (Birmingham, UK) compared the potential paracrine benefits of intravitreally grafted human-derived mesenchymal stem



cells such as **dental pulp stem cells (DPSC)**, bone marrow-derived mesenchymal stem cells (BMSC) and adipose-derived stem cells (ADSC) as neuroprotective cell therapies for glaucomatous adult rats. As compared to BMSC and ADSC, intravitreally implanted DPSC **failed to penetrate and engraft within the retina but allowed significant increase of RGC survival rate** by releasing of neurotrophic factors, providing a supportive neuroprotective trophic environment for compromised RGC.

Angiotensin 2 receptor inhibitor: Losartan, may have a neuroprotective effect. Dr

H. A. Quigley (Baltimore, MD, USA) assessed the **neuroprotective effect of the angiotensin 2 receptor inhibitor Losartan**, in a murine model of experimental glaucoma. Losartan, which also inhibits transforming growth factor β activity, may play a role in the remodelling of the peripapillary scleral matrix components and have promising subsequent neuroprotective effect upon ganglion cell axons.

Blockade of gap junctions as a novel therapeutic approach for Glaucoma. Dr S.

A. Stewart (NY, USA) showed that **blockade of retinal gap junctions offers significant neuroprotection** in an experimental mouse model of glaucoma. Since secondary cell death via gap junctions plays a critical role in the progression of retinal ganglion cells as well as amacrine cells in experimental glaucoma, a pharmacologic or genetic blockade of gap junctions forms a novel therapeutic approach for neuroprotection in glaucomatous retinae.

- **SURGERY AND WOUND HEALING**

A novel *in-vitro* model of tissue contraction and inflammation. Modulation of wound healing is challenging as both inflammation and fibroblast-mediated tissue contraction underly scarring and treatment failure following glaucoma filtration surgery. G. Sharma *et al* (London, UK) presented a **novel in-vitro model of tissue contraction and inflammation in fibrosis after glaucoma filtration surgery**. This new device could shed light into the role of fibroblasts and macrophages in scarring, and provide the possibility to assess the effect of anti-scarring agents on this process.



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