

## Author Response: Comparison of MicroRNA Expression in Aqueous Humour of Normal and Primary Open-Angle Glaucoma Patients Using PCR Arrays: A Pilot Study

We thank Li and Wang<sup>1</sup> for their interest in our work and thoughtful comments about our recent pilot study on the differential expression of microRNA (miRNA) within the aqueous humor (AH) of patients with primary open angle glaucoma (POAG) and normal controls.<sup>2</sup>

Until recently, the study of miRNA expression within biofluids was hindered by small sample volumes, which led to pooling of samples. However, this can now be overcome either through the use of targeted sample preamplification with low-density polymerase chain reaction (PCR) arrays, as we demonstrated, or through next-generation sequencing with adapter-driven amplification.<sup>3</sup> Analyzing individual AH samples paves the way for future studies that can benefit from increased statistical power and precision. This will further our understanding of the mechanistic role of miRNAs upon the aqueous outflow facility and advance the pursuit of molecular biomarkers of outflow function.

Li and Wang<sup>1</sup> highlight two pertinent issues. First, they point out that comorbidity of dry age-related macular degeneration (AMD) may be a confounding factor in the analysis of AH miRNA expression. Confounding factors, from known and unknown sources, always are a concern in medical research. Our pilot study was not of sufficient size to examine formally whether the presence of AMD may or may not be a confounding factor, since AMD was present only in three of 14 samples analyzed. However, when considering whether a factor is likely to be a confounder, it is important to consider whether its presence provides a reasonable explanation for the observed outcome. The predicted biological impact of altered miRNA expression in these patients was to reduce cell proliferation, extracellular matrix remodeling, endocytosis, Wnt signaling, ubiquitin-mediated proteolysis, and adherens junction function. These biological processes have been described frequently in relation to trabecular meshwork (TM) dysfunction, but are not consistent with contemporary thinking regarding the pathogenesis of AMD. Thus, it is unlikely that the presence of AMD is a confounder for these TM-specific processes. Furthermore, our study specifically identified upregulation of miR-143 in glaucomatous AH. Since the publication of our report, targeted deletion of miR-143 in mice increased outflow facility and reduced IOP, while knockdown of miR-143 in human TM cells regulated actin dynamics.<sup>4</sup> Thus, their results are consistent with ours and independently validate the important role of miR-143 in outflow regulation. Nonetheless, future studies with larger study populations can address this question more thoroughly through the stratification of miRNA expression not only by AMD status, but also by other ocular abnormalities to generate adjusted stratum-specific measures of relative miRNA expression.

Secondly, Li and Wang<sup>1</sup> argue that miRNA expression within plasma is equally important to that of AH. Only approximately 1% of plasma-derived proteins (and presumably miRNAs) are present in AH due to the blood-aqueous barrier.<sup>5</sup> Thus, it is not surprising that we detected six miRNAs that were common to AH and plasma. However, it remains unclear whether the majority of plasma-derived miRNAs affect TM function and outflow regulation. Thus, we would propose, for the study of outflow regulation and IOP, that it would be more prudent to place the emphasis upon identifying AH miRNAs. However, if the intention is to discover novel systemic mechanisms that may underpin the highly complex pathogenesis of POAG, then the study of circulating miRNAs within plasma may be preferable, to include miRNAs known to affect retinal cell function.

Hari Jayaram<sup>1,2</sup>  
Diana C. Lozano<sup>2</sup>  
Elaine C. Johnson<sup>2</sup>  
Tiffany E. Choe<sup>2</sup>  
William O. Cepurna<sup>2</sup>  
Julie A. Saugstad<sup>3</sup>  
John C. Morrison<sup>2</sup>  
Kate E. Keller<sup>2</sup>

<sup>1</sup>Glaucoma Service, National Institute for Health Research Biomedical Research Centre for Ophthalmology, UCL Institute of Ophthalmology & Moorfields Eye Hospital, London, United Kingdom; <sup>2</sup>Casey Eye Institute, Oregon Health & Science University, Portland, Oregon, United States; and the <sup>3</sup>Anesthesiology & Perioperative Medicine, Oregon Health & Science University, Portland, Oregon, United States.  
E-mail: h.jayaram@ucl.ac.uk.

### References

1. Li X, Wang J. Comparison of microRNA expression in aqueous humor of normal and primary open-angle glaucoma patients using PCR arrays: a pilot study. *Invest Ophthalmol Vis Sci.* 2017; 58:4988.
2. Jayaram H, Phillips JI, Lozano DC, et al. Comparison of microRNA expression in aqueous humor of normal and primary open-angle glaucoma patients using PCR arrays: a pilot study. *Invest Ophthalmol Vis Sci.* 2017;58:2884–2890.
3. Wecker T, Hoffmeier K, Plötner A, et al. MicroRNA profiling in aqueous humor of individual human eyes by next-generation sequencing. *Invest Ophthalmol Vis Sci.* 2016;57:1706–1713.
4. Li X, Zhao F, Xin M, et al. Regulation of intraocular pressure by microRNA cluster miR-143/145. *Sci Rep.* 2017;7:915.
5. Fredde TF. A contemporary concept of the blood-aqueous barrier. *Prog Retin Eye Res.* 2013;32:181–195.

Citation: *Invest Ophthalmol Vis Sci.* 2017;58:4989.  
doi:10.1167/iovs.17-22866

