Comparison of Extracellular Vesicle Subpopulations Among Congenital and Adult Glaucoma via Aqueous Humor

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Background

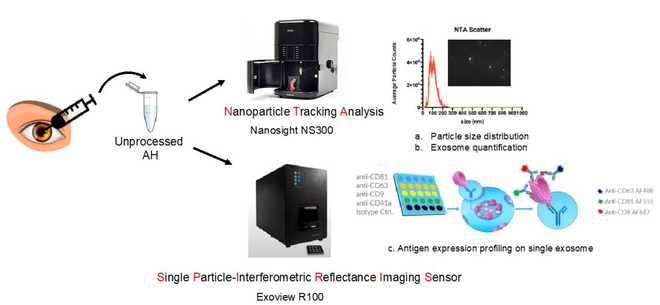
Aqueous humor (AH), the clear liquid in the front of the eye, maintains ocular pressure and provides nutrition. The fluid can be accessed through the clear cornea, and extracellular vesicles (EVs) and small extracellular vesicles (sEVs) can be detected within it. These properties allow AH to be used as a liquid biopsy source of biomarkers for intraocular disease. Such an approach is especially relevant for patients with glaucoma (GLC), as the disease creates a buildup of ocular pressure by blocking AH drainage. Thus, AH extraction serves as treatment as well as sample collection. While both pediatric and adult GLC patients' AH have been explored independently, comparison between the two groups has not been extended to EV analysis. In this study, we use Nanoparticle Tracking Analysis (NTA) and Single Particle-Interferometric Reflectance Imaging Sensor (SP-IRIS) analysis to compare the EV populations of congenital and adult GLC.

Methods

AH samples were taken from 5 pediatric GLC eyes and 5 adult GLC eyes, totaling to 10 processing-free AH samples from 8 GLC patients overall.

10µl unprocessed AH samples underwent Nanoparticle Tracking Analysis using NanoSight. Results were displayed as particle count per size distribution, which also provides quantification of EVs.

Another 10 µl of unprocessed AH underwent Single Particle Interferometric Reflectance Imaging Sensing by Exoview R100. This analysis was performed using the ExoView Human Tetraspanin Kit. The data was then analyzed using ExoView Analyzer 3.0 to obtain antigen expression profiles on each individual exosome.



Results

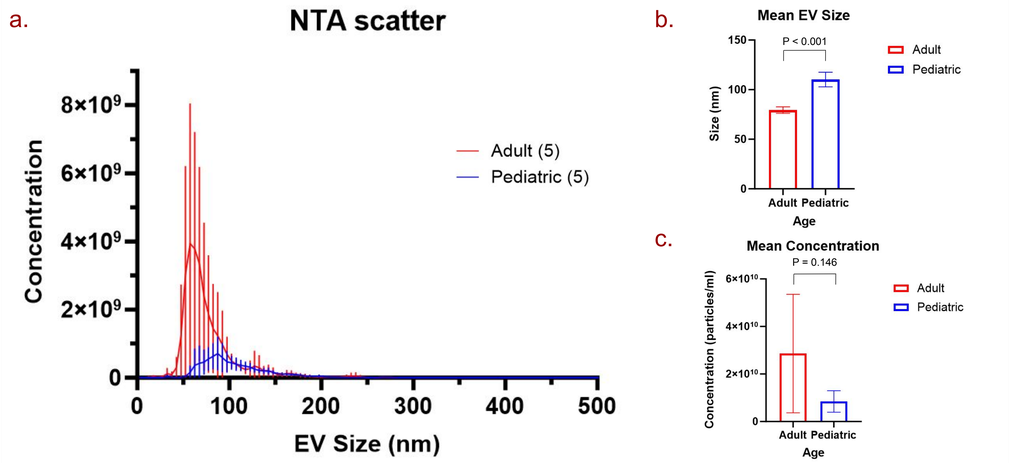


Figure 1. Particle concentration shown by EV size (a). Adult GLC AH exhibits smaller mean EV size than pediatric GLC AH (b), as well as higher mean EV concentration (c). Error bars represent +/- 1 SD.

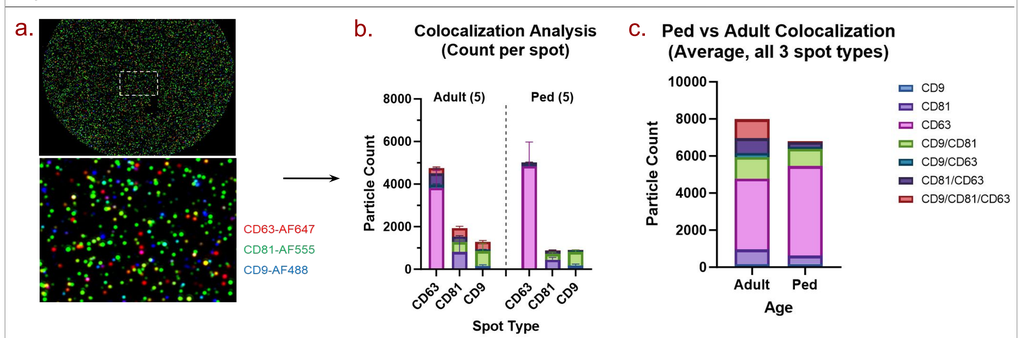


Figure 2. SP-IRIS explored coexpression of tetraspanins via fluorescence on capture beads (a). CD63 is predominant in both populations (b); however, adult AH shows more heterogeneity, with more EVs exhibiting coexpression than in pediatric AH (c).

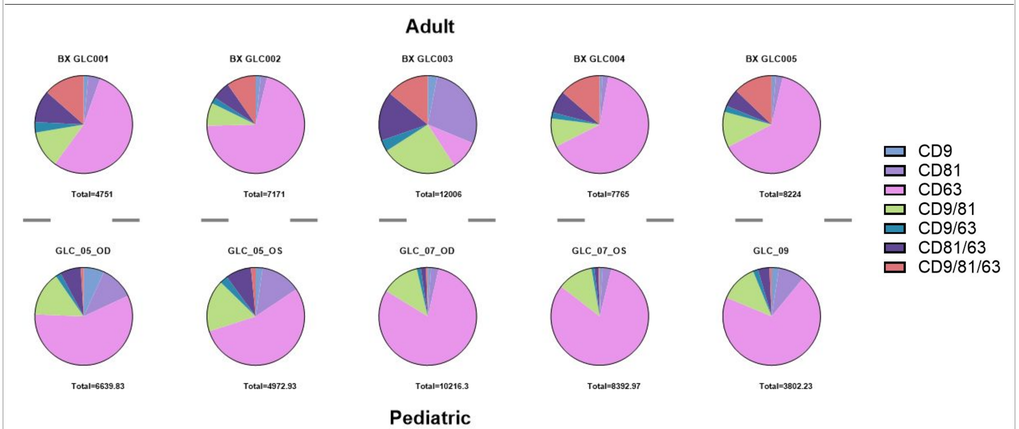


Figure 3. EV population demographics sorted by eye. Adults exhibit more coexpression, particularly of CD9/81/63. BX GLC003 has an unusually smaller CD63 subpopulation.

Conclusions and Relevance

- NTA and SP-IRIS analysis were used to compare the EV subpopulations in AH from pediatric and adult GLC patients.
- NTA revealed that mean EV size in pediatric GLC is significantly larger than mean EV size in adult GLC ($p < 0.001$). However, the difference in average EV concentration between pediatric and adult GLC was not significant ($p = 0.146$) (Fig. 1).
- SP-IRIS shows that CD63 is the predominant tetraspanin, consistent with prior findings. It also indicates that the EV population demographics in adult and pediatric GLC are significantly different ($p < 0.001$), with tetraspanin coexpression being more prevalent in adult GLC AH than in pediatric GLC AH (Fig. 2).
- Some variation in EV demographics exists between the patients of the same group. There is one notable outlier: adult patient BX GLC003, with much less mono-CD63 than any other patient in either group. This could be a major contributor to the discrepancy in average EV population between adults and children described previously (Fig. 3).
- Overall, this study indicates variation in glaucoma's expression in pediatric and adult patients at the EV level. Future studies of this sort with larger sample sizes could better illuminate differences, refining our understanding of GLC's AH EV characteristics. Ultimately, they could further increase the potential of EVs as biomarkers for diagnosis and prognosis.

References

[1]Edward DP, Mahale A, Hao H, Khan A, Hameed S, Al Jadaan I, Owaidhah O, Kondkar A, Al Shamrani M, Al Shahwan S, & K Abu-Amero K (2016). Aqueous humor microRNA profiling in primary congenital glaucoma. *Journal of Translational Science*, 3. 10.15761/JTS.1000169

[2]An HJ, Cho HK, Song DH, Kee C. Quantitative analysis of exosomes in the aqueous humor of Korean patients with pseudoexfoliation glaucoma. Sci Rep. 2022 Jul 27;12(1):12875. doi: 10.1038/s41598-022-17063-9. PMID: 35896586; PMCID: PMC9329372

[3]Peng CC, Im D, Sirivolu S, Reiser B, Nagiel A, Neviani P, Xu L, Berry JL. Single vesicle analysis of aqueous humor in pediatric ocular diseases reveals eye specific CD63-dominant subpopulations. J Extracell Biol. 2022 Apr;1(4):e36. doi: 10.1002/jex2.36. Epub 2022 Apr 20. PMID: 36339649; PMCID: PMC9632627.

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