Reviewer 1

Comment 1.1:

Raphtis et al compare the ocular pulse amplitude, as measured by a standard pneumotonometry technique, in control eyes and eyes from a well-characterized naturally occurring ADAMTS10 mutant Beagle model of glaucoma. The authors found a statistically significant lower OPA in the glaucoma model, which they relate to previous ex vivo studies showing a reduced scleral stiffness in these affected eyes. They also suggest a lower OPA may be significant to the clinical observation that these ADAMTS10 mutants are capable of handling elevated IOP with less glaucomatous damage relative to ocular hypertensive eyes without the mutation (a convincing reference for this is not available).

Response 1.1:

Yes, this is based in clinical observation without any supportive scientific data. This is the focus of currently ongoing investigations. No edits were made in the manuscript to address this comment.

Comment 1.2:

The strengths of the study include the sample size (relatively high for a large animal model of glaucoma), measurement of contributable variables to OPA (e.g., blood pressure, IOP, axial length), wide range of animal ages, exclusion of eyes receiving topical glaucoma therapies (particularly PGAs that may alter biomechanics) and the use of a potentially clinically relevant and easily obtained measurement (i.e., OPA) as a surrogate measure of ocular biomechanics. The authors provide an excellent review of OPA findings in human eyes. The finding of a reduced OPA in the glaucomatous eyes is important, especially given the elevated IOP in these eyes with the expectation of larger OPA. However, additional clarification is needed, particularly in regard to the age breakdown of the affected animals.

Response 1.2:

We thank the Reviewer for the overall positive assessment of our work.

Comment 1.3 (Weakness #1):

The introduction and discussion make some strong statements about the role of ocular biomechanics in glaucoma. Some of these statements need to be rephrased or “toned down” as direct evidence is still lacking. It may also be useful to discuss OPA a bit more in the introduction.

Response 1.3:

…need to modify Introduction…

Comment 1.4 (Weakness #2):

Although previous studies have shown the ADAMTS10 mutant Beagles to have a lower complex modulus, without a more direct mechanical test in these eyes, it may be difficult to claim the difference in OPA is definitively related to scleral biomechanics. The authors do make a compelling argument given their measurements of other variables known to influence OPA. One concern is the influence of the cornea on OPA. Some of these animals develop IOP elevation at a young age. If this is true, and the eyes are on the spectrum of buphthalmia, the cornea may also be larger. A larger cornea, or a cornea contributing to a greater extent of the corneosceral shell surface areas may also reduce OPA. Measurement of corneal diameter in these animals may be revealing.

Response 1.4:

…need to ask Christine about corneal diameters…

Comment 1.5 (Weakness #3):

I would mention that prior ex vivo testing showed a lower normalized ocular rigidity in the ADAMTS10 group compared to normal eyes. This is consistent with your findings.

Response 1.5:

…need to add information…

Comment 1.6 (Weakness #4):

The abstract mentions averaging two eyes for each animal, but this is not seen in the methods section. I have concerns over the approach of averaging both eyes for some of the correlations and statistics. This may be okay when assessing a systemic variable with its relationship to OPA (e.g., VPA), but less so when analyzing the other ocular variables. At the very least, the degree of correlation of the ocular metrics between the left and right eyes should be discussed, and corrected for in the stats if this correlation is low.

Response 1.6:

…need CSTAT…

…see also Comment 2.4…

Comment 1.7 (Weakness #5):

Do the correlations that were tested between the ocular variables include all eyes together (normal and glaucomatous). I would be curious to see if more or stronger correlations exist looking at just the glaucomatous eyes. Particularly between IOP and axial length or OPA and axial length.

Response 1.7:

…need CSTAT…

Comment 1.8 (Weakness #6):

What was the age of the youngest eyes? This is important, and a more detailed age breakdown should be presented. If these animals were not near adulthood, how much did their shorter axial lengths (from not being fully developed) influence the mean axial length measurements, especially for the affected eyes. I would expect longer axial lengths than presented for adult affected eyes. Also, given that a lower OPA would be expected in a younger eye, and it appears there are more young eyes in the affected group – how would it influence your results if the young eyes were excluded (depending on how young they are)?

Response 1.8:

…need to look at ages… …CSTAT…?

Comment 1.9 (Weakness #7):

Is cumulative IOP data available? Even if infrequent, this would strengthen the conclusion - given that remodeling of the sclera is likely somewhat influenced by the cumulative IOP insult over a single measurement.

Response 1.9:

…look for cumulative IOP data…

Reviewer 2

Comment 2.1:

This is a very interesting and important study reporting ocular pulse amplitude (OPA) in a canine model of spontaneous glaucoma. This study showed that the ADAMTS10-mutant dogs with glaucoma had a significantly lower OPA despite a higher, untreated IOP. The authors postulated that this lower OPA is a result of the previous reported biomechanically weaker posterior sclera in the mutant dogs.

Response 2.1:

We thank the Reviewer for the overall positive assessment of our work.

Comment 2.2:

The study has a straightforward experimental design. A few aspects show the rigor. For example, blood pressure and vascular pulse amplitude were also measured in the same animal, providing useful data for interpreting the OPA results. The comparison between TonoVet and Pneumotonometer measurements of IOP is also helpful. The statistical analyses provide details of how the data was analyzed.

Response 2.2:

We thank the Reviewer for the overall positive assessment of our work.

Comment 2.3:

There is however not much discussion about the sample size. Is the sample size suitable (sufficient power) to test the hypothesis (detect difference or correlations)? The mutant group has a much larger sample size than the normal control. Would that create any issue for comparison between groups?

Response 2.3:

…need CSTAT…

Comment 2.4:

Another aspect to clarify is the potential association of the two eyes of the same animal. Most animals had both eyes included in the study. It seemed they were treated as independent samples in the analysis. The outcome may or may not change when the association is considered, but it would be good to check.

Response 2.4:

…need CSTAT…

See also Comment 1.6…

Comment 2.5:

In terms of study design and data analysis, a few other questions need some clarification:

* Was OPA just measured once in each animal?
* How repeatable is the measurement?
* Is age different between the mutant and normal groups?\

Response 2.5:

…

Comment 2.6:

Another strength of this manuscript is the comprehensive citation of the literature related to OPA and ocular rigidity. The connection between OPA and ocular rigidity has been well-recognized, and the current study provides new insight and data that shows ocular rigidity could have a dominant effect on OPA in some cases.

Response 2.6:

We thank the Reviewer for the overall positive assessment of our work.

Comment 2.7:

However, OPA and ocular rigidity/biomechanical properties are quite distinct entities, whereas OPA is influenced also by IOP and the pulsatile blood flow. Thus, this reviewer has difficulty agreeing with statements such as “eye pressure depends on the connective tissue’s mechanical properties or the eye’s rigidity;” “ocular pulse amplitude could be a valuable clinical and diagnostic tool to assess an individual’s scleral biomechanics.” I agree that ocular rigidity has a significant influence on OPA, but it is not the only dominant player. In the case of the mutant dogs, their OPA may be largely influenced by sclera biomechanics. However, it is quite conceivable that there are patients of low OPA due to low pulsatile blood flow rather than low ocular rigidity. Another example that shows ocular rigidity and OPA does not necessarily go hand in hand is that sclera stiffness increases significantly over age, but there is no detectable increase in OPA over age.

Response 2.7:

…

Comment 2.8:

However, OPA and ocular rigidity/biomechanical properties are quite distinct entities, whereas OPA is influenced also by IOP and the pulsatile blood flow. Thus, this reviewer has difficulty agreeing with statements such as “eye pressure depends on the connective tissue’s mechanical properties or the eye’s rigidity;” “ocular pulse amplitude could be a valuable clinical and diagnostic tool to assess an individual’s scleral biomechanics.” I agree that ocular rigidity has a significant influence on OPA, but it is not the only dominant player. In the case of the mutant dogs, their OPA may be largely influenced by sclera biomechanics. However, it is quite conceivable that there are patients of low OPA due to low pulsatile blood flow rather than low ocular rigidity. Another example that shows ocular rigidity and OPA does not necessarily go hand in hand is that sclera stiffness increases significantly over age, but there is no detectable increase in OPA over age.

Response 2.8:

…

Comment 2.9:

One of the main conclusions stated in the paper is: “This study shows that the softer ADAMTS10-mutant sclera results in a significantly smaller OPA than normal.” This reviewer feels this conclusion is not fully supported by the current data. Reasons are:

1. Pulsatile blood flow was not measured in this study. Is there evidence that blood flow was not reduced in the mutant dogs? This should be discussed/acknowledged.

2. From a biomechanical point of view, the larger axial lengths and thus larger eyes (buphthalmos) have a large baseline volume. Even if the pulsatile blood flow (delta\_volume) and stiffness are the same, it could create a smaller OPA (delta\_pressure). The mutant dogs have a larger axial length, which should also partially explain the lower OPA. With that said, it is possible that sclera biomechanics is the dominant factor; but current evidence does not conclusively demonstrate that it “resulted” in lower OPA.

Response 2.9:

…thank the Reviewer for the useful comment…

Comment 2.10:

As stated earlier, this manuscript provides a comprehensive reference list that covers the relevant literature. A few places however may require further attention/clarification.

Response 2.10:

…thank the Reviewer for the useful comment…

Comment 2.11:

Line 208-209: Dastiridou et al 2009 was cited. The study showed a larger OPA in the same eye when IOP was artificially increased. Due to tissue nonlinear biomechanics, ocular rigidity is also increased in the same eye at higher IOP. In essence, that study showed OPA increased in the same eye when IOP was transiently increased and so was ocular rigidity. However, that relationship between OPA and ocular rigidity across different eyes may not be the same and is much more complex, because many other factors impact OPA.

Response 2.11:

…thank the Reviewer for the useful comment…

Comment 2.12:

Line 211-212: The average OPA in this study seems higher than those reported in human eyes (~3 mmHg). This may be related to the generally higher pressure readings by pneumotonometer. Would be good to acknowledge.

Response 2.11:

…thank the Reviewer for the useful comment…

Comment 2.13:

Line 229-233: Sturmer and Kniestedt, 2015 was cited for the opposite groups of studies. That study showed reduced OPA in more severe glaucoma, and thus should only be sited in the latter group. Vulsteke et al., 2008 seems to fit the latter group too. The list of citations needs to be checked.

Response 2.13:

…thank the Reviewer for the useful comment… check references…

Comment 2.14:

Line 302-303: Robert et al 2012 study was cited and stated that “OPA and blood pressure variances ration was a strong diagnostic indicator for early human POAG.” That study only had 10 normal and 11 glaucoma patients and is pilot in nature. The conclusion of being “a strong diagnostic indicator” may be an overstatement.

Response 2.14:

…thank the Reviewer for the useful comment… check references…

Comment 2.15:

A few minor questions for figures: Figure 2: Is Y-axis “mean OPA” or just OPA?

Response 2.15:

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Comment 2.16:

A few minor questions for figures: Figure 3: The trend line may be affected by the few outliers at older age?

Response 2.16:

…

Comment 2.17:

A few minor questions for figures: Figure 4: What is ScanAge?

Response 2.17:

…

1. Age distribution histogram.
2. Axl length and age
3. Excluded young eyes(<1yrs age)
4. Two eye correlations
5. Stratify normal and glaucomous to do correlations.
6. Cumulative IOP

Post hoc power calculation using the observed effect size is not providing any more information other than p-value. In other words, the calculated post-hoc power is completely determined by p-value(Althouse, 2021). High p-value leads to low power, low p-value leads to high power. Therefore, it is not meaningful to calculate post-hoc power. The study was subjected to the constraint of low sample size of normal dogs. The included normal dogs were all the available ones. Furthermore, it is not sensible to discard data from mutant dogs sample to achieve balanced sample size, which discards useful information for mutant dogs parameters’ estimation.

Althouse AD. Post Hoc Power: Not Empowering, Just Misleading. J Surg Res. 2021 Mar;259:A3-A6. doi: 10.1016/j.jss.2019.10.049. Epub 2020 Aug 16. PMID: 32814615.