Peer Review File

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Reviewer A

Comment 1: Throughout the manuscript, the authors grossly over-interpret their results. Examples include but are not limited to:

Reply 1: Thank you very much for your suggestions. We have revised the manuscript according to your comments as followings.

a. L363 – "showed that occult ischemia activated proinflammatory pathways producing massive acute ROS"; the authors have no direct evidence of ischemia or massive amounts of ROS

Changes in the text: We have revised the statement as followings. (Page 19 Line 412-414)

"Our results and previous studies (43,48) indicated that ocular ischemia might activate inflammation pathways and produce massive amounts of ROS, finally cause the damage of RGCs."

b. Lines 370-372 – "we fond increases in Ca2+, the production of ROS and activation of death receptor signaling in the late group"; the authors have not directly measured any of these 3 items.

Changes in the text: We have revised the statement as followings. (Page 20 Line 420-422)

"The IPA function analysis results showed Quantity of Ca^{2+}, Superoxide Radicals Degradation, Death receptor signaling pathway were up-regulated in the late group."

c. Lines 393-397: Both of these sentences do not accurately reflect what was actually found in their study

Changes in the text: We have revised the statement as followings. (Page 21 Line 444-449)

"In this study, the IPA functional analysis indicated that ocular hemodynamic changes and oxidative damage related pathways, such as occlusion in blood vessels, vasoconstriction and Production of NO and ROS, were up-regulated. Therefore, the up-regulation of PTGDS in the late stage might play a protective role during AACG to improve ocular blood perfusion and promote ROS scavenging."

Comment 2: This study would be more meaningful if a control group (age matched

individuals without acute angle-closure glaucoma) was also included in the overall analysis.

Reply 2: Thank you for your suggestion. In this study, we want to investigate the AH proteomic pattern related to VFI and try to find objective biomarker of optic nerve damage. Another control group without AACG might improve the understanding of AACG. This study is a pilot work for AH proteomic analysis of glaucoma, and a large-scale analysis including different glaucoma and control group will be presented in our future work.

Changes in the text: We have added above statements in the manuscript. (Page 22 Line 461-464) "in this study the samples were from one centre, thus a large-scale analysis including different glaucoma and control group (cataract) from multi-centers should be used to validate the conclusions."

Comment 3: Certain experimental details are missing, including:

a. Detail on the acquisition of AH samples; Line 150 - how was total protein concentration measured?; how did the authors exclude the potential for obtaining secondary AH due to breakdown of the blood aqueous barrier?

Reply:

① All study participants underwent an ophthalmic evaluation, including IOP measurement, VFI values measurement, best corrected visual acuity measurement, gonioscopy testing, and fundus examination. The inclusion criteria for AACG were as follows: most of the angle was closed, intraocular pressure was increased, fundus changes and visual field defects were found in glaucoma optic nerve injury. Patients with autoimmune diseases, malignant tumors, severe liver disease and previous ocular surgery were excluded. AH samples were obtained from glaucoma patients during surgery. Each sample was approximately 50 to 200 μL and was aspirated from the anterior chamber using a 26-gauge needle before the start of surgery. After collection, the AH samples were immediately centrifuged at 2,500 ×g for 10 minutes at 4°C to remove the cellular components and debris, and the supernatants were stored at −80°C until further analysis.

②The protein concentrations of AH samples were determined by spectrophotometry based on the Bradford method.

③According to previous study (1) the blood aqueous barrier (BAB) might be damaged in APACG, and related components might be released into AH. In this study the patients were not accept the BAB test (laser flare cell photometry) as previous study (1), therefore, we could not know whether the AH samples were secondary AH. When processing the results, we supposed the number of patients with BAB damage were similar in early and late group, so the proteomic changes from BAB damage would be excluded. This study is a pilot study of glaucoma AH proteome, and to find

the pattern related optic nerve damage. We will include the BAB test in our future work.
Above statements were added to revised manuscript.

Reference：

1. Kong X, Liu X, Huang X, Mao Z, Zhong Y, Chi W. Damage to the blood-aqueous barrier in eyes with primary angle closure glaucoma. *Mol Vis*. 2010;16:2026-2032.

Changes in the text: We have revised our text according to your comments. (Page 8 Line 169-177) "All study participants underwent an ophthalmic evaluation, including IOP measurement, VFI values measurement, best corrected visual acuity measurement, gonioscopy testing, and fundus examination. The inclusion criteria for AACG were as follows: most of the angle was closed, intraocular pressure was increased, fundus changes and visual field defects were found in glaucoma optic nerve injury. Patients with autoimmune diseases, malignant tumors, severe liver disease and previous ocular surgery were excluded. AH samples were obtained from glaucoma patients during surgery. Each sample was approximately 50 to 200 μL and was aspirated from the anterior chamber using a 26-gauge needle before the start of surgery."
And (Page 9 Line 190) "The protein concentrations of AH samples were determined by spectrophotometry based on the Bradford method."
And (Page 22 Line 464-466) "present study did not evaluate the impact factors of AH proteome, therefore, related factors, including age, sex, blood aqueous barrier, etc. should be comprehensively analyzed."

b. Were any of the patients on anti-glaucoma medications? This therapy may have effects on the AH proteome.

Reply: Thank you for your comments. In this study to avoid the drug effects on AH proteome, the AACG patients recruited were treated with the same drug (topical alpha receptor agonists, topical carbonic anhydrase inhibitors, topical beta-blockers and systemic carbonic anhydrase inhibitors). Therefore, the differential proteins were mainly due to the pathological changes of disease.
Changes in the text: We have revised our manuscript. (Page 7 Line 151-153) "All AACG patients were on anti-glaucoma medication with same drugs (topical alpha receptor agonists, topical carbonic anhydrase inhibitors, topical beta-blockers and systemic carbonic anhydrase inhibitors)"

c. Lines 147-148 and line 153: What was the pH of these buffers?

Reply: The pH of lysis buffer was 8, and the pH of 25 mM NH_4HCO_3 buffer was 8,

too.

Changes in the text: We have revised our manuscript. (Page 9 Line 190) "the pellets were centrifuged at 10,000 ×g for 30 minutes and resuspended in lysis buffer (7 M urea, 2 M thiourea, 0.1 M of DTT, and 5 mM of Tris, pH=8)."

And (Page 9 Line 196) "the samples were digested with trypsin (4 μg) in 25 mM NH_4HCO_3 buffer (pH=8)"

d. Line 154: What elution solution was used in this HLPC technique?

Reply: The peptides were washed with 500 μL of 0.1% formic acid and eluted with 500 μL of 100% ACN, then vacuum-dried.

Changes in the text: We have revised our manuscript. (Page 9 Line 198) "the peptides were desalted with a C18 solid-phase extraction column (Waters Oasis, Ireland), washed with 500 μL of 0.1% formic acid and eluted with 500 μL of 100% ACN"

e. Line 145: define "pooled sample"

Reply: Each AH sample was taken in 5μL to pool into a pooled sample. The pooled sample was used as quality control (QC). QC sample was injected frequently to monitoring reproducibility of the LC/MS/MS.

Changes in the text: We have revised our manuscript. (Page 9 Line 185-187) "Each AH sample was taken in 5μL to pool into a pooled sample. The pooled sample was used as quality control (QC). QC sample was injected frequently to monitoring reproducibility of the LC/MS/MS."

f. Line 162-163: unclear how sample fractions were combined

Reply: The eluted peptides were collected at one fraction per minute, and a total of sixty fractions were collected from 1 to 60. Then, the sixty fractions were vacuum-dried. Dried fractions were resuspended in 0.1% formic acid and pooled into 20 samples by combining fractions 1, 21, and 41; 2, 22, 42; and so on.

Changes in the text: We have revised our manuscript. (Page 10 Line 205-208) "The eluted peptides were collected at one fraction per minute, and a total of sixty fractions were collected from 1 to 60. Then, the sixty fractions were vacuum-dried. Dried fractions were resuspended in 0.1% formic acid and pooled into 20 samples by combining fractions 1, 21, and 41; 2, 22, 42; and so on."

g. Need better explanation on statistical analyses; were p-values adjusted for multiple comparisons?

Reply: We have added a statistical section in the manuscript according to your suggestions.

Changes in the text: We have revised our manuscript. (Page 13 Line 271-276) "For DIA

results, differential proteins were defined as up-regulated where abundance was ≥ 1.5-fold increased, or as down-regulated where abundance was ≤ 0.67-fold reduced relative to early stage group. For PRM results, the abundance change of peptides was inspected. Besides a cut-off value of 1.5-fold change relative to early stage group, an adjusted p-value (Bonferroni method) < 0.05 was applied to define differential peptides."

Comment 4: What is the rationale for assuming an equal number of up-regulated and down-regulated peptides, especially given the data presented in Lines 259 and 289 showing a considerable skew in this regulation?

Reply: Thank you for your comments. The normalization method was used in DIA data processing. This normalization method has often been defined as "global" normalization in microarray analyses (1). For a finite RNA sample, when representation of one RNA species increases, representation of other species must decrease. Consequently, approximately the same number of molecules from each sample should hybridize to the arrays and, therefore, the total hybridization intensities summed over all elements in the arrays should be the same for each sample (1). Recent years this normalization technique was used for differential proteomic analysis (2), which the peptide number of up-regulated and down-regulated was similar.

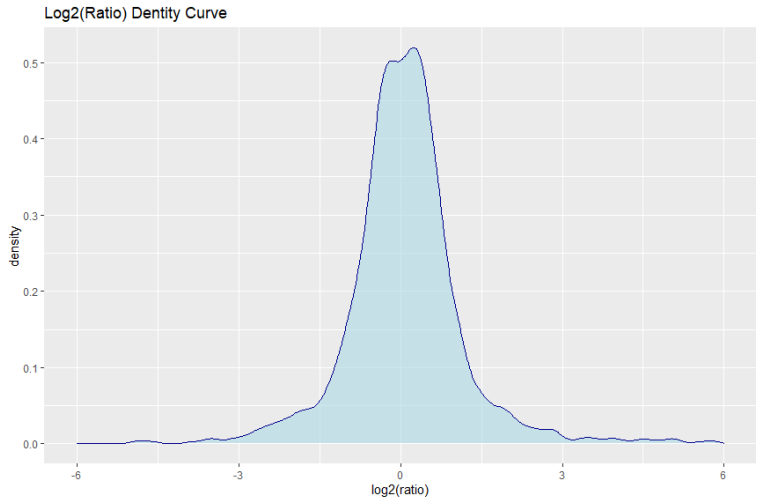
In DIA results of this study, total 369 peptides were found to be up-regulated and 309 peptides down-regulated (Overall distribution of all peptides was shown in following figure). The result was consistent with above assumption. For one protein its abundance was calculated by summing the abundance of all peptides from the protein. Due to the number of peptides from different protein was different, the number of up-regulated and down-regulated proteins showed a skew.

For PRM analysis, only a part of differential proteins and peptides was used to validate DIA results. Above normalization method was not applied in PRM analysis, therefore, the PRM results was not in accordance with above assumption.

Reference：

1. Quackenbush J. Microarray data normalization and transformation. *Nat Genet*. 2002;32 Suppl:496-501.

2. Callister SJ, Barry RC, Adkins JN, et al. Normalization approaches for removing systematic biases associated with mass spectrometry and label-free proteomics. *J Proteome Res*. 2006;5(2):277-286



Changes in the text: There is no changes in the text.

Comment 5: There is a skew in gender demographics (many more females in both groups). Are there known gender differences in the development of acute angle-closure glaucoma?

Reply 5: Thank you for your comment. Previous clinical studies reported that higher incidence of PAACG in female patients in multi surveys, about 2.4-2.6 fold than that in male patients (1-3). Therefore, the AH samples showed a gender skew.

Reference:

1. Seah SK, Foster PJ, Chew PT, et al. Incidence of acute primary angle-closure glaucoma in Singapore. An island-wide survey. *Arch Ophthalmol*. 1997;115(11):1436-1440.
2. Teikari J, Raivio I, Nurminen M. Incidence of acute glaucoma in Finland from 1973 to 1982. *Graefes Arch Clin Exp Ophthalmol*. 1987;225(5):357-360.
3. Park SJ, Park KH, Kim TW, Park BJ. Nationwide Incidence of Acute Angle Closure Glaucoma in Korea from 2011 to 2015. *J Korean Med Sci*. 2019;34(48):e306.

Changes in the text: There is no changes in the text.

Comment 6: Is ocular angiogenesis common in acute angle closure glaucoma? What is the evidence for this occurring in their patient cohorts, other than their interpretation of the AH proteome? The IOPs reported in Table 1 would not be high enough to cause retinal ischemia.

Reply 6: Thank you very much for your comments.

① We tried to looked up related references, but found no clinical reports about angiogenesis in AACG. In ocular hypertension animal model the development of tortuous and dilated retinal vessels throughout the whole retina was observed,

indicating that the increased IOP might account for ocular neovascularization (1). In this study AH proteome data showed that angiogenesis related proteins were found to be differential ones in optic nerve damage. Whether angiogenesis was an important factor for AACG needs more clinical and molecular biology researches.

② In this study, all patients were medicated by anti-glaucoma medication before surgery to control the IOP. And the IOP data was measured before surgery, so it was not significantly high.

Reference:

1. Mukai R, Park DH, Okunuki Y, et al. Mouse model of ocular hypertension with retinal ganglion cell degeneration. *PLoS One*. 2019;14(1):e0208713.

Changes in the text: We have revised our manuscript. (Page 17 Line 366-368)

"Previous study observed the development of tortuous and dilated retinal vessels throughout the whole retina in ocular hypertension mouse model (5). These findings indicated the AACG might be related to ocular neovascularization."

Comment 7: PEDF is considered to be anti-angiogenic, which is the opposite of the authors' interpretations that upregulation of PEDF might promote neovascularization. Figure 6 – how is PEDF involved in pathological neovascularization? It also should be noted that PEDF has been shown to be neuroprotective for RGCs.

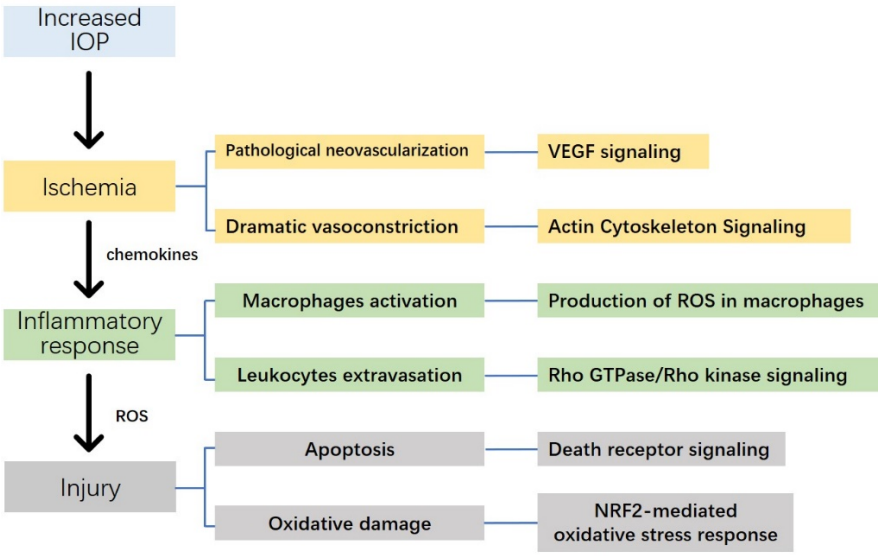
Reply 7: Thank you very much for your comments. We agreed with your options that PEDF was a neuroprotective feature. In Figure 6 we just wanted to show it was related to neovascularization, but it might mislead the readers. Therefore, we deleted it from Figure 6.

The IPA annotated VEGF signaling in pathway analysis which closely related to neovascularization, therefore, we replaced PEDF signaling with VEGF signaling in Figure 6.

Changes in the text: We have revised our manuscript. (Page 18 Line 377)

"The upregulation of PEDF in the late-stage group might promote the regression of neovascularization"

And we revised Figure 6.



Comment 8: Figure 2A: what does the sphere represent?

Reply 8: Thank you for your comments. Figure 2A showed the result of principal component analysis of all samples. PCA reduces the dimensionality of a multivariate data to two or three principal components, which can be visualized graphically with minimal loss of information. The ellipse represents a 95% confidence interval using Hotelling's T2 statistic. Hotelling's T2 statistic is a classical test for testing the location of a multivariate population or for testing the mean difference for two multivariate populations. Observations situated far outside the ellipse are outliers.

Changes in the text: We have revised our manuscript. (Page 32 Line 685) "The ellipse represents a 95% confidence interval using Hotelling's T2 statistic. Observations situated far outside the ellipse are outliers."

Comment 9: Figure 3B-C: what is the authors' interpretation of changes in cell adhesion proteins?

Reply 9: Thank you for your comments. The upregulated cell adhesion proteins in this study were related to cell-ECM adhesions, including Fibrillin-1, Vitronectin, Cadherin. According to previous study (1) in glaucoma the retinal ganglion cells loss is associated with the extracellular matrix (ECM) in the optic nerve head prelaminar region. In the prelaminar region of the optic nerve head the connective tissue sheaths around the capillaries thickened, which would influence the oxygen and nutrition transport, and finally contributing to the retinal ganglion cell loss (2).

Reference:

1. Schneider M, Fuchshofer R. The role of astrocytes in optic nerve head fibrosis in glaucoma. *Exp Eye Res*. 2016;142:49-55.
2. Tektas OY, Lütjen-Drecoll E, Scholz M. Qualitative and quantitative morphologic

changes in the vasculature and extracellular matrix of the prelaminar optic nerve head in eyes with POAG. *Invest Ophthalmol Vis Sci*. 2010;51(10):5083-5091.

Changes in the text: We have revised our manuscript. (Page 14 Line 312-318) "The upregulated cell adhesion proteins in this study were related to cell-ECM adhesions, including Fibrillin-1, Vitronectin, Cadherin. According to previous study in glaucoma the retinal ganglion cells loss is associated with the extracellular matrix (ECM) in the optic nerve head prelaminar region (28). In the prelaminar region of the optic nerve head the connective tissue sheaths around the capillaries thickened, which would influence the oxygen and nutrition transport, and finally contributing to the retinal ganglion cell loss (29)"

Comment 10: Lines 86-88: How can changes in the optic nerve head lead to alterations in the AH proteome? Suggest using the following reference as a potential rationale (PMID: 27453343)

Reply 10: Thank you very much for your comments. According to previous study in glaucoma, the elevated IOP might influence the ciliary muscle and the choroid function (1).

The choroid might stiffen and the tensional forces at the optic nerve head increased, and finally caused optic nerve damage (2). Optical flow analysis showed vitreous fluid move toward optic nerve head (2), therefore, the optic nerve might release damage related components to vitreous fluid (VF). Due to the inter-change between VF and AH, the changes in VF might be reflected in aqueous humor. Therefore, the changes in the optic nerve head might lead to the alterations of AH proteome.

Reference:

1. Aggarwala KRG. Ocular Accommodation, Intraocular Pressure, Development of Myopia and Glaucoma: Role of Ciliary Muscle, Choroid and Metabolism. *Med Hypothesis Discov Innov Ophthalmol*. 2020;9(1):66-70.
2. Croft MA, Lütjen-Drecoll E, Kaufman PL. Age-related posterior ciliary muscle restriction - A link between trabecular meshwork and optic nerve head pathophysiology. *Exp Eye Res*. 2017;158:187-189.

Changes in the text: We have revised our manuscript. (Page 4 Line 101-105) "In glaucoma, elevated IOP would cause the damage to optic nerve (20), and the optic nerve head may release related components to reflect vitreous fluid (21), which might be found in AH due to the vitreous-aqueous exchange. Therefore, it was possible to find optic nerve damage biomarker in AH proteome."

Comment 11: Table 1: were there any statistical differences between groups?

Reply 11: Thank you for your comments. Table 1 showed the clinical information of enrolled patients. All samples were randomly divided into test and validation group. The VFI values between early and late group showed statistical difference. We have added above information in Table 1.
Changes in the text: We have revised our manuscript. (Page 30 Line 669) "[a] $p < 0.05$ for two stages, [b] $p < 0.001$ for two stages."

Comment 12: Table 1: why is the IOP higher in the late disease discovery group, while IOP is higher in the early disease validation group?
Reply 12: Thank you for your rigorous consideration. In this study the IOP was measured before the patients underwent glaucoma surgery. All samples were randomly divided into test and validation group. The IOP between early and late group showed a little difference, but there was no statistical difference between two groups.
Changes in the text: There is no changes in the text.

Reviewer B

Comment 1: It is not clear when aqueous humor was acquired. Aqueous humor was acquired when the authors treated acute angle closure attack? Or after an AAC attack? One year after? 2 years? This information is very important.
Reply 1: Thank you for your comments. Most patients were repeated attacked, and some could not be well controlled. Therefore, they were received surgery. AH samples were obtained from glaucoma patients during surgery.
Changes in the text: We have revised our manuscript. (Page 8 Line 175) "AH samples were obtained from glaucoma patients during surgery."

Comment 2: Samples were randomly divided into two groups, the experimental group (31 patients) and the validation group (20 patients). If the authors divided randomly, the numbers of each group should be almost equal.
Reply 2: Thank you for your comment. In disease biomarker discovery study all samples would be randomly divided into experimental and validation group. To achieve a representative result usually more samples (60-70%) would be used to find the differences and less (30-40%) to validate the results. This strategy had been widely used in biomarker discovery (1,2). In this study we also used above strategy, 31 (60.7%) was used to find the differential proteins, and 20 (29.3%) to validate the results.
Reference:

1. Shi L, Westwood S, Baird AL, et al. Discovery and validation of plasma proteomic biomarkers relating to brain amyloid burden by SOMAscan assay. *Alzheimers Dement*. 2019;15(11):1478-1488.

2. Xuan Q, Ouyang Y, Wang Y, et al. Multiplatform Metabolomics Reveals Novel Serum Metabolite Biomarkers in Diabetic Retinopathy Subjects. *Adv Sci (Weinh)*. 2020;7(22):2001714.

Changes in the text: There is no changes in the text.

Reviewer C:

Comment 1: Even APACG induced in visual loss and the changes of AH proteins. It's not sure that the changes of AH proteins are only factor to induce in VF loss. How to prove it?

Reply 1: Thank you for your comment. In this study we found the AH proteins related to visual loss by a proteomic analysis. For the role of these proteins in visual loss the protein functional analysis in animal model was necessary by overexpressing or knocking out related genes. This study was a pilot study for visual loss, and the function analysis will be presented in the future.

Changes in the text: We have added above statements in the manuscript (Page 22 Line 466-468). "for the protein roles in visual field loss the molecular biology and related animal model should be used, which might find the possible mechanism of VF loss and improve the understanding of VF loss."

Comment 2: AACG patients with a VFI lower than 50 or higher than 80 should be written in the materials and methods parts not in the results parts.

Reply 2: Thank you so much for your suggestions. We have added the description of clinical information to Materials and Methods.

Changes in the text: We have added the description of Clinical information to Materials and methods section.

Comment 3: VFI data was obtained before or after AACG attack?

Reply 3: Thank you for your comments. All study participants underwent an ophthalmic evaluation, including IOP measurement, VFI values measurement, best corrected visual acuity measurement, gonioscopy testing, and fundus examination. The inclusion criteria for AACG were as follows: most of the angle was closed, intraocular pressure was increased, fundus changes and visual field defects were found in glaucoma optic nerve injury. Patients with autoimmune diseases, malignant tumors, severe liver disease and previous ocular surgery were excluded. VFI data was obtained from patients before glaucoma surgery.

Changes in the text: We have added this information in the revised manuscript. (Page 7 Line 155). "VFI data was also obtained before surgery."

Comment 4: All AACG patients were well controlled or not?
Reply 4: Thank you for your comments. Most patients were repeated attacked, and some could not be well controlled. Therefore, they were received surgery.
Changes in the text: There is no changes in the text.

Comment 5: IOP after AACG within both stages was not controlled evenly
Reply 5: Thank you for your comments. All patients were received anti-glaucoma medication before surgery, and the IOP was measured before the glaucoma surgery. The IOP values between early and late group seemed different, but they did not show statistical difference.
Changes in the text: There is no changes in the text.

Comment 6: The AACG patients have been performed cataract or not? All of them should be recorded
Reply 6: Thank you for your comments. None of the AACG patients had cataract.
Changes in the text: We have added above information in manuscript (Page 7 Line 153). "None of them had cataract."

Comment 7: The mean age of the early and late stages in validation groups seems different. Whether age factor might influence AH proteins?
Reply 7: Thank you very much for your comment. Up to now there was no report about the age influence the AH proteome, therefore, we did not know whether it would interference with our results. In the future we will evaluate the possible impact factors on AH proteome, including sex, age and etc.
Changes in the text: We have added above information in manuscript. (Page 22 Line 464-466). "present study did not evaluate the impact factors of AH proteome, therefore, related factors, including age, sex, blood aqueous barrier, etc. should be comprehensively analysed."

Comment 8: What is the collection time of AH? Before or after AACG attack?
Reply 8: Thank you very much for your comments. Most patients were repeated attacked and taken the same drug treatment. All patients underwent a thorough ophthalmic evaluation and met the inclusion criteria. Each sample was approximately 50 to 200 μL and was aspirated from the anterior chamber using a 26-gauge needle before the start of glaucoma surgery. Therefore, AH samples were collected after glaucoma attacked.
Changes in the text: We have added above information in manuscript (Page 8 Line 175). "AH samples were obtained from glaucoma patients during surgery."

Comment 9: Whether AH could reflect changes in different stages of VF loss should be validated more. The study results are not enough supporting the title. Also, other factors such anti-glaucoma drugs, ages, lens condition, IOP level may contribute to the changes of AH proteins.

Reply 9: Thank you very much for your comments.

①In this study we tried to find the AH proteins related to visual loss by DIA analysis, and validated the DIA results by PRM approach. And the functional annotation of differential proteins found they were related to nerve damage, such as PEDF, PTGDS and CDH2. Therefore, AH proteome might reflect the change of visual loss.

But this work was only a pilot study with limit samples from one center. We totally agreed with your comments to validate the results with more experiments, including more samples from multiple centers, functional analysis of differential protein roles in glaucoma by animal model, etc. We are trying to collect more samples to validate the results and to analyze the protein function in animal model. We will provide these data in the future.

②We tried to control the possible impact factors of AH proteomes. All patients were taken the same drug treatment (Page 7 Line 151-153), the lens conditions were similar, and age and IOP showed no statistical significance between two groups (Table 1). Whether other factors might impact the AH proteome will be evaluated in our future work.

Changes in the text: We have added above information in manuscript. (Page 22 Line 461-468). "First, in this study the samples were from one centre, thus a large-scale analysis including different glaucoma and control group (cataract) from multi-centers should be used to validate the conclusions. Second, present study did not evaluate the impact factors of AH proteome, therefore, related factors, including age, sex, blood aqueous barrier, etc. should be comprehensively analysed. Third, for the protein roles in visual field loss the molecular biology and related animal model should be used, which might find the possible mechanism of VF loss and improve the understanding of VF loss."

Comment 10: Line 113-"The goal of this study was to reveal the possible mechanism of VF loss in AACG." However, the conclusion of the abstract is not supporting the title. The given results and conclusions used in this study but not the possible mechanism of the observed results with acute glaucoma (mechanism should be validation by the pathway validation such as protein antibody blocker or other signal transduction assay. Including a sentence on the observed proteomics alterations and their importance in glaucoma may link the conclusions of this study with the title of the manuscript.

Reply 10: Thank you for your suggestions. We totally agreed with your comments and had deleted the related statements in the manuscript.
Changes in the text: We deleted "The goal of this study was to reveal the possible mechanism of VF loss in AACG."

Comment 11: The content of the Introduction part is deficient. It should be discussed more detailed.
Reply 11: Thank you for your comments. We have comprehensively revised the Introduction part in the manuscript.
Changes in the text: We have comprehensively revised the Introduction. (Page 3 Line 68)

"Glaucoma is a progressive optic neuropathy resulting in retinal ganglion cell loss, optic nerve atrophy, and visual field (VF) loss (1). Acute angle-closure glaucoma (AACG) is an ophthalmologic emergency characterized by a rapid increase in intraocular pressure (IOP) due to an impaired outflow of aqueous humor (2) and it usually has a higher incidence in Asians (3). Reduced drainage leads to raised IOP, which potentially causes damage to the optic nerve (4). Despite adequate treatment, 3–12% of patients with acute angle closure develop long-term severe visual impairment, mainly as a result of glaucomatous optic neuropathy (4). Moreover, VF loss is not obvious though under high IOP when AACG attack, it progressively develops after AACG attack (3,5). Glaucomatous optic nerve damage occurs due to retinal ganglion cell (RGC) death (6). To date, many studies have pointed out that ocular hemodynamic changes and vascular pathological changes tend to cause optic nerve ischemic reperfusion injury, eventually resulting in RGC death (7,8). Some molecules injure RGCs in various ways, such as NO, which induces apoptosis and aggravates retinal damage; a high level of glutamate is also closely related to RGC death (9-11). However, a deep understanding of the RGC death mechanism in response to glaucoma is still lacking. Besides, patients with glaucoma can suffer optic nerve damage, progressively and slowly, even in the face of well-controlled IOP (12). Biomarkers reflecting optic nerve damage could be high clinical value. Optic nerve damage secondary to angle-closure leads to vision loss, and it has been traditionally determined by light microscopic evaluation of optic nerve cross sections (12). Currently there has been no reliable biomarkers found to evaluate optic nerve damage. Therefore, an improved molecular investigation may illustrate the relative changes in AACG and offer the evidence for screening biomarkers.

Aqueous humor (AH) is an integral component in many ocular health functions, including nutrients and oxygen supply, removal of metabolic waste, ocular immunity, and ocular shape and refraction (13). The major constituents of AH are proteins (including proteins derived from the protein exchanges across the AH, vitreous fluid,

retina, and optic nerve head), water, and electrolytes (14,15). Although proteins in AH are present in relatively low concentrations compared to blood serum, they are vital for the maintenance of anterior segment homeostasis (15). Proteins secreted from anterior segment tissues play a role in various eye diseases, such as oedema, neovascularization, cataracts, and glaucoma (16-19). In glaucoma, elevated IOP would cause the damage to optic nerve head (20), and the optic nerve head may release related components to reflect vitreous fluid (21), which might be found in AH due to the vitreous-aqueous exchange. Therefore, it was possible to find optic nerve damage biomarker in AH proteome. Technological advancements have allowed for high-throughout proteomic studies examining biofluids such as aqueous humor, vitreous humour, tear, and serum (22). A better understanding of the AH proteomic changes that occur during eye diseases development may provide clues for searching AH biomarkers.

Previous studies have suggested that the AH proteome could reflect alterations in glaucomatous eyes. As early as 2010, Izzotti, A. et al. reported AH proteome alterations in primary open angle glaucoma (23). In 2016, Kliuchnikova, A.A. et al. investigated 29 human AH samples from cataract and glaucoma with and without pseudoexfoliation syndrome patients. They identified 215 proteins in AH from glaucoma samples using high-resolution LC-MS/MS and found that AH proteins could reflect the neural origin of the eye, decreased apolipoprotein D was also defined as a marker of the pseudoexfoliation syndrome (16). Kaeslin, M.A. et al. defined 87 proteins differentially expressed between glaucomatous and control AH, and the differentially expressed proteins were found to be involved in cholesterol-related, inflammatory, metabolic, antioxidant and proteolysis-related processes (24). Recently, in 2019, Wang et al. analyzed the differential expression of AH proteins between acute primary angle-closure glaucoma (APACG) combined with cataracts and cataracts alone and found that the change in proteins in AH was related to the APACG (25). Previous studies have shown that the AH proteome could reflect proteomic changes in glaucoma and provide potential AH biomarkers. To our knowledge, AH proteome studies of VF loss in glaucoma are still unavailable. Therefore, in this study, we investigated the functions of AH proteins in VF loss in glaucoma by applying a proteomic strategy, but more than that, we intended to identify biomarkers to assess optic nerve damage from AH proteins.

AH samples were obtained from early-stage and late-stage patients. The data-independent acquisition (DIA) method was performed to define the differential proteins. The functions of the DEPs were annotated by GO and IPA. Furthermore, parallel reaction monitoring (PRM) was used to validate the key AH proteins. The goal of this study was to investigate the proteomic alterations in AACG and provide helpful clues for finding potential VF loss biomarkers in AH proteins."

Comment 12: It would be a good way to validate the conclusion if the clinical samples

are sufficient to do some molecular biology experiments such as qPCR and western blot analysis.

Reply 12: Thank you for your suggestions. We totally agreed with you that more experiments, such as qPCR and WB would validate our conclusion. However, the total volume of aqueous humor in the anterior chamber is around 150–200 μL. Only about 50-150 μL could be collected for proteomic analysis. Therefore, we could not do related experiments with present volume. We are trying to collect more samples, and will provide molecular biology experiments in our future work.

Changes in the text: We have revised the manuscript. (Page 22 Line 461-468) "First, in this study the samples were from one center, thus a large-scale analysis including different glaucoma and control group (cataract) from multi-centers should be used to validate the conclusions. Second, present study did not evaluate the impact factors of AH proteome, therefore, related factors, including age, sex, blood aqueous barrier, etc. should be comprehensively analyzed. Third, for the protein roles in visual field loss the molecular biology and related animal model should be used, which might find the possible mechanism of VF loss and improve the understanding of VF loss."