• Basic Research •

Impacts of angiotensin II on retinal artery changes in apolipoprotein E deficient mice

Li-Hui Meng[1,2], Shi-Yu Cheng[1,2], He Chen[1,2], Yue-Lin Wang[1,2], Wen-Fei Zhang[1,2], Huan Chen[1,2], Xin-Yu Zhao[1,2], You-Xin Chen[1,2]

[1]Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China

[2]Key Lab of Ocular Fundus Diseases, Chinese Academy of Medical Sciences, Beijing 100730, China

Co-first authors: Li-Hui Meng and Shi-Yu Cheng

Correspondence to: You-Xin Chen and Xin-Yu Zhao. Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China. chenyx@pumch.cn; zhaoxinyu@pumch.cn

Received: 2023-03-14 Accepted: 2023-10-20

Abstract

● **AIM:** To investigate the impacts of angiotensin II (Ang II) on retinal artery changes in apolipoprotein E deficient (*apoE*$^{-/-}$) mice.

● **METHODS:** *ApoE*$^{-/-}$ male mice were infused by minipumps with Ang II at 1000 ng/kg·min (Ang II group) or saline (control group) for 28d. They were underwent ophthalmic fundus examination on day 0, 14, and 28 of infusion. Histopathologic examination, ribonucleic acid (RNA) sequencing and local Ang II measurement of retinas were conducted.

● **RESULTS:** Ophthalmic fundus examination showed Ang II infusion promoted the formation of retinal arterial aneurysm-like lesions on day 28. Optical coherence tomography revealed the ganglion cell and inner plexiform layer (GCIPL) thickness in the control group was significantly thinner than that in Ang II group (P<0.001). Hematoxylin-eosin staining demonstrated diffused swelling of GCIPL layer and its disordered structure in Ang II group. Transmission electron microscopy showed Ang II infusion caused aggravation of atherosclerotic lesions, including increased swelling, roughness, disorganization of the retinal vasculature, and vacuoles formation. RNA-sequencing and gene ontology enrichment analysis demonstrated that the structure and function of cellular membrane might be disturbed and visual function might be compromised by Ang II. The local level of Ang II was higher in Ang II infusion group but did not show significant differences compared to the control group (P=0.086).

● **CONCLUSION:** Ang II infusion promotes the formation of retinal arterial aneurysm-like lesions in *apoE*$^{-/-}$ mice, causing aggravation of atherosclerotic lesions, more severe disorganization of the retinal vasculature and disturbance of the cellular membrane.

● **KEYWORDS:** angiotensin II; retinal artery; aneurysm; *apoE*$^{-/-}$ mice

DOI:10.18240/ijo.2024.01.03

Citation: Meng LH, Cheng SY, Chen H, Wang YL, Zhang WF, Chen H, Zhao XY, Chen YX. Impacts of angiotensin II on retinal artery changes in apolipoprotein E deficient mice. *Int J Ophthalmol* 2024;17(1):16-24

INTRODUCTION

Retinal arterial macroaneurysm (RAM) is an acquired pathological condition characterized by saccular or fusiform dilatation of a retinal artery, relatively common in elderly females with systemic hypertension and arteriosclerotic vascular changes[1-2].

RAM is usually asymptomatic and resolves spontaneously in most cases. However, when hemorrhage and/or exudation affect the macula, severe vision loss occurs despite aggressive treatments including photocoagulation, vitrectomy, or anti-vascular endothelial growth factor intravitreal injections[3-5]. The main pathophysiology of RAM involves weakness of the vascular wall owing to aging and arteriosclerosis, which makes it more susceptible to the increased hydrostatic pressure seen in hypertension, leading to the dilatation of a major retinal arteriole[6-8].

For several decades, the renin-angiotensin system (RAS) has been considered to be crucial in the pathogenesis of many types of vascular diseases, such as hypertension, aneurysms, and vascular injury[9-10]. The main effector peptide of the RAS, angiotensin II (Ang II) plays a critical role in facilitating cell proliferation, apoptosis, fibrosis, oxidative stress, and inflammation which contribute to the remodeling

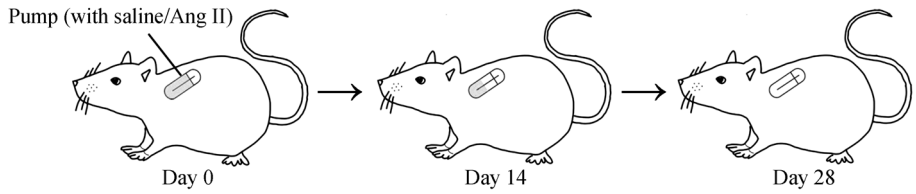

Figure 1 The diagram of the angiotensin II (Ang II)/saline infusion system Sustained-release of saline or 1000 ng/min·kg of Ang II for 28d was accomplished by implantation of minipumps subcutaneously between two scapulae at the back.

of the vasculature[11-12]. It also promotes the development and progression of retinal vascular diseases without exception, presumably through local changes in blood flow and initiation of components of the inflammatory cascade to induce vascular damage[13-14]. Experimental studies have shown that the occurrence of RAM is associated with increased Ang II. Chen *et al*[15] found that infusion of Ang II induced aneurysm formation in the mouse retina, contributing to increased vascular permeability, thickening of ganglion cell and inner plexiform layer (GCIPL), and upregulation of interleukin-1β, platelet-derived growth factor receptor-β, and metalloproteinase-9 expression. However, the specific mechanism of how Ang II causes aneurysms remains elusive and needs further study.

Apolipoprotein E (*apoE*) that locates on chromosome 19 encodes a secreted protein with canonical roles in lipid metabolism[16]. While *apoE* deficient ($apoE^{-/-}$) mice may develop spontaneous hyperlipidemia and atherosclerosis on chow diet[17]. It is well known that chronic infusion of Ang II in the $apoE^{-/-}$ mice could induce the formation of typical abdominal aortic aneurysms (AAA)[18]. However, there has been no research focusing on the role of Ang II in retinal changes of $apoE^{-/-}$ mice.

Therefore, the present study was designed to explore whether Ang II would be important in the development of dilatation of retinal blood vessels and other related changes of the retina in the Ang II-infused $apoE^{-/-}$ mouse model. Hopefully, it could provide clues or reference for further studies about the pathogenesis of RAM.

MATERIALS AND METHODS

Ethical Approval All experimental procedures involving animals were approved by the Animal Care and Use Committee of Peking Union Medical College Hospital (approval number: XHDW-2020-009).

Animals $ApoE^{-/-}$ mice with a C57BL/6J background and male gender were obtained from Charles River Laboratories (Beijing, China). Forty mice at 7mo of age were used in this study. All mice were maintained under barrier conditions with water and a normal laboratory diet available ad libitum.

Infusion of Ang II Infusion of saline or 1000 ng/min·kg of Ang II (Sigma, Missouri, USA) delivered for 28d was accomplished by implantation of Alzet osmotic minipumps (Model 2004; ALZA Scientific Products, California, USA) subcutaneously between two scapulae at the back. Ten mice in the control group were infused with saline, and the other 30 in the experimental group were infused with Ang II (Figure 1).

Determination of Body Weight and Blood Pressure Body weight and blood pressure were measured in all mice at the baseline (day 0) and on day 14 and 28 of infusion. A noninvasive tail-cuff system (MadLab-4C/5H, Beijing, China) was used to measure blood pressure. The mice were trained first to adapt to the device to ensure reproducible measurements. The blood pressure in each mouse was recorded as the average of three consecutive measurements.

Ophthalmic Examination and Images Evaluation Mice were underwent ophthalmic fundus examination on day 0, 14, and 28 of infusion. They were anesthetized and their pupils were dilated before the examination. The fundus photographs were taken first using the MCOLOR mode of Confocal Retina Ophthalmoscope (Suzhou MicroClear Medical Instruments Co., Ltd, Jiangsu, China). Then the optical coherence tomography (OCT) images were obtained with a swept-source OCT device (VG200, SVision Imaging, Ltd., Luoyang, China). At last, 5% fluorescein sodium (50 μL/30 g) was injected intraperitoneally into mice and consecutive fundus fluorescein angiography (FFA) imaging was conducted immediately (Heidelberg Engineering, Heidelberg, Germany). Each mouse had both eyes examined.

The images of these fundus examinations were evaluated by two independent ophthalmologists (Meng LH and Cheng SY) to assess the incidence of retinal aneurysms, vascular changes, and characteristics. The thickness GCIPL was measured by these two ophthalmologists of the central retina on day 28 of infusion. The whole process of evaluation was blinded to the study groups.

Histopathology The whole eyes were fixed with 4% paraformaldehyde and embedded in paraffin. Then 3- to 4-μm histological sections along the cornea-optic nerve axis were cut and stained with hematoxylin-eosin (H&E). The histological slides were coded for blind assessment by two ophthalmologists independently.

Transmission Electron Microscopy After the eyes were enucleated, the cornea was perforated using a needle to create a small hole. The eyes were fixed with 2.5% glutaraldehyde

in 0.1 mol/L cacodylate buffer (pH 7.4). Subsequently, the eyes were post-fixed in 1% OsO_4 for 1h and were then stained with uranyl acetate and resin-embedded. Then eye blocks were sectioned into 90 nm ultra-thin sections and imaged under JEM-1400 electron microscope at 80 kV (JEOL, Japan). The ultrastructure of retinal arteries was assessed by two independent ophthalmologists.

RNA Extraction and Sequencing The whole mouse retinas were harvested from freshly enucleated eyes and immediately frozen in liquid nitrogen. Then the samples were stored at -80℃ until ribonucleic acid (RNA) extraction using the RNeasy kit from Qiagen (Qiagen, Hilden, Germany). Total amounts and integrity of RNA were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technol) before sequencing.

Next, complementary DNA libraries were constructed and quantified by Qubit2.0 Fluorometer and Agilent 2100 bioanalyzer to ensure their quality. Then the library was sequenced on the NovaSeq 6000 System with the basic principle of Sequencing by Synthesis, generating 100-bp paired-end reads. Data was provided in FASTQ format.

For statistical analysis, the clean data were obtained by removing reads that contained adapter, N base and low-quality reads from raw data. All the subsequent analyses were based on clean data with high quality. The RNA sequencing (RNA-seq) reads were mapped on the mouse reference genome (GRCm38) using the program Hisat2 (v 2.0.5). Then the reads numbers mapped to each gene were counted by featureCounts (v 1.5.0-p3). Differential expression analysis of two groups was performed using the DESeq2 R package (1.20.0). For the continuation of subsequent analyses, $P \leq 0.05$ and $|\log2(\text{foldchange})| \geq 0.0$ were set as the threshold for significantly differential expression. Gene ontology (GO) enrichment analysis of differentially expressed genes (DEGs) was implemented by the clusterProfiler R package (3.8.1). $P \leq 0.05$ were considered statistically significant.

Enzyme-linked Immunoabsorbent Assay The eyeballs were quickly extracted after the mice were killed. The whole mouse retinas were gently isolated and stored in the -80℃ refrigerator until protein extraction. The retinas were homogenized under liquid nitrogen in RIPA Lysis Buffer (Servicebio) which contained 100 mmol phenylmethylsulphonyl fluoride. Then samples were sonicated on ice for 20min and then centrifuged at 6000 g for 10min. The supernatants were collected and placed on a 48-well plate. The concentrations of Ang II were measured using the mouse (Ang II) enzyme-linked immunoabsorbent assay (ELISA) kit (#DRE30154, Daucell Biotechnology Co., Ltd).

Statistical Analysis Other statistical analyses except sequencing data was conducted with SPSS software version 22.0 (IBM-SPSS, Chicago, IL, USA). Data were presented as mean±SD. The Student's *t*-test or Mann-Whitney test was performed to compare two sample groups. A *P* value <0.05 was considered statistically significant.

Table 1 The changes of body weight and blood pressure in Ang II infusion group and control group during 28d

Group	Baseline	14d	28d
Body weight			
Control	29.35±1.27	29.67±2.32	30.43±1.54
Ang II	30.15±1.29	30.46±1.49	30.56±0.47
Blood pressure			
Control	81.49±1.29	82.82±0.31	82.4±0.51
Ang II	80.32±1.92	114.62±4.15[a,b]	121.49±6.78[a,b]

[a]*P*<0.05 *vs* baseline; [b]*P*<0.05 *vs* control. Ang II: Angiotensin II.

RESULTS

Body Weight and Blood Pressure Changes The weight and blood pressure was monitored on day 0, 14 and 28. As is shown in Table 1, the body weight gradually increased during the experiment period but did not have significant differences between the two groups. While the infusion of Ang II caused a significant increase in systolic blood pressure compared with the control group and baseline value.

Retinal Arterial Aneurysm-like Lesions in *apoE*$^{-/-}$ Mice Induced by Ang II Infusion Vascular beading indicates alternating areas of constriction in the retinal arteries that appear on the FFA with a repeating pattern of bulging then narrowing. In our study, we found that Ang II infusion could lead to retinal arterial beading in *apoE*$^{-/-}$ mice (Figure 2), whereas nothing occurred in mice infused with saline (Figure 3). In total, 10% (3/30) of *apoE*$^{-/-}$ mice with Ang II infusion developed retinal arterial beadings at day 28. Noteworthy, all of these beads occurred along the major arterial trunks and they became prominent after day 14. The beads were detected by FFA, while color fundus photography and OCT did not show obvious signs. And no obvious vascular leakage was detected during the FFA examination.

Changes of GCIPL Induced by Ang II Infusion Besides, we observed that the thickness of GCIPL was significantly increased by Ang II infusion compared with the control group on OCT images (Figure 4A, 4C, and 4E). The thickness of GCIPL had no significant difference on day 0 between control group (64.35±1.33 μm, *n*=10) and Ang II group (65.31±1.74 μm, *n*=30). While it was significantly thinner in control group than that in Ang II group on day 14 (65.27±2.31 μm, *n*=10 *vs* 71.42±0.87 μm, *n*=30, *P*<0.001) and day 28 (65.41±2.03 μm, *n*=10 *vs* 79.33±2.233 μm, *n*=30, *P*<0.001). Histological examination demonstrated diffused swelling of GCIPL layer and its disordered structure in Ang II infusion group (Figure 4B and 4D).

Ultrastructure Abnormalities As shown in Figure 5, TEM images revealed Ang II infusion caused aggravation of

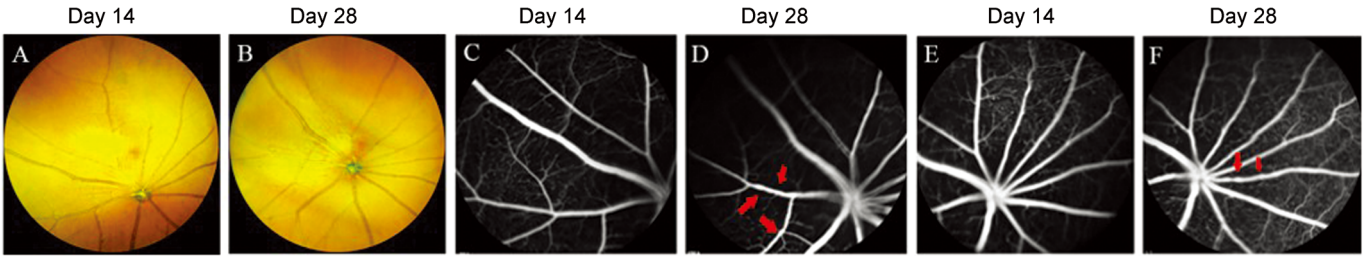

Figure 2 The retinal arterial aneurysm-like lesion (red arrows) in apolipoprotein E-deficient mice with Angiotensin II infusion A: Fundus photograph on day 14; B: Fundus photograph on day 28; C, E: Fluorescein angiography on day 14; D, F: Fluorescein angiography on day 28.

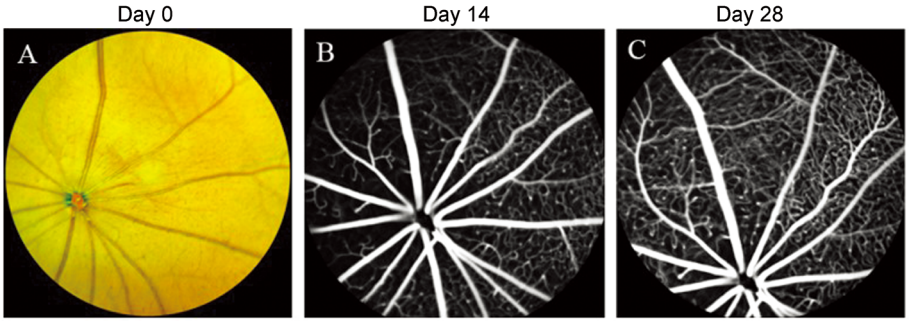

Figure 3 Ophthalmic fundus examination of control group A: Fundus photograph on day 0; B: Fluorescein angiography examination on day 14; C: Fluorescein angiography examination on day 28.

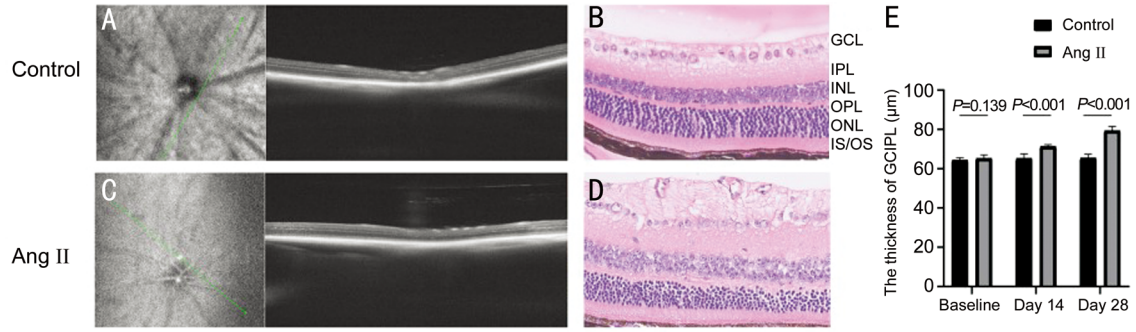

Figure 4 Optical coherence tomography (OCT) and histologic examination of retina on day 28 A: OCT examination of the control group on day 28; B: Hematoxylin-eosin (H&E) stained sections of the retina in the control group; C: OCT examination of the angiotensin II (Ang II) infusion group on day 28; D: H&E stained sections of the retina in the Ang II infusion group demonstrated disorganized ganglion cell and inner plexiform layer (GCIPL); E: The GCIPL thickness in control group was significantly thinner than that in Ang II group on day 14 (P<0.001) and day 28 (P<0.001). GCL: Ganglion cell layer; IPL: Inner plexiform layer; OPL: Outer plexiform layer; ONL: Outer nuclear layer; IS/OS: Inner segment/outer segment.

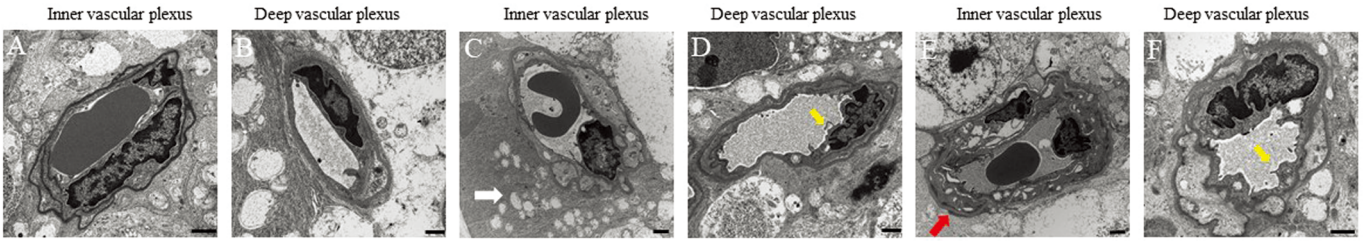

Figure 5 Ultrastructure of the retina on day 28 Transmission electron microscopy demonstrated the swelling retinal vascular wall (red arrow), the roughness of the inner surface (yellow arrow), the thickened and disordered basement membrane as well as formation of many vacuoles around the vessels (white arrow) in angiotensin II (Ang II) infusion group (C-F) compared to control group (A, B). Scale bars: 1 μm (A, C, D, E, F); 500 nm (B).

atherosclerotic lesion of the *apoE*$^{-/-}$ mice. In Ang II group, the retinal vascular wall became much more swelling and the inner surface of the vessels increased the roughness and irregularity. The basement membrane became thickening and disordered. And there was formation of many vacuoles around the vessels.

Differential Retinal Gene Expression Induced by Ang II Infusion RNA-seq was conducted to identify DEGs between Ang II and control groups. The raw sequencing data can be found in GEO database (GSE206970). In total, 4 samples in Ang II group and 3 samples in control group were analyzed. A

total of 962 DEGs (P≤0.05 and |log2(foldchange)|≥0.0) were acquired, including 645 significantly upregulated DEGs and 317 significantly downregulated DEGs. The top ten DEGs which were significantly upregulated by Ang II infusion were *Tyr*, *Gm44250*, *Slc39a12*, *Slc22a8*, *Entpd4b*, *Slc4a5*, *Tbc1d1*, *Flvcr2*, *Wls* and *Apold1*. And the downregulated were *Ier2*, *Ckmt1*, *Gm3756*, *Vcp-rs*, *Egr1*, *Gm10154*, *Igfbp3*, *Hspd1-ps3*, *Slco4a1* and *Gm9826*.

Functional Enrichment Analyses of DEGs GO enrichment analysis was performed to explore the functional characteristics of the DEGs. The results suggested that in upregulated DEGs, the top three GO terms that were most significantly enriched in Biological Process (BP) were “anion transport”, “organic anion transport”, and “camera-type eye development”. For Cellular Components (CC), the top three were “basolateral plasma membrane”, “extracellular matrix”, and “basement membrane”. And for Molecular Function (MF), they were “secondary active transmembrane transporter activity”, “symporter activity”, and “cytokine binding” (Figure 6A). In downregulated genes, the top three GO terms in BP were “visual perception”, “sensory perception of light stimulus”, and “purine ribonucleoside monophosphate metabolic process”. For CC, the top three terms were “photoreceptor outer segment”, “photoreceptor cell cilium”, and “9+0 non-motile cilium”. And for MF, they were “3’,5’-cyclic-nucleotide phosphodiesterase activity”, “cyclic-nucleotide phosphodiesterase activity”, and “3’,5’-cyclic-GMP phosphodiesterase activity” (Figure 6B). The GO enrichment dot plots of the significantly upregulated genes and the downregulated genes were shown in Figure 6C and 6D.

Levels of Ang II in the Retina To evaluate the effect of systemic Ang II infusion on the local level of Ang II in retinas, we performed ELISA to detect its exact concentration in Ang II group and control group. As is demonstrated in Figure 7, the mean level of Ang II in control group (43.17±41.34 ng/L, n=5) was lower than that in Ang II group (82.84±18.39 ng/L, n=5) but the difference was not statistically significant (P=0.086).

DISCUSSION

This study aimed to establish whether an infusion of Ang II is associated with retinal aneurysm formation. All experiments were performed in *apoE*$^{-/-}$ mice that presented hyperlipidemia when a normal diet was fed. Seven-month-old age mice were chosen since the previous study found that the development of aortic aneurysm lesions was most prominent at this time[17]. We hypothesized that hyperlipidemia in *apoE*$^{-/-}$ mice could cause atherosclerosis, which increases the rate of retinal aneurysm formation. However, the result showed that only 10% of mice eventually developed arterial bead-like changes and no definite retinal aneurysm formation was observed. Hyperlipidemia is not a necessary factor for RAM formation since it was also successfully induced in wild-type C57BL/6 mice with normal lipid metabolism[15]. Another possible cause is that the formation of RAMs may be longer in a hyperlipidemic state, as we only found bead-like changes after 28d of Ang II infusion, rather than typical aneurysm manifestations. Further studies are needed to investigate whether *apoE*$^{-/-}$ and wild-type C57BL/6 mice affect the incidence, the size and the shape of RAMs.

Ang II is a potent hypertensive agent, and 73% of patients with retinal aneurysms were reported to be accompanied by hypertension[1]. Our report showed a sustained increase in arterial blood pressure over 28d of Ang II infusion which was more pronounced in the first 14d than in the last 14d. However, this is in contrast to past studies in which the results of Daugherty *et al*[19] showed that Ang II infusion of anesthetized mice caused no significant change in systolic blood pressure compared with vehicle-infused controls. It is presumed that effects including elastin degradation and macrophage accumulation of Ang II would occur independently of elevations in blood pressure[20]. Studies of cerebral aneurysms have shown that more than 50% of aneurysms ruptures are associated with transient hypertension, emphasizing the significance of mechanical events and indicating that such events may play a part in RAM. Cassis *et al*[21] found that mean arterial pressure increased to a similar extent in *apoE*$^{-/-}$ mice infused with Ang II or norepinephrine and that 50% of the Ang II-infused group developed AAA. In addition, hydralazine administration to the Ang II-infused group reduced systolic blood pressure without preventing AAA formation of atherosclerosis, suggesting that aneurysms formation induced by Ang II infusion is independent of the elevated blood pressure. Taken together, the role of hypertension in aneurysm formation remains to be explored, and it can be suggested that the elevated hemodynamic effect acts on the structurally abnormal vessels caused by Ang II, indirectly leading to the development of RAMs.

FFA is one of the most important diagnostic criteria for patients with RAMs. FFA of fusiform RAMs demonstrates rapid filling in the early arterial phase, while saccular RAMs show complete filling in the middle to late phases. The fluorescence of RAMs is generally irregular inhomogeneous filling, which is possibly related to clot formation or endothelial cell proliferation[22]. Our results showed significant arterial bead-like changes which became apparent over time without vascular leakage. The changes most commonly arise on the first or second order of the arterial tree, where the perfusion pressure is higher and thus causing the weak stretched vessels to relatively easily perforated. No significantly morphologic changes were found in fundus color photography and OCT. This study observed the segmental changes in arteries, which

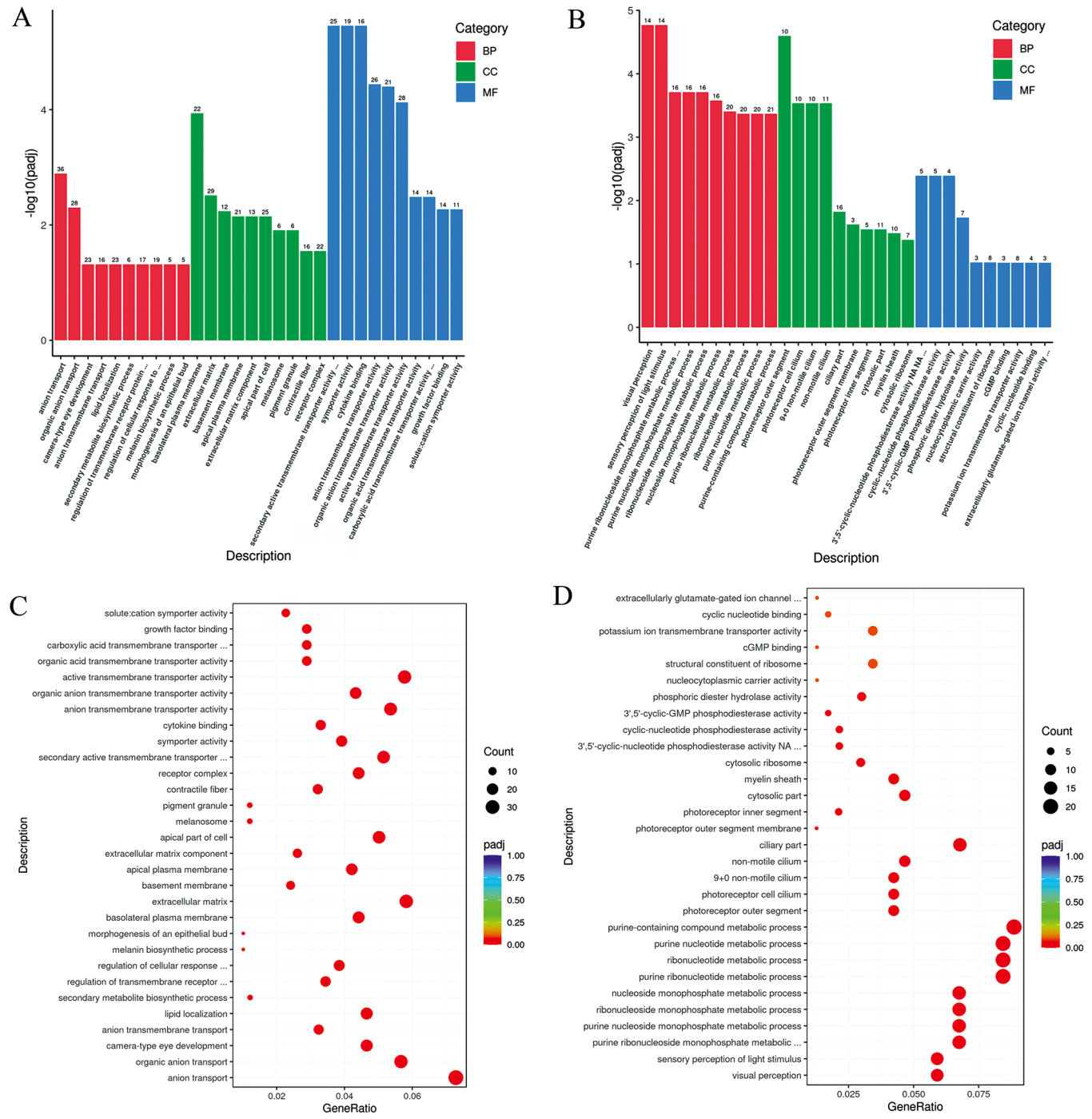

Figure 6 Functional enrichment analysis of DEGs A: GO enrichment analysis histogram of the significantly upregulated DEGs; B: GO enrichment analysis histogram of the significantly downregulated DEGs; C: GO enrichment dot plot of the significantly upregulated DEGs; D: GO enrichment dot plot of the significantly downregulated DEGs. BP: Biological Process; CC: Cellular Components; MF: Molecular Function; DEG: Differentially expressed gene; GO: Gene ontology.

is similar to the histopathologic findings in human RAMs in which many aneurysms of varying sizes are present along the retinal arteries morphologically[23].

Ang II-induced vascular abnormalities are well demonstrated. We have noticed thickening of the GCIPL through OCT measurement and H&E staining, hypothesizing a diffuse swelling as an early sign of Ang II-induced mice. The possible pathway of GCIPL thickening might be through the vasculature, that is, the development of leaking vessels and diffuse edema. Wang *et al*[24] found an increase in retinal vascular permeability and arteriolar tortuosity in Ang II-infused wild-type mice, which caused central retina thickening, especially in the inner plexiform layer and inner nuclear layer. Histopathological studies revealed thickened vessel walls with hyaline, fibrin, and foamy macrophages in the RAM area. Aneurysms developed progressively from wall thickening to hemorrhagic aneurysms with linear division of the vessel wall, which elucidate the well-accepted clinical course of RAM[23].

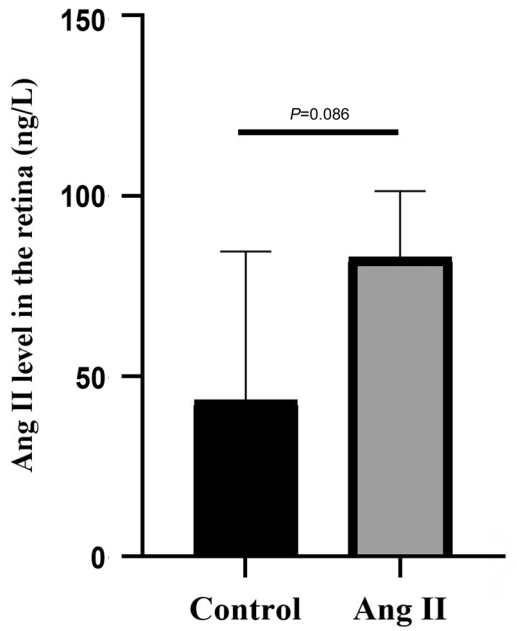

Figure 7 The mean level of angiotensin II (Ang II) in control group (*n*=5) was lower than that in Ang II group (*n*=5) but the difference was not significant (*P*=0.086).

Ang II infusion was found to cause vascular inflammasome activation, leading to endothelial dysfunction and vascular remodeling through *ex vivo* and *in vitro* experiments[25]. Perumal *et al*[26] found that exogenous Ang II administration significantly induces dynamics of proteins implicated in actin cytoskeleton-mediated remodeling of the ophthalmic artery. Our results also showed that Ang II significantly leads to the vascular pathological structural features, including the degeneration of vascular wall and disruption of endothelial cells, as well as basement membrane structures, resulting in segmental weakness of the vessel wall. Despite the well-characterized vascular injury induced by Ang II, many unresolved issues exist in the mechanisms involved.

The underlying mechanisms of Ang II infusion promoting retinal aneurysm-like lesions formation in *apoE*$^{-/-}$ mice are not well understood. To explore the mechanisms behind it, we conducted the RNA-seq and GO enrichment analysis to identify the DEGs and biological function enrichment genes. Our results showed that "anion transport", "basolateral plasma membrane" and "secondary active transmembrane transporter activity" represented significant GO terms for the upregulated DEGs in aspects of BP, CC, and MF respectively. In the literature, there has been some direct and indirect evidence related to the changes in the above functions caused by Ang II. It has been reported that Ang II receptor antagonists had a potent inhibitory effect on the urate/anion transport in the human renal proximal tubule[27]. In the myocardium, researchers found Ang II could activate protein kinase C and increase anion exchange activity, contributing to the development of cardiac hypertrophy[28]. The epithelial cell lines from the proximal tubule of kidney showed that Na/H exchanger activity is regulated by Ang II[29]. As for the down-regulated DEGs, "visual perception", "photoreceptor outer segment" and "3',5'-cyclic-nucleotide phosphodiesterase activity" represented significant GO terms in aspects of BP, CC and MF respectively. These terms have a close relationship to visual function, which suggested that the visual function might be impaired due to Ang II. In fact, RAS plays an important role in visual function from various aspects[30-32]. Researchers have found that RAS could promote age-related macular degeneration in mouse models and angiotensin II type 1 receptor (AT1R) blocker could reversed the retinal pigmented epithelial cell condition and visual function[30]. AT1R blockade might prevent light-induced retinal neural tissue damage[33]. In retinal inflammation, retinal protein expression and visual function are disturbed. It was reported that AT1R blocker demonstrated neuroprotective effects and prevented these signs through the reduction of local Ang II expression[34]. Our results were consistent with these findings and hinted that the structure and dynamic changes of cellular membrane and visual function test like electroretinogram might be the future research directions.

In addition, we measured the Ang II concentrations in the retina. The results showed that the Ang II level in Ang II infusion group was higher than that in control group but there was no statistically significant difference between the two groups. Due to the small sample size, we could not discard one of the data which appeared probably abnormal. Besides, according to the reference by Senanayake *et al*[35], in human retinas, Ang II levels had a wider range compared with the vitreous, ranging from 1 to 329 pg/mL and 5 to 367 pg/mL in non-diabetic and diabetic samples, respectively. We hypothesized the mice might have the same condition. Further research will be conducted to validate this with larger sample size. We deduced that his phenomenon might be due to the following reasons. On the one hand, systemic Ang II infusion had an impact on its local concentration in the retina. The elevated Ang II caused retinal arterial changes and aneurysm-like lesion formation in *apoE*$^{-/-}$ mice. On the other hand, the local RAS system might be disturbed by the systemic infusion of Ang II. It has been reported that the components of RAS, such as renin and angiotensin-converting enzyme, whose messenger RNA and protein were found both in the retinal pigmented epithelial cell and neural retina of the eye[36]. And some researchers found that the existence of local production of angiotensin peptides because ocular angiotensin concentrations were too high to be caused by blood-borne peptides[37]. Since we could not distinguish the source of Ang II, we hypothesized that the endogenous Ang II production might be slightly compromised by the exogenous Ang II. Further studies about the relationship

between endogenous and exogenous Ang II were needed.

There were some limitations in this study. First, we focused on the effects of Ang II infusion on the retina, while we did not observe the changes in other organs at the same time. Since the model we used was mature in AAA formation, further studies could investigate whether there were potential associations between AAA and retinal changes. Second, the local changes of ocular Ang II might be better to explore its specific role than systemic infusion. Intravitreal or subretinal injection of Ang II could be considered as a tool to make local changes to RAS components. Third, a treatment group, like using an AT1R blocker, might be needed to further confirm the role of Ang II in aneurysm-like lesion formation. Besides, the differences of RAM formation between *apoE*$^{-/-}$ mice and wild type mice and investigating the role of hyperlipidemia are interesting points to study in the future.

In conclusion, this study investigated the impacts of Ang II infusion on the retina in *apoE*$^{-/-}$ mice. We demonstrated that Ang II infusion induced the formation of retinal arterial aneurysm-like lesions and aggravated atherosclerotic lesion of retinal vessels in *apoE*$^{-/-}$ mice. Structures and function of cellular membrane might be disturbed and visual function might be impaired by Ang II. Further studies about how Ang II exerts its effects are needed.

ACKNOWLEDGEMENTS

Authors' contributions: Meng LH and Cheng SY carried out the devise, the experiment and manuscript drafting. Chen H contributed to statistical analysis and manuscript drafting. Zhang WF and Wang YL helped devise the study. Chen H helped revise the manuscript. Chen YX and Zhao XY conceived of the study, coordinated and participated in the entire process of drafting and revising the manuscript. All authors read and approved the final manuscript.

Foundation: Supported by Peking Union Medical College Hospital Deposit Integration Commission Funds (No. ZC201904168).

Conflicts of Interest: Meng LH, None; **Cheng SY,** None; **Chen H,** None; **Wang YL,** None; **Zhang WF,** None; **Chen H,** None; **Zhao XY,** None; **Chen YX,** None.

REFERENCES

1 Chen H, Zhao XY, Meng LH, Zhang WF, Chen YX. Clinical characteristics of retinal arterial macroaneurysms and prognosis of different interventions. *Graefes Arch Clin Exp Ophthalmol* 2022;260(2):439-450.

2 Gamal El-Deen AM, Gamal-Edeen AM. Triple retinal arterial macroaneurysms in a hypertensive patient with hypothyroidism. *BMC Ophthalmol* 2023;23(1):207.

3 Kim JH, Kim JW, Kim CG, Lew YJ, Cho HJ. Influence of bevacizumab therapy and intraretinal hemorrhage in long-term outcomes of hemorrhagic retinal arterial macroaneurysm. *Sci Rep* 2021;11(1):14246.

4 Zhang S, Wang CH, Cao GF, Xu XZ, Wang JA. Outcomes of combined treatments in patients with retinal arterial macroaneurysm. *Indian J Ophthalmol* 2021;69(12):3564.

5 Wang YL, Du H, Zhao XY, Meng LH, Chen YX. Observation, laser photocoagulation or anti-VEGF therapy in the management of retinal arterial macroaneurysms. *BMC Ophthalmol* 2022;22(1):417.

6 Speilburg AM, Klemencic SA. Ruptured retinal arterial macroaneurysm: diagnosis and management. *J Optom* 2014;7(3):131-137.

7 Zienkiewicz A, Francone A, Cirillo MP, Zompa T, Charles M. Near-infrared reflectance imaging to detect an incipient retinal arterial macroaneurysm. *Case Rep Ophthalmol* 2021;12(1):150-153.

8 Chen J, Zou YL. Advances in the diagnosis and treatment of retinal artery macroaneurysms. *Guoji Yanke Zazhi* (*Int Eye Sci*) 2023(3):395-399.

9 Bekassy Z, Lopatko Fagerström I, Bader M, Karpman D. Crosstalk between the renin-angiotensin, complement and kallikrein-kinin systems in inflammation. *Nat Rev Immunol* 2022;22(7):411-428.

10 Xia HQ, Yang JR, Zhang KX, Dong RL, Yuan H, Wang YC, Zhou H, Li XM. Molecules related to diabetic retinopathy in the vitreous and involved pathways. *Int J Ophthalmol* 2022;15(7):1180-1189.

11 van Thiel BS, van der Pluijm I, te Riet L, Essers J, Jan Danser AH. The renin-angiotensin system and its involvement in vascular disease. *Eur J Pharmacol* 2015;763:3-14.

12 Phipps JA, Dixon MA, Jobling AI, Wang AY, Greferath U, Vessey KA, Fletcher EL. The renin-angiotensin system and the retinal neurovascular unit: a role in vascular regulation and disease. *Exp Eye Res* 2019;187:107753.

13 Wilkinson-Berka JL, Suphapimol V, Jerome JR, Deliyanti D, Allingham MJ. Angiotensin II and aldosterone in retinal vasculopathy and inflammation. *Exp Eye Res* 2019;187:107766.

14 Igić R. Four decades of ocular renin-angiotensin and kallikrein-kinin systems (1977-2017). *Exp Eye Res* 2018;166:74-83.

15 Chen H, Zhao XY, Chen YX, Deng TT. Angiotensin II is a crucial factor in retinal aneurysm formation. *Exp Eye Res* 2021;213:108810.

16 Yang LG, March ZM, Stephenson RA, Narayan PS. Apolipoprotein E in lipid metabolism and neurodegenerative disease. *Trends Endocrinol Metab* 2023;34(8):430-445.

17 Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb* 1994;14(1):133-140.

18 Xue F, Yang J, Cheng J, Sui W, Cheng C, Li H, Zhang M, Zhang J, Xu X, Ma J, Lu L, Xu J, Zhang M, Zhang Y, Zhang C. Angiotensin-(1-7) mitigated angiotensin II-induced abdominal aortic aneurysms in apolipoprotein E-knockout mice. *Br J Pharmacol* 2020;177(8):1719-1734.

19 Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E–deficient mice. *J Clin Invest* 2000;105(11):1605-1612.

20 Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin

II-infused, apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2003;23(9):1621-1626.

21 Cassis LA, Gupte M, Thayer S, Zhang X, Charnigo R, Howatt DA, Rateri DL, Daugherty A. ANG II infusion promotes abdominal aortic aneurysms independent of increased blood pressure in hypercholesterolemic mice. *Am J Physiol Heart Circ Physiol* 2009;296(5):H1660-H1665.

22 Moosavi RA, Fong KS, Chopdar A. Retinal artery macroaneurysms: clinical and fluorescein angiographic features in 34 patients. *Eye (Lond)* 2006;20(9):1011-1020.

23 Fichte C, Streeten BW, Friedman AH. A histopathologic study of retinal arterial aneurysms. *Am J Ophthalmol* 1978;85(4):509-518.

24 Wang S, Li J, Bai J, Li JM, Che YL, Lin QY, Zhang YL, Li HH. The immunoproteasome subunit LMP10 mediates angiotensin II-induced retinopathy in mice. *Redox Biol* 2018;16:129-138.

25 Cau SB, Bruder-Nascimento A, Silva MB, Ramalho FNZ, Mestriner F, Alves-Lopes R, Ferreira N, Tostes RC, Bruder-Nascimento T. Angiotensin-II activates vascular inflammasome and induces vascular damage. *Vascul Pharmacol* 2021;139:106881.

26 Perumal N, Straßburger L, Herzog DP, Müller MB, Pfeiffer N, Grus FH, Manicam C. Bioenergetic shift and actin cytoskeleton remodelling as acute vascular adaptive mechanisms to angiotensin II in murine retina and ophthalmic artery. *Redox Biol* 2020;34:101597.

27 Momoniat T, Ilyas D, Bhandari S. ACE inhibitors and ARBs: managing potassium and renal function. *Cleve Clin J Med* 2019;86(9):601-607.

28 Bhullar SK, Dhalla NS. Angiotensin II-induced signal transduction mechanisms for cardiac hypertrophy. *Cells* 2022;11(21):3336.

29 Woost PG, Orosz DE, Jin WW, Frisa PS, Jacobberger JW, Douglas JG, Hopfer U. Immortalization and characterization of proximal tubule cells derived from kidneys of spontaneously hypertensive and normotensive rats. *Kidney Int* 1996;50(1):125-134.

30 Nagai N, Kawashima H, Toda E, Homma K, Osada H, Guzman NA, Shibata S, Uchiyama Y, Okano H, Tsubota K, Ozawa Y. Renin-angiotensin system impairs macrophage lipid metabolism to promote age-related macular degeneration in mouse models. *Commun Biol* 2020;3(1):767.

31 Choudhary R, Shree J, Singh A, Bodakhe SH. Role of the renin–angiotensin system in the development of cataract formation in angiotensin-II-induced experimental rats. *J Biochem Mol Toxicol* 2021;35(7): e22789.

32 Hirooka K, Kiuchi Y. The retinal renin-angiotensin-aldosterone system: implications for glaucoma. *Antioxidants* 2022;11(4):610.

33 Narimatsu T, Ozawa Y, Miyake S, Nagai N, Tsubota K. Angiotensin II type 1 receptor blockade suppresses light-induced neural damage in the mouse retina. *Free Radic Biol Med* 2014;71:176-185.

34 Kurihara T, Ozawa Y, Shinoda K, Nagai N, Inoue M, Oike Y, Tsubota K, Ishida S, Okano H. Neuroprotective effects of angiotensin II type 1 receptor (AT1R) blocker, telmisartan, via modulating AT1R and AT2R signaling in retinal inflammation. *Invest Ophthalmol Vis Sci* 2006;47(12):5545.

35 Senanayake PD, Drazba J, Shadrach K, Milsted A, Rungger-Brandle E, Nishiyama K, Miura SI, Karnik S, Sears JE, Hollyfield JG. Angiotensin II and its receptor subtypes in the human retina. *Invest Ophthalmol Vis Sci* 2007;48(7):3301.

36 Wagner J, Jan Danser AH, Derkx FH, de Jong TV, Paul M, Mullins JJ, Schalekamp MA, Ganten D. Demonstration of renin mRNA, angiotensinogen mRNA, and angiotensin converting enzyme mRNA expression in the human eye: evidence for an intraocular renin-angiotensin system. *Br J Ophthalmol* 1996;80(2):159-163.

37 Danser AHJ, van den Dorpel MA, Deinum J, Derkx FHM, Peperkamp E, de Jong PTVM, Schalekamp MADH. Prorenin in vitreous and subretinal fluid of the human eye. *Clin Exp Hypertens A* 1988;10(6):1297-1299.