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Original article

# APOE2 promotes the development and progression of subretinal neovascularization in age-related macular degeneration via MAPKs signaling pathway

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## ABSTRACT

**Background:** Neovascular age-related macular degeneration (nvAMD) is one of the main pathological features of wet AMD. Apolipoprotein E2 is involved in the formation of nvAMD but the molecular mechanism has not been reported.

**Methods:** The APOE alleles in AMD patients were detected by genotyping. Mouse models were divided into 4 groups according to transfection different gene segments and laser-induced treatment. APOE2, VEGF, PDGF-BB, b-FGF and inflammatory cytokines (including p-NF-κB, TNF-α, IL-1β and IL-6) were tested by ELISA in mice retinal lysate. The formation of nvAMD in the indicated treatment groups at 3rd, 7th and 14th day after laser-induced damage were detected by FFA. Besides, qRT-PCR was used to determine the mRNA levels of p38, JNK and ERK in ARPE-19 cells. Finally, the inflammatory cytokines and MAPK proteins (including P38, p-P38, JNK, p-JNK, ERK and p-ERK) were detected by western blot.

**Results:** The statistics of APOE alleles showed that APOE2 allele carriers were more likely to nvAMD. VEGF, PDGF-BB, b-FGF and related inflammatory cytokines were up-regulated significantly after treatment with APOE2, which were reduced after silencing the MAPK family genes, however. Further, the expression levels of neovascular growth factors and inflammatory cytokines were highly consistent between mouse models and ARPE-19 cells. Besides, the phosphorylation levels of p38, JNK and ERK were affected by APOE2.

**Conclusion:** nvAMD was affected directly by the overexpression of VEGF, PDGF-BB and b-FGF, which were regulated by APOE2 through activating MAPKs pathway.

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## 1. Introduction

In age-related macular degeneration (AMD), especially exudative AMD, choroidal neovascularization (CNV) is one of the major pathological features that causes serious complications including leakage of fluid, lipids and blood in retina leading to irreversible blindness (Yanagi et al., 2019; Li et al., 2020). According to the

survey, 8.7% of the worldwide population suffered from AMD in 2014 (Wong et al., 2014). In addition, one of the critical forms of advanced AMD is neovascular AMD (nvAMD), which shows proliferative growth and leakage of extraretinal vessels (SanGiovanni and Lee, 2013).

Apolipoprotein E (APOE) confers genetic risk of many diseases related to aging. In three specific APOE isoforms (APOE2, APOE3 and APOE4) of humans, APOE2 allele increases the risk of developing AMD (Toops et al., 2016; Yang et al., 2017). Serum APOE regulates the expression of inflammatory cytokines and vascular endothelial growth factor (VEGF) family of cytokines in retinal pigment epithelial (RPE) cells (Qureshi and Ambreen, 2017). Higher possibilities of developing AMD in patients carried with APOE2 allele has been verified, APOE2 increases the content of VEGF in retinas, which further promotes the development of nvAMD (Wickremasinghe et al., 2011; Levy et al., 2015a,b).

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nvAMD is directly affected by the VEGF family including VEGF, placental growth factor (PGF) and platelet-derived growth factor (PDGF) (Siedlecki et al., 2017; Balser et al., 2019). Fibroblast growth factor (FGF) directly regulates the growth of lymphatic and capillaries vessels as well (Li et al., 2020). In the eyes, visual signaling is predominately mediated by dimerization of PDGF-BB (Siedlecki et al., 2017). Clinically, inhibitors targeting VEGF and FGF have been applied in the treatment of retinal leakage and neovascularization (Blasiak et al., 2013; Balser et al., 2019). VEGF is essentially modulated by mitogen-activated protein kinase (MAPK) signaling pathways including ERK1/2, JNK1/2/3, p38 MAPK and ERK5 (Kyosseva 2016). These MAPK pathways mediate cell differentiation, movement and proliferation through activating transcription factors (Nagai et al., 2009; SanGiovanni and Lee, 2013). Besides, activated ERK1/2 and p38 MAPK have pro-inflammatory and proangiogenic properties. Studies have shown that MAPKs pathway promotes the upregulation of VEGF related factors, which leads to nvAMD (Nagai et al., 2009; Kyosseva, 2016; Busch et al., 2018). The accumulation of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  reduces after the addition of inhibitors of MAPKs which confirms MAPK is an upstream activator of inflammation-related NF- $\kappa$ B pathway (Vyasa et al., 2014; Zhang et al., 2019). However, whether APOE influences nvAMD via MAPK pathway is unclear.

Immortalized APRE19 cell line and mice are usually used as model systems to determine the expression of cytokines and growth factors in AMD (Sullivan et al., 1997; Goverdhan et al., 2013; SanGiovanni and Lee, 2013; Levy et al., 2015a,b; Liu et al., 2017; Yang et al., 2017). In the present study, we evaluated the enhancement of ocular neovascularization by APOE2 through activating MAPK signaling pathway in RPE cells and mice separately.

## 2. Materials and methods

### 2.1. Samples and fundus fluorescein angiography (FFA)

342 AMD patients diagnosed by FFA and 105 health were selected in the department of ophthalmology in Heze Municipal Hospital. Before FFA examination, 5 ml of venous blood was drawn without empty stomach and centrifuged at room temperature and stored at  $-80^{\circ}\text{C}$ . The contrast agent was injected into elbow vein and a fundus camera with a set of color filters was used for continuous shooting and tracing the contrast agent in a dynamic developing process. The location and area of the lesions were measured by software generated grids and measurement tools. The informed consents were obtained from all patients selected and this study was approved by the Affiliated Hospital of Heze Medical College Ethics Committee. All experiments were performed in accordance with the Declaration of Helsinki.

### 2.2. Mice and nvAMD mouse models

All experimental procedures complied with the rules of Laboratory and the Affiliated Hospital of Heze Medical College Animal Ethics Committee approved the study protocol. C57BL/6J mice were operated for the verification of animal model. APOE Knockout (APOE-KO) C57BL/6J mice were purchased from Biocytogen (C57BL/6-Apoetm1Bcgen, Beijing, China). The homologous recombination of human APOE2 gene through embryonic stem cells (ES cells). Human APOE2 gene was expressed after selection of homozygous positive progeny. In brief, the human APOE2 vector was constructed and transplanted into the 4–6-week-old APOE-KO surrogate mothers, and the homozygous human APOE2 recombinant mice were obtained by hybridization (h-APOE2-TR).

Laser coagulation was performed on the mouse models with 532 nm green laser. The mice were abdominal anesthetized, and

the pupils were dilated by agentia injection before laser coagulation. Laser irradiations of mice peripheral optic nerve (energy 125 mw, duration 100 ms and spot size 100  $\mu\text{m}$ ) were performed seven times according to previous research (Balser et al., 2019) and the following procedures were performed on 3rd, 7th and 14th day. Each group contained at least 5 mice. And the formation of laser-induced nvAMD was confirmed by FFA. The mice were anesthetized and the pupils were dilated. 2.5% fluorescein (Alcon, USA) was diluted with normal saline before intraperitoneal injection. And images were taken after 10 min of fluorescein administration. The mice were divided into the following 4 groups: APOE-KO, APOE-KO+Laser, h-APOE2-TR and h-APOE2-TR+Laser.

### 2.3. GeneChip assay

Genomic DNA was extracted from the collected venous blood samples using TIANamp Blood DNA kit (Tiangen, Beijing, China). This kit provided a DNA purification method based on centrifugal column adsorption. Genotype of 112 sites (rs429358) and 158 sites (rs7412) in APOE gene were detected with APOE genotype detection kit (GeneChip Assay, Sinochips, Zhuhai, China). Briefly, the DNA samples were amplified by C1000 thermal cycler (Bio-rad, USA) (45 cycles,  $94^{\circ}\text{C}$  for 30 s and  $65^{\circ}\text{C}$  for 45 s). Then, the amplified products were detected with an automatic Gene chip detection system and the genotyping results were listed.

### 2.4. ELISA

The levels of serum APOE2 was detected by ELISA (RayBio<sup>®</sup> Human ApoE2 ELISA Kit, CA, USA) and phospho-p38 MAPK (p-p38), phospho-JNK1/2/3 (p-JNK), phospho-ERK1/2 (p-ERK), phospho-NF- $\kappa$ B (p-NF- $\kappa$ B), TNF- $\alpha$ , IL-1 $\beta$ , IL-6, VEGF and PDGF-BB in retinal lysate of mice were also measured by ELISA kit (Sigma-Aldrich, USA). In addition, b-FGF ELISA kit was purchased from ThermoFisher (ThermoFisher scientific, shanghai, China). According to manufacturer's protocol, the corresponding standards or samples were added into 96-well plates separately and incubated for 2.5 h at room temperature. After washing, biotin antibody was added and the plates were incubated for 1 h. Then streptavidin solution was added into the plate and incubated for 45 min. Next, the TMB One-Step Reagent and Stop Solution were added in order and the light intensity was measured by enzyme-labeled instrument (Varioskan LUX, ThermoFisher USA).

### 2.5. Human RPE cell culture

Human RPE ARPE-19 cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 100  $\mu\text{g}/\text{ml}$  streptomycin (Invitrogen, Carlsbad, USA) and incubated in a humidified atmosphere with 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$  for 48 h. Knockdown ARPE 19 cells were established using si-p38, si-JNK and si-ERK. Briefly, 20 nM siRNA (including p38, JNK and ERK) and 2 $\mu\text{L}$  transfection reagent were added to serum-free growth medium and incubated for 5 min at room temperature (Wang et al., 2014). The combined solution was mixed with cells and cells were transfected for 24 h. Then the ARPE-19 cells were transferred into growth medium to incubate for 24 h. Next, the transfection efficiency of siMAPKs was detected by qRT-PCR.

### 2.6. Methylthiazol tetrazolium (MTT) assay

The routine MTT assay (Sigma-Aldrich, USA) was used to determine the cell viability. In brief, ARPE 19 cells were inoculated into 96-well-plates, incubated at 5%  $\text{CO}_2$   $37^{\circ}\text{C}$  until the plate covering with cells. APOE2 (Sigma-Aldrich, USA) was dissolved with 0.1% BSA buffer. Fresh culture medium was prepared with different

concentrations of APOE2 (50, 100, 200, 300, 400, 500 nM) and the cells were maintained for another 24 h. After that, the supernatant was removed and fresh culture medium and MTT solution were added in turn, following continuous incubation for 4 h. Removed the supernatant, formazan solution was added into the culture. The absorbance was detected at 490 nm by enzyme-labeled instrument (Varioskan LUX, ThermoFisher, USA).

### 2.7. Quantitative real-time RCR (qRT-PCR)

RNA Isolation Kit (Trizol Reagents, ThermoFisher, USA) was used for the extraction of total RNA from ARPE 19 cells. Then the Reverse Transcriptase Assay Kit (EnzChek®, ThermoFisher, USA) was applied to reverse-transcribe RNA into cDNA. Green PCR Master Mix (Power SYBR®, ThermoFisher, USA) was chosen and the StepOne 7500 real-time PCR system (Applied Biosystems, USA) was applied in qRT-PCR processes. The synthetic mRNA primer (Sangon Biotech, China) sequences were used as shown in Table 1. The expression levels of mRNA was normalized to  $\beta$ -actin and the relative level was calculated via  $2^{-\Delta\Delta Ct}$  method.

### 2.8. Western blot

The antibodies related to NF- $\kappa$ B and MAPK signaling pathway were purchased from ThermoFisher (USA). VEGF, PDGF-BB and b-FGF were purchased from Santa Cruz Biotechnology (USA). ARPE 19 cells were lysed on ice with pyrolysis liquid RIPA for the extraction of cell total protein. SDS-PAGE was used to isolate proteins transferred from the strips to polyvinylidene difluoride (PVDF). The PVDF membrane was blocked by 5% milk for 1 h before incubation with specific primary antibodies. The antibodies were diluted as follows: APOE2 antibody (1:1000), IL-1 $\beta$  Antibody (1:1000), IL-6 antibody (1:500), TNF- $\alpha$  Antibody (1:500), p-NF- $\kappa$ B (1:1000), phospho-ERK1/2 (p-ERK) Antibody (1:1000), ERK1/2 Antibody (1:1000), phospho-JNK1/2/3 (p-JNK) Antibody (1:1000), JNK1/2/3 Antibody (1:1000), phospho-p38 MAPK (p-p38) Antibody (1:1000), p38 MAPK Antibody (1:1000), VEGF Antibody (1:800), PDGF-BB Antibody (1:800) and b-FGF Antibody (1:800). PVDF membrane was washed again with ice-cold PBS followed by incubation with horseradish peroxidase-conjugated goat antibodies against rabbit IgG (1:500, Sigma, USA). Then ELC solution was

added to expose the strip. The grey value was quantified and normalized with control group by ImageJ software.

### 2.9. Statistical analysis

The Fisher exact test was conducted to obtain the probabilities of whether there was an association between the two kinds of AMD and different genotypes or alleles. Each experiment was repeated three times. The figures were plotted by Graphpad Prism 8. Mean  $\pm$  SD model was used for statistics and the  $P$  value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. APOE2 allele increased the susceptibility to nvAMD

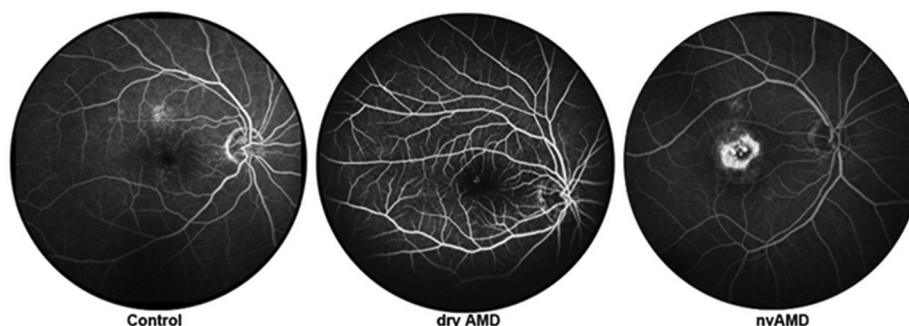
Clinical nvAMD symptom was often observed with FFA. This was evaluated by measuring lesion size and leakage area, and used to evaluate clinical care and the response to treatment in clinical trials. The patients were divided into dry AMD and nvAMD through joint diagnosis of FFA (Fig. 1) and ICGA. The occurrence frequencies of APOE alleles presented in AMD patients and controls were concluded in Table 2. We observed a significantly higher occurrence frequency of APOE2 allele in nvAMD patients (15.04%) compared with control (6.19%) and dry AMD groups (8.45%) ( $p < 0.05$ ). The results of  $\epsilon 3$  and  $\epsilon 4$  alleles analysis showed that there was no significant difference between occurrence frequencies of the two alleles and AMD risk. Meanwhile, the occurrence frequency of APOE2 homozygous genotype ( $\epsilon 2/\epsilon 2$ , 9.76%) in nvAMD patients was higher compared with control (0.00) and dry AMD groups (0.91%) ( $p < 0.05$ ). These findings indicated that the APOE2 allele brought a high risk of nvAMD than other alleles.

### 3.2. Laser injury accelerated the formation of nvAMD and the MAPKs pathway was over-activated in nvAMD mice

To confirm the construction of nvAMD mice model induced by laser injury, FFA was used to detect the vessel leakage of mouse eyeballs on the 3rd, 7th or 14th days of laser injury. The development of nvAMD in these groups was shown in Table 3. The results

**Table 1**  
Primers.

	forward primer	reverse primer
ERK	5'-CCTAAGGAAAAGCTCAAAGA-3'	5'-AAAGTGGATAAGCCAAGAC-3'
p38	5'-TGACTTGC TTCCTGTTCTTGA-3'	5'-TTTGAAAATGTGTCCACAGAGG-3'
JNK	5'-GGATATAGCTTTGAGAACTCTTCC-3'	5'-TCTAACTGCTGTGTCAGGGATCTT-3'
$\beta$ -actin	5'-CTTAGTTGCGTTACACCTTCTTG-3'	5'-CTGTACCTTCCAGTTCAGTTC-3'



**Fig. 1.** FFA images of Control (left), dry AMD (middle) and nvAMD (right).

**Table 2**  
Frequencies of APOE Alleles and Genotypes in AMD patients and Controls.

	Control n(%)105	Dry AMD n(%)219	nvAMD n(%)123	P value
Allele				
ε2	13(6.19)	37(8.45)	37(15.04)	0.0492
ε3	167(79.52)	341(77.85)	177(71.95)	0.0832
ε4	30(14.29)	60(13.70)	32(13.01)	0.156
Genotype				
ε2/ε2	0(0.00)	2(0.91)	12(9.76)	0.012
ε2/ε3	11(10.48)	27(9.59)	9(7.32)	0.052
ε3/ε3	71(67.62)	140(63.93)	74(60.16)	0.351
ε4/ε2	2(1.90)	6(2.74)	4(3.25)	0.369
ε4/ε3	14(13.33)	34(15.52)	20(16.26)	0.698
ε4/ε4	7(6.67)	10(4.57)	4(3.25)	0.215

**Table 3**  
The formation of nvAMD with different treatments.

	0d	3d	7d	14d
APOE-KO	normal	normal	normal	normal
APOE-KO+Laser	normal	normal	early AMD	dry AMD
h-APOE2-TR	normal	early AMD	early AMD	nvAMD
h-APOE2-TR+Laser	normal	nvAMD	leakage	leakage

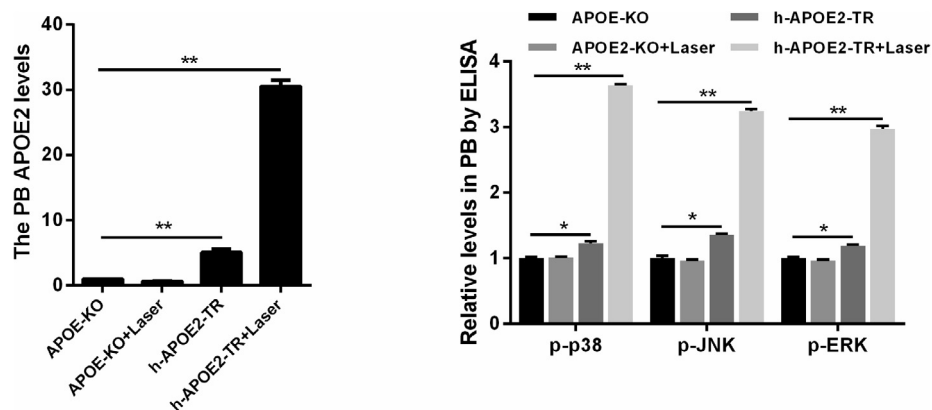
showed a smaller number of nvAMD mice and there was no leakage in h-APOE2-TR group. Besides, extensive nvAMD leakage and a greater number of nvAMD mice were detected in h-APOE2-TR+Laser group, which proved laser injury accelerated the formation of nvAMD. To confirm the correlation between APOE2 and proteins of MAPKs pathway in nvAMD mice, the levels of APOE2 and phospho-MAPKs proteins in peripheral blood were detected by ELISA (Fig. 2), the results showed a slight increase of these proteins expression levels in h-APOE2-TR mice, and these proteins were significantly upregulated in h-APOE2-TR+Laser mice compared with APOE-KO and APOE-KO+laser group. All these findings suggested that laser-induced the aberrant activation of MAPKs pathway, thereby accelerating nvAMD formation.

### 3.3. APOE2 promoted the expression and secretion of inflammatory cytokines

ARPE-19 cells were cultured and simulated the generation of nvAMD in retina. Cells were treated with different concentration of APOE2 (50, 100, 200, 300, 400, 500 nM), and the results of MTT which represented the corresponding cell viability were normalized in Fig. 3A. The cell viability decreased with the increase of APOE2, which indicated significant correlation between them ( $p < 0.01$ ). Besides, more than 50% cells lost their viability when treated with 400 nM or 500 nM of APOE2. Thus, 300 nM was

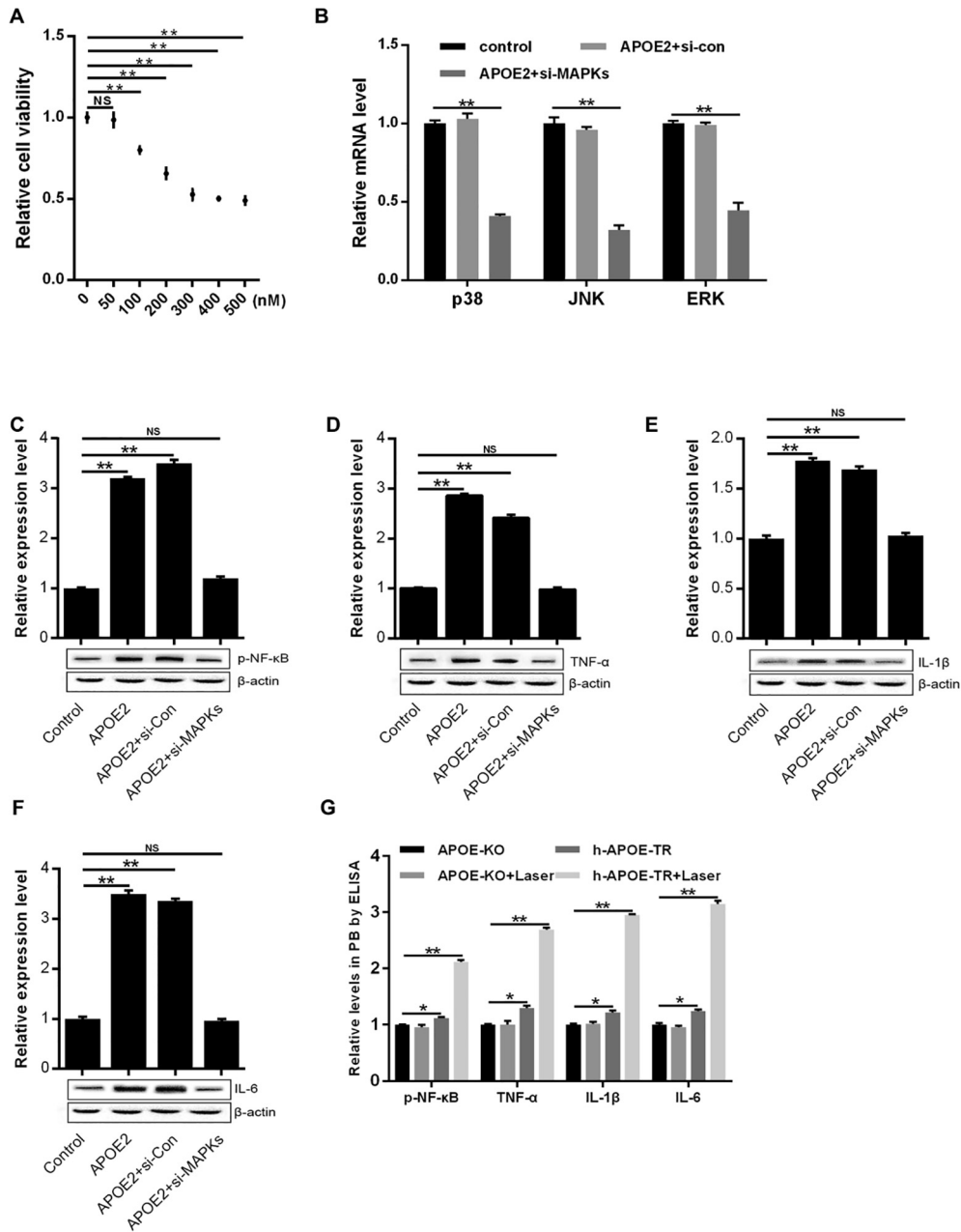
selected as the appropriate concentration of APOE2. Then the ARPE-19 cells were divided into APOE2 treated groups (including APOE2, APOE2+si-Con and APOE2+si-MAPKs group) and a control group (without APOE2 treatment). The transfection efficiency of si-p38, si-ERK and si-JNK detected by qRT-PCR was shown in Fig. 3B, the expression of p38, ERK and JNK decreased sharply in APOE2+si-MAPKs group than those in control and APOE2+si-con group.

The inflammatory cytokines in ARPE-19 cells culture medium and nvAMD mice retinal lysate were detected by western blot and ELISA, respectively. In ARPE-19 cells, the results of western blot showed much higher levels of p-NF-κB in APOE2 and APOE2+si-Con groups than APOE2+si-MAPKs and control groups (Fig. 3C). And the downstream inflammatory cytokines (TNF-α, IL-1β and IL-6) mediated by p-NF-κB were correspondingly up-regulated in APOE2 and APOE2+si-Con groups compared with the other two groups (Fig. 3D-F,  $p < 0.01$ ). The similar results were shown in ELISA of nvAMD mice retinal lysate which proved that the levels of p-NF-κB, TNF-α, IL-1β and IL-6 in h-APOE2-TR+Laser mice were up-regulated compared with APOE-KO Laser and APOE-KO mice (Fig. 3G,  $p < 0.01$ ). These findings indicated that APOE2 decreased cell viability and increased the expression of inflammatory cytokines, but the knockdown group with si-MAPKs blocked the expression and secretion of the IL-1β, IL-6, and TNF-α, which demonstrated that APOE2 stimulated the



**Fig. 2.** APOE2 was overexpressed and the MAPKs pathway was activated in nvAMD mice.





**Fig. 3.** APOE2 affected RPE cell viability and promoted expression of inflammatory cytokines.

expression and secretion of inflammatory cytokines via MAPKs pathway.

**3.4. APOE2 up-regulated the expression of angiogenic factors**

Previous reports (Levy et al., 2015a,b; Liu et al., 2017; Balser et al., 2019) have proved the laser-induced nvAMD mouse models are applied widely. To evaluate the effects of APOE2 on nvAMD, the expression levels of VEGF, PDGF-BB, b-FGF and APOE2 in mice model retinal lysate were tested by ELISA, and western blot was used for the test of expression in ARPE-19 cells. As shown in Fig. 4A-C, in ARPE-19 cells, the expression levels of VEGF, PDGF-BB and b-FGF were significantly up-regulated in APOE2 and APOE2+si-Con groups compared with control and APOE2+si-MAPKs groups ( $p < 0.01$ ). The level of APOE2 in retinal lysate was much higher in h-APOE2-TR+Laser mice than that of APOE-KO+Laser and APOE-KO mice (Fig. 4D,  $p < 0.01$ ). The levels of VEGF, PDGF-BB

and b-FGF in retinal lysate of mouse models were consistent with the APOE2 levels in mice (Fig. 4E-G). To summarize, the expression of VEGF, PDGF-BB and b-FGF was significantly up-regulated in h-APOE2-TR+Laser mice and APOE2-treated ARPE-19 cells, while the results of ARPE-19 cells with si-MAPKs showed low expression of these angiogenic factors. These findings indicated that APOE2 promoted the expression of angiogenic factors and the up-regulatory effects were inconspicuous without the involvement of MAPKs.

**3.5. APOE2 accelerated nvAMD via activation of MAPKs pathway**

MAPK family mainly includes ERK1/2, JNK1/2/3 and p38 MAPK (Kyoosseva, 2016). To explore the effects of APOE2 on MAPKs pathway, we detected the expression of MAPK proteins (including ERK, JNK and p38) and phospho-MAPK proteins (p-MAPKs). We found that the levels of mRNA of ERK, JNK and p38 MAPK in APOE2 and

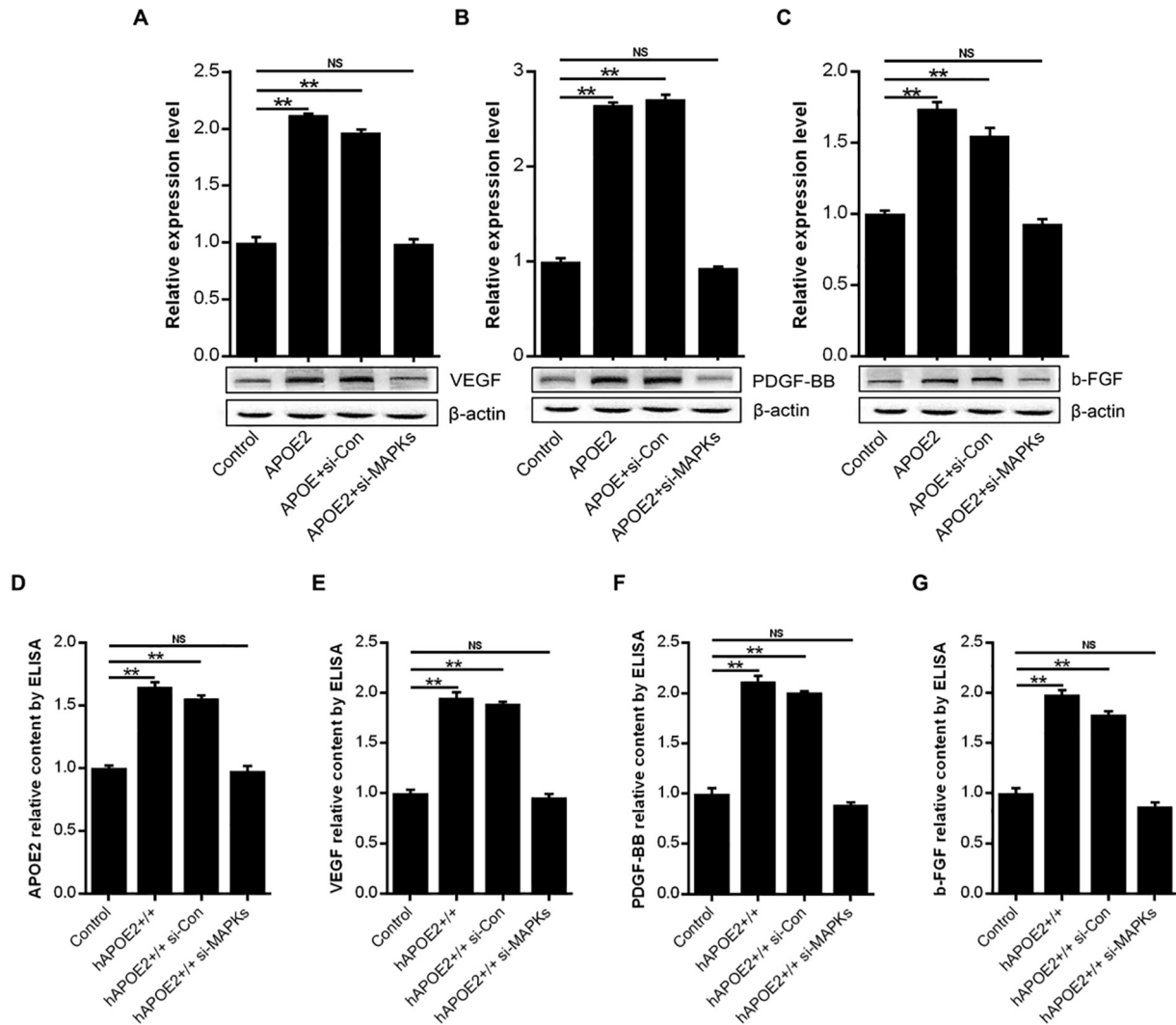


Fig. 4. APOE2 up-regulated angiogenic factors via MAPKs pathway.

control groups were higher compared to APOE2+si-MAPKs group (Fig. 5A-C). Similarly, the expression of ERK,

JNK and p38 MAPK were down-regulated compared with other groups.

However, the ratios of p-MAPKs and MAPKs were at the same level with APOE2 and APOE2+si-Con groups, which was much higher than control group without APOE2 treatment (Fig. 5D-F). Thus, the expression levels of p-ERK, p-JNK and p-p38 in APOE2 group were up-regulated compared with control group, which suggested that APOE2 regulated the expression of cytokines by activating MAPKs pathway.

#### 4. Discussion

In the present study, we have proved that APOE2 induced the activation of MAPKs pathway in human RPE cells and promoted the up-regulation of downstream target genes to cause inflammation in eye tissues and the formation and leakage of nvAMD. In laser-induced nvAMD, the expression and secretion of inflammatory cytokines increased accompanying with the up-regulation of APOE2. ARPE cells showed an inflammatory response, and the expression of neovascular growth factors (such as VEGF, PDGF-BB and b-FGF) was also up-regulated. Interestingly, significant differences in phospho-MAPK proteins but not total levels were found between the APOE2 group and control group.

Moreover, the inflammatory cytokines and vascular growth factors in APOE2+si-MAPKs group and control group were all reduced compared to the APOE2 group. To conclude, our results showed that APOE2 had no effect on the expression level of MAPKs, but it activated vascular growth factor and inflammatory cytokine signaling pathway by increasing the phosphorylation level of MAPKs. FFA is a useful diagnostic method and an important clinical research tool. It is a dynamic testing method involved in the diagnosis of nvAMD which takes a series of photos to show the movement of injected-dye over time (Novais et al., 2016). In FFA images, the fluorescence intensity of lesion was determined by the size and thickness of the lesion, the size and extent of new vessels and leakage (Shah et al., 2006). As shown in FFA images (Fig. 1), the area of fluorescent was varied in dry AMD and nvAMD, which indicated that FFA might be used not only for early diagnosis but also for the evaluation of the efficacy of antiangiogenic drugs in AMD treatment.

Lee et al., have proved that APOE2 induced nvAMD by altering the expression of angiogenic cytokines including VEGF and b-FGF (Lee et al., 2007). PDGF-BB, a primary stimulator of pericyte proliferation, promotes endothelial cell proliferation, migration and stabilization via autocrine and paracrine effects (Stewart, 2013). Our results demonstrated that the expression of APOE2, VEGF, b-FGF and PDGF-BB was significantly up-regulated in both human APOE2 mouse models and APRE cells.

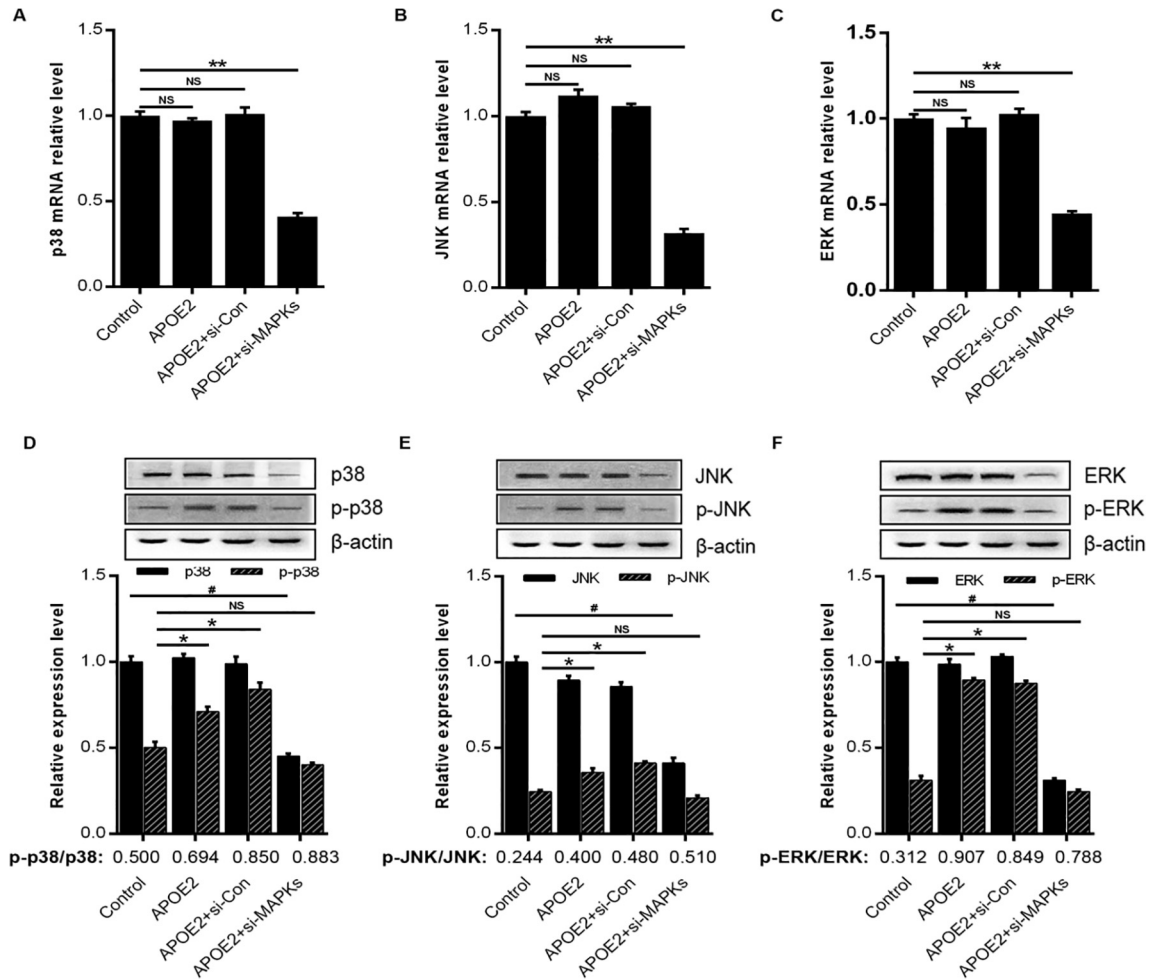


Fig. 5. APOE2 accelerated nvAMD via activation of MAPKs pathway.

MAPKs pathway plays a role in generation and maturation of blood vessels (Tate et al., 2013). Roduit et al., have proved that MAPK proteins including ERK, JNK, and p38 Kinases were activated when the human RPE cells induced by UV laser (Roduit and Schorderet, 2008), and these MAPK proteins controlled the activities of downstream transcription factors. The previous study has demonstrated that MAPK-activated inhibitors reduced the expression of NF- $\kappa$ B, IL-1 $\beta$ , IL-6, and TNF- $\alpha$  (Feng et al., 2017). As shown in Figs. 2–5, the high levels of p-MAPKs multiplied the expression of inflammatory and angiogenic cytokines several times compared to that without activated MAPKs, which revealed the key role of MAPKs pathway in pathogenesis of nvAMD.

Several reports have introduced the application of targeting inhibitors (especially VEGF targeting drug) in the treatment of nvAMD, the therapeutic effects including retinal thickness and leakage of new vessels were proved (Wong et al., 2014; Feng et al., 2017; Siedlecki et al., 2017). The present study provides a novel idea for nvAMD treatment and proposes possible approaches of nvAMD therapy via the use of drugs targeting upstream genes. However, this study is restricted to animal experiments, and it is necessary to confirm the clinical feasibility in the further study.

## 5. Conclusion

In summary, we analyzed the correlation between APOE2 allele and the susceptibility of nvAMD. Subsequently, we explored the relationship between the level of APOE2 and the severity of nvAMD, and we suggested that the overexpression of APOE2

activated MAPKs pathway. It revealed that serum APOE2 may be a new biomarker of early nvAMD and a new field of targeting inhibitors for nvAMD treatment is found.

## Declaration of Competing Interest

All the authors hereby agreed and confirm that there is no conflict of interest for this research work and publication of this paper.

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