Minor allele of rs12976445 polymorphism in the promoter region of microRNA-125a is associated with the severity of primary open-angle glaucoma (POAG)

Type

Research paper

Keywords

inflammation, POAG, miR-125a, IL-6R, rs12976445

Abstract

Introduction
The signaling pathway of IL-6 has been reported to be associated with the severity of glaucoma. And the rs12976445 SNP in miR-125 could alter the expression of miR-125, which is directly targeted by IL-6R.

Material and methods
In this study, we recruited 88 POAG patients and grouped them according to their genotype of rs12976445 as GG group, GC group and CC group to study the association between the miR-125a polymorphism and POAG. We collected demographic characteristics and peripheral blood samples from 88 subjects. Then, rs12976445 genotypes in these subjects were determined to evaluate their relationship with the POAG index. THP-1 and U937 cells were transfected with miR-125a mimic or IL-6R siRNA to assess the relationship between miR-125a and IL-6R/ACHE.

Results
IL-6R is a downstream target of miR-125a and the overexpression of miR-125a showed significantly decreased mRNA and protein levels of IL-6R. The expression levels of miR-125a were the highest in the GG group and the lowest in the CC group, while the activity of IL-6 was comparable in the three groups. Moreover, the mRNA and protein levels of IL-6R were the lowest in the GG group and the highest in the CC group. Additionally, significantly thinner RNFL, larger average cup disc ratio, larger vertical cup disc ratio, and depressed visual field were observed in POAG patients carrying the CC genotype.

Conclusions
In summary, our data suggested that the rs12976445 polymorphism was significantly associated with the risk of POAG.

Minor allele of rs12976445 polymorphism in the promoter region of microRNA-125a is associated with the severity of primary open-angle glaucoma (POAG)

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Abstract

The signaling pathway of IL-6 has been reported to be associated with the severity of glaucoma. And the rs12976445 SNP in miR-125 could alter the expression of miR-125, which is directly targeted by IL-6R. In this study, we recruited 88 POAG patients and grouped them according to their genotype of rs12976445 as GG group, GC group and CC group to study the association between the miR-125a polymorphism and POAG. We collected demographic characteristics and peripheral blood samples from 88 subjects. Then, rs12976445 genotypes in these subjects were determined to evaluate their relationship with the POAG index. THP-1 and U937 cells were transfected with miR-125a mimic or IL-6R siRNA to assess the relationship between miR-125a and IL-6R/ACHE. IL-6R is a downstream target of miR-125a and the overexpression of miR-125a showed significantly decreased mRNA and protein levels of IL-6R. The expression levels of miR-125a were the highest in the GG group and the lowest in the CC group, while the activity of IL-6 was comparable in the three groups. Moreover, the mRNA and protein levels of IL-6R were the lowest in the GG group and the highest in the CC group. Additionally, significantly thinner RNFL, larger average cup disc ratio, larger vertical cup disc ratio, and

depressed visual field were observed in POAG patients carrying the CC genotype. In summary, our data suggested that the rs12976445 polymorphism was significantly associated with the risk of POAG.

Running title: rs12976445 is associated with severity of POAG

Keywords: POAG, miR-125a, IL-6R, inflammation, rs12976445

Abbreviation

POAG: primary open-angle glaucoma;

PBMC: peripheral blood monocyte;

ACHE: acetylcholinesterase;

RNFL: retinal nerve fiber layer;

IL-6R: interleukin-6 receptor;

RA: rim area;

C/D: cup/disc.

Introduction

As a kind of optic neuropathy featured by the loss of ganglion cells in the retina, glaucoma is a leading contributor of blindness. As the most frequently diagnosed type of glaucoma, primary open-angle glaucoma (POAG) impacts the lives of more than 40 million patients worldwide [1]. More importantly, the visual impairment induced by POAG is irreversible, making the early diagnosis of POAG an urgent need in its treatment [2]. It was previously shown that the inflammation of the trabecular meshwork accelerates the progression of POAG, suggesting that the oxidative stress and inflammation of the conjunctival stroma play an essential role in the diagnosis and treatment of POAG [2, 3].

MicroRNAs (miRNAs) have been detected in many biological species and can regulate the expression of their target mRNAs at the post-transcriptional level by interacting with the 3' untranslated region (3’ UTR) of these mRNAs, thus participating in the pathogeneses of

many diseases [4]. As a miRNA highly expressed in many types of mammalian cells, miR-125 has three homologs, i.e., miRNA-125b-2, miRNA-125b-1 and miR-125a. In particular, miRNA-125b-2 was recently demonstrated to be implicated in various immune reactions [5, 6]. MiRNA-125b-2 has also been shown to play important roles in stabilizing the activities of signal transducer and activator of transcription 3 (STAT3) in antigen-presenting cells [7]. The overexpression of miR-125a reduced the expression of various pro-inflammatory cytokines such as IL-12, p40, IL-6, and TNF-α in human monocytes [8]. MiR-125a can also inhibit the polarization of M1 macrophages [9-11].

Past experiments demonstrated that the rs12976445 single nucleotide polymorphism (SNP) in pre-miR-125a can affect the maturation of pre-miR-125a [12]. Past studies also demonstrated that the rs12976445 SNP can affect receptor tyrosine-protein kinase erbB-2 (ERBB2) expression in patients with breast cancer. As a miR-125a target, ERBB2 expression is increased in patients with esophageal cancer [13, 14]. Existing data also suggested that the rs12976445 SNP T allele affects the maturation of miRNA-125a, leading to increased susceptibility to autoimmune disorders [12, 15]. Furthermore, the genotypes of rs12976445 SNP also regulate miRNA-125a expression and the expression of its target against decapentaplegic homolog 2 (SMAD2) and transforming growth factor-beta 1 (TGFB1).

Both interleukin-6 receptor (IL-6R) and interleukin-6 (IL-6) have been linked to POAG-induced autoimmune disorder [16]. In addition, upon the increased intraocular pressure in POAG patients, IL-6R expression in the trabecular meshwork also increases, suggesting that the allele frequency, as well as the genotypes of IL-6R and IL-6 are apparently affected by the onset of POAG [16, 17]. Moreover, the serum levels of IL-6 in POAG are decreased along with an apparently elevated level of fibrinogen, which induces a high level of hemorheological viscosity [16].

The deregulation of the IL-6 signaling pathway is associated with the severity of glaucoma, while IL-6R is a direct target of miR-125a [18]. Furthermore, rs12976445 SNP located in miR-125 has been shown to alter the expression of miR-125 [19]. In this study, we hypothesized

that the rs12976445 polymorphism could be associated with the severity of POAG, which enables miR-125a to be a potential biomarker for the susceptibility to POAG. Accordingly, we collected blood and peripheral blood monocyte (PBMC) samples from POAG subjects and studied the association between the polymorphism in miR-125a and the severity of POAG.

Materials and Methods

Clinical data of patients and sample collection

This study enrolled a total of 88 POAG patients. After enrollment, peripheral blood samples were collected from each POAG subject and the genotype of rs12976445 in each sample was analyzed by Taqman genotyping assays to determine its genotype of rs12976445. At the same time, the clinical-pathological data, demographic characteristics and PBMC samples were collected from all POAG subjects. Among these 88 POAG patients, 35 POAG subjects (the GG group, N=35) carried the GG genotype of rs12976445, 28 POAG subjects (the GC group, N=28) carried the GC genotype of rs12976445, and 25 POAG subjects (the CC group, N=25) carried the CC genotype of rs12976445. No significant difference in respect to age and gender were spotted after these randomly-selected patients were grouped (Page > 0.05, P gender > 0.05). According to the studies by Lehmann et al. [20], rs12976445 might not a somatic tumor-origin mutation since its ratio varies in different study populations. Generally, the ratio of some genotype group might by rare in the general population. However, since our study was a functional study with a relatively small sample size instead of an association study, to balance the sample size of each genotype group, we recruited a comparable number of participants in each group. The presence of systemic diseases (hypertension, diabetes mellitus and hyperlipidemia) also did not differ among the subjects in the three groups (P>0.05). All patients have signed written informed consent before the study begins.

Calculation of the cup-disc (C/D) ratio using a method based on slit-lamp ophthalmoscopy

In this study, the C/D ratio of each POAG subject was independently calculated by 3 experienced clinicians specialized in the treatment of POAG. The determination of the C/D ratio was done using a method based on slit-lamp ophthalmoscopy by scanning the optic nerve head in each POAG patient. The severity of POAG in these subjects was classified as mild or severe depending on their C/D ratio measurement. In brief, mild POAG indicated the POAG patients were in the early to medium stage of the disease and their C/D ratio was ≥0.3 and ≤0.7. On the other hand, severe POAG indicated the POAG patients were in the advanced stage of the disease and their C/D ratio was ≥0.7 and ≤1.0. At the same time, alterations of the optic nerve disc were also assessed. During the determination of the severity of POAG and the assessment of the optic nerve disc, the 3 clinicians would cast their votes respectively and the final results were obtained via consensus.

Genotyping using Taqman assay

The genotypes of rs12976445 SNP, i.e., genotypes GG, GC and CC, were determined using a Taqman method. First, genomic DNA was separated from collected PBMC samples using a DNA extraction kit (Tiangen, Beijing, China) following the standard protocol provided by the manufacturer. Then, the isolated DNA samples were amplified on an ABI 7300 Real Time PCR instrument (Applied Biosystems, Foster City, CA) using a Taqman method to determine the genotype of rs12976445 SNP in each sample.

ELISA assay

The concentration of IL-6 in peripheral blood samples collected from each POAG subject was assayed using an IL-6 ELISA assay kit purchased from Bio-Rad Laboratory (Hercules, CA) and the assay was carried out per the standard protocol provided on kit instruction.

RNA isolation and real-time PCR

First, total RNA in collected peripheral blood samples and PBMC samples from each POAG patient as well as in cultured THP-1 and U937 cells was extracted using a Trizol experimental assay (Invitrogen, Carlsbad, CA) according to the standard method

recommended by the manufacturer. In the next step, the isolated RNA was converted to cDNA using a Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA) before the relative expression of miR-125a, IL-6R mRNA as well as acetylcholinesterase (ACHE) mRNA in each sample was detected using Taqman Universal Master Mix (Applied Biosystems, Foster City, CA) on the ABI 7300 Real Time PCR instrument (Applied Biosystems, Foster City, CA). The quantification of miR-125a, IL-6R mRNA as well as ACHE mRNA was carried out using the standard $2^{-\Delta\Delta CT}$ method, while the expression of GAPDH and U6 was used as the internal standard for miR-125a and IL-6R/ACHE mRNA, respectively. The sequence of the primer pairs used are: miR-125a-F: 5'-CCTGAGACCCTTTAACC -3'; miR-125-R: 5'- GAACATGTCTGCGTATCTC -3'; IL-6R-F: 5'-GACTGTGCACTTGCTGGTGGAT -3'; IL-6R-R: 5'- ACTTCCTCACCAAGAGCACAGC -3'; ACHE-F: 5'- GTTCTCCTTCGTGCCTGTGGTA -3'; ACHE-R: 5'- ATACGAGCCCTCATCCTTCACC -3'; GAPDH-F: 5'- GTCTCCTCTGACTTCAACAGCG -3'; GAPDH-R: 5'-ACCACCCTGTTGCTGTAGCCAA -3'; U6-F: 5'- CTCGCTTCGGCAGCACAT -3'; U6-R: 5'-TTTGCGTGTCATCCTTGCG -3'.

Cell culture and transfection

THP-1 and U937 cells were acquired from the American Type Culture Collection (ATCC, Manassas, VA) and stored in a liquid nitrogen tank. Prior to the experiment, the cells were thawed and passaged in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Thermo Fisher Scientific, Waltham, MA) added with 10% (v/v) fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Waltham, MA) and appropriate concentrations of penicillin and streptomycin (Sigma Aldrich, St. Louis, MO). The cells were incubated in a regular tissue culture incubator containing 5%CO_2 and 95% air. The culture conditions were saturated humidity and 37°C. The cells were passaged once every 2 days using trypsin-EDTA (Gibco, Thermo Fisher Scientific, Waltham, MA) under they reached logarithmic growth. Then, the cells were randomly divided into the following 3 groups: 1. the scramble control group, in which the cells were treated with PBS during the subsequent transfection experiment; 2. the group of miR-125a mimics, in which the cells were transfected with miR-125a mimics using Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA)

following the standard procedure provided by the manufacturer; 3. the group of IL-6R siRNA, in which the cells were transfected with IL-6R siRNA using the Lipofectamine 3000 transfection reagent. At 48 after transfection, the cells were harvested to assay the expression of target genes.

Vector construction, mutagenesis and luciferase assay

To determine the effect of miR-125a on the expression of IL-6R and ACHE, the 3' UTRs of IL-6R and ACHE mRNAs carrying the miR-125a binding sites were respectively inserted into pcDNA vectors (psiCHECK™-1, Promega, Madison, WI) to create the plasmids of wild type 3' UTRs of IL-6R and ACHE mRNAs. Then, site-directed mutagenesis was carried out using a Quick Change mutagenesis kit (Stratagene, San Diego, CA) following the kit instruction to induce site-directed mutations in the miR-125a binding sites located on the 3' UTRs of IL-6R and ACHE mRNAs, respectively. The full length 3' UTR of IL-6R and ACHE mRNA carrying the mutant miR-125a binding sites were also inserted into separate pcDNA vectors to create the plasmids of mutant 3' UTRs of IL-6R and ACHE mRNAs, respectively. In the next step, THP-1 and U937 cells were co-transfected with the vectors of wild type/mutant 3' UTRs of IL-6R or ACHE mRNA in conjunction with miR-125a or scramble control using the Lipofectamine 3000 transfection agent. At 48 after transfection, the luciferase activity of transfected cells was determined using a Bright-Glo luciferase assay kit (Promega, Madison, WI).

Western blot

The protein expression of IL-6R and ACHE in collected peripheral blood and PBMC samples from each POAG patient as well as in cultured THP-1 and U937 cells was determined using a standard Western blot assay.

Statistical analysis

Categorical variables were tested using Chi Square tests, while continuous variables were tested using Student's t tests. The categorical results were shown as percentages while the continuous variables were shown in mean ± standard deviations. For comparisons

between multiple groups, one-way ANOVA was used with Tukey's test as the post hoc test. All statistical analyses were carried out using SPSS 21.0 (SPSS, Chicago, IL). The level of statistical significance was set to $P < 0.05$.

Results

Validation of downstream targets of miR-125a

To find potential targets of miR-125a, we searched the TargetScan, Pictar-Vert, and Microrna.Org databases, and found IL-6R and ACHE as potential targets of miR-125a. As shown in Fig.1A and Fig.1C, both IL-6R and ACHE carried a miR-125a binding site, indicating that IL-6R and ACHE might be direct targets of miR-125a. To verify that the miR-125a binding sites in IL-6R and ACHE were responsible for miR-125a regulation, we constructed vectors containing wild-type or mutant IL-6R and ACHE directly fused to the firefly luciferase gene. Then, the wild-type or mutant vectors of IL-6R/ACHE were co-transfected into THP-1/U937 cells with miR-125a or miR-125a NC. The results showed that the relative luciferase activity of miR-125a sharply decreased in cells transfected with wild type IL-6R (Fig.1B). However, the relative luciferase activity of miR-125a displayed no differences in cells either transfected with wild type ACHE or mutant type ACHE (Fig.1D). Taken together, these findings indicated that IL-6R, but not ACHE, is a direct target for miR-125a.

To further verify above results, THP-1/U937 cells were transfected with miR-125a mimic or IL-6R siRNA. Then, RT-qPCR and Western blot were performed to evaluate the mRNA and protein levels of IL-6R and ACHE in THP-1/U937 cells. As shown in Fig.2A and 3A, the mRNA and protein levels of IL-6R in cells transfected with miR-125a mimic or IL-6R siRNA were significantly decreased. Meanwhile, the mRNA and protein levels of ACHE (Fig.2B and Fig.3B) showed no significant difference among the three groups in THP-1/U937 cells.

Distribution of different genotypes of rs12976445 in POAG patients

The peripheral blood samples of POAG patients were collected to determine their genotypes of rs12976445. The demographic characteristics of subjects were described in

Table 1. Among all POAG subjects, 35 cases carried the GG genotype of rs12976445, 28 cases carried the GC genotype of rs12976445, and 25 cases carried the CC genotype of rs12976445. These groups were well matched for age and gender (Page > 0.05, P gender > 0.05). The presence of systemic diseases (hypertension, diabetes mellitus and hyperlipidemia) did not differ among the subjects in the three groups (P>0.05).

Expression levels of miR-125a and IL-6R in the PMBC of POAG patients carrying different genotypes of rs12976445

The peripheral blood samples collected from GG, GC and CC groups were analyzed using RT-qPCR and ELISA. The expression level of miR-125a (Fig.4A) was the highest in the GG group and the lowest in the CC group, while the serum activity of IL-6 was comparable in three groups (Fig.4B).

Subsequently, the mRNA level of miR-125a (Fig.5A) and the activity of IL-6R (Fig.5B) in peripheral monocytes collected from the three groups were tested. As shown in Fig.5, the results were consistent with those obtained using peripheral blood samples.

Moreover, the mRNA and protein levels of IL-6R and ACHE in peripheral blood samples of POAG patients were detected by RT-qPCR and Western blot. Accordingly, the mRNA and protein levels of IL-6R were the lowest in the GG group and the highest in the CC group (Fig.6), while the mRNA and protein levels of ACHE were comparable among the three groups (Fig.7).

Comparison of demographic and clinical data among POAG patients carrying the GG, GC and CC genotypes of rs12976445 SNP

The glaucoma indexes of POAG patients in the three groups were collected. As shown in Fig.8, these groups were well matched on central corneal thickness (Fig.8A) and IOP (Fig.8B) during enrollment. However, the POAG patients carrying the CC genotype of rs12976445 SNP showed significant thinner retinal nerve fiber layer (RNFL) (Fig.8C), larger vertical cup disc ratio (Fig.8E), larger average cup disc ratio (Fig.8F), and depressed visual field.

Discussion

It has been indicated that the minor allele of rs12976445 apparently changes the ratio between mature miR-125a expression and pre-miR-125a expression, which indicated that rs12976445 could affect the maturation of miR-125a [21]. Moreover, miR-125a expression in the TT group was similar to that in the CT group and was apparently elevated in the CC group, suggesting a dominant role of rs12976445 minor allele [19]. In this study, the glaucoma indexes of the POAG patients in the three groups indicated that the POAG patients carrying the CC genotype of rs12976445 SNP showed significant thinner RNFL, larger average cup disc ratio, larger vertical cup disc ratio, and depressed visual field. Meanwhile, the expression level of miR-125a was the highest in the GG group and the lowest in the CC group, which also led to the most suppressed mRNA and protein levels of IL-6R in the GG group. Meanwhile, the mRNA and protein levels of ACHE were comparable among the three groups. Therefore, the above results all supported our hypothesis that the allele type of rs12976445 apparently influenced the severity of POAG via regulating expression of miR-125a and IR-6R.

Both miRNA-125b and miR-125a are members of the miRNA-125 family, which was shown to play important roles in various processes such as the apoptosis, growth and differentiation of cells [22]. MiR-125a is inhibited during inflammation, while miRNA-125b can reduce inflammatory reactions by targeting TNF-α [23-26]. Nevertheless, a past study demonstrated that miR-125a promotes the pro-inflammatory adaptation of macrophages while increasing their response to IFN-α stimulation [27]. It was also shown that miR-125a inhibits LPS-induced expression of TNF-α, iNOS as well as IL-12, suggesting that while miR-125a-5p can target TNF-α, its anti-inflammatory role is mediated via other regulators. It was also shown that KLF13, a transcriptional factor and a target of miR-125a-5p, can inhibit inflammation and decrease the activation level of T cells [28]. In a past study, Graff et al. showed that the over expression of miR-125a-5p induces the activation of THP-1 cells, while another report demonstrated that miR-125a-5p can activate NF-kB signaling in cells of diffuse large B-cell lymphoma [29, 30]. It was also demonstrated that miR-125a-5p can mediate the IL-6-induced Treg cell sensitivity. In the absence of stimulation by IL-6,

the change in miR-125a-5p expression failed to affect FOXP3 expression or Treg activity [31]. The IL-6 signaling is crucial for iTreg differentiation. Nevertheless, altered miR-125a-5p expression in naïve T cells exerted no effects on iTreg and naïve T cell polarization [31]. In this study, IL-6R and ACHE were shown to contain miR-125a binding sites. The relative luciferase activity of miR-125a sharply decreased in cells transfected with wild type IL-6R, while the relative luciferase activity of miR-125a displayed no differences in cells either transfected with wild type ACHE or mutant type ACHE. In addition, mRNA and protein levels of IL-6R and ACHE were evaluated in THP-1 and U937 cells transfected with miR-125a mimic or IL-6R siRNA. The mRNA and protein levels of IL-6R in cells transfected with miR-125a mimic or IL-6R siRNA were significantly decreased, while the mRNA and protein levels of ACHE showed no significant differences among the three groups.

Released from adipocytes, macrophages, as well as other types of cells such as fibroblasts, skeletal muscle cells as well as endothelial cells, IL-6 plays important roles in the regulation of lipid metabolism as well as body weight [32-35]. IL-6 is also involved in the formation of obesity as well as insulin resistance. Nevertheless, the functions of IL-6 can be complicated [36-38]. For example, IL-6 can play an anti-inflammatory role to block the functions of TNF-α, to promote the polarization of M2 macrophages, as well as to alleviate insulin resistance [39, 40]. Other studies showed that obesity can elevate the level of IL-6 as well as IL-6R in adipose tissues to elevate the levels of IP-10, MCP-1, as well as TNF-α in these tissues [41]. Past studies also demonstrated that metabolic synthesis is a POAG risk factor and is involved in alternating the allele frequency of certain genes. For example, during the onset as well as development of POAG, the functions of various factors were modified by metabolic synthesis, such as the effect of Serpine1 on the trabecular meshwork, the effect of ENPP1 on the proliferation of cells in the trabecular meshwork, the effects of IL-6R, IL-6, and E-Sel on autoimmune reactions, the effect of LIPC and FGB on hyper-viscosity, as well as the effect of ADIPOQ on NOS/NO synthesis. Past studies also showed that the expression of IL-6 in the serum of POAG patients is elevated [42]. In addition, the G allele of the single nucleotide polymorphism (SNP) located at position (–

174) of the IL-6 gene was shown to elevate the expression of IL-6 proteins in POAG patients [43].

However, despite the results obtained which supported our hypothesis, the conclusion could be quite limited due to the small sample size recruited in this study. In this study, only 88 POAG patients were recruited and subjected to genotyping, therefore, this limited sample size will influence the accuracy of the correlation analysis between allele type and POAG severity. In our future study, large sample size is necessary, preferably with varied nations.

Conclusion

In summary, our data suggested that the rs12976445 polymorphism was significantly associated with the risk of POAG. To our knowledge, this is the first study investigating the association between miR-125a rs12976445 polymorphisms and POAG. The miR-125a rs12976445 SNP may be used as a biomarker to determine the susceptibility to POAG after further validation with larger scale population.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None

Authors' contributions

Wenjia Zhang and Hai Liu planned the study, Yan Li and Hongqin Ke collected the literatures, Wenjia Zhang, Yan Li, Hongqin Ke, Yingting Wang and Cong Duan collected

and analyzed the data, Qin Zhu and Hai Liu visualized the data, Wenjia Zhang and Hai Liu composed the manuscript, and all the other co-authors approved the final manuscript.

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Not applicable

Figure legends

Table1. Clinical pathological data of POAG patients carrying different genotypes of rs12976445.

Fig.1

Luciferase assay confirmed the target genes of miR-125a in THP-1 and U937 cells (* P value < 0.05 vs. miR-NC group; number of replicas = 3)

A: predicted binding site of miR-125a in IL-6R

B: luciferase activities of miR-125a in THP-1 cells co-transfected with wild/mutant type of IL-6R and miR-125a /NC.

C: predicted binding site of miR-125a in ACHE

D: luciferase activities of miR-125a in THP-1 cells co-transfected with wild/mutant type of ACHE and miR-125a /NC.

Fig.2

mRNA and protein levels of IL-6R and ACHE in THP-1 cells transfected with NC, miR-125a mimics or IL-6R siRNA (* P value < 0.05 vs. scramble control group; number of replicas = 3)

A: mRNA and protein levels of IL-6R in the three THP-1 groups.

B: mRNA and protein levels of ACHE in the three THP-1 groups.

Fig.3

mRNA and protein levels of IL-6R and ACHE in U937 cells transfected with NC, miR-125a mimics or IL-6R siRNA (* P value < 0.05 vs. scramble control group; number of replicas = 3)

A: mRNA and protein levels of IL-6R in the three U937 groups.

B: mRNA and protein levels of ACHE in the three U937 groups.

Fig.4

Relative expression of miR-125a mRNA and activity of IL-6R in blood samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).

A: Relative expression of miR-125a in the three groups.

B: IL-6 activity in the three groups.

Fig.5

Relative expression of miR-125a mRNA and activity of IL-6R in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).

A: Relative expression of miR-125a in the three groups.

B: IL-6 activity in the three groups.

Fig.6

mRNA and protein levels of IL-6R in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).

A: mRNA levels of IL-6R in the three groups

B: protein levels of IL-6R in the three groups

Fig.7

mRNA and protein levels of ACHE in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (number of replicas = 3).

A: mRNA levels of ACHE in the three groups

B: protein levels of ACHE in the three groups

Fig.8

Comparison of demographic and clinical data among POAG patients carrying GG, GC and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).

A: central corneal thickness in the three groups

B: IOP at recruitment in the three groups

C: retinal nerve fiber layer thickness in the three groups

D: rim area in the three groups

E: vertical cup disc ratio in the three groups

F: average cup disc ratio in the three groups

G: mean deviation in the three groups

H: pattern SD in the three groups

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Demographic characteristics	GG (N=35)	GC (N=28)	CC (N=25)	*P* value
Sex (n, %)				0.421
Male (54)	23 (42.6)	16 (29.6)	15 (27.8)	
Female (34)	12 (35.3)	12 (35.3)	10 (29.4)	
Age, years (mean, SD)	65.3 (5.3)	64.8 (8.2)	65.5 (7.5)	0.625
Systemic diseases (n, %)				
Hypertension (55)	28 (50.9)	16 (29.1)	11 (20.0)	0.356
Diabetes mellitus (28)	15 (53.6)	9 (32.1)	4 (14.3)	0.563
Hyperlipidemia (26)	15 (57.7)	8 (30.8)	3 (11.5)	0.835
Body mass index, kg/m2 (mean, SD)	25.6 (5.5)	24.9 (6.2)	24.5 (7.8)	0.642

Table 1. Demographic data of the subjects in this study

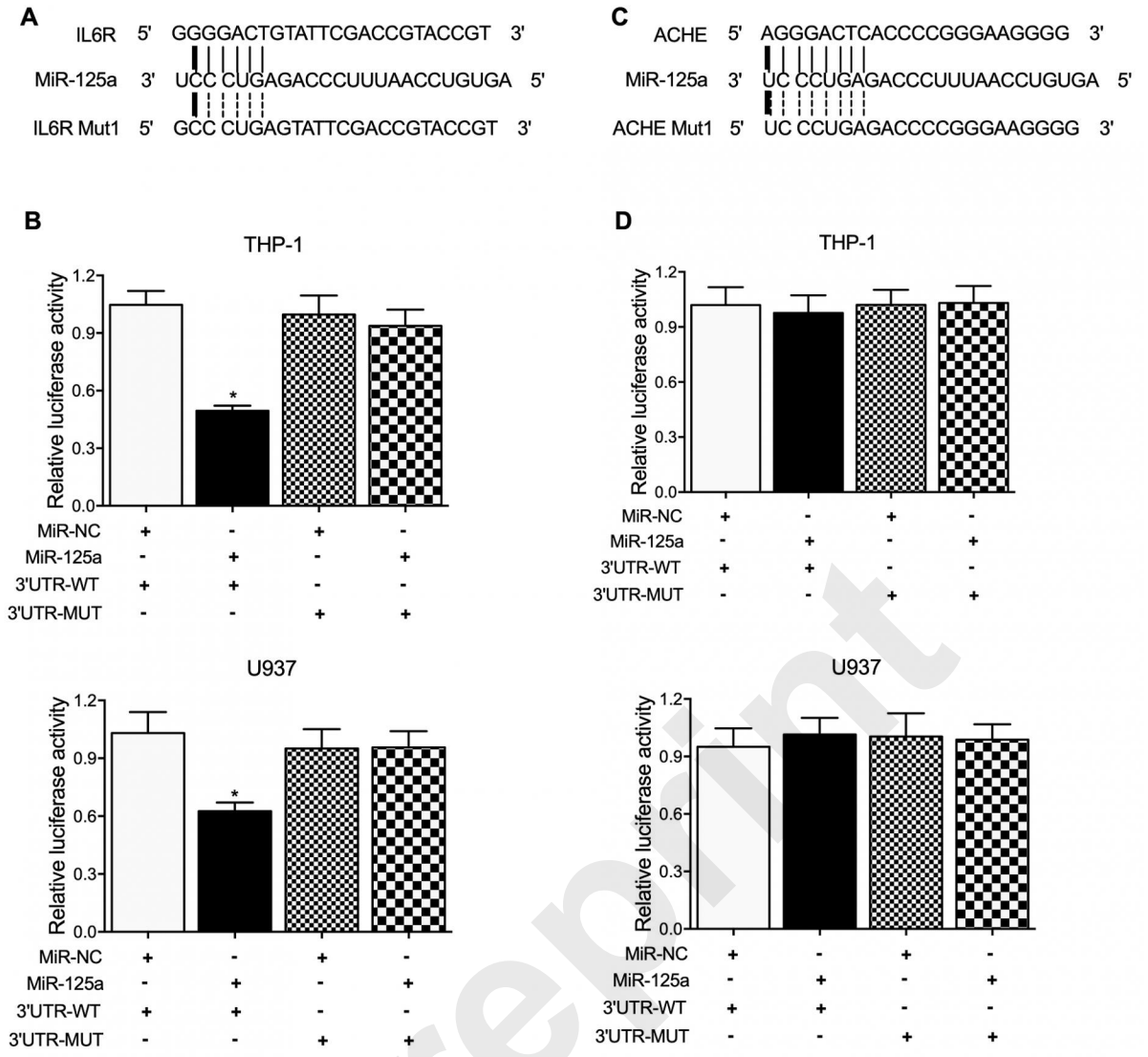

Fig.1
Luciferase assay confirmed the target genes of miR-125a in THP-1 and U937 cells (* P value < 0.05 vs. miR-NC group; number of replicas = 3)
A: predicted binding site of miR-125a in IL-6R
B: luciferase activities of miR-125a in THP-1 cells co-transfected with wild/mutant type of IL-6R and miR-125a /NC.
C: predicted binding site of miR-125a in ACHE
D: luciferase activities of miR-125a in THP-1 cells co-transfected with wild/mutant type of ACHE and miR-125a /NC.

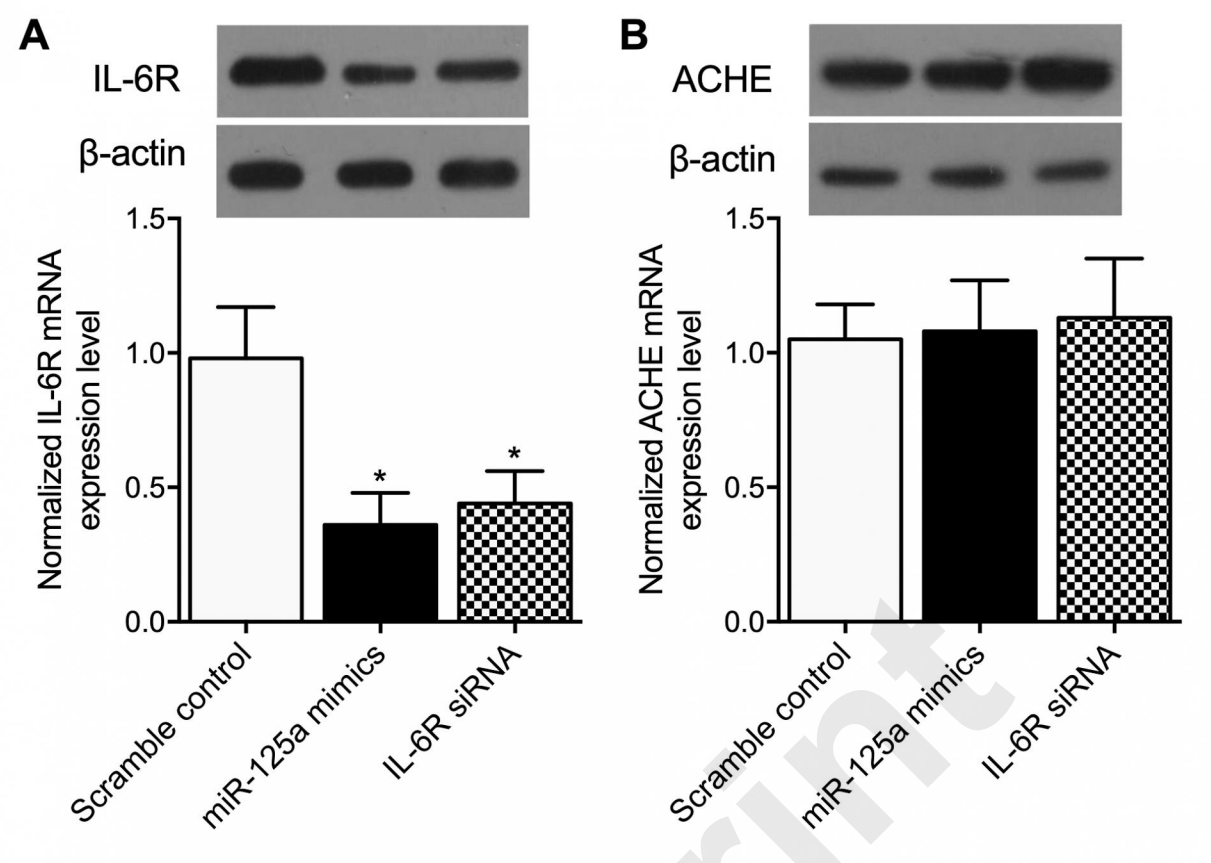

Fig.2 mRNA and protein levels of IL-6R and ACHE in THP-1 cells transfected with NC, miR-125a mimics or IL-6R siRNA (* P value < 0.05 vs. scramble control group; number of replicas = 3) A: mRNA and protein levels of IL-6R in the three THP-1 groups. B: mRNA and protein levels of ACHE in the three THP-1 groups.

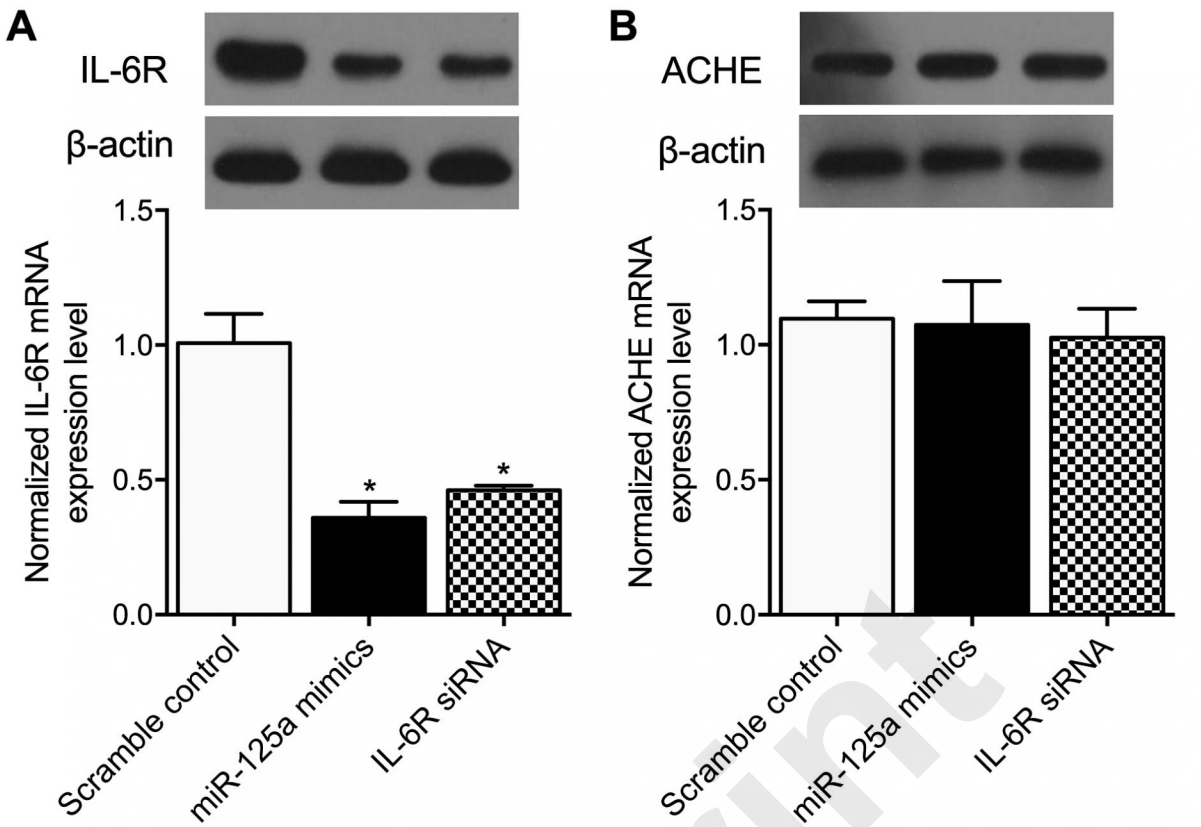

Fig.3 mRNA and protein levels of IL-6R and ACHE in U937 cells transfected with NC, miR-125a mimics or IL-6R siRNA (* P value < 0.05 vs. scramble control group; number of replicas = 3) A: mRNA and protein levels of IL-6R in the three U937 groups. B: mRNA and protein levels of ACHE in the three U937 groups.

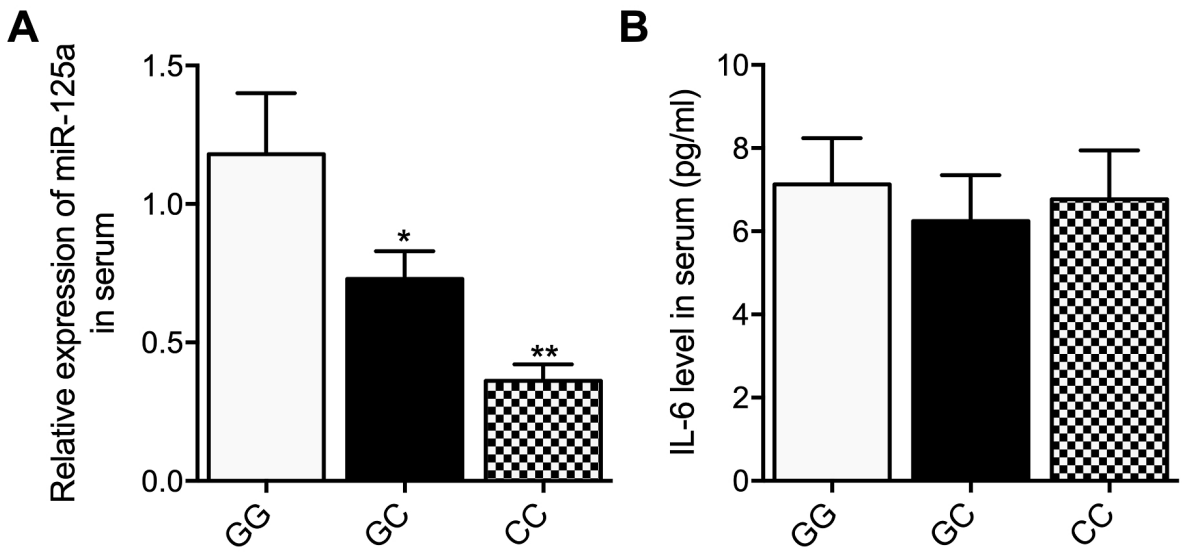

Fig.4
Relative expression of miR-125a mRNA and activity of IL-6R in blood samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).
A: Relative expression of miR-125a in the three groups.
B: IL-6 activity in the three groups.

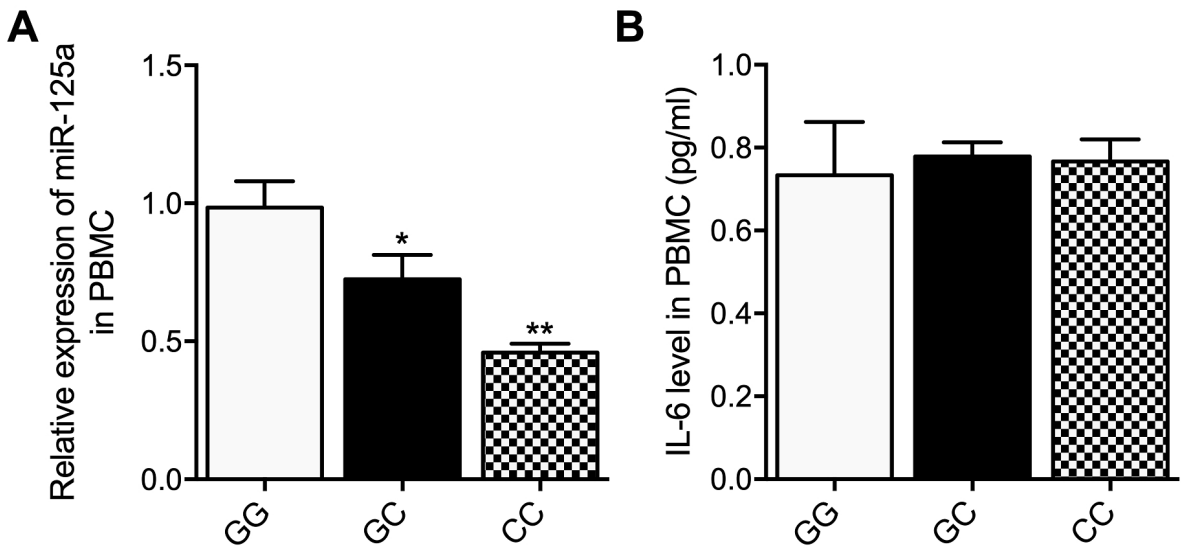

Fig.5
Relative expression of miR-125a mRNA and activity of IL-6R in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).
A: Relative expression of miR-125a in the three groups.
B: IL-6 activity in the three groups.

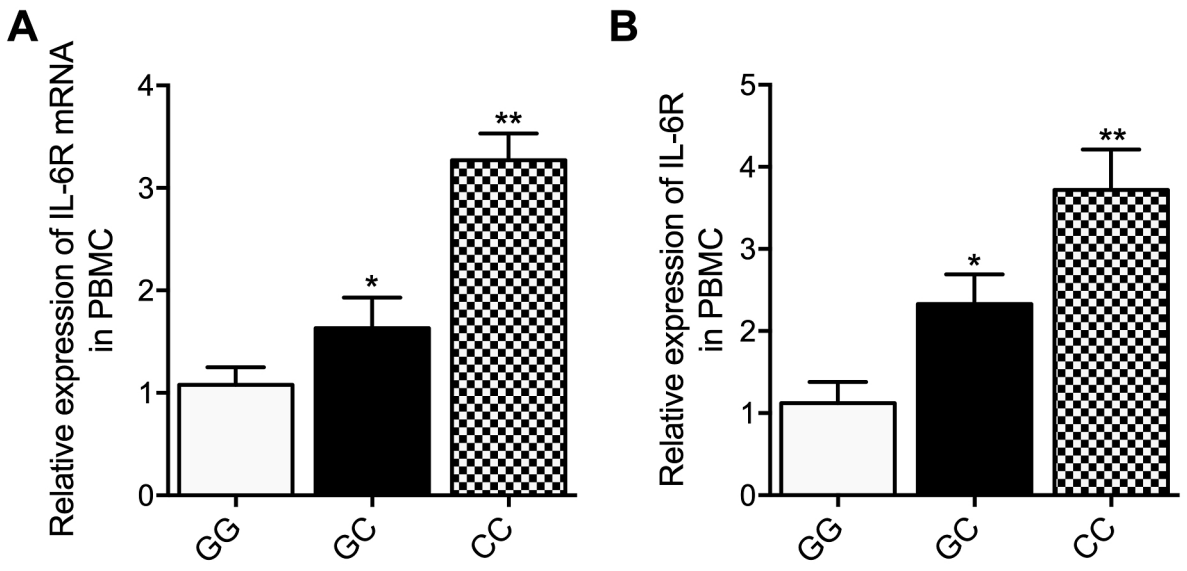

Fig.6
mRNA and protein levels of IL-6R in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).
A: mRNA levels of IL-6R in the three groups
B: protein levels of IL-6R in the three groups

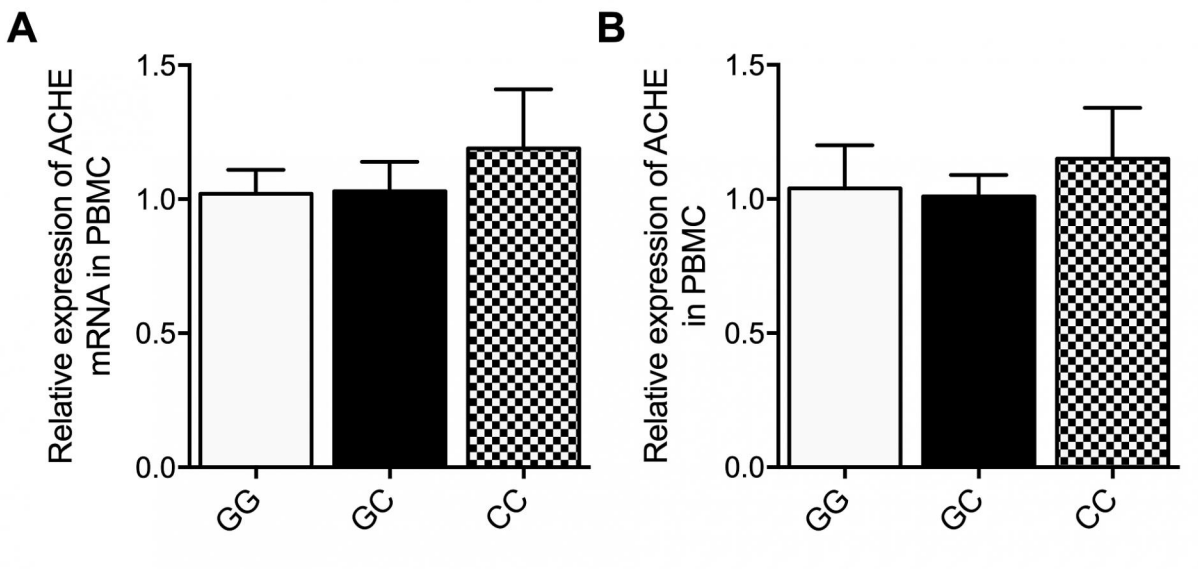

Fig.7
mRNA and protein levels of ACHE in PBMC samples collected from POAG patients carrying GG, GC, and CC genotypes of rs12976445 (number of replicas = 3).
A: mRNA levels of ACHE in the three groups
B: protein levels of ACHE in the three groups

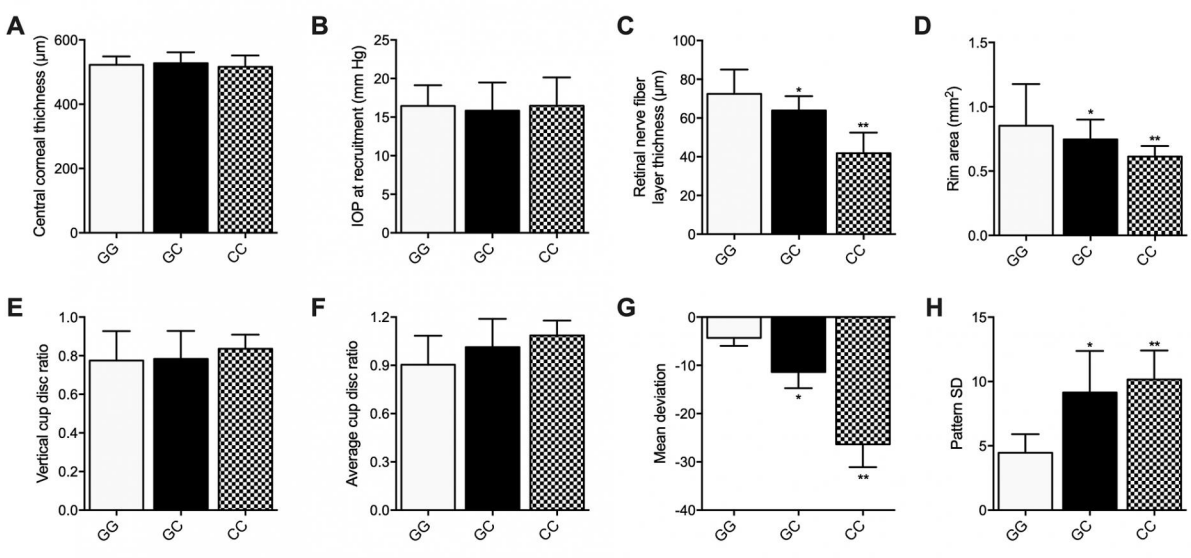

Fig.8
Comparison of demographic and clinical data among POAG patients carrying GG, GC and CC genotypes of rs12976445 (* P value < 0.05 vs. GG group; ** P value < 0.05 vs. GC group; number of replicas = 3).
A: central corneal thickness in the three groups
B: IOP at recruitment in the three groups
C: retinal nerve fiber layer thickness in the three groups
D: rim area in the three groups
E: vertical cup disc ratio in the three groups
F: average cup disc ratio in the three groups
G: mean deviation in the three groups
H: pattern SD in the three groups