ORIGINAL ARTICLE

PPAR-γ and CYP46A1 genes polymorphism is associated with Primary Open Angle Glaucoma (POAG) in hypertensive North Indians

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KEYWORDS
PPAR-γ; CYP46A1; Gene polymorphism; POAG; Hypertension

Abstract

Background: Involvement of genetic factors like gene polymorphisms was found to contribute significantly to development and progression of Primary Open Angle Glaucoma (POAG) in the last few decades.

Aim of study: The present study was carried out to investigate association of PPAR-γ (rs10865710) and CYP46A1 (rs754203) gene polymorphism with development of POAG in hypertensive North Indians.

Patients and methods: Study included 328 individuals, 226 as POAG cases and 102 controls. PPAR-γ and CYP46A1 gene polymorphism was evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotypic and allelic frequency distribution in patients and controls was analyzed by SPSS (version 12.0).

Results: In CYP46A1 gene polymorphism, TT genotype and T allele were found to be associated with a significantly decreased risk of POAG whereas the CT, CC genotypes and C allele were associated with an increased risk of POAG in both hypertensive and normotensive individuals. In PPAR-γ gene polymorphism, only GG genotype was nearly associated with POAG in only hypertensive cases.

Abbreviations: POAG, Primary Open Angle Glaucoma; IOP, intraocular pressure; PPAR-γ, peroxisome proliferator-activated receptor gamma; CYP46A1, cholesterol-24S-hydroxylase; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; GHT, glaucoma hemifield test; DBP, diastolic blood pressure; SBP, systolic blood pressure

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1. Introduction

Glaucoma is a multifactorial, complex disease considered to be the second leading cause of blindness around the globe. It is estimated that by 2020 over 11.1 million people will be bilaterally blind from primary glaucoma [1]. Primary Open Angle Glaucoma (POAG) is the most common type of glaucoma, characterized by an elevated intraocular pressure (IOP > 21 mmHg) which can ultimately destroy the optic nerve cells resulting in gradual visual loss. However, high IOP alone does not cause glaucoma apart from being a significant risk factor since patients with normal range IOP were also found to develop glaucoma. The other risk factors proposed to play a role in the development of POAG are older age, positive family history, ethnicity, myopia, diabetes, hyperension and genetic mutations [2]. Various population-based studies covering different ethnic backgrounds have consistently found an association between high blood pressure and IOP [3–6]. A study reported that the risk of developing POAG was 17% in hypertensive population as compared to healthy population [7]. However, despite the clear association between blood pressure and IOP, the exact relationship between blood pressure and open-angle glaucoma is still unclear. Over the past, genetic studies have substantially contributed to the study of glaucoma. Various genetic loci including a number of genes have also been reported to contribute to POAG susceptibility like MYOC, ASB10, WDR36, NTF4, and TBK1 genes. In this study we focused on peroxisome proliferator-activated receptor gamma (PPAR-γ) and cholesterol-24S-hydroxylase (CYP46A1) genes with the risk of POAG in hypertensive North Indians.

PPARs were initially identified as a peroxisome proliferator “binding-protein” capable of inducing hepatocyte peroxisome proliferation. PPAR-γ is a type II nuclear receptor which is encoded by the PPAR-γ gene in humans. Like other PPAR subtypes, PPAR-γ is constitutively expressed in the whole retina [8], most specifically in the retinal pigment epithelia [9]. It is involved in optic nerve damage and glaucomatous retinal dysfunction. PPAR-γ polymorphisms were found to be significantly associated with diabetic retinopathy [10,11]. Activation of PPAR-γ may play an important role in regulating the expression of target genes that are involved in lipid and fatty acid metabolism in the photoreceptor renewal process [12]. CYP46A1 is a cholesterol-metabolizing enzyme which is expressed in the neuronal part of the retina, specifically in the retinal ganglion cells [13,14]. It holds importance in context to POAG since it was found that cholesterol and cholesterol metabolism are somewhere involved in the pathogenesis of glaucoma [15]. CYP46A1 gene had been found to be associated with Alzheimer’s disease [16,17] and neurodegeneration. A single-nucleotide polymorphism (SNP) in CYP46A1 (rs754203) was proposed to be a genetic risk factor for POAG and a recent study showed a positive association between an intronic SNP (rs754203) in CYP46A1 gene with POAG in a French population [18].

The purpose of the present study was to explore the association of PPAR-γ and CYP46A1 gene polymorphism along with the effect of hypertension on POAG risk in the North Indian population.

2. Subjects and methods

2.1. Patient selection

After approval of the study protocol by the Institutional Ethics Committee and obtaining patients consent, a total of 226 blood samples of POAG cases (where 108 were hypertensives and 118 were normotensives) and 102 controls were collected from the Department of Ophthalmology at Era’s Lucknow Medical College & Hospital, Lucknow. Data collection was done for each patient on clinical variables including age, alcohol consumption, body mass index, height, weight, cigarette smoking and family history. Each subject underwent a complete ophthalmological examination.

Patients with POAG were defined by the presence of an open angle, pathological cupping of the optic disc, a glaucoma hemifield test (GHT) outside normal limits with reproducible visual field defects at the same location on two consecutive visits, and an IOP > 21 mmHg without anti-glaucoma drugs. Cup-to-disc ratios were between 0.4 and 0.9. Patients with a history of eye surgery before the diagnosis of glaucoma, evidence of secondary glaucoma such as exfoliation, pigment dispersion or uveitis and other causes were excluded.

POAG patients with a sustained diastolic blood pressure (DBP) > 90 mmHg that is accompanied by an elevated systolic blood pressure (SBP) > 140 mmHg were considered as hypertensives (according to the WHO diagnostic criteria of hypertension). These patients had a prior history of hypertension before the commencement of POAG. We also included age, sex and ethnicity matched control group who were non-smokers and had neither hypertension nor any other systemic illness. They had no family or personal history of glaucoma. Our work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. DNA extraction

Five milliliters of peripheral blood was collected from all the subjects in 0.5 M EDTA tubes. Genomic DNA was isolated from whole blood using the standard phenol–chloroform extraction method [19]. The DNA concentration was determined by spectrophotometer and stored at −20°C.
2.3. Analysis of polymorphisms

2.3.1. PPAR-\(\gamma\) polymorphism

Genotyping was performed using PCR-RFLP with the following primer: The forward primer 5'-CAA GCC CAG TCC TTT CTG TG-3' and the reverse primer 5'-AGT GAA GGA ATC GCT TTC CG-3' [20]. All reactions were performed in a total volume of 50 \(\mu\)L containing 10 mmol/L Tris HCl (pH 8.8), 50 mmol/L KCl, 1.5 mmol/L MgCl\(_2\), 0.2 mmol of each dNTP, 50 pmol of each primer, 1 U Taq DNA polymerase and 200 \(\mu\)g of genomic DNA. PCR was performed using an MJ mini personal thermal cycler (Bio-Rad). The amplification program included: Initial pre-cycling denaturation by holding at 94 °C for 3 min, denaturation for 40 cycles at 94 °C for 30 s followed by annealing at 53 °C for 30 s, extension at 72 °C for 1 min and a final extension period at 72 °C for 9 min. Restriction fragment length polymorphism (RFLP) will be detected after digestion over night with 2 U of the restriction enzyme \(Hpa\ II\) (New England Biolabs Inc., USA) which cuts the mutant allele at a site introduced by the reverse primer. Samples were applied to 3% agarose gel and subjected to electrophoresis for about half an hour and visualized on a UV transilluminator after ethidium bromide staining.

PPAR-\(\gamma\) homozygous for CC genotype corresponded to the 106-bp fragment, whereas the heterozygous CG was characterized by two fragments of 106 and 130 bp (Fig. 1).

2.3.2. CYP46A1 polymorphism

Genotyping was performed using PCR-RFLP with the following primer: forward primer: 5'-TGAAAACGAGTTTCCCTGCC-3'; reverse primer: 5'-GTGTGACCAGGTAA CAGTCA-3' [18] in a 12.5-\(\mu\)L volume reaction with 1.25 U of Taq DNA polymerase, 1.5 mM of MgCl\(_2\), 1.25 \(\mu\)L of 10X NH\(_4\) buffer, and 200 \(\mu\)M dNTPs. PCR was performed using an MJ mini personal thermal cycler (Bio-Rad). The amplification program included: initial denaturation at 95 °C for 8 min, after which the reaction mixture was subjected to 50 cycles of 1-min denaturation at 95 °C, 1-min annealing at 53 °C, and a 2-min extension at 72 °C, followed by a final extension at 72 °C for 5 min. The PCR products were digested by \(MspI\) (New England Biolabs Inc., USA) over night. The fragment amplification and digestion results were revealed by 1.8% agarose gel electrophoresis and visualized on a UV transilluminator after ethidium bromide staining. The CYP46A1*T allele corresponded to the uncut 285-bp fragment, whereas the CYP46A1*C allele was characterized by two fragments of 209 and 76 bp (Fig. 2).

We validated 25% of our genotyping results through Direct sequencing technique.

2.4. Statistical analysis

Clinical data are expressed as mean ± SD. The genotyping data were compared between cases and controls using Chi-square test. \(p\)-values \(\leq 0.05\) were considered as significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to test the relative risk for association. Other variables will be compared using Student’s \(t\)-test for normally-distributed variables. All statistical tests were performed using SPSS (Statistical Package for the Social Sciences) version 12 software.

3. Results

Our study included 328 individuals, 226 as POAG cases (108 with and 118 without hypertension) and 102 age, sex and
ethnicity matched controls. Clinical and biochemical parameters of cases and controls are shown in Table 1.

3.1. Hardy–Weinberg equilibrium test

*PPAR-γ* and *CYP46A1* genotype distributions in all cases and controls were in line with Hardy–Weinberg equilibrium (all \( p > 0.05 \), data not shown).

3.2. Power of study

Powers were calculated for various values of the Bonferroni corrected \( p \)-values (alpha) for the specified sample size and proportions using the formula given by Indrayan [21]. Then an average value of such powers is taken. Genetic power estimation showed that cases and controls had >80% power to detect association between *PPAR-γ* and *CYP46A1* gene polymorphism and POAG in the hypertensive and normotensive North Indian population.

3.3. Genetic polymorphism analysis

3.3.1. *PPAR-γ* polymorphism analysis

Compared to the healthy control group (40%), the frequency of GG genotype was significantly lower in hypertensive POAG patients (26.85%; \( p = 0.04 \)) but it was not significant in pure POAG patients (27.97%; \( p = 0.056 \)). No significant association was found between frequencies of C and G alleles in hypertensive (48.15%, \( p = 0.101 \) and 51.85%, \( p = 0.101 \) respectively) and normotensive POAG cases (47.03%,

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**Table 1** Biochemical and clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>POAG with hypertension cases ((N = 108))</th>
<th>Normotensive POAG cases ((N = 118))</th>
<th>Controls ((N = 102))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>61/47</td>
<td>56/62</td>
<td>52/50</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45.02 ± 17.65</td>
<td>47.03 ± 12.88</td>
<td>40.41 ± 11.29</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>144.96 ± 15.82</td>
<td>100 ± 11.54</td>
<td>112.23 ± 9.07</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>90.12 ± 8.57</td>
<td>75 ± 9.7</td>
<td>74.85 ± 10.01</td>
</tr>
<tr>
<td>S. Cholesterol (mg/dl)</td>
<td>181.11 ± 56.88</td>
<td>166.73 ± 24.17</td>
<td>170.52 ± 18.27</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>178.06 ± 110.55</td>
<td>137.35 ± 44.16</td>
<td>140 ± 36.72</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>45.767 ± 8.7929</td>
<td>55.23 ± 9.59</td>
<td>50.252 ± 8.53</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>33.53 ± 16.7</td>
<td>30.83 ± 11.64</td>
<td>30.41 ± 18.54</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>103.78 ± 31.926</td>
<td>89.60 ± 18.53</td>
<td>91.82 ± 43.45</td>
</tr>
</tbody>
</table>

Values are means ± S.D.

\( N \), number of subjects; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, Triglycerides; HDL, High Density Lipoproteins; VLDL, Very Low Density Lipoproteins; LDL, Low Density Lipoproteins.

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**Figure 2** 1.8% agarose gel picture showing PCR product for *CYP46A1* gene polymorphism. Lane L7 shows C/T genotype (285, 209, 76 bp), lanes L3 and L4 show C/C genotype (209, 76 bp), lanes L2, L6, and L8 show T/T genotype (285 bp) and lane L5 shows 100 bp ladder.
p = 0.149 and 52.97%, p = 0.149 respectively) as compared to controls (40.2% and 59.8% respectively). No significant differences were observed in genotype and allele frequencies on comparing hypertensive POAG study group with pure POAG study group as shown in Table 2.

3.3.2. CYP46A1 polymorphism analysis

The frequencies of CT; CC genotypes and C allele were significantly higher in both hypertensive POAG (51.85%, p = 0.002; 19.44%, p = 0.002 and 45.37%, p < 0.001) and pure POAG cases (49.15%, p = 0.002; 18.65%, p < 0.001 and 43.22%, p < 0.001) as compared to controls (28.43%, p = 0.002; 3.92%, p < 0.001 and 18.14%, p < 0.001) respectively. In contrast, the frequencies of TT genotype and T allele were significantly lower in both hypertensive POAG (26.85%, p < 0.001 and 54.63%, p < 0.001) and normotensive POAG cases (32.2%, p < 0.001 and 56.78%, p < 0.001) as compared to controls (67.65%, p < 0.001 and 81.86%, p < 0.001), respectively. No significant differences were observed in genotype and allele frequencies on comparing hypertensive POAG study group with pure POAG study group as shown in Table 3.

4. Discussion

POAG is a heterogeneous disease where genetic factors and environmental factors interact in predisposing an individual to disease risk. The etiology of POAG is still not well understood and the present studies in this field aimed to develop techniques for early identification of patients before the onset of clinical phenotype manifestation. A number of genes have been found to be associated with POAG but the studies failed to detect a substantial biomarker gene which confers significantly high-risk of developing glaucoma in future. This is the first association study between PPAR-γ and CYP46A1 gene polymorphism and the risk of POAG in the North Indian hypertensive population.

Activation of PPAR-γ gene might be involved in neurodegeneration that occurs during diabetic retinopathy. The anti-inflammatory effect of PPAR-γ helps in preventing the possible deleterious effect of fatty acid accumulation in the retina leading to neuroprotection. In this context, a significant association was found between PPAR-γ gene (Gly482Ser) polymorphism and diabetic retinopathy disease mechanism in Caucasians [11]. The PPAR-γ gene (rs1797912) allele frequencies were found to be almost same in POAG cases and controls in a Chinese population (p = 0.16) but differed in Asian report [22]. In our study, it was observed that GG genotype frequency of PPAR-γ gene polymorphism (rs10865710) was higher in controls (40%) as compared to normotensive POAG cases (27.97%, p = 0.056) and was further lowered in hypertensive POAG cases (26.85%, p = 0.04). Similarly, the frequency of C allele was higher in POAG cases both with and without hypertension as compared to controls but the difference was statistically insignificant. On the other hand the frequencies of G allele were found to be higher in control group than both the studied POAG cases but this difference

Table 2 Genotypes and alleles frequencies of PPAR-γ gene in cases and controls.

<table>
<thead>
<tr>
<th>GENOTYPE/ALLELE</th>
<th>Controls (102)</th>
<th>Normotensive POAG cases (118) OR 95% CI c2 p values Bonferroni corrected p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>CG</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>CC</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>GG</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>82</td>
<td>40.2</td>
</tr>
<tr>
<td>G</td>
<td>122</td>
<td>59.8</td>
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<td></td>
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</table>

Table 3 CYP46A1 gene polymorphism in POAG

<table>
<thead>
<tr>
<th>GENOTYPE/ALLELE</th>
<th>Controls (102)</th>
<th>POAG cases with hypertension (108) OR 95% CI c2 p values Bonferroni corrected p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>CG</td>
<td>40</td>
<td>39</td>
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<tr>
<td>CC</td>
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<td>C</td>
<td>82</td>
<td>40.2</td>
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<tr>
<td>G</td>
<td>122</td>
<td>59.8</td>
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Table 4 Genotypes and alleles frequencies of CYP46A1 gene in cases and controls.

<table>
<thead>
<tr>
<th>GENOTYPE/ALLELE</th>
<th>Normotensive POAG cases (118) OR 95% CI c2 p values Bonferroni corrected p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>CT</td>
<td>54</td>
</tr>
<tr>
<td>TT</td>
<td>25</td>
</tr>
<tr>
<td>CC</td>
<td>29</td>
</tr>
<tr>
<td>C</td>
<td>112</td>
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<tr>
<td>T</td>
<td>104</td>
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was also not found to be significant ($p > 0.001$). It could be thus inferred that the GG genotype was significantly associated with a negative risk for POAG in hypertensive POAG cases.

*CYP46A1* gene encodes for the enzyme involved in cholesterol-metabolism and it has been demonstrated that cholesterol metabolism is involved in the progression of POAG. *CYP46A1* gene polymorphism was shown to be associated with the risk of POAG in French [18] but not in Caucasian and Chinese populations [23,24]. In our study, the frequency of CT genotype in normotensive POAG cases (49.15%) was similar to its frequency in French moderate POAG cases (42.9%) and Chinese POAG cases (48.8%) respectively [18,23], but this frequency increased to 51.85% in the presence of hypertension in our study. Similarly, the frequency of minor C allele in our study was found to be higher in our normotensive and hypertensive POAG cases (43.22% and 45.37% respectively) as compared to its frequency in Chinese POAG cases (30.4%) [24]. In our study, the frequency of minor C allele was found to be significantly higher in POAG cases with hypertension (45.37%, $p < 0.001$) and normotensive POAG patients (43.22%, $p < 0.001$) as compared to control group (18.14%) where as on the other hand, the frequency of major T allele was nearly same in both the cases of POAG with (54.63%, $p < 0.001$) and without (56.78%, $p < 0.001$) hypertension but was significantly lower as compared to the control group (81.86%). This result was contradictory to the observation in French population where, T allele in the *CYP46A1* rs754203 SNP was associated with a higher risk for primary open-angle glaucoma [18]. The frequency of TT genotype in our study was found to be much lower as compared to the French population (61.3%) in both normotensive (32.2%) and hypertensive (28.7%) POAG cases [18]. The results of this study thus show association of *CYP46A1* gene polymorphism (rs754203) with POAG where CT, CC genotypes and C allele increase the risk of POAG while the TT genotype and T allele show a negative association with POAG.

### 5. Conclusion

In summary, in the North Indian population *CYP46A1* (rs754203) gene polymorphism confers the risk susceptibility to the development of POAG both in normotensive and hypertensive populations. In context to *PPAR-* γ (rs10865710) gene polymorphism only the GG genotype was found to be nearly significant with POAG risk in the Hypertensive North Indian population. Further association studies and screening of other candidate gene polymorphisms are required to elucidate the precise genetic susceptibility of POAG and understanding the associated polymorphism is expected to increase the understanding of the course of disease.

### Conflict of interest

The authors declare no conflicts of interest.

![Table 3](image-url)
Acknowledgments

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References

[12] Ciudin A, Hernández C, Simó R. Molecular Implications of the PPARα in the Diabetic Eye PPARα activation might participate in abrogating the two most important events that occur in DR: neurodegeneration and microangiopathy. PPAR Res 2013:11 686525.