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**Original Research Article**

# Prevalence of genetic variants among primary open angle glaucoma patients in North West Rajasthan

**Raunaq Poonia**[1], Anju Kochar[*2], Nabab Ali[3] and Deepchand Saini[4]

*[1]Senior Resident, [2]Senior Professor, [3]Associate Professor, [4]Medical Officer*
*Department of Ophthalmology, Sarder Patel Medical College (SPMC), SP Medical College Rd, PBM Hospital, Bikaner, Rajasthan 334001 India*

## Abstract

**Introduction:** Glaucoma is defined as progressive optic neuropathy leading to irreversible blindness if not treated on time. Primary open angle glaucoma (POAG) is most common form of glaucoma. Mutation in any one of Myocillin (MYOC), Optineurin (OPTN) or WDR36 gene contributes to nearly 4% of the glaucoma cases.
**Objective:** To establish the disease causative role of the Myocillin, Optineurin and WDR36 genes mutations in POAG.
**Methodology:** A hospital based observational study was carried out among POAG patients attending OPD. 90 consecutive cases of glaucoma and 35 healthy first degree relatives recruited for study. All patient underwent complete ophthalmic examination followed by genomic DNA (deoxyribonucleic acid) isolation from peripheral blood and quantification of DNA on spectrophotometer. Samples were amplified with each primer by PCR (Polymerase Chain Reaction) technique and amplified DNA and primer sequence checked again by electrophoresis for confirmation of specified gene mutation.
**Results:** Frequency of MYOC gene mutation was 17.78% (16/90). Out of 35 healthy relatives 3 controls were positive for pathogenic MYOC gene variant. Frequency of OPTN gene mutation was 18.89% (17/90). No control was positive for pathogenic OPTN gene variant. No case was positive for pathogenic WDR36 gene variant.
**Conclusion:** This study is first of its kind in North India. Early diagnosis and management of high risk family members is needed to prevent the development of vision loss by regular monitoring, thereby making the prognosis better.
**Keywords:** Primary open angle glaucoma, myocillin, optineurin, WDR36, mutation.

***Correspondence Info:**
Dr. Anju Kochar
B-3/94, Sudarshana Nagar,
Bikaner- 334003, Rajasthan, India

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## 1. Introduction

Glaucoma is defined as chronic, progressive optic neuropathy caused by group of ocular conditions, which lead to damage of optic nerve with loss of visual function and often but not invariably associated with increased intraocular pressure. It is the leading cause of irreversible blindness and the second leading cause of blindness after cataract, affecting 80 million people worldwide.[1] Majority of cases would be attributed to primary open angle glaucoma (POAG)[2]. India has high prevalence of glaucoma which affects around 12 million people aged above 40 years and is responsible for 12.8% of the total blindness in the country with POAG being the most common form of glaucoma. [3]

POAG being a complex disease, the underlying molecular mechanisms are still unclear and it exhibits multifactorial aetiology. Along with elevated IOP, other common risk factors for POAG are age, race, family history, thin cornea, myopia, diabetes, hypertension and oxidative stress. Among various risk factors, a positive family history of POAG is considered a major risk factor. Approximately, 16%–22% of first degree relatives of POAG patients develop disease

POAG is a genetically complex trait due to the involvement of many potential candidate genes which are involved in the development, structure and function of the trabecular meshwork and optic nerve head. Four genes, including Trabecular meshwork inducible glucocorticoid response/Myocillin (TIGR/MYOC), Cytochrome P450,

family 1, subfamily B, polypeptide 1 (CYP1B1), Optineurin (OPTN), and WD repeat domain 36 (WDR36), have been identified as glaucoma associated genes.[4] It was found that mutation in any one of MYOC, OPTN or WDR36 gene contribute to nearly 4% of the glaucoma cases.[5] Around the world, an average mutation frequency of ~ 1.4–4.6% was observed in MYOC among POAG subjects.[6-9] Mutations in OPTN were found in 16.7% of the hereditary forms of NTG.[10] It has been suggested that WDR36 may participate in T-cell activation.[11] T-cell responses may be involved in optic nerve degeneration in glaucoma.

The present study was aimed to implicate or rule out the involvement of mutations in the MYOC, OPTN and WDR36 genes in disease causation among POAG patients and to access the prevalence of genes causing POAG in the North-West, Rajasthan, India. The secondary objective of the study was to understand the role of these genes as a cause of POAG and identification of high risk patients in susceptible population i.e. first degree relatives of established POAG patients.

## 2. Methodology

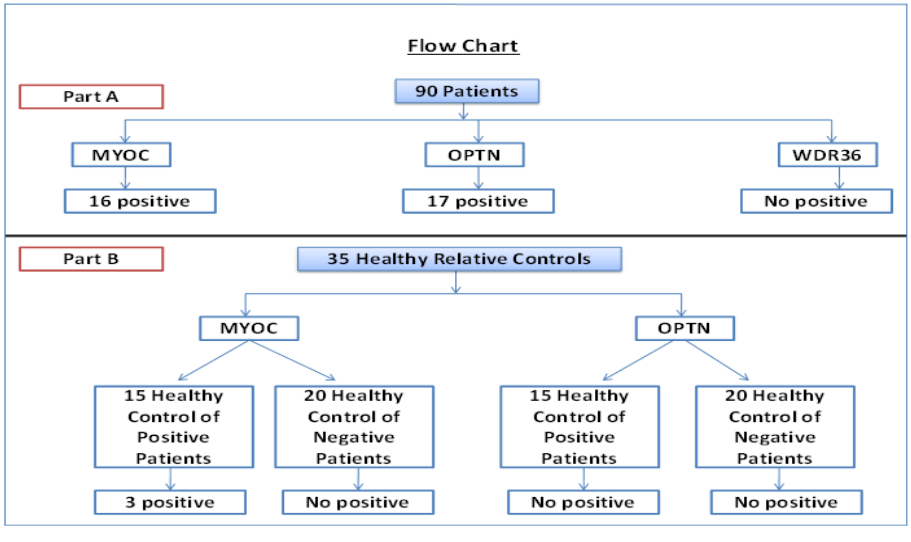
A hospital based observational study was carried out among POAG patients attending OPD. Cases studied in terms of clinical examinations, relevant investigations, appropriate treatment and documentation. All patients having the diagnosis of adult/juvenile-onset POAG, normal tension glaucoma and ocular hypertension of either sex were studied.

This study was done in two parts. In first part initially 90 consecutive cases of POAG given consent to participate were enrolled in study. In second part of study 35 healthy first degree relatives were recruited for study to confirm mutant gene transmission in family. All the POAG patients of either sex of age group ranging from 15 to 80 years answered a predesigned questionnaire that included their personal, demographic and other systemic and clinical details. Each patient underwent a complete ophthalmic examination including best corrected visual acuity, measurement of IOP by applanation tonometer, gonioscopic evaluation of the angle, fundus examination by direct ophthalmoscope after dilatation of pupil, visual field testing with automated perimeter, fundus photography and central corneal thickness by specular microscopy.

For this study inclusion criteria was POAG patients and healthy first degree relatives who gave informed consent to participate in the study. Both sporadic and familial POAG cases and those with optic disc and visual field changes suggestive of glaucoma or patients with an IOP greater or less than 21 mm of Hg with treatment were enrolled for the study. Exclusion criteria was patients with a history of ocular inflammation, a history of ocular trauma, angle closure in any quadrant or those not giving informed consent.

### 2.1 Analysis of the MYOC gene:

After confirmation of diagnosis and obtaining informed consent from the patients and healthy related control, 2 ml blood was drawn in EDTA vial and sent to Multi-Disciplinary Research Units for analysis. Genomic DNA was isolated from the blood samples of patients and healthy related controls using genomic extraction kits and quantification of DNA was done at 260/280 nm on spectrophotometer then quantified DNA was used for further analysis. Primer sets were designed to amplify the gene in fragments of <800 base pairs so as to enable analysis of the amplicons. PCR amplifications were carried out in 25 µl reaction volumes containing about 25 nMol of genomic DNA, 0.5 µl of each primer dilute to 10 pMol in PCR master mix. Amplification was carried out under the following conditions: initial denaturation 94 °C for 5 min, followed by 30 cycles of denaturation 94 °C for 30 s, annealing for 30 s, extension 72 °C for 30 s, followed by a final extension at 72 °C for 2 min and finally the PCR products were analysed by electrophoresis in 1.2% agarose gel.



## 3. Results

In present study it was found that 20% (18/90) patients were of ≤ 50 years of age and 80% (72/90) patients were of >50 years of age.68.9% (62/90) were males and 31.1% (18/90) females were enrolled in study. Mean age was 57 ±12 years. Minimum age was 22 years and maximum age was 79 years. The study included 90 patients, of which 77 (85.6%) patients were of adult onset POAG, 7 (7.8%) of juvenile onset POAG, 4 (4.4%) of NTG and 2 (2.2%) of ocular hypertension.

**Table 1: Demographic distribution of types of Primary Open Angle Glaucoma**

| Parameters | | POAG | | JOAG | | NTG | | Ocular HTN | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % |
| | **Total** | 77 | 85.6 | 7 | 7.8 | 4 | 4.4 | 2 | 2.2 | 90 | 100 |
| **Sex** | **Male** | 52 | 83.9 | 4 | 6.4 | 4 | 6.4 | 2 | 3.2 | 62 | 68.9 |
| | **Female** | 25 | 89.3 | 3 | 10.7 | 0 | 0 | 0 | 0 | 28 | 31.1 |
| **Age groups** | **21-30** | 0 | 0 | 3 | 60.0 | 1 | 20.0 | 1 | 20.0 | 5 | 5.5 |
| | **31-40** | 2 | 40.0 | 2 | 40.0 | 0 | 0 | 1 | 20.0 | 5 | 5.5 |
| | **41 -50** | 6 | 66.7 | 2 | 22.2 | 1 | 11.1 | 0 | 0 | 9 | 10 |
| | **51-60** | 33 | 97.1 | 0 | 0 | 1 | 2.9 | 0 | 0 | 34 | 37.7 |
| | **61-70** | 31 | 96.9 | 0 | 0 | 1 | 3.1 | 0 | 0 | 32 | 35.5 |
| | **71-80** | 5 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5.5 |
| **Comorbidity** | **HTN** | 22 | 81.5 | 4 | 14.8 | 0 | 0 | 1 | 3.7 | 27 | 30 |
| | **DM** | 3 | 75 | 0 | 0 | 1 | 25 | 0 | 0 | 4 | 4.4 |
| | **HTN, DM** | 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2.2 |
| | **Nil** | 50 | 89.3 | 3 | 5.4 | 3 | 5.4 | 1 | 1.8 | 57 | 63.3 |

**Table 2: Distributions of different parameters between sexes in study population**

| Parameter / Group | IOP | | | CCT | | | AL | | |
|---|---|---|---|---|---|---|---|---|---|
| | **M** | **F** | **Overall Mean** | **M** | **F** | **Overall Mean** | **M** | **F** | **Overall Mean** |
| **Adult onset POAG (77)** | 19.8±3.4 (n=52) | 19.9±3.5 (n=25) | 19.9±3.5 | 502±24 | 518± 22 | 510±23 | 23.38± 0.99 | 23.27± 0.79 | 23.33±0.89 |
| | P = 0.91 | | | P= 0.006 | | | P<0.001 | | |
| **JOAG (7)** | 18.0± 3.2 (n=4) | 22.3±4.4 (n=3) | 20.2±3.8 | 516±13 | 489±40 | 503±27 | 23.64±0.66 | 22.65±0.61 | 23.15±0.64 |
| | P = 0.15 | | | P= 0.25 | | | P = 0.099 | | |
| **NTG (4)** | 18.3± 5.2 | | 18.3±5.2 | 500±9 | | 500±9 | 22.53±0.77 | | 22.53±0.77 |
| **OCULAR HTN (2)** | 25.6± 1.6 | | 25.6±1.6 | 546±13 | | 546±13 | 22.84±1.10 | | 22.84±1.10 |

**Table 3: Pathogenic MYOC, OPTN & WDR36 gene variants distribution in defined study group**

| Gene | Mutated primer | POAG | JOAG | NTG | Ocular HTN | Total |
|---|---|---|---|---|---|---|
| **MYOC** | MYOC 1F/MYOC 1R to MYOC 3F/MYOC 3R | NIL | NIL | NIL | NIL | NIL |
| | MYOC 4F/MYOC 4R | 12 | 3 | NIL | 1 | 16 |
| | MYOC 5F/MYOC 5R to MYOC 12F/MYOC 12R | NIL | NIL | NIL | NIL | NIL |
| **OPTN** | OPTN4bF/OPTN4bR | 6 | 2 | NIL | NIL | 8 |
| | OPTN5F/OPTN5R | 1 | NIL | NIL | NIL | 1 |
| | OPTN6F/OPTN6R | 7 | 1 | NIL | NIL | 8 |
| | OPTN7F/OPTN7R | NIL | NIL | NIL | NIL | NIL |
| | OPTN8F/OPTN8R | NIL | NIL | NIL | NIL | NIL |
| **WDR 36** | 6 WDR36 gene PCR primers | No case was positive for pathogenic WDR36 gene variant. | | | | |

Frequency of MYOC gene mutation was 17.77% (16/90) in defined study group. 4 healthy related controls (11.42%) out of 35 were also positive for MYOC gene mutation. Frequency of OPTN gene mutation was 18.89% (17/90) in study groups. Out of 35 healthy relatives, No control was positive for pathogenic OPTN gene variant. And no case was positive for pathogenic WDR36 gene variant.

**Table 4: Parameters distribution in defined gene study population**

| Group<br>Parameter | Mutant gene positive patients | Mutant gene negative patients | Healthy control of positive patients (n=15) | Healthy control of negative patients (n=20) |
|---|---|---|---|---|
| **IOP(mm hg)** | 19.9 ±3.2 | 19.1±4.2 | 15.7±2.2 | 15.1±2.2 |
| **CCT(µm)** | 509.7 ±21.2 | 511.2±28.3 | 538.5±18.1 | 530.1±16.1 |
| **Axial Length (AL) (mm)** | 23.8±0.7 | 23.1±1.2 | 21.9±0.8 | 23.0±0.9 |

## 4. Discussion

In our study 68.9% (62/90) cases were male and 31.1% (28/90) were female (Table 1). All four groups showed male predominance. In this study male-female ratio was 2.2 which were similar to other studies done worldwide. These findings are well comparable to Framingham Eye study which showed increased prevalence of POAG among men compared to women [12]. Rotterdam eye study done in 1994 at Netherlands showed men had a more than three times higher risk of having POAG than women (odds ratio, 3.6)[13].

In our study 30% cases had POAG with hypertension (Table 1). These are findings like Beaver Dam Eye study[14]in which a significant correlation was found between changes in systemic blood pressure and changes in IOP, reduced systemic blood pressure was found associated with reduction of intraocular pressure. A similar study done by Newman-Casey *et al* [15] has shown that systemic hypertension alone is responsible for 17% of increased risk of developing POAG.

In the present study frequency of MYOC gene mutation was 17.78% (16/90) in open angle glaucoma cases (Table 3). Our results are similar to Allingham *et al* [16] (1998) study at United States shows MYOC mutations account for 17.22.2% of POAG cases studied. But other studies showed lesser prevalence like Mukhopadhyay *et al* [17] (2002) at Kolkata, India which shows that MYOC mutations frequency were found in 7.1% of the population studied. In a study done by Kanagavalli *et al* [18] in 2003 at Madurai, India showed that mutation frequency of the MYOC gene was 2% in the Indian population affected with POAG. Bhattacharjee *et al* [19] in 2007 at Kolkata, India it was concluded that MYOC mutations account for 2.2% of POAG cases.

Out of 90 patients, we detected pathogenic OPTN gene variant in 17 (18.9%) open angle glaucoma cases (Table 3). Out of 17 cases pathogenic OPTN gene variant was found in 14 adult onset POAG cases, 3 in juvenile onset POAG. Yen *et al* [20] study (2008) shows that fifteen variants of OPTN were found in the 51 JOAG patients and 51 unrelated normal controls. Other studies like Willoughby *et al*[21] (2004), Sirohi *et al*[5], Yuan *et al*[22] study (2008) also suggest that OPTN gene mutations are associated with the occurrence of primary open angle glaucoma.

Out of 90 cases of open angle glaucoma, No case was positive for pathogenic WDR36 gene variant (Table 3).Our results are similar to other studies like Liu *et al* [23] study (2017) in which Five WDR36 polymorphisms were meta-analyzed, none of them was significantly associated with POAG, HTG, or NTG. In another study Huang C *et al* [24] (2018) investigated MYOC, OPTN, NTF4, WDR36 and CYP1B1 in JOAG patients. No mutation was detected in NTF4 or WDR36. Frezzotti *et al* [25] study (2011) revealed that WDR36 sequence variance is only a rare cause of glaucoma in Italian families. Weisschuh *et al* [26] study (2007) also indicates that WDR36 gene variants may be only rare causes of normal tension glaucoma in the German population.

In contrary of our results some studies shows the association of mutation in WDR36 gene in glaucoma cases like, Footz *et al*[27] study (2009) shows that WDR36 sequence variants can lead to an altered cellular phenotype. Su *et al* [28] study (2017) also says that the WDR36 gene is reckoned as one of the major causative genes of POAG.

## 5. Conclusion

This study is unique since all variants of open angle glaucoma like adult onset POAG, JOAG, OHT and NTG have been enrolled in this study. The results of this study provide evidence to prove that DNA screening is a useful method with high specificity and sensitivity for early detection of the at-risk individual in a glaucoma pedigree.

Understanding the molecular basis of glaucoma is important to several aspects of glaucoma diagnosis and management. Identifying novel pathways could be used to design more specific and effective therapies. Thus, gene screening can be used for pre-symptom diagnosis and forewarning in familial open-angle glaucoma patients, especially in pedigrees with early-onset.

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