Optineurin Gene Polymorphism and Its Association With Glaucoma Onset

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Research Article

Keywords: Glaucoma, OPTN polymorphism, Genotyping, Haplotype, Linkage disequilibrium.

Posted Date: March 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1375307/v1>

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Abstract

Glaucoma is a polygenic trait, and an irreversible visual impairment disorder caused by elevation of IOP (Intraocular Pressure), apoptotic death of the retinal Ganglion cells, excavation of optic nerve and ultimately vision loss if remain untreated. It is inherited in next generation in carrier families. In this study we aimed to identify the genetic polymorphism in *OPTN* gene and its association with glaucoma. Three SNPs (rs11258194, rs75654767, rs2234968) of *OPTN* gene were evaluated with their restriction enzymes *StuI*, *Acil* and *HpyChIV* respectively for their genetic association. For this purpose, blood samples were carried out from 100 patients and 100 controls with their clinical characteristics. Genotyping was done by DNA sequencing and PCR-RFLP. Overall the genotypic frequencies of two SNPs rs11258194 “TA” and rs75654767 “AA” were (0.21) and (0.42) in patients respectively while these genotypes were absent in controls. So rs75654767 (AA) and rs11258194 were significantly associated ($p < 0.05$) with Glaucoma in our population. No association was detected with rs2234968. Haplotype analysis indicated that TGG, AAG, and TAG were strongly associated ($p < 0.05$) with the disease onset while the haplotype AGG was significant in controls and has a protective role against disease development. Moreover, no linkage disequilibrium was seen between the rs2234968 and rs2234968 except the rs11258194 ($D' = 0.75$). In conclusion, the polymorphism in *OPTN* was significantly associated with the glaucoma. A large scale genetic screening of families is required for the evaluation of their role in disease development and early diagnosis.

Introduction

Primary Open Angle Glaucoma (POAG) is the most common form of primary glaucoma, characterized by an open angle with or without visible glaucomatous changes rather than people had an increased intraocular pressure (IOP) > 21 mmHg on gonioscopic examination (López-Martínez et al., 2007; Ray et al., 2003). POAG is more prevalent in those individuals which have age > 40 years. POAG genetically appears as a complex set of Mendelian inheritance pattern and sporadic manifestations in which a specific gene at their loci not only necessary but sufficient to induce the phenotype (Kumar et al., 2007; López-Martínez et al., 2007). Multiple genes are involved in the onset of POAG disease (by monogenic, autosomal dominant trait) (Kwon et al., 2009). The candidate genes linked with POAG includes Myocilin (MYOC), *Optineurin* (*OPTN*), Cytochrome P450 Family 1, subtype B polypeptide 1 (CYP1B1), WD repeat domain 36 (WDR36), ankyrin repeat and SOCS-box containing 10 (ASB10) etc. (Monemi et al., 2005; Rezaie et al., 2002; Stone et al., 1997). Moreover, according to the Genome wide association studies (GWAS) a number of variants were investigated at different loci including CAV1/CAV2, TMC01, CDKN2B-AS1, CDC7-TGFB3, SIX1/SIX6, GAS7 and ATOH7 have been associated with POAG (Abu-Amero et al., 2015).

Optic neuropathy inducing gene was reported on chromosome 10p15-p14 at the gene locus GLC1E and this gene was regarded as the *Optineurin* gene (*OPTN*) i.e. optic neuropathy inducing protein (Sarfarazi et al., 1998; Rezaie et al., 2002). The *OPTN* gene contains total 16 exons of which the 3 noncoding exons are in the 5' untranslated region (UTR) and the remaining 13 exons are in the coding region which code

for a 577 amino acids protein having a molecular weight of about 66Kda (Leung et al., 2003; Rezaie and Sarfarazi 2005).

The *OPTN* gene codes four transcripts which differ from each other according to their 5' UTR region (Wiggs et al., 2003; Ying and Yue, 2012) and their protein products expressed not only in the optic tissues but also in the non-optic tissues such as the heart, brain, liver, skeletal muscle, kidney, placenta and pancreas. In the eye *OPTN* coded transcript expressed in the trabecular meshwork, non-pigmented ciliary epithelium, retina, the Schlemm's canal and the aqueous humor (Li et al., 1998; Rezaie et al., 2002; Sarfarazi and Rezaie, 2003). The *OPTN* products by interacting with other proteins are involved in apoptotic pathway, inflammatory response and vasoconstriction (Sripriya et al., 2006). According to Ying and Yue, (2012) the *OPTN* gene had a basic role in the antimicrobial signaling, proper functioning of Golgi apparatus, trafficking of protein at cellular level and any knockdown or overexpression in the *OPTN* gene can lead to the neurodegenerative diseases like POAG. In a Chinese population Leung et al., (2003) investigated 16 SNP's (*OPTN*) were studied in which the 3 mutations (T34T, M98K and R545Q) reported in other populations and the other 13 ones were the novel Fan et al., (2005) also reported the exonic (T34T) and intronic (IVS5 + 38T > G and IVS7 + 24G > A) polymorphs in *Optineurin* gene associated with the onset of POAG in Chinese population. Mukhopadhyay et al., (2005) reported three polymorphs Thr34Thr (rs2234968) on exon 4, Met98Lys (rs rs11258194) on exon 5 (Kumar et al., 2007; Sripriya et al., 2006) and Arg545Gln (rs75654767) on exon 16 of the *OPTN* gene in Indian population.

Pakistan is among the developing countries where the genetic diseases are increasing day by day. Our genetic pool is becoming narrow due to frequent intra caste and cousin marriages and that's the reason genetic polymorphs are transmitted from parents to offspring generation by generations. This is because of lack of awareness and loopholes in health care facilities. However, many more have to be disclosed about the exact genetic cause of glaucoma (POAG) disease. Therefore, the current study is to identify the SNP in *OPTN* gene and the association of *OPTN* gene with POAG and to evaluate its role in glaucoma development in Pakistanis.

Materials And Methods

Subjects

The patients with glaucoma (POAG) were recruited from the population of Lahore Punjab, Pakistan. The sampling was carried out from the public and private hospitals of Lahore. The current study was approved by the Board of Advance Research, department of Zoology, GCU, Lahore. All of the patients were clinically diagnosed with glaucoma according to WHO criteria. The inclusion and exclusion criteria for the selection of POAG among the patients included Age > 30 years, IOP > 21mmHg, Cup-disc ratio > 0.7 (optic disc damage), Open angle on gonioscopy, Glaucomatous visual field loss on Goldman Perimetry. The subjects with secondary causes (ocular trauma, ocular hypertension, uveitis and NTG) were excluded from the current study.

In the current study 100 patients with glaucoma (POAG) and 100 healthy controls participated. All the subjects were interviewed for their gender, age, disease onset and complications etc. The pre-informed consent forms were signed by the patients/guardian and controls prior to sampling. The blood was collected with 5cc Syringes and carried out in EDTA (Atlas-Medo-O-Vae Francisco Vacuum tubes) coated tubes and stored at 4°C for further use.

DNA Isolation and PCR

The genomic DNA was extracted from the blood by standard organic chloroform and ethanol precipitation method (Sambrook *et al.*, 1989). Concentration and purity of all the DNA samples was measured by Nanodrop. The genetic study for candidate gene of POAG was selected with reported SNPs from NCBI (National Centre for Biotechnology Information) to check their association Table 1.

Table 1

Selected SNP's for genetic association study of POAG with the respective primers and RFLP enzymes.

SNP ID	Exon	Primer	PCR product (bp)	RFLP Enzyme
rs2234968	4	F 5'-TGAAAGCACAGGAAATGGAC-3' R 5'-AGGGATGGCATTCTTGCAG-3'	177	<i>HpyCh4IV</i>
rs11258194	5	F 5'-GTCCACTTTCCTGGTGTGTGACT-3' R 5'-CAACATCACAATGGATCG-3'	277	<i>StuI</i>
rs75654767	16	F 5'-GAAACAAACACAACCTGCCTGC-3' R 5'-CCATCACGTGAATCTGTAACG – 3'	166	<i>AccI</i>
bp = base pair; F = Forward primer; R = Reverse primer; RFLP = Restriction Fragment Length Polymorphism				

Table 1
Allelic and Genetic association study of POAG with *OPTN* gene.

Frequency Patients		Frequency Controls	Chi2 Value	Fisher's P value
STU				
Alleles				
T	0.106	0.00	2.811	0.094
C	0.894	1.00		
Genotypes				
TT	0.00	0.00	3.139	0.076
TC	0.212	0.00		
CC	0.788	1.00		
ACI				
Alleles				
T	0.418	0.00	16.007	6.40e-005
C	0.582	1.00		
Genotypes				
TT	0.418	0.00	8.009	0.004
TC	0.000	0.00		
CC	0.582	1.00		
HPY				
Alleles				
C	1.00	1.00	-	-
Genotype				
CC	1.00	1.00	-	-
Significance level 0.01				

For Polymerase Chain Reaction (PCR) reported oligonucleotide primers were used (Alward *et al.*, 2003; Funayama *et al.*, 2004 and Baird *et al.*, 2004) and applied for amplification of required gene segment. All primers double checked for the sake of validity and authenticity with the sequences from NCBI website (<https://www.ncbi.nlm.nih.gov/snp/>). All of the primer sets along with their SNP, ID product size and Restriction Fragment Length Polymorphism (RFLP) enzyme are given Table 1. For PCR a reaction mixture of 25µl (Master mix: 8µl, Forward primer: 1.5µl; reverse primer: 1.5µl; DNA template: 3µl; DEPC water:

11µl) was loaded for the amplification of DNA in PCR tube. The PCR conditions of primers with an annealing temperature 58.3°C for rs2234968, 58.5°C for rs11258194 and 59.1°C for rs7565476 within (Q Cycler) PCR machine. All of the steps/conditions involved within PCR were denaturation (5 min at 96°C), initial denaturation (30 sec at 95°C), annealing (45 sec at optimized temperatures), extension (45 sec at 72°C) and storage was at 4°C.

Genotyping

Genotyping was carried out by DNA sequencing and PCR-RFLP. The PCR products were sequenced from Applied Bioscience International (ABI). Forward primers were used for DNA sequencing in reaction mixture.

After PCR, RFLP was performed to determine *OPTN* gene single nucleotide polymorphisms/substitutions at *StuI* (rs11258194-), *Acil* (rs75654767) and *HpyCh4IV* (rs2234968) with their restriction enzymes respectively. The reaction mixture (16µl) of pcr tubes containing PCR product (5µl), Nuclease free water (9µl), buffer (1µl) and enzyme (1µl: *StuI*-incubation at 37 5–6 hours, *Acil* incubation at 37 5–6 hours, *HpyCh4IV* incubation at 65 5–6 hours and deactivation at 80°C) were incubated at 37°C for 5–6 hours according to the manufacturer instructions in water bath followed by final deactivation at 65°C for 20 min. The mixture (5µl reaction mixture and 2µl loading dye) of each sample run against 1µl of DNA ladder on a 2% agarose gel (dissolving 2g of agarose in 100ml of 1X of TAE buffer) and observed under UV Transilluminator.

Statistical Analysis

All of the sequences were visualized in Bio Edit software and in order to determine the mutation sequences of interest were multi-aligned with other sequences using NCBI. The sequences were also aligned through MEGA5 software for determining their expected amino acid sequences changes. All data of cases and controls was passed from the Hardy-Weinberg equilibrium. Genetic Data of SNP was carried out by SHESIS and Plink available online to evaluate the linkage disequilibrium and haplotype analysis. Genes and allele frequencies were calculated and respective genetic significance level was determined by Fisher exact test and Chi square test.

Results

The clinical characteristics of 100 glaucoma patients (male = 50, female = 50) and 100 are as followed. The mean age of male and female patients was 51.4 and 53.5 years respectively while in controls it was observed 49.4 and 53.4 years so the difference was not significant. The mean age of diagnosis of glaucoma within male was 47.3 years whereas in female was 52.5 years. However the observed disease duration among the male and females was 4.1 and 1.0 years respectively. Moreover, mean age of inclusion of male and female patients was 51.6 years and 54.5 years respectively. The mean BMI of the male and female patients was 24.29 (Kg/m²) and 23.23 (Kg/m²) respectively which was considered normal while it was observed 20.78 (Kg/m²) in male and 24.29 (Kg/m²) in female of control subjects.

Current study demonstrated that 52% of patients were with positive family history out of patients while all of the controls were with negative family history of the glaucoma disease.

The sequencing results showed that heterozygous (TA) at rs11258194 (*StuI*) polymorphic site was frequent in patients (0.21) as compared to controls (0.00) shown in (Fig. 1). Similarly a homozygous sequence variant (AA) was found at rs75654767 (*AccI*) polymorphic site in subjects (0.42). No mutation was observed in cases and controls at rs2234968 (*HpyChIV*) polymorphic site shown (Fig. 2).

The association of genetic polymorphism with POAG at case/control allelic level was shown in table 4.2. The case/control genetic frequencies of three SNP's were summarized in table 2. It was observed that all SNPs (rs11258194, rs75654767 and) followed the Hardy Weinberg Equilibrium (HWE) i.e., $p > 0.5$ whereas rs2234968 violated the HWE. It was observed that the rs11258194 and rs75654767 significantly associated with the Glaucoma (POAG) as P value is ($P < 0.05$). It was estimated that the rs2234968 polymorphism was not significantly associated with onset of POAG in Pakistani population.

The Haplotype analysis was performed for selected SNP's of *OPTN* gene and it was found that the frequency of haplotypes T G G (0.560), A A G (0.085), T A G (0.335) was higher in patients as compared to the controls and strongly associated with the onset of Glaucoma. On the other hand the Haplotype A G G was not associated with the disease onset.

Linkage disequilibrium (LD) estimated the possible recombination's (D) among the *OPTN* gene variants associated with POAG shown in Fig. 3 (a, b). No linkage disequilibrium was observed between the rs2234968 and rs75654767 either they transfer in two combinations except the rs11258194.

The amino acid sequence of proteins were aligned on MEGA 6 and it was observed that the polymorphism at rs11258194 lead to the change of amino acid Tryptophan into Arginine. However, the polymorphisms at rs75654767 lead to the change of Glycine into Serine amino acid.

Discussion

Glaucoma is a group of diseases characterized by degeneration of the optic nerves and loss of visual field resulting in blindness if left untreated (Fan *et al.*, 2005). Glaucoma (POAG) referred to as complex multifactorial disease caused by both the genetic and environmental risk factors (Shigeeda *et al.*, 2002). *Optineurin* is identified as the 2nd susceptible gene for POAG and it was reported that *OPTN* mutations accounts for 16.7% POAG patients throughout the world (Rezaie *et al.*, 2002; Sarfarazi *et al.*, 1998). About 70 million people (40-80years) are affected by glaucoma (POAG) throughout the world but it will be increased to 79.6 million by the year 2020 as the population increases (Barkana and Dorairaj, 2015).

The current study demonstrated that the polymorphism at *StuI* site (rs11258194 T > A) on exon 5, significantly associated ($p = 0.01$) with the development of POAG. The allelic and genotypic frequencies of *StuI* polymorphism showed significance difference in both cases and controls ($p < 0.01$). A study by Rezaie *et al.*, (2002), showed that *StuI* polymorphism was higher (13.6%) among the patients in

comparison to controls (2.1%) and was statistically significant ($p = 0.002$) with the POAG. The association of rs11258194 polymorphic site with the onset of POAG had been reported in different populations. A significant association ($p = 0.009$) among the Japanese population was observed between with *Stul* polymorphic site and POAG (Fuse *et al.*, 2004). The studies conducted in Japan, India, London and China it was reported as the risk associated factor to the glaucoma onset (Alward *et al.*, 2003; Aung *et al.*, 2017; Leung *et al.*, 2003; Tang *et al.*, 2003). The polymorphism from different cohorts varied with respect to significance level i.e. ($p < 0.05$) due to the ethnicity differences, different recruitment strategies and population specific variants (Baird *et al.*, 2004). In contrast to the current study rs11258194 was not significantly associated ($p > 0.05$) with the POAG in United States (Wiggs *et al.*, 2003). Our present study demonstrated that homozygous genotype (AA) of *Acil* (rs75654767 G > A) polymorphic site significantly associated ($p = 0.03$) with POAG at genotypic level.

The *Acil* polymorphic site (rs75654767) was reported to be strongly associated ($p < 0.05$) with POAG onset due to alterations in *OPTN* gene within the US population (Rezaie *et al.*, 2002). A study in Indian population reported that rs75654767 had an association with the glaucoma (POAG) (Mukhopadhyay *et al.*, 2005). Whereas in Caucasian population reported that polymorphic site (rs75654767) did not have any association with the development glaucoma (Alward *et al.*, 2003). The homozygous (AA) genotype ($p = 0.77$) was also reported among the Japanese population but it did not shown the significance level with glaucoma disease. This mutation was reported in many independent studies covering different ethnic origins including Canada, the United States, Germany and Australia and considered as non significant risk factor for disease onset (Alward *et al.*, 2003; Baird *et al.*, 2004; Fuse *et al.*, 2004; Willoughby *et al.*, 2004).

In the current study, rs2234968 G > A was highly non-significant with glaucoma onset and no mutation was found among the patients of Pakistan. Similarly no significant association ($p > 0.05$) was evaluated between the rs2234968 and *OPTN* gene to develop POAG within Hispanic population (McDonald *et al.*, 2010). In another study, Leopez-Martinez *et al.* (2007) reported that rs2234968 was non disease causing polymorphic site ($p = 0.06$) of *OPTN* gene among the Spanish individuals. The rs2234968 polymorphism was strongly associated ($p = 0.011$) in Japanese patients and the allele frequency (A) was higher (0.003) in patients as compared to controls (Funayama *et al.*, 2004).

The current study revealed that TGG, AAG and TAG Haplotype have higher frequencies as compared to the controls and was significantly associated ($p < 0.05$) in disease development. While the Haplotype AGG played a protective effect against the disease development.

Conclusion

In conclusion, the present study evaluated that glaucoma (POAG) is a rapidly emerging disease in the Pakistani population and people after the age of 50–60 years are at high risk of disease development. The SNPs rs11258194 and rs75654767 in *OPTN* gene were significantly associated with onset of Glaucoma. Furthermore there is need of large scale genetic screening to build a set of genetic markers a

for Pakistani population and these genetic markers can be used for genetic testing so that susceptible individuals can be tested and cured prior the onset of disease.

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Figures

(a)

(b)

Figure 1

Chromatographic representation of rs11258194 of *OPTN* gene: a. Control genotype (TT), b. Patient genotype (TA)

(a)

(b)

Figure 2

Chromatographic representation of rs2234968 of *OPTN* gene: a. Control genotype (GG), b. Patient genotype (GG)

(a) Patient

(b) Controls

Figure 3

Location and map of Linkage Disequilibrium (LD) of SNP's at chromosome 10 presented (a) Patient (b) Control. The SNP numbers are represented at the top of the Haploview. Red color showed the disease onset; white color showed no disease transfer.