RESEARCH ARTICLE

A novel single nucleotide polymorphism in exon 3 of MYOC enhances the risk of glaucoma

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Abstract

Genetic polymorphisms of MYOC alter the myocilin protein, which leads to disruption of the normal regulation of intraocular pressure (IOP) that ultimately causes glaucoma. The aim of the present study was to identify the polymorphism in exon 3 of the MYOC gene of the glaucoma patients in Lahore, Pakistan. We conducted a case-control study with 100 patients and 100 controls subjects. We extracted DNA from blood samples, amplified the target DNA fragment by PCR, and identified polymorphisms through sequencing. We observed that the allelic and genotypic frequencies of rs74315341 and rs879255525 were associated with glaucoma in our patient population. The polymorphism at rs74315341 led to the substitution of serine for arginine, whereas the polymorphism at rs879255525 led to the substitution of asparagine for lysine. The haplotype TGAAGCCATTTC was associated with disease onset, whereas the haplotype GGAAGCCA TTTC was protective against disease development. In conclusion, we identified MYOC gene polymorphisms in susceptible regions that were associated with glaucoma onset among the Lahore patient population. This is the first report to identify a novel mutation in rs879255525 in exon 3 of the MYOC gene that is associated with glaucoma.

Introduction

Glaucoma is a progressive optic neuropathy that leads to visual field impairment[1]. Optic neuropathy is caused by rimming up or regression of the optic nerve, which prompts the loss of fringe vision; if not cured, it can lead to irreversible visual impairment[2]. Primary open angle glaucoma (POAG) is a complex disorder with a major heritable component. The candidate genes associated with POAG onset are myocilin (MYOC); WD repeat domain 36; optineurin; cytochrome P450 family 1, subtype B, polypeptide 1; ankyrin repeat and SOCS-box containing 10 and neurotrophin 4[3,4]. The first locus associated with POAG was
positioned in chromosome 1; the GLC1A, now known as MYOC, locus encodes the protein myocilin. Disease-related myocilin mutations are commonly found in juvenile or early adult patients with very high levels of IOP [5].

MYOC has 3 exons; most mutations have been found in the third exon, which encodes the olfactomedin-like domain [5]. Myocilin forms part of the main structure of the eye, the trabecular meshwork, which regulates IOP [6–8]. Mutations that change the structure of myocilin disrupts the normal regulation of IOP. Disease-related forms of myocilin undergo altered protein trafficking, leading to intracellular aggregation of the misfolded protein. The inability to properly release the protein enhances the IOP [9].

Genetic diseases are increasingly prevalent in Pakistan due to its relatively genetically heterogeneous population. Common consanguineous marriages result in frequent transmission of mutations through the generations. The glaucoma incidence rate in Pakistan is 3.9% [10], but the exact genetic cause of this disease remains a mystery because of the unavailability of baseline data. Therefore, we designed a case-control study with the aim to determine the polymorphism in exon 3 of MYOC in Lahore glaucoma patients.

Materials and methods

Sampling

The study was ethically approved by the Board of Studies of the University of the Punjab, Lahore. Sampling was carried out at Layton Rahmatullah Benevolent Trust, Lahore. After we obtained written, informed consent from the patients or their guardians on the prescribed forms, we collected blood samples (3 ml) from 100 glaucoma patients and 100 healthy individuals in EDTA-coated tubes; we recorded the clinical characteristics of the subjects on performa. The inclusion and exclusion criteria for patient selection include IOP (tonometry), optic nerve damage (ophthalmoscopy), complete field of vision (perimetry), angle where the iris meets the cornea (gonioscopy), and thickness of the cornea (pachymetry).

Genotyping

We extracted genomic DNA from each blood sample using the modified organic method [11]; we quantified the DNA using a NanoDrop™ spectrophotometer. We amplified the target sequence using previously reported primers [12].

We optimized the primers by gradient PCR and amplified the targeted sequence of 960 bp in 25-μl PCR mixtures containing 3 μl DNA template, 4 μl MgCl₂(25 mM), 4 μl 10× PCR buffer, 3 μl dNTP mix (2.0 mM), 1.5 μl forward and reverse primers (10 pM), 0.5 μl Taq Polymerase (500 U; Thermo Fisher Scientific), and 7.5 μl DEPC water. The PCR cycle included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 30s of denaturation at 95°C, 45s of annealing at 67.5°C, and 45s of extension at 72°C. This was followed by final extension at 72°C for 10 min. We sent the PCR products to Advance Biosciences International for sequencing.

Sequence and statistical analysis

We visualized the sequences with BioEdit software and analyzed them using the Basic Local Alignment Search Tool from the National Center for Biotechnology (NCBI) and the University of California, Santa Cruz Genome Browser to identify single nucleotide polymorphisms (SNPs). All SNPs were assessed for Hardy–Weinberg Equilibrium (HWE). We calculated the allelic and genetic frequencies and determined the association of the MYOC gene polymorphisms with disease onset with the chi-squared test and Fisher’s test. We determined the linkage disequilibrium (LD) and performed haplotype analysis online with SHEsis software (http://...
shesisplus.bio-x.cn/SHEsis.html). We evaluated changes in amino acid sequence using MEGA 6 software.

**Results**

Among the 100 patients, 40 males and 55 females had positive family histories of glaucoma, whereas none of the control subjects had a positive family history. The mean age at the time of glaucoma diagnosis was 47.3 years for males and 52.5 years for females. The mean age of inclusion of disease for male and female patients was 51.6 years and 54.5 years, respectively. We identified rs74315341 by genotyping. rs74315341 comprises the replacement of guanine with thymidine. We also identified a novel SNP that comprises the replacement of guanine with thymidine. We submitted the sequence to the ClinVar NCBI database and the SNP was assigned the novel number rs879255525. Both SNPs were in HWE (p > 0.05). The allelic and genotypic frequencies of the SNPs are presented in Tables 1 and 2, respectively. The allelic and genotypic frequencies of rs74315341 and the novel SNP rs879255525 varied significantly between the patients and controls, and were significantly (p < 0.01) associated with glaucoma onset. The change in the nucleotide sequence of rs74315341 resulted in the substitution of serine for arginine and the change in rs879255525 resulted in the substitution of asparagine for lysine. The SNPs rs74315335, rs121909193, rs74315334, rs74315329, rs74315330, rs74315336, rs74315338, rs74315328, rs74315331, and rs74315332 were associated with glaucoma onset in our population, but the associations did not reach statistical significance.

Haplotype analysis indicated that the sequences GTAAGCCTTTC and TGAAGCATTTC appeared at higher frequencies in patients than in controls, and that TGAAGCATTTC was strongly associated with the onset of glaucoma (p = 0.005). On the other hand, GGAAGCCA TTTC appeared at higher frequency in the controls than in the patients, indicating that it exerted a protective role against glaucoma onset.

The LD value for rs74315341 and rs879255525 was 0.703, suggesting that they are significant risk factors for glaucoma development. We did not observe significant LD between the SNPs, with the exception of rs879255525 (Fig 1A and 1B).

**Discussion**

More than 60 million people have been diagnosed with glaucoma, a complex group of optic neuropathies [13]. Mutations of the MYOC gene are associated with POAG onset in Chinese,
French, Spanish, American, Australian, Indian, Swiss, and Japanese populations [14–16].

We demonstrated that mutations in exon 3 of myocilin at the rs74315341 and rs879255525 polymorphic sites were associated with glaucoma onset in a Lahore patient group, which was consistent with the role of rs74315341 in POAG development in an Australasian population [17]. Studies in Caucasian and Brazilian populations also found a significant association of this SNP with glaucoma [18,19].

We found that rs74315335, rs121909193, rs74315334, rs74315330, rs74315336, rs74315338, rs74315331, and 74315332 were not significantly associated with glaucoma onset in our patient population. However, a previous study reported that rs74315335 was significantly associated with POAG [20]. Similarly, rs74315334, rs74315329, and rs74315330 are significantly associated with glaucoma onset in an Australasian population [17]. rs74315329 is a risk factor for disease onset in a Tasmanian population [21]. Furthermore, rs74315336 is significantly associated with hereditary glaucoma onset in the United States [22] and rs74315328 and rs74315331 have also been associated with glaucoma onset [23].

In the present study, we observed that the SNPs changed the amino acid sequences and would, ultimately, alter the myocilin protein structure. Consistent with our results, previous studies have reported that the mutated myocilin protein becomes entangled in the cell in its altered forms [24,25]. Heterodimers and heteromultimers with wild-type myocilin form with altered myocilin proteins [26]. Large proteins aggregate in the endoplasmic reticulum as a consequence of misfolded, disease-causing myocilin mutants. Altered myocilin secretion is also sensitive to temperature, in support of the hypothesis that myocilin-induced glaucoma is a protein conformational disease [27,28].

**Conclusions**

Thus, polymorphisms in exon 3 of MYOC at the rs74315341 and rs879255525 polymorphic sites are significantly associated with POAG onset in a Pakistani population. A large-scale survey should be conducted to evaluate the genetic factors associated with POAG to facilitate the identification and treatment of susceptible communities.

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Author Contributions

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