# Molecular analysis of *myocilin* and *optineurin* genes in Korean primary glaucoma patients

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Received September 20, 2015; Accepted June 27, 2016

DOI: 10.3892/mmr.2016.5557

**Abstract.** To investigate the underlying genetic influences of primary glaucoma in Korea, molecular analysis was performed in 112 sporadic cases, and results compared with healthy controls. The *myocilin* (*MYOC*) and *optineurin* (*OPTN*) genes were directly sequenced in 112 unrelated patients, including 17 with primary open-angle glaucoma, 19 with juvenile open-angle glaucoma, and 76 with normal tension glaucoma. Healthy unrelated Korean individuals (n=100) were used as the non-selected population control. A total of three *MYOC* and four *OPTN* variants potentially associated with primary glaucoma were identified in 4 and 18 patients, respectively. A novel variant of *MYOC*, *p.Leu255Pro*, was predicted to be potentially pathogenic by *in silico* analysis. Another, *p.Thr353Ile*, has been previously reported. These two missense variants were detected in patients with a family history of glaucoma. Combined heterozygous variants *p.[Thr123=;Ile288=]* were identified in 2 of 112 (2%) patients but not in healthy controls. Among *OPTN* variants, a novel variant *p.Arg271Cys* was identified. Homozygous *p.[Thr34=;Thr34=]* (4/112, 4%), homozygous *p.[Met98Lys;Met98Lys]* (4/112, 4%), or combined heterozygous *p.[Thr34=;Arg545Gln]* (9/112, 8%) was significantly associated with the development of primary glaucoma [odds ratio (OR)=8.768, 95% confidence interval (CI)=1.972-38.988; relative risk=1.818, 95% CI=1.473-2.244; P=0.001]. The present study provides insight into the genetic or haplotype variants of *MYOC* and *OPTN* genes contributing to primary glaucoma. Haplotype variants identified in the present study may be regarded as potential contributing factors of primary glaucoma in Korea. Further studies, including those on additional genes, are required to elucidate the underlying pathogenic mechanism using a larger cohort to provide additional statistical power.

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*Key words:* primary glaucoma, molecular analysis, *myocilin*, *optineurin*, mutation

## Introduction

Glaucoma is a complex, heterogeneous ocular disorder with multifactorial etiology characterized by structural damage to the optic nerve, visual field defects (1) and commonly relatively high intraocular pressure (IOP) (2). It is a leading cause of irreversible blindness worldwide (3,4) with ~20% of cases occurring secondary to other ocular or systemic diseases (1). Based on anatomical changes in the anterior chamber angle, primary glaucoma may be classified as primary angle closure glaucoma (PACG) or primary open-angle glaucoma (POAG), which may be further subdivided into juvenile open-angle glaucoma (JOAG) and adult onset POAG (1,5). Glaucoma is a treatable disease if detected early; however, many patients are diagnosed during routine examinations or only following advanced field loss, as glaucoma is typically asymptomatic in the early stages. Therefore, the development of an accurate test for the detection of presymptomatic carriers at risk is important for the management of glaucoma.

A family history of glaucoma is a well-known risk factor. Therefore genetic background is considered important for the development of the disease (6-8). The following genes have been reported to be associated with primary glaucoma: *myocilin* (*MYOC*) (9), *optineurin* (*OPTN*) (10), *WD repeat domain 36* (11,12), *neurotrophin 4* (13), *optic atrophy 1* (14,15), *cytochrome P450 family 1 subfamily B member 1* (16) and *latent transforming growth factor β* (17). To date, mutations in these genes account for only ~5% of patients with POAG (18), and the influence of mutations in these genes on patients with PACG remain controversial (19). The *MYOC* gene, located at the GLC1A locus on chromosome 1q24.3-q25.2, has been confirmed to be associated with JOAG and POAG, although mutation frequencies vary between ethnic groups (20). The altered protein product secreted into the extracellular matrix

of the trabecular meshwork causes a severe form of autosomal dominant JOAG associated with very high IOP (9). Although up to 20% of JOAG may be associated with *MYOC* mutations, mutations in this gene have been identified in only 3 to 5% of POAG patients (7,21). Mutations in the *OPTN* gene, located at the GLC1E locus on chromosome 10p14-p15, result in normal tension glaucoma (NTG) without elevated IOP, a subtype of POAG (10).

To investigate the underlying genetic influences of primary glaucoma, molecular analysis of the *MYOC* and *OPTN* genes was performed in 112 Korean sporadic primary glaucoma patients, with the results compared to healthy controls.

## Patients and methods

*Patients*. All 112 patients with primary glaucoma from unrelated Korean patients were recruited from the ophthalmology clinic of Seoul St. Mary's Hospital (Seoul, Korea), including 17 POAG, 19 JOAG and 76 NTG patients. Healthy, unrelated Korean individuals (n=100) served as the non-selected population control. All patients and healthy controls in the study provided written informed consent for clinical and molecular analyses, and the study protocol was approved by the institutional review board of The Catholic University of Korea (Seoul, Korea). The diagnostic criteria of POAG were an IOP >21 mmHg by Goldmann applanation tonometry (GAT), presence of glaucomatous visual field defect or glaucomatous optic neuropathy and open angle. Where onset occurred at 5-35 years of age, patients were diagnosed with JOAG (22). The diagnostic criteria of NTG were an IOP <22 mmHg by GAT, presence of glaucomatous visual field defect or glaucomatous optic neuropathy and open angle. Patients with secondary glaucoma resulting from trauma, uveitis, neovascular or steroid-induced glaucoma (SIG), and other associated ocular or systemic anomalies were excluded from the present study. Healthy controls had IOP <20 mmHg, a normal optic disc appearance without suspicious glaucomatous changes, and no family or personal history of primary glaucoma.

*Molecular analysis of the MYOC and OPTN genes*. Genomic DNA samples were extracted from the peripheral blood using the QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany). Entire coding exons and flanking intronic sequences of *MYOC* and *OPTN* were amplified by polymerase chain reaction (PCR) using different combinations of forward and reverse primers designed by the authors (Table I). Briefly, the PCR reaction had a total volume of 15 $\mu$l, comprising 10 $\mu$l *Taq* PCR Master Mix kit (Qiagen GmbH) containing *Taq* DNA Polymerase, 2X Qiagen PCR Buffer, 3 mM $MgCl_2$, and 400 $\mu$M of each deoxynucleotide, 2 $\mu$l 5X Q-Solution, 1 $\mu$l primer mix, and 2 $\mu$l genomic DNA (50 ng/$\mu$l). The PCR was amplified with a C1000 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) under the following conditions: 5 min at 95˚C, followed by 35 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 1 min, and a final step of 72˚C for 5 min. Direct sequencing of PCR products was performed with the BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and the products were resolved on ABI 3130XL Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The resulting sequence electropherogram was analyzed using Sequencher software version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequence data were analyzed using reference sequences in GenBank (www.ncbi.nlm.nih.gov/genbank/). The RefSeq IDs NM_000261.1 for *MYOC* and NM_001008211.1 for *OPTN* were used for cDNA nucleotide numbering. All identified variants were confirmed by bidirectional resequencing.

Genetic variations were contrasted with Human Gene Mutation Database Public (www.hgmd.cf.ac.uk), 1,000 genomes project browser (browser.1000genomes.org) and the single nucleotide polymorphism database (www.ncbi.nlm.nih.gov/projects/SNP). Mutation pathogenicity was predicted through PolyPhen-2 version 2.2.2 (genetics.bwh.harvard.edu/pph2) (23) and PROVEAN version 1.1 (provean.jcvi.org) (24), the first predictor that includes in-frame insertions/deletions. To assess the extent of conservation of a novel variant of *MYOC* and *OPTN* hypothesized to be associated with disease, the amino acid sequence was aligned with protein sequences of various mammalian species using Clustal Omega software (www.ebi.ac.uk/Tools/msa/clustalo/) (25).

*Haplotype analysis of the MYOC and OPTN genes*. Haplotypes of each gene were constructed based on the genotype data of the *MYOC* and *OPTN* variants obtained from the 112 patients and 100 healthy controls. Haplotype reconstruction and frequency estimation were performed using haplo.em, an implementation of an Expectation-Maximization algorithm included in the R package haplo.stats, which computes maximum likelihood estimates of haplotype probabilities from unphased genotypes measured on unrelated individuals (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org). The frequencies of variant status between the patients and healthy controls were compared by $\chi^2$ or Fisher's exact test using MedCalc software version 12.7.2 (MedCalc Software bvba, Ostend, Belgium). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

A total of three *MYOC* and four *OPTN* variants potentially associated with primary glaucoma were identified in 4 and 18 patients, respectively (Table II).

*Molecular analysis of MYOC*. Missense variants of *MYOC* were identified in two JOAG patients (P107 and P044) with a family history of primary glaucoma (pedigree analysis of P107 is presented in Fig. 1A). One was determined to be a novel missense variant, *p.Leu255Pro*, (P107; Fig. 1B) which was predicted to be 'probably damaging' with a score of 0.970 by PolyPhen-2 and was expected to be 'deleterious' with a score of -2.887 by PROVEAN. The position of changed amino acid was conserved among other mammalian species (Fig. 1C). No healthy control exhibited the same variant. The other variant identified, *p.Thr353Ile*, has previously been reported in Korean POAG (26). In addition, combined heterozygous variants *p.[Thr123=;Ile288=]* were identified in 2 of 112 (2%) patients but not in healthy controls; *p.Thr123=*[rs75682756;

Table I. Oligonucleotide primer sequences used for mutation analysis of *MYOC* and *OPTN* genes.

| Gene | Exon | Primer sequence (5' to 3') Forward | Reverse | Size (bp) |
|---|---|---|---|---|
| *MYOC* | 1a | TCTTGCTGGCAGCGTG | CTGGTCCAAGGTCAATTGGT | 626 |
| *MYOC* | 1b | AGCACCCAACGCTTAGACCT | TCTGTCTTGTGCTAGCTGTGC | 497 |
| *MYOC* | 2 | TGCCACCACATCCAGCTAAT | CTCTGCTCCCAGGGAAGTTA | 495 |
| *MYOC* | 3a | ACCCAGACGATTTGTCTCCA | GCCTCATCGGTGCTGTAAAT | 586 |
| *MYOC* | 3b | CGCTGAGTCCAGAACTGTCA | CGCCCTCAGACTACAATTCC | 685 |
| *OPTN* | 1 | CGGACAGCGAGGGTGGGTA | GCGGGTACCGTTTTCAGG | 445 |
| *OPTN* | 2 | TCCACATGGATGCCTCTACA | TTCCCATGCAAATCTTCAAA | 459 |
| *OPTN* | 3 | TGTTAGCCAGGATGGTCTCA | AGAGGTTGATGGGACATTGC | 391 |
| *OPTN* | 4 | CACACACACACTTTTCTGAAGC | CCCCACCAGCTACCACCTAT | 497 |
| *OPTN* | 5 | CTTCGTCTTTTTGCTGCTGA | CTTCCAAGACCAGGCAAAAC | 492 |
| *OPTN* | 6 | TGTAAAGATGGGGGTCTTGC | GAAAATGAGAGCCAATTTATCTTTG | 491 |
| *OPTN* | 7 | CTTGGGTTGCATGTCACAAA | CAGTGTGAGCCAAACAGGAA | 398 |
| *OPTN* | 8 | GACCAGCTGTGCTTGTTCAC | CAGACAGTGAGTGCTGTTTGG | 495 |
| *OPTN* | 9 | TTTCACTTGCCTTTTACCTCTG | GACACAGAGCAGGACAAGGA | 498 |
| *OPTN* | 10 | TTGGGGTATTGTCAAAGTTGG | ATGCCCCTAAATGGCAGAAT | 486 |
| *OPTN* | 11 | TCATAAACCCTACAGCCCTAAAA | TGCTAGGACTCCTTCAGATAAGTG | 398 |
| *OPTN* | 12 | GCTAGTAGGTCGTGGGGTGA | GGAAAACAACCTTTGAAACCA | 346 |
| *OPTN* | 13 | CCGGCCAGAGCTGATAAT | TTTTAATACACTCACGGGTGAAA | 394 |
| *OPTN* | 14 | AGCAGGATTGTGCATCTGTG | GCGCGAACACAGCTATTCTT | 369 |
| *OPTN* | 15 | GGTTTTTATGAACCTTGGCAGT | GATTCGGTGGGTAATGGATG | 381 |
| *OPTN* | 16 | TGCATCGTGATGACTTCAGTT | CTCAAACCCTGACCCCAAGT | 500 |

Table II. Frequencies of *MYOC* and *OPTN* variants identified in primary glaucoma.

| Gene | Variant | Zygosity | Primary glaucoma, n=112 POAG, n=17 | JOAG, n=19 | NTG, n=76 | Healthy control, n=100 |
|---|---|---|---|---|---|---|
| *MYOC* | c.-83G>A and c.764T>C;*p.Leu255Pro* | Combined heterozygous | 0 | 1 | 0 | 0 |
| *MYOC* | c.1058C>T;*p.Thr353Ile* | Heterozygous | 0 | 1 | 0 | 0 |
| *MYOC* | c.369C>T;*p.Thr123=* and c.864C>T;*p.Ile288=* | Combined heterozygous | 0 | 1 | 1 | 0 |
| *OPTN* | c.102G>A; *p.Thr34=* | Homozygous | 1 | 1 | 2 | 0 |
| *OPTN* | c.293T>A;*p.Met98Lys* | Homozygous | 1 | 1 | 2 | 0 |
| *OPTN* | c.811C>T;*p.Arg271Cys* | Heterozygous | 0 | 0 | 1 | 0 |
| *OPTN* | c.102G>A;*p.Thr34=* and c.1634G>A;*p.Arg545Gln* | Combined heterozygous | 2 | 2 | 5 | 2 |

JOAG, juvenile open-angle glaucoma; POAG, primary open-angle glaucoma; NTG, normal tension glaucoma; *MYOC*, *myocilin*; *OPTN*, *optineurin*.

minor allele frequency (MAF)/minor allele count (MAC) by 1000 Genomes=0.0014/7], *p.Ile288=*(rs181923440; MAF/MAC=0.0010/5). The *MYOC* promoter variant c.-83G>A (rs2075648; MAF/MAC=0.1380/691) was detected only as a heterozygous state in a JOAG patient with *p.Leu255Pro* and in five NTG patients, but not in the 100 healthy controls (Table III). Clinical manifestations of primary glaucoma with *MYOC* are summarized in Table IV.

*Molecular analysis of OPTN*. *OPTN* variants were identified in 18 patients with primary glaucoma. One patient (P086) harbored a novel variant, *p.Arg271Cys* as a heterozygous state (Fig. 2A). This variant was predicted to be 'benign' with a score of 0.047 by PolyPhen-2 and was expected to be 'neutral' with a score of -1.726 by PROVEAN. This protein sequence was not highly conserved across the compared species, and the sequence in chicken had a cysteine in that position (Fig. 2B).

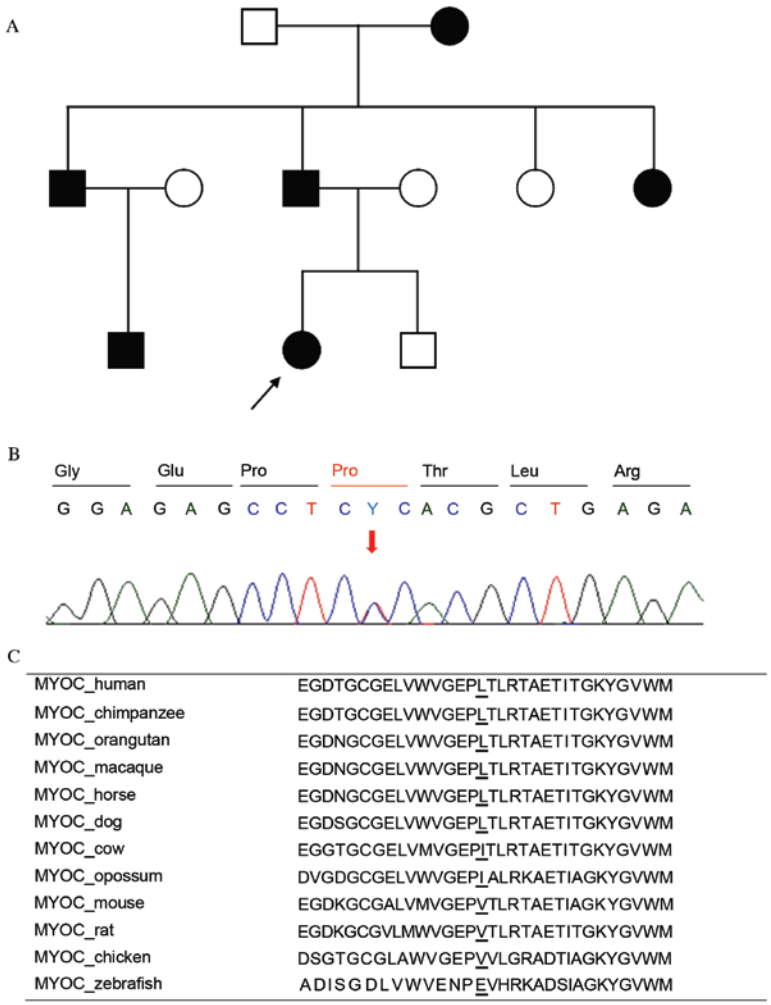



Figure 1. (A)Pedigree analysis of patient P107 diagnosed with juvenile open-angle glaucoma, with a novel missense variant *p.Leu255Pro*. Proband (indicated by the arrow) and affected family members revealed the same mutation in the heterozygous state. (B) Partial sequence of exon3 of *MYOC* revealed a heterozygous single-base substitution (c.764T>C) leading to missense variant *p.Leu255Pro* in patient P107. (C) Alignment of *MYOC* amino acid sequences in human and other species. The position of the changed amino acid in the patient with glaucoma identified in the present study is underlined. The conservation of *p.Leu255Pro* was assumed by protein alignment of various *MYOC* orthologs using Clustal Omega software. *MYOC*, *myocilin*.

One novel synonymous variant, *p.Leu568=*; c.1704A>G was identified in heterozygosity. These two variants were not detected in healthy controls.

In addition, three reported variants associated with primary glaucoma (10,27), including *p.Thr34=*(rs2234968), *p.Met98Lys* (rs11258194) and *p.Arg545Gln* (rs75654767) were identified (Table V). The *p.Thr34=*, *p.Met98Lys* and *p.Arg545Gln* mutations were detected in 40 (36%), 19 (17%), and 10 (9%) patients with primary glaucoma as well as in 25 (25%), 18 (18%), and 2 (2%) healthy controls, respectively, in a heterozygous or homozygous state. The odds ratio (OR) was not statistically different for *p.Thr34=* and *p.Met98Lys* by the $\chi^2$ test (P=0.061 and P=0.492, respectively); however, it was significant for *p.Arg545Gln* by Fisher's exact test [OR=4.804, 95% confidence interval (CI)=1.026-22.482; relative risk=1.634, 95% CI=1.226-2.178; P=0.037]. The presence of one of homozygous *p.[Thr34=;Thr34=]* (4/112; 4%), homozygous *p.[Met98Lys;Met98Lys]* (4/112; 4%) or combined heterozygous *p.[Thr34=;Arg545Gln]* (9/112, 8%) was significantly associated with the development of primary glaucoma (OR=8.768, 95% CI=1.972-38.988; relative risk=1.818, 95% CI=1.473-2.244; P=0.001). Clinical manifestations of primary glaucoma with *OPTN* variants are summarized in Table VI.

## Discussion

The present study reports a molecular analysis of *MYOC* and *OPTN* genes in 112 patients with primary glaucoma. One novel *MYOC* variant, *p.Leu255Pro*, in patient P107 with a family history of glaucoma was predicted to have a deleterious effect by *in silico* analysis. This variant is a possible pathogenic mutation associated with the development of primary glaucoma. The second *MYOC* variant, *p.Thr353Ile*, in patient P044 with a family history of glaucoma has been previously reported as a pathologic mutation for POAG and resides in the olfactomedin-homology region. It may be involved in the regulation of IOP in trabecular-meshwork cells (26). The presence of heterozygous *p.Thr123=* and *p.Ile288=* was observed only in patients with JOAG and NTG. Thus, *p.[Thr123=;Ile288=]* may be a part of the haplotype variant associated with the

Table III. Genotype frequencies of *MYOC* gene identified in korean patients with primary glaucoma.

| Location | cDNA change | Protein change | Genotype frequencies (%) | | | | | | rs IDs |
|---|---|---|---|---|---|---|---|---|---|
| | | | Primary glaucoma, n=112 | | | Normal control, n=100 | | | |
| 5'-UTR | c.-83G>A | - | GG 106/112 (94.6) | GA 6/112 (5.4) | AA 0/112 (0) | GG 100/100 (100) | GA 0/100 (0) | AA 0/100 (0) | rs2075648 |
| Exon1 | c.57G>T | *p.Gln19His* | GG 110/112 (98.2) | GT 2/112 (1.8) | TT 0/112 (0) | GG 100/100 (100) | GT 0/100 (0) | TT 0/100 (0) | rs2234925 |
| Exon1 | c.227G>A | *p.Arg76Lys* | GG 102/112 (91.1) | GA 10/112 (8.9) | AA 0/112 (0) | GG 96/100 (96) | GA 4/100 (4) | AA 0/100 (0) | rs2234926 |
| Exon1 | c.369C>T | *p.Thr123=* | CC 110/112 (98.2) | CT 2/112 (1.8) | TT 0/112 (0) | CC 100/100 (100) | CT 0/100 (0) | TT 0/100 (0) | rs75682756 |
| Exon2 | c.624C>G | *p.Asp208Glu* | CC 109/112 (97.3) | CG 3/112 (2.7) | GG 0/112 (0) | CC 98/100 (98) | CG 2/100 (2) | GG 0/100 (0) | rs2234927 |
| Exon3 | c.764T>C | *p.Leu255Pro* | TT 111/112 (99.1) | TC 1/112 (0.9) | CC 0/112 (0) | TT 100/100 (100) | TC 0/100 (0) | CC 0/100 (0) | - |
| Exon3 | c.864C>T | *p.Ile288=* | CC 110/112 (98.2) | CT 2/112 (1.8) | TT 0/112 (0) | CC 100/100 (100) | CT 0/100 (0) | TT 0/100 (0) | rs181923440 |
| Exon3 | c.1058C>T | *p.Thr353Ile* | CC 111/112 (99.1) | CT 1/112 (0.9) | TT 0/112 (0) | CC 100/100 (100) | CT 0/100 (0) | TT 0/100 (0) | rs137853277 |
| Exon3 | c.1464C>T | *p.Ala488=* | CC 111/112 (99.1) | CT 1/112 (0.9) | TT 0/112 (0) | CC 100/100 (100) | CT 0/100 (0) | TT 0/100 (0) | rs2234929 |
| 3'-UTR | c.*73G>C | - | GG 103/112 (92) | GC 8/112 (7.1) | CC 1/112 (0.9) | GG 98/100 (98) | GC 1/100 (1) | CC 1/100 (1) | rs74403899 |

*MYOC*, *myocilin*; UTR, untranslated region; rs IDs, reference single nucleotide polymorphism identification numbers.

Table IV. Clinical manifestations in four primary glaucomas with *MYOC* variants.

| Patient | Diagnosis | Variant 1 | Variant 2 | Sex | Age at Dx (year) | BCVA | | Max IOP (mmHg) | | VCDR | | VFMD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OD | OS | OD | OS | OD | OS | OD | OS |
| P107 | JOAG | c.-83G>A | c.764T>C;*p.Leu255Pro* | F | 14 | 1 | 0.04 | 39 | 40 | 0.6 | 0.8 | -5.17 | -5.32 |
| P044 | JOAG | c.1058C>T;*p.Thr353Ile* | None | F | 12 | 1 | 0.8 | 27 | 26 | 0.6 | 0.6 | -3.33 | -4.24 |
| P006 | JOAG | c.369C>T;*p.Thr123=* | c.864C>T;*p.Ile288=* | M | 28 | 1 | 1 | 23 | 23 | 0.5 | 0.6 | -0.26 | -1.11 |
| P039 | NTG | c.369C>T;*p.Thr123=* | c.864C>T;*p.Ile288=* | F | 22 | 1 | 1 | 16 | 15 | 0.6 | 0.5 | -1.07 | -1.02 |

*MYOC*, *myocilin*; JOAG, juvenile open-angle glaucoma; NTG, normal tension glaucoma; M, male; F, female; Dx, diagnosis; BCVA, best corrected visual acuity; Max IOP, maximum intraocular pressure; VCDR, vertical cup-to-disc ratio; VFMD, visual field mean deviation; OD, oculus dexter; OS, oculus sinister.

Table V. Genotype frequencies of *OPTN* gene identified in Korean patients with primary glaucoma.

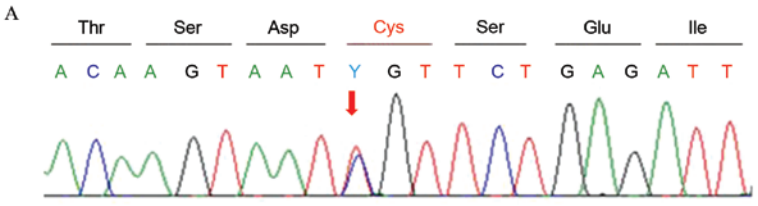
| Location | cDNA change | Protein change | Genotype frequencies (%) | | | | | | rs IDs |
|---|---|---|---|---|---|---|---|---|---|
| | | | Primary glaucoma, n=112 | | | Normal control, n=100 | | | |
| Exon4 | c.102G>A | *p.Thr34=* | GG 72/112 (64.3) | GA 36/112 (32.1) | AA 4/112 (3.6) | GG 75/100 (75) | GA 25/100 (25) | AA 0/100 (0) | rs2234968 |
| Exon4 | c.147C>T | *p.Thr49=* | CC 111/112 (99.1) | CT 1/112 (0.9) | TT 0/112 (0) | CC 95/100 (95) | CT 5/100 (5) | TT 0/100 (0) | rs187734249 |
| Exon5 | c.293T>A | *p.Met98Lys* | TT 93/112 (83.1) | TA 15/112 (13.4) | AA 4/112 (3.5) | TT 82/100 (82) | TA 18/100 (18) | AA 0/100 (0) | rs11258194 |
| Intron6 | c.552+63C>T | - | CC 112/112 (100) | CT 0/112 (0) | TT 0/112 (0) | CC 99/100 (99) | CT 1/100 (1) | TT 0/100 (0) | rs184333348 |
| Intron6 | c.553-10G>A | - | GG 84/112 (75) | GA 20/112 (17.9) | AA 8/112 (7.1) | GG 66/100 (66) | GA 34/100 (34) | AA 0/100 (0) | rs11258210 |
| Intron6 | c.553-5C>T | - | CC 20/112 (17.9) | CT 39/112 (34.8) | TT 53/112 (47.3) | CC 7/100 (7) | CT 45/100 (45) | TT 48/100 (48) | rs2244380 |
| Intron7 | c.626+24G>A | - | GG 103/112 (92) | GA 7/112 (6.3) | AA 2/112 (1.8) | GG 90/100 (90) | GA 10/100 (10) | AA 0/100 (0) | rs11258211 |
| Intron8 | c.780-53T>C | - | TT 88/112 (78.6) | TC 22/112 (19.6) | CC 2/112 (1.8) | TT 89/100 (92) | TC 10/100 (10) | CC 1/100 (1) | rs765884 |
| Exon9 | c.811C>T | *p.Arg271Cys* | TT 111/112 (99.1) | CT 1/112 (0.9) | TT 0/112 (0) | TT 100/100 (100) | CT 0/100 (0) | TT 0/100 (0) | - |
| Intron9 | c.882+19C>T | - | CC 111/112 (99.1) | CT 1/112 (0.9) | TT 0/112 (0) | CC 99/100 (99) | CT 1/100 (1) | TT 0/100 (0) | rs2277219 |
| Exon10 | c.964G>A | *p.Glu322=* | GG 111/112 (99.1) | GA 0/112 (0) | AA 1/112 (0.9) | GG 100/100 (100) | GA 0/100 (0) | AA 0/100 (0) | rs523747 |
| Intron11 | c.1149-86G>T | - | GG 28/112 (25) | GT 33/112 (29.5) | TT 51/112 (45.5) | GG 10/100 (10) | GT 41/100 (41) | TT 49/100 (49) | rs676302 |
| Intron15 | c.1613-48C>A | - | CC 64/112 (57.1) | CA 30/112 (26.8) | AA 18/112 (16.1) | CC 34/100 (34) | CA 50/100 (50) | AA 16/100 (16) | rs10906310 |
| Exon16 | c.1634G>A | *p.Arg545Gln* | GG 102/112 (91.1) | GA 10/112 (8.9) | AA 0/112 (0) | GG 98/100 (98) | GA 2/100 (2) | AA 0/100 (0) | rs75654767 |
| Exon16 | c.1704A>G | *p.Leu568=* | AA 111/112 (99.1) | AG 1/112 (0.9) | GG 0/112 (0) | AA 100/100 (100) | AG 0/100 (0) | GG 0/100 (0) | - |

*OPTN*, *optineurin*; rs IDs, reference single nucleotide polymorphism identification numbers.

Table VI. Clinical manifestations in 18 primary glaucomas with *OPTN* variants.

| Patient | Diagnosis | Variant 1 | Variant 2 | Sex | Age at Dx (year) | BCVA | | Max IOP (mmHg) | | VCDR | | VFMD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OD | OS | OD | OS | OD | OS | OD | OS |
| P059 | JOAG | c.102G>A;*p.Thr34=* | c.102G>A;*p.Thr34=* | M | 7 | 1 | 1 | 28 | 28 | 0.8 | 0.7 | -1.52 | -1.81 |
| P060 | POAG | c.102G>A;*p.Thr34=* | c.102G>A;*p.Thr34=* | F | 47 | 0.1 | 0.1 | 25 | 25 | 0.6 | 0.6 | -10.47 | -12.93 |
| P098 | NTG | c.102G>A;*p.Thr34=* | c.102G>A;*p.Thr34=* | M | 25 | 1 | 1 | 13 | 13 | 0.7 | 0.8 | -4.08 | -3.09 |
| P109 | NTG | c.102G>A;*p.Thr34=* | c.102G>A;*p.Thr34=* | M | 34 | 1 | 1 | 16 | 17 | 0.7 | 0.6 | -0.69 | -1.38 |
| P014 | POAG | c.293T>A;*p.Met98Lys* | c.293T>A;*p.Met98Lys* | M | 40 | 1 | 1 | 24 | 22 | 0.8 | 0.8 | -12.88 | -32.71 |
| P029 | JOAG | c.293T>A;*p.Met98Lys* | c.293T>A;*p.Met98Lys* | M | 7 | 0.4 | 1 | 24 | 27 | 0.7 | 0.7 | -8.32 | -6.63 |
| P054 | NTG | c.293T>A;*p.Met98Lys* | c.293T>A;*p.Met98Lys* | M | 29 | 0.8 | 0.8 | 19 | 19 | 0.6 | 0.4 | -16.36 | -2.2 |
| P058 | NTG | c.293T>A;*p.Met98Lys* | c.293T>A;*p.Met98Lys* | M | 26 | 1 | 1 | 21 | 21 | 0.5 | 0.6 | -0.04 | -1.93 |
| P086 | NTG | c.811C>T;*p.Arg271Cys* | None | M | 17 | 1 | 1 | 19 | 19 | 0.7 | 0.6 | -1.46 | -1.25 |
| P003 | POAG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | M | 49 | 0.8 | 1 | 23 | 22 | 0.8 | 0.7 | -3.42 | -2.48 |
| P010 | NTG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | M | 36 | 1 | 1 | 16 | 16 | 0.6 | 0.6 | 0.23 | -0.95 |
| P023 | NTG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | F | 28 | 0.8 | 1 | 17 | 18 | 0.7 | 0.5 | -1.18 | -3.25 |
| P034 | JOAG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | F | 29 | 1 | 1 | 29 | 28 | 0.4 | 0.8 | NA | NA |
| P049 | NTG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | F | 29 | 1 | 1 | 20 | 20 | 0.7 | 0.7 | -3.75 | -3.3 |
| P066 | NTG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | F | 32 | 1 | 1 | 15 | 15 | 0.2 | 0.2 | -4.66 | -3.77 |
| P081 | JOAG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | M | 15 | 1 | 1 | 23 | 22 | 0.5 | 0.5 | -0.08 | -0.54 |
| P095 | POAG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | M | 43 | 1 | 1 | 31 | 31 | 0.6 | 0.7 | -6.48 | -4.35 |
| P105 | NTG | c.102G>A;*p.Thr34=* | c.1634G>A;*p.Arg545Gln* | F | 43 | 1 | 1 | 21 | 21 | 0.6 | 0.6 | -0.34 | 0.18 |

*OPTN*, *optineurin*; JOAG, juvenile open-angle glaucoma; POAG, primary open-angle glaucoma; NTG, normal tension glaucoma; M, male; F, female; Dx, diagnosis; BCVA, best corrected visual acuity; Max IOP, maximum intraocular pressure; VCDR, vertical cup-to-disc ratio; VFMD, visual field mean deviation; OD, oculus dexter; OS, oculus sinister; NA, not available.




B

| | |
|---|---|
| OPTN_human | EAKERVSDFEKKTSNRSEIETQTEGSTEKEN |
| OPTN_chimpanzee | EAKERVSDFEKKTSNRSEIETQTEGSTEKEN |
| OPTN_orangutan | EAKERVSDFEKKASNRSEIETQTEGSTEKEN |
| OPTN_macaque | EAKERVSDFEKKASNRSEIETQTEGSTEKEN |
| OPTN_horse | EAKERISYFEKKVNYRSEIETQTEESKEKEK |
| OPTN_dog | EAKERVLYFEKKASGGSEIETQTEESTEKEK |
| OPTN_cow | EAKERISDFEKKAKDHSETETQTEEHTEQEK |
| OPTN_opossum | AAKDKLSDLEKKAEDHSENATQTEEGPGEEK |
| OPTN_mouse | EAKERISDFEKKANGHSSTEKQTARRADRE– |
| OPTN_rat | EAKERISDFEKKANGHSAIETQTEGSTQKE– |
| OPTN_chicken | DHKERLSKMENETSNCLESGTQT-------N |
| OPTN_zebrafish | EARGRIAELESKLEH–ADSSAQTSLPS---- |

Figure 2. (A) Partial sequence of exon9 of *OPTN* revealed a heterozygous single-base substitution (c.811C>T) leading to missense variant *p.Arg271Cys* in patient P086 with normal tension glaucoma. (B) Alignment of *OPTN* amino acid sequences in human and other mammalian species. The position of the changed amino acid in the patient with glaucoma identified in the present study is underlined. The conservation of *p.Arg271Cys* was assessed by protein alignment of various *OPTN* orthologs using Clustal Omega. *OPTN*, *optineurin*.

development of primary glaucoma. Further extended haplotype analysis is required to confirm the association of *MYOC* haplotype variants. The variant in the 5'-UTR region, c.-83G>A, which was initially reported in Western countries (20,28), and subsequently observed in Hong Kong (29) and the Philippines (30), was identified only in primary glaucoma patients in the present study. The association of c.-83G>A and the risk of primary glaucoma remains controversial due to the variable frequency and the non-significant differences between POAG patients and controls observed in previous studies (28,31,32).

Genetic analysis of the *OPTN* coding region was performed in all patients with primary glaucoma. Notably, *OPTN* variants (10/76, 13.2%) were identified more frequently than *MYOC* variants (1/76, 1.3%) in NTG patients, which is consistent with previous studies (33,34). The three *OPTN* variants *p.Thr34=*, *p.Leu40=* and *p.Glu89His* were identified in 7 of 53 Korean NTG patients (33), whereas only one patient with the *MYOC* variant *p.Leu411=* was reported from 32 separate Korean NTG patients (34). Supporting the findings of the present study, Rezaie *et al* (10) suggested that mutations in *OPTN* may be responsible for 16.7% of the hereditary forms of NTG and that there is an additional risk factor of 13.6% in familial and sporadic cases. Furthermore, Sohn *et al* (35) demonstrated that the *MYOC* gene itself was not associated with OAG, including POAG, NTG and SIG. Their results do not support the hypothesis that MYOC induction may be linked to IOP variation and that promoter variants of MYOC may be a risk factor for the pathogenesis of OAG.

A novel *OPTN* variant, *p.Arg271Cys*, of unknown significance was identified in patient P086 with NTG (1/112, 1%). This variant led to replacement of arginine with cysteine at codon 271, however, the protein sequence was not highly conserved across species. Although this variant was predicted to be 'benign' or 'neutral', it is potentially pathogenic as it was not present in the healthy controls. Segregation analysis and/or functional studies are required to verify the pathogenicity or neutrality of this variant.

Of the *OPTN* haplotype variants, *p.Thr34=*, *p.Met98Lys* and *p.Arg545Gln* have been previously reported as possible glaucoma-causing mutations (10,27). However, they were present at reduced frequencies as a heterozygous state in healthy controls.

The *p.Thr34=* variant was detected in 40 of 112 (36%) patients with primary glaucoma and in 25 of 100 (25%) healthy controls in a heterozygous or homozygous state in the present study. Homozygosity for *p.Thr34=* was observed only in four patients with primary glaucoma (P059, P060, P098 and P109). Funayama *et al* (27) reported that *p.Thr34=* was weakly associated with patients with OAG with elevated IOP, whereas *p.Met98Lys* was weakly associated with patients with OAG with normal IOP. In interaction analysis between olfactomedin 2 (*OLFM2*) and *OPTN* genes in patients with OAG, the c.317G>A; *p.Arg106Gln* of *OLFM2* and c.412G>A; *p.Thr34=* of *OPTN* and the c.1281C>T; *p.Arg427Arg* of *OLFM2* and c.412G>A; *p.Thr34=* of *OPTN* were significantly associated with OAG with elevated IOP. These results suggest that these variants in *OLFM2* and *OPTN* contribute interactively to OAG, indicating a polygenic etiology with different properties for *p.Thr34=* and *p.Met98Lys* variants of *OPTN*.

The *p.Met98Lys* variant was identified in 19 of 112 (17%) patients with primary glaucoma as well as in 18 of 100 (18%) healthy controls as a heterozygous or homozygous state in the present study. Alward *et al* (36) and Fuse *et al* (37) reported a significant association between *p.Met98Lys* and glaucoma in Japanese patients. By contrast, Toda *et al* (38) identified similar frequencies of *p.Met98Lys* in Japanese glaucoma patients and controls. Notably, *p.Met98Lys* has been demonstrated to be a polymorphic variant in German, French and Moroccan patients (39,40). By contrast to previous studies (10,41,42), the

frequency difference in the present study (4 vs. 0%) between the patients with homozygous *p.Met98Lys* (P014, P029, P054 and P058) and healthy controls was highly significant. *p.Met98Lys* is located within a putative basic leucine zipper domain and is conserved in macaques; it may represent a risk associated factor or a dominant susceptibility allele (10). Wild-type OPTN protein, operating through the tumor necrosis factor α pathway, may have a neuroprotective role in the eye and optic nerve; however when defective, it produces visual loss and optic neuropathy as typically observed in normal and high-pressure glaucoma (10).

The *p.Arg545Gln* variant was reported previously in POAG families with normal IOP (10). Although the *p.Arg545Gln* variant is not part of a known protein domain, it is situated near the only zinc finger motif within OPTN. This motif is typically observed in transcription factors. *p.Arg545Gln* has been detected in similar frequencies in Japanese glaucoma patients and healthy controls (38). Alward *et al* (36) suggested that *p.Arg545Gln* may not be a disease-causing polymorphism. Results concerning the effect of *OPTN* on glaucoma have been equivocal (10,32,43,44); however, the expression of *MYOC* may be regulated by *OPTN* (45). Notably, results from the present study support the view that the three variants of *OPTN* may be involved in the development of primary glaucoma, as the OR for the haplotype harboring homozygous *p.Thr34=* or *p.Met98Lys* as well as a simultaneous presence of heterozygous *p.Thr34=* and *p.Arg545Gln* was statistically significant by the Fisher's exact test (OR=8.768, 95%, CI=1.972-38.988; relative risk=1.818, 95% CI=1.473-2.244; P=0.001).

In conclusion, haplotype variants identified in the present study may be regarded as potential contributing factors for primary glaucoma in Korea. The present study provides insight into the genetic or haplotype variants contributing to primary glaucoma. Further studies, including on additional genes, are required to elucidate the underlying pathogenic mechanism using larger cohorts to provide additional statistical power.

## Acknowledgements

The authors would like to thank The Catholic Genetic Laboratory Center (Seoul, Korea) for its assistance in conducting the present study and compiling this report. The present study was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (Daejeon, Korea; grant no. NRF-2013R1A1A2006801) funded by the Ministry of Education (Sejong, Korea).

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