

# Clinical and genetic analysis of Ser341Pro *MYOC* variant in a Korean family with primary open angle glaucoma

Sangwoo Moon<sup>1,2</sup>, Namhee Kim<sup>3</sup>, Jiwoong Lee<sup>1,2</sup>

<sup>1</sup>Department of Ophthalmology, Pusan National University College of Medicine, Busan 49241, Republic of Korea

<sup>2</sup>Biomedical Research Institute, Pusan National University Hospital, Busan 49241, Republic of Korea

<sup>3</sup>Department of Laboratory Medicine, Dong A University College of Medicine, Busan 49201, Republic of Korea

**Correspondence to:** Jiwoong Lee. Department of Ophthalmology, Pusan National University College of Medicine, 179 Gudeok-ro, Seo-gu, Busan 49241, Republic of Korea. glaucoma@pusan.ac.kr

Received: 2020-01-13 Accepted: 2020-07-29

## Abstract

• **AIM:** To report the first discovery of Ser341Pro *myocilin* (*MYOC*) variant in Korea and analyze its clinical characteristics and genetic significance.

• **METHODS:** Ten family members from three generations participated in this study and received the thorough ophthalmologic examination. Focused exome sequencing on a proband was performed to confirm the target mutations (*MYOC* c.1021T>C) in the family members, and the direct sequencing was conducted. Variant was analyzed according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines.

• **RESULTS:** A nucleotide change from thymine to cytosine at c.1021T>C was found in eight family members. Three members diagnosed with primary open angle glaucoma (POAG) were characterized by severe clinical presentations, high intraocular pressure, and poor response to medical treatment (100% of the patient required filtering surgery). On variant analysis by ACMG/AMP guidelines, Ser341Pro is not found in normal population. Multiple computational predictive programs support a deleterious effect of Ser341Pro variant (PolyPhen 2, SIFT, Mutation Taster). Ser341Pro could be involved in moderate (PM) and supporting (PP) criteria (PM1, PM2, PP2, PP3). Combining the criteria, Ser341Pro has a combination of 2 moderate (PM1+PM2) and 2 supporting (PP2+PP3) criteria, which is interpreted to “likely pathogenic”.

• **CONCLUSION:** The Ser341Pro variant is correlated with severe phenotype of POAG. There are similar clinical

aspects to previous studies: autosomal dominant inheritance, incomplete penetrance (62.50% and 66.67%), and proportion of patients requiring trabeculectomy (100% in both study). According to ACMG/AMP guidelines and the previous basic researches, the Ser341Pro variant had a “strong evidence of pathogenicity (PS3)” and then it could be interpreted to “pathogenic (PS3, PM1, PM2, PP2, PP3)”. Additionally, Ser341Pro variant can be reported as “c.1021T>C (p.Ser341Pro), likely pathogenic, POAG, autosomal dominant” according to guideline.

• **KEYWORDS:** Ser341Pro variant; primary open angle glaucoma; Korean

**DOI:10.18240/ijo.2020.11.02**

**Citation:** Moon SW, Kim NH, Lee JW. Clinical and genetic analysis of Ser341Pro *MYOC* variant in a Korean family with primary open angle glaucoma. *Int J Ophthalmol* 2020;13(11):1689-1696

## INTRODUCTION

**G**laucoma is an irreversible and chronic progressive optic neuropathy characterized by progressive degeneration of retinal ganglion cells and affects more than 70 million people worldwide<sup>[1]</sup>. It is the second most common cause of visual impairment and blindness worldwide<sup>[2]</sup>. Glaucoma is accompanied by high intraocular pressure (IOP), optic disc cupping, optic nerve fiber atrophy, and visual field defect. Elevated IOP is a major risk factor for most types of glaucoma. Primary open angle glaucoma (POAG) is the most common type of glaucoma in developed countries, accounting for >50% of glaucoma cases<sup>[3]</sup>.

Glaucoma can occur at all ages, with early onset disease (before the age of 40y) exhibiting Mendelian inheritance and adult onset forms (after the age of 40y) inherited as complex traits<sup>[4]</sup>. A family history of glaucoma is a well-known risk factor and a familial aggregation study reported that the likelihood of developing POAG in relatives of patients with POAG was 7- to 10-fold higher than that in the general population<sup>[5]</sup>.

*Myocilin* (*MYOC*), also known as the trabecular meshwork inducible glucocorticoid response gene (TIGR), is the first causative gene identified in juvenile open angle glaucoma (JOAG)<sup>[6]</sup>. This gene is located at chromosome 1q24.3-q25 and

has three exons which consist of 604, 126 and 782 base pairs. Most mutations are found in exon 3. The product, myocilin protein, is composed of 504 amino acids<sup>[6]</sup>. The pathogenesis of POAG is associated with *MYOC* mutation site with 90% at the 3<sup>rd</sup> exon of the olfactomedin homology region<sup>[7-8]</sup>. This domain is an active site of protein function, which plays an important role in the expression of myocilin protein.

There are many theories on how the myocilin protein produced by this gene mutation causes glaucoma<sup>[9-10]</sup>. Myocilin protein with a mutation accumulates in the vesicle and causes toxic effects in the trabecular meshwork cells and thus develops into POAG<sup>[9]</sup>. There is another theory that the modified myocilin protein increases resistance to aqueous humor drainage through the trabecular meshwork, which increases IOP<sup>[10]</sup>.

*MYOC* variants on POAG including JOAG have been reported in various studies<sup>[11-17]</sup>. Most of the variants were reported in the conserved olfactomedin domain of exon 3 (codons 242-504) with either missense mutation including Pro370Leu, Gly246Arg, Tyr437His, Ser341Pro, Pro13Leu, Cys245Tyr, Thr353Ile, Gly252Arg, Glu300Lys, Ser313Phe, Asn450Tyr, Tyr471Cys, Thr455Lys, Val53Ala, Arg82Cys, Thr293Lys, Asp378Gly, Gly387Asp, Leu486Phe, Tyr437His, Thr377Met, Lys423Glu, Cys25Arg or nonsense mutation such as Gln368Stop, Gln337Stop<sup>[11-17]</sup>.

A few studies about *MYOC* gene variant for POAG have been reported in Korea<sup>[18-20]</sup>. Kee and Ahn<sup>[18]</sup> reported that the Pro334Ser variant was found in open angle glaucoma patient and siblings (glaucoma not yet been detected). Yoon *et al*<sup>[19]</sup> reported the Thr353Ile variant in patients with POAG and Park *et al*<sup>[20]</sup> reported a novel Leu255Pro and Thr353Ile variants in a JOAG and normal tension glaucoma patients.

Ser341Pro variant, one of the *MYOC* gene variants, has been reported in China<sup>[11-12,21]</sup>. Huang *et al*<sup>[12]</sup> and Qin and Li<sup>[21]</sup> found this variant in one family pedigrees with POAG. Wang *et al*<sup>[11]</sup> also represented a pedigree analysis of this variant in a POAG family with basic research including restriction endonuclease site, protein secondary structure prediction, and homology analysis of protein.

However, Ser341Pro variant has not been reported in Korean POAG patient. In the present study, we clinically followed up a Korean POAG patient with Ser341Pro variant over a period of years with a pedigree analysis, molecular biology, and interpretation of sequence variant.

## SUBJECTS AND METHODS

**Ethical Approval** This study was performed in accordance with the tenets of the Declaration of Helsinki for Research Involving Human Subjects and approved by the Institutional Review Board of the Pusan National University Hospital (approval No.1907-010-080). Informed consent was obtained from all patients when they were enrolled.

**Clinical Aspects of Family Members** The data were collected from the Glaucoma Clinic & Laboratory Medicine of Pusan National University Hospital.

In this study, there were twenty-nine family members over 4 generations. Ten family members from 3 generations participated in this study and were numbered from I-1 to III-2. II-1 was a family member-in-law. The ten family members received thorough ophthalmologic examination including best corrected visual acuity (BCVA), slit lamp examination, IOP measurement by Goldmann applanation tonometry (GAT), angle evaluation by gonioscopy, dilated fundus examination, stereoscopic optic disc and red-free retinal nerve fiber layer (RNFL) photography (AFC-210; Nidek, Aichi, Japan), visual field examination with a 24-2 test pattern, size III white stimulus with the Swedish Interactive Threshold Algorithm standard strategy (Humphrey Field Analyzer, Carl Zeiss Meditec, Inc. Dublin, CA, USA), and spectral-domain optical coherence tomography (Cirrus HD-OCT, Carl Zeiss Meditec). POAG was diagnosed by the presence of glaucomatous optic disc changes [a rim notch with a rim width  $\leq 0.1$  disc diameters or a vertical cup-to-disc ratio (VCDR) of  $>0.7$ , VCDR between the 2 eyes  $\geq 0.2$  and/or a RNFL defect] and corresponding visual field defects as confirmed by reliable visual field examinations and open anterior chamber angle.

Glaucomatous visual field was defined as visual field that meets at least one of Anderson-Patella's criteria<sup>[22]</sup>. The criteria are as follows: 1) a cluster of  $\geq 3$  points in the pattern deviation plot in a single hemifield (superior/inferior) with  $P < 0.05$ , one of which must have been  $P < 0.01$ ; 2) glaucoma hemifield test result outside of normal limits; or 3) abnormal pattern standard deviation with  $P < 0.05$ .

The patients with POAG who were  $< 35$  years old were subclassified as JOAG. Patients with any other ocular or systemic disorders known to influence the optic nerve head, macula, or visual field were excluded. Individuals with IOP  $> 21$  mm Hg, normal optic disc and visual field but without ocular sign or systemic abnormality causing an increase of IOP were diagnosed with ocular hypertension, which was grouped under glaucoma suspect. Glaucoma suspect was defined as those who have VCDR  $\geq 0.7$  or VCDR asymmetry  $\geq 0.2$ , or optic disc hemorrhages with no definite field defect<sup>[23]</sup>. Individuals with IOP  $< 21$  mm Hg, normal optic disc and visual field were categorized as "unaffected".

**Exome Sequencing** We performed focused exome sequencing on a proband using the SureSelect Focused Exomes Kit (SureSelect Focused Exomes, Agilent Technologies, Santa Clara, CA, USA) to screen approximately 4800 human disease-associated genes and regions. The sample was prepared using the SureSelect Target Enrichment System (Agilent Technologies) and sequenced using paired-end, 100-cycle

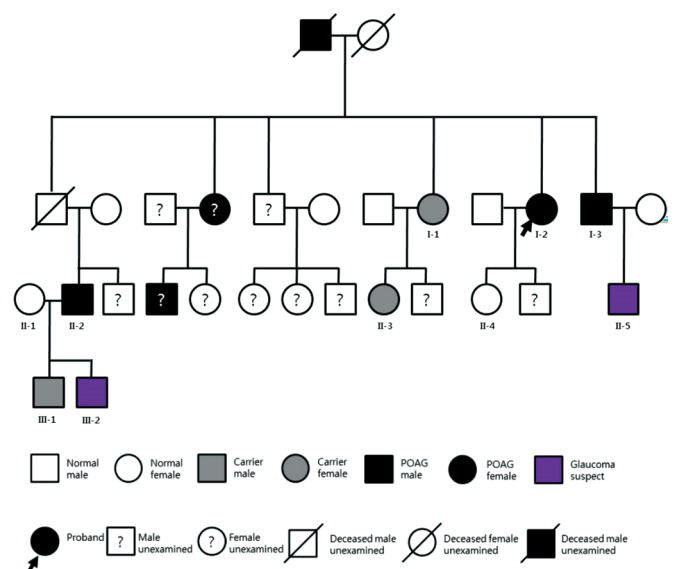
chemistry on the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA, USA). Sequence data were aligned to the reference human genome (GRCh37), and variant calls were generated using VarDict. Stepwise filtering included the removal of common single-nucleotide polymorphisms, intergenic and 3'/5' UTR variants, nonsplice-related intronic variants, and synonymous variants. Variants were filtered further based on family history and possible inheritance models. All variants were annotated by SnpEff (version 1.9.6) and classified following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) standards and guidelines<sup>[24]</sup>.

**Variant Screening** To confirm the target variant (*MYOC* c.1021T>C, Accession NM\_000261.1) in the family members, direct sequencing for *MYOC* exon 3 was performed. DNA was extracted from whole blood using the Quick Gene-800 (FUJIFILM, Tokyo, Japan). Gene amplification was performed with the AmpliTaq Gold 360 Master Mix Kit (Applied Biosystems, Foster City, CA, USA) using the forward (5'-CGGGTGGTAGAGCCTAGCTT-3') and reverse (5'-GGATTCCTTCTCAGCCTTCA-3') primers on a GeneAmp 9700 PCR system (Applied Biosystems) under the following conditions: 10-min predenaturation and 30s denaturation at 95°C, annealing at 55°C, and 60s chain extension at 72°C; the above steps were cycled 32 times. Polymerase chain reaction products were sequenced using BigDye terminator version 3.1 (Applied Biosystems) sequencing chemistry and run on an ABI3730XL genetic analyzer (Applied Biosystems). The sequences were analyzed using Sequencher software, version 4.2 (Gene Codes Corporation, Ann Arbor, MI, USA).

**Interpretation of the Ser341Pro: Population Database and Missense Variant Prediction** Genetic variations were contrasted with the Genome Aggregation database (gnomAD), Exome Aggregation Consortium and 1000 Genome project. Pathogenicity of the variant was predicted by computational analyses such as SIFT, PolyPhen-2, Mutation taster, Align GVD, CADD. As pathogenic variants, ACMG/AMP 2015 guideline proposed evidence of pathogenicity for the interpretation of sequence variants; very strong (PVS), strong (PS), moderate (PM), supporting (PP). This guideline provides rules of combining above criteria to classify sequence variants into five-tier terminology: pathogenic, likely pathogenic, uncertain significance, benign, likely benign.

## RESULTS

**Pedigree of the POAG Family** This family consisted of twenty-nine members from 4 generations and ten members from 3 generations took part in this study. Three members (two men and one woman) were diagnosed with POAG in the proband generation and the next generation. Other three



**Figure 1** Pedigree structure of the POAG family.

members (father of proband, proband's sister and proband's nephew) who did not participate in this study were also diagnosed with glaucoma according to the proband's statement. In this study, as we know, glaucoma did tend to pass vertically from generation to generation regardless of gender, which suggests that it is an autosomal dominant disorder (Figure 1).

Three members (I-2, I-3, II-2) were diagnosed with POAG, and two members (II-5, III-2) were diagnosed with glaucoma suspect. Three members (I-1, II-3, III-1) were considered carriers, who had a variant on the *MYOC* gene without clinical manifestations. Two members (II-1, II-4) were diagnosed as "unaffected". One (II-4) is a daughter of the proband, another (II-1) is a family member-in-law but her two sons (III-1, III-2) were diagnosed as carrier and glaucoma suspect, respectively.

**Clinical Characteristics** Three members including proband (I-2, I-3, II-2) were diagnosed with POAG, and two members (II-5, III-2) were diagnosed with glaucoma suspect (reduced inferior rim with 0.7 of VCDR, which is not relevant to the inferior  $\geq$  superior  $\geq$  nasal  $\geq$  temporal [ISNT] rule<sup>[25]</sup> that normal eyes showed a characteristic configuration for disc rim thickness (Table 1).

The proband (I-2) was diagnosed with POAG in 2013 at the age of 61y. BCVA was 0.8/1.0 (right/left) and IOP was 25/27 mm Hg at baseline examination and refractory to medical treatment. Disc excavation was noted for both eyes (VCDR 0.84/0.75). Visual field mean deviation (MD) was -16.33/-4.68 dB. She had received trabeculectomy in both eyes and additional glaucoma surgeries (bleb revision and second trabeculectomy in the left eye) were needed for following-up period (Figure 2A1, 2A2, 2B1, 2B2).

The I-3 was diagnosed with POAG in 2015 at the age of 60y. BCVA was 1.0/0.02 and IOP was 11/15 mm Hg at baseline examination. Disc excavation was noted for both eyes

Table 1 Demographic and ophthalmic characteristics of 10 family members in this study

Pedigree ID No.	Gender/age (y)	Diagnosed at age (y)	BCVA OD/OS baseline		IOP OD/OS baseline (mm Hg)		IOP OD/OS last visit (mm Hg)		VCDR OD/OS baseline		VCDR OD/OS last visit		VF OD/OS	Treatment OD/OS	Diagnosis baseline/last visit	S341P
			OS	OD	OS	OD	OS	OD	OS	OD	OS	OD				
I-1	F/70		0.8/0.8		17/16				0.48/0.51				Normal VF		Carrier	Yes
I-2	F/67	61	0.8/1.0	1.0/0.5	25/27	16/11			0.84/0.75	0.85/0.85			Yes/Yes	T/T, BR, T	POAG/POAG	Yes
I-3	M/64	60	1.0/0.02	0.8/FC	11/15	11/8			0.78/0.92	0.85/0.9			Yes/Yes	T/T	POAG/POAG	Yes
II-1	F/49		1.0/1.0		12/12				0.62/0.58				Normal VF		Unaffected	No
II-2	M/49	44	0.02/1.0	0.02/0.63	42/11	20/12			0.9/0.85	0.9/0.85			Yes/Yes	T <sup>a</sup> , A <sup>a</sup> , BR/T <sup>a</sup> , T <sup>a</sup>	POAG/POAG	Yes
II-3	F/44		1.0/1.0		15/14				0.37/0.19				Normal VF		Carrier	Yes
II-4	F/41		1.0/1.0		15/13				0.54/0.38				Normal VF		Unaffected	No
II-5	M/37		1.0/1.0		19/20				0.6/0.7				Normal VF		GS (reduced inferior rim)	Yes
III-1	M/22		1.0/1.0		17/17				0.37/0.34				Normal VF		Carrier	Yes
III-2	M/20		1.0/1.0		18/17				0.7/0.7				Normal VF		GS (reduced inferior rim)	Yes

M: Male; F: Female; BCVA: Best corrected visual acuity; OD: Right eye; OS: Left eye; FC: Finger count; IOP: Intraocular pressure; VCDR: Vertical cup disc ratio; VF: Visual field; T: Trabeculectomy; A: Ahmed valve implantation; BR: Bleb revision; GS: Glaucoma suspect. <sup>a</sup>Performed at another hospital.

(VCDR 0.78/0.92). Visual field MD was -12.03/-30.87 dB. Despite medical treatment, the visual field had considerable glaucomatous change and the visual acuity of the left eye had decreased insidiously. He had received trabeculectomy in both eyes (Figure 2C1, 2C2, 2D1, 2D2).

The II-2 was diagnosed with POAG at the age of 44y. He is believed to be the youngest member of this pedigree with glaucoma. Three years before his first visit to our hospital, he had received trabeculectomy in both eyes and Ahmed valve implantation in the right eye at another hospital. At baseline examination, BCVA was 0.02/1.0 and IOP was 42/11 mm Hg. Disc excavation was nearly total cupping on both eyes (VCDR 0.9/0.85). Visual field showed highly considerable glaucomatous change. Visual field MD was -33.01/-29.34 dB. During following-up period, he had received bleb revision surgery in the right eye and second trabeculectomy in the left eye. He was highly likely to have JOAG with genetic mutation and was the most severely affected among the three patients (Figure 2E1, 2E2, 2F1, 2F2).

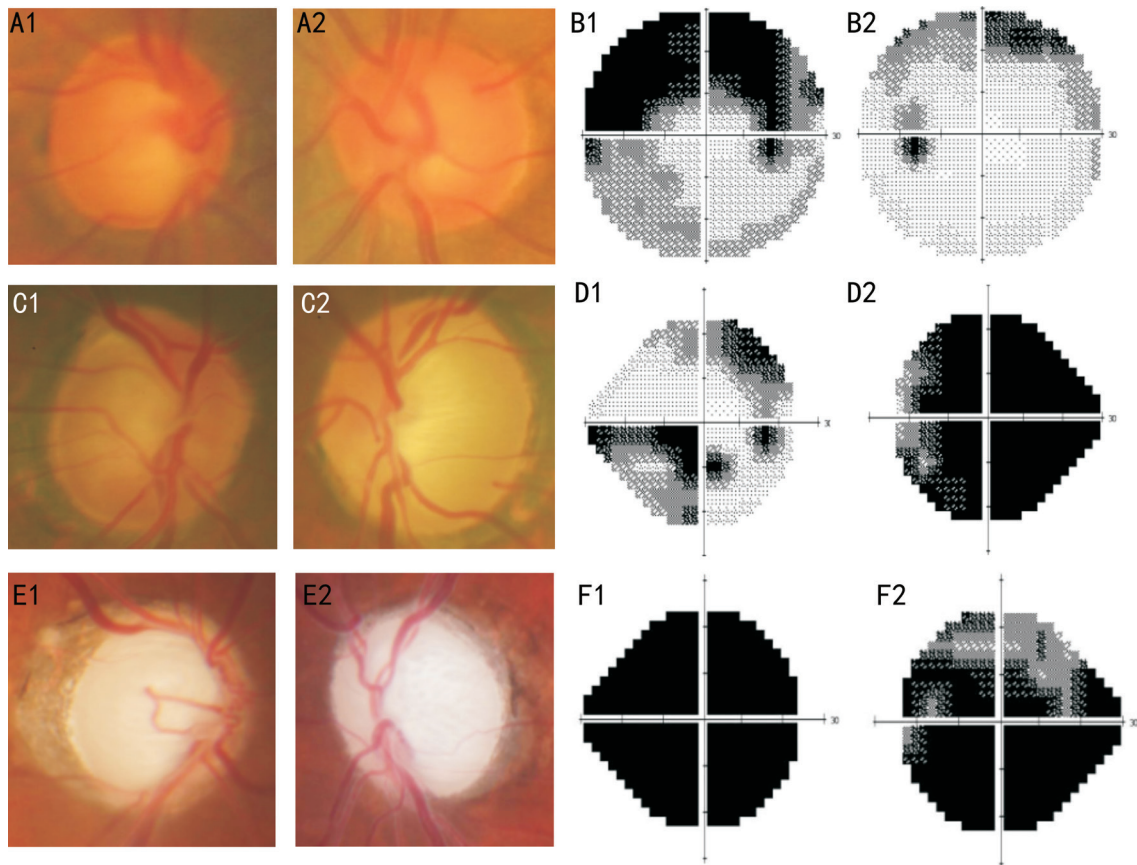
**Sequencing and Variant Analysis** We sequenced the MYOC exon3 for proband and family members. In our study, nucleotide change from thymine to cytosine at c.1021T>C was found in eight members (I-1, I-2, I-3, II-2, II-3, II-5, III-1, III-2). The codon changed from TCC to CCC, and thus amino acids at position 341 were changed. Therefore, the myocilin protein was altered from serine (Ser) to proline (Pro; p.S341P; Figure 3).

The missense variant c.1021T>C (p.Ser341Pro) was not listed in the population database. In silico analyses revealed that the following variant was predicted: [(Align Class): Class C65 (GV 0.00 GD 73.35)]; (PolyPhen 2): probably damaging (0.998); (SIFT): deleterious (score:0), affect protein function; Mutation Taster: disease causing; CADD: 9.969.

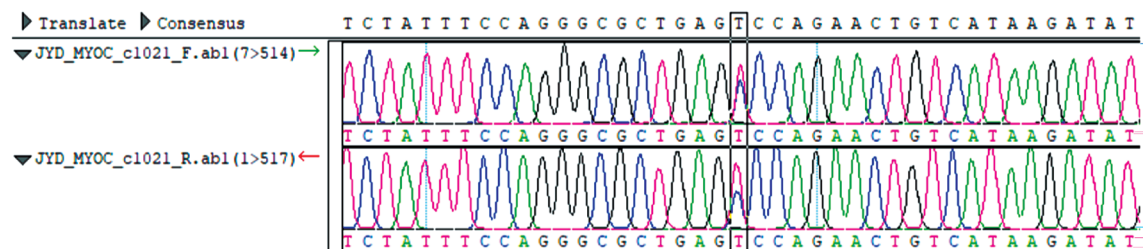
Ser341Pro could be involved in following criteria of ACMG/AMP 2015 Guideline<sup>[24]</sup>. 1) PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation; 2) PM2: Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium; 3) PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; 4) PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).

Combining the above criteria, Ser341Pro has a combination of 2 moderate (PM1+PM2) and 2 supporting (PP2+PP3) criteria, which is interpreted to “likely pathogenic”. This guideline proposed that the term “likely pathogenic” be used to mean greater than 90% certainty of a variant with being disease-causing<sup>[24]</sup>.





**Figure 2 Images were acquired from three POAG patients** A, B: Proband (I-2). VCDR was 0.84 in the right eye and 0.75 in the left eye at baseline examination (A1, right eye, A2, left eye). The inferior rim width of the right eye was highly reduced, which is relevant to the visual field defect. There were superior arcuate scotomas in both eyes (B1, right eye, B2, left eye). Particularly in the right eye, the visual field had already progressed beyond the horizontal raphe. Mean deviation was -16.33/-4.68 dB at baseline examination. C, D: Proband's brother (I-3). VCDR was 0.78 in the right eye and 0.92 in the left eye at baseline examination (C1, right eye, C2, left eye). The superior and inferior rim width of the left eye was significantly reduced, which is relevant to the visual field defect (temporal island). There was glaucomatous visual field loss in both eyes. In the left eye, with progression of glaucoma, only a temporal island was left. Mean deviation was -12.03/-30.87 dB at baseline examination (D1, right eye, D2, left eye). E, F: Proband's nephew (II-2). VCDR was 0.9 in the right eye and 0.85 in the left eye at baseline examination. Disc excavation was nearly total cupping in both eyes (E1, right eye, E2, left eye). There was considerable glaucomatous visual field loss in both eyes. Mean deviation was -33.01/-29.34 dB at baseline examination (F1, right eye, F2, left eye).



**Figure 3 Validation and DNA sequence chromatograms** The sequence of the third exon of *MYOC* is highlighted. Heterozygote sequence (vertical square box) shows a T/C transition in codon 341(c.1021T>C) that changed serine (TCC) to proline (CCC).

## DISCUSSION

The purpose of this study is to report the first discovery of Ser341Pro variant in Korea and analyze its clinical characteristics and genetic significance. According to our study, the Ser341Pro variant is associated with severe phenotype of POAG. In this study, eight members had

Ser341Pro variant which passed vertically with autosomal dominant inheritance with incomplete penetrance; three of them were diagnosed with POAG and two of them were diagnosed with glaucoma suspect. The 62.50% (5/8) of the members carrying the Ser341Pro variant had developed POAG or glaucoma suspect. The patients with POAG had

moderate or severe stage of glaucoma at initial visit to our hospital. Because the disease progressed aggressively and was refractory to medical treatment, all of three POAG patients underwent trabeculectomy and required additional glaucoma surgery. In the case of the II-2, the one with early onset open angle glaucoma progressed faster and required more additional glaucoma surgeries than others.

In accordance with previous study by Wang *et al*<sup>[11]</sup>, all of POAG patients with Ser341Pro variant in this study had trabeculectomy in both eyes. The results of our study are similar to the previous study in terms of autosomal dominant inheritance and incomplete penetrance (62.50% and 66.67%)<sup>[11]</sup>. Moreover, the younger patient had the faster progression and worse prognosis. In the case of the II-2 (diagnosed at the age of 44y) and a proband of previous study (diagnosed at the age of 25y) had more severe symptoms than other members<sup>[11]</sup>. In contrast to the previous study<sup>[11]</sup>, patients diagnosed with POAG in our study were older (55.00±9.54 vs 48.33±20.82y) and more severe glaucomatous optic disc change (0.84 vs 0.65, VCDR). However, there was a lack of data such as MD on visual field test in previous study, so comparison was impossible for functional test.

Wang *et al*<sup>[11]</sup> found that the c.1021T>C (Ser341Pro) variant caused loss of the restriction enzyme sites of CviKI-1 in the corresponding DNA sequence. The homologous chromosomes of family members who had the variant were cleaved into four fragments (257, 209, 203 and 48 bp), while the homologous chromosomes of other members were cleaved into three fragments (209, 203 and 48 bp)<sup>[11]</sup>. They also found that the MYOC c.1021T>C base change caused amino acid switch from Ser to Pro at sites 333, 337 and 342. The coil structure of amino acids 333 was replaced with the  $\beta$ -sheet structure and the  $\beta$ -sheet structure of amino acids 337 and 342 were replaced with the coil structure<sup>[11]</sup>.

Qin and Li<sup>[21]</sup> reported that Ser341Pro variant was found in one pedigree and there were no variants in unaffected relatives and normal control. Huang *et al*<sup>[12]</sup> also reported this variant in one pedigree and all members including proband and three siblings with this variant were diagnosed with POAG. However, these studies did not address detailed clinical or genetic aspect of this variant<sup>[12,21]</sup>.

ACMG/AMP 2015 guideline<sup>[24]</sup> recommended the term “variant” instead of the term “mutation (permanent change in the sequence)” and “polymorphism (variant with a frequency above 1%)” with the 5-tier terminology as follows: 1) pathogenic, 2) likely pathogenic, 3) uncertain significance, 4) likely benign, 5) benign. They also provided criteria for classification of pathogenic or likely pathogenic variants and benign or likely benign variants. Each pathogenic criterion is weighted as very strong (PVS1), strong (PS1-PS4), moderate

(PM1-PM6), or supporting (PP1-PP5), and each benign criterion also is weighted as stand-alone (BA1), strong (BS1-BS4), or supporting (BP1-BP6).

We analyzed the genetic variant found in our study based on the ACMG/AMP 2015 guideline<sup>[24]</sup>. First, we utilized population databases for obtaining the frequencies of Ser341Pro variant in large populations. As a result, this variant is not found in normal population. Second, we performed multiple computational (in silico) predictive programs and these programs support a deleterious effect of Ser341Pro variant. Third, third exon of the olfactomedin region is well-established functional domain and known as a mutational hot spot. Furthermore, this domain is an active site for expression of myocilin protein<sup>[6-8]</sup>. This variant was located in third exon of the olfactomedin region. Lastly, most of variants about POAG were missense variants including some nonsense variants. With combining the evidence of pathogenicity (PM1, PM2, PP2, PP3), Ser341Pro variant is interpreted as “likely pathogenic”. If we take into account the results of previous basic researches<sup>[11]</sup>, the Ser341Pro variant had a “strong evidence of pathogenicity (PS3)” and then it could be interpreted to “pathogenic (PS3, PM1, PM2, PP2, PP3)”. In addition, ACMG/AMP 2015 guideline recommended that all assertions of pathogenicity (including “likely pathogenic”) be reported with respect to a condition and inheritance pattern. As to this proposal, Ser341Pro variant can be reported as “c.1021T>C (p.Ser341Pro), likely pathogenic, POAG, autosomal dominant”.

As to the Myocilin Allele-Specific Glaucoma Phenotype Database (myocilin.com), the majority of variant type is missense variant (85.1%) and other variant types such as nonsense variant (5%), small deletion (5%), small insertion (4%), small indel (1%) are also presented. Ser341Pro variant is listed as “glaucoma causing mutation” in Myocilin Allele-Specific Glaucoma Phenotype Database with mean age at diagnosis of 22.2±11.2y, mean maximum recorded IOP of 23.9±7.5 mm Hg, the proportion requiring trabeculectomy of 42.9%, and the penetrance of 22.2% at age 25y, 33.3% at age 50y, 55.6% at age 75y. Our study showed that the proportion of patients requiring trabeculectomy was 60% (3/5), mean age at diagnosis of glaucoma was 44.4±17.1y, mean maximum recorded IOP was 20.5±9.2 mm Hg, and penetrance was 12.5% at age 25y, 37.5% at age 50y, 62.5% at age 75y.

Gln368Stop variant is reported as the most prevalent variant and leads to milder POAG manifestation with late onset<sup>[14]</sup>, while Pro370Leu, Gly246Arg, or Tyr437His variants are associated with the most severe glaucoma phenotypes with early onset<sup>[17]</sup>. Pro370Leu has been found in various ethnicity, which was analyzed with Chinese pedigree. The previous study suggested that eight of twenty two participants with

POAG had Pro370Leu variant and Pro370Leu variant caused severe phenotype of POAG<sup>[26]</sup>. Seven patients with Pro370Leu variant (87.5%) had trabeculectomy in both eyes<sup>[26]</sup>. Mean age at diagnosis was 17y (range 9-28y), mean maximum recorded IOP was 34.18±2.97 (range 29-40) mm Hg<sup>[26]</sup>.

Pro370Leu variant is also listed as “glaucoma causing mutation” in Myocilin Allele-Specific Glaucoma Phenotype Database with mean age at diagnosis of 13.3±2.4y, mean maximum recorded IOP of 30.9±3.0 mm Hg, proportion requiring trabeculectomy of 41.6% and penetrance of 47.1% at age 25y, 65% at age 50y, 65% at age 75y.

Unlike previous studies, the proportion of patients who had trabeculectomy in our study was greater than the databases of Ser341Pro and Pro370Leu. One possible explanation for this difference may be that the POAG patients in our study had already moderate to severe stage at initial visit, and the diagnosis might be delayed without appropriate treatment. Therefore, the mean age at diagnosis (44.4y) is much higher than that of database about Ser341Pro (22.2y).

Clinically and genetically compared with Ser341Pro variant and Pro370Leu variant, Pro340Leu variant is responsible for severe phenotype with much higher maximal IOP and earlier onset than that of Ser341Pro variant. But the proportion of trabeculectomy is similar or higher in phenotype of Ser341Pro variant. So far, Ser341Pro variant has only been found in patients of Korea and China, whereas Pro340Leu has been found in various ethnicities (Indian, English, French, North America, Japanese, and German populations) and widely reported<sup>[26-27]</sup>.

Previous experiment studies found that mutant myocilin protein becomes hydrophobic with lower solubility altered by misfolding and accumulates in the endoplasmic reticulum (ER)<sup>[9,28-30]</sup>. These aggregates may cause the parade of ER stress and give rise to cytotoxic effect<sup>[9,28-30]</sup>.

Another experimental study demonstrated that myocilin was proteolytically cleaved at the COOH-terminus of Arg226 and the endoproteolytic processing of myocilin was regulated by diverse mutations with variable efficacy<sup>[31]</sup>. The Pro370Leu is known as the highest suppression of endoproteolytic cleavage<sup>[31]</sup>.

The Ser341Pro variant is a heterozygous mutation that turns Ser into Pro, and has different physical and chemical properties<sup>[32]</sup>. Ser is a mixed hydrophilic amino acid with molecular weight of 105.09 g/mol, an isoelectric point of 5.68, and 6 codons<sup>[32]</sup>. In contrast, Pro is a highly hydrophilic amino acid with a molecular weight of 115.13 g/mol, an isoelectric point of 6.30, and 4 codons<sup>[32]</sup>. Consequently, the corresponding myocilin protein can be altered by misfolding and becomes hydrophobic with lower solubility<sup>[32-33]</sup>. As a result, like other mutant protein such as Pro370Leu variant, it may block the trabecular meshwork and affect the outflow of the aqueous humor. It may also accumulate in the vesicle and then

cause toxic effects in the trabecular meshwork cells. However, there is a lack of experimental research on Ser341Pro, so there seems to be a limit to interpret this variant. Therefore, further basic researches are required to elucidate the pathogenic roles played by the Ser341Pro variant in the pathogenesis of POAG with severe phenotype.

As we know, there are various factors (genetic or environmental) involved in the occurrence of glaucoma<sup>[34]</sup>, even if there is the same genetic variant. While some phenotypes are associated with a single gene, others are associated with multiple genes. The clinical significance of any given sequence variants ranges from those in which the variant is almost certainly pathogenic for a disease to those that are almost certainly benign. Therefore, genetic analysis through basic experiments and predictive programs, including clinical analysis can be helpful in interpreting “pathogenicity”.

From a genetic point of view, the importance of this study is as follows. Increasingly, phenotype and genotype are important for disease diagnosis, and the genetic causes of diseases are becoming clear. It has been difficult to conduct genetic tests for glaucoma because of the various and complex genes involved. However, the development of genetic testing techniques has made it easier and faster to test several genes at a time. By identifying the causative genes, it is possible to identify and test the genes of family members. Screening, early detection, and follow-up can be performed by classifying whether a high-risk person is a carrier. In particular, molecular diagnosis should be considered actively for familial and early-onset glaucoma.

#### ACKNOWLEDGEMENTS

We would like to thank all of patients for their participation in this study and Editage (www.editage.co.kr) for English language editing.

**Foundations:** Supported by Patient-Centered Clinical Research Coordinating Center funded by the Ministry of Health & Welfare, Republic of Korea (No.HI19C0481; No.HC19C0276).

**Authors' contributions:** Conceptualization: Lee JW, Moon SW. Methodology: Lee JW, Kim NH. Investigation: Moon SW. Data curation: Lee JW, Moon SW, Kim NH. Writing original draft: Moon SW. Writing, review, and editing: Lee JW, Kim NH.

**Conflicts of Interest:** Moon SW, None; Kim NH, None; Lee JW, None.

#### REFERENCES

- 1 Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90(3):262-267.
- 2 Hogewind BF, Gaplovska-Kysela K, Theelen T, Cremers FP, Yam GH, Hoyng CB, Mukhopadhyay A. Identification and functional characterization of a novel MYOC mutation in two primary open angle glaucoma families from The Netherlands. *Mol Vis* 2007;13:1793-1801.



- 3 Fan BJ, Leung YF, Wang N, *et al.* Genetic and environmental risk factors for primary open-angle glaucoma. *Chin Med J (Engl)* 2004;117(5):706-710.
- 4 Allen KF, Gaier ED, Wiggs JL. Genetics of primary inherited disorders of the optic nerve: clinical applications. *Cold Spring Harb Perspect Med* 2015;5(7):a017277.
- 5 Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol* 1998;116(12):1640-1645.
- 6 Avisar I, Lusky M, Robinson A, Shohat M, Dubois S, Raymond V, Gatton DD. The novel Y371D myocilin mutation causes an aggressive form of juvenile open-angle glaucoma in a Caucasian family from the Middle-East. *Mol Vis* 2009;15:1945-1950.
- 7 Hewitt AW, MacKey DA, Craig JE. Myocilin allele-specific glaucoma phenotype database. *Hum Mutat* 2008;29(2):207-211.
- 8 Mohanty K, Mishra S, Dada R, Dada T. Genetic complexity of primary open-angle glaucoma. *JOCGP* 2011;5:31-39.
- 9 Sohn S, Hur W, Joe MK, *et al.* Expression of wild-type and truncated myocilins in trabecular meshwork cells: their subcellular localizations and cytotoxicities. *Invest Ophthalmol Vis Sci* 2002;43(12):3680-3685.
- 10 Borrás T, Rowlette LL, Tamm ER, Gottanka J, Epstein DL. Effects of elevated intraocular pressure on outflow facility and TIGR/MYOC expression in perfused human anterior segments. *Invest Ophthalmol Vis Sci* 2002;43(1):33-40.
- 11 Wang FY, Li Y, Lan L, Li B, Lin L, Lu XH, Li JP. Ser341Pro MYOC gene mutation in a family with primary open-angle glaucoma. *Int J Mol Med* 2015;35(5):1230-1236.
- 12 Huang X, Li M, Guo X, *et al.* Mutation analysis of seven known glaucoma-associated genes in Chinese patients with glaucoma. *Invest Ophthalmol Vis Sci* 2014;55(6):3594-3602.
- 13 Gupta V, Somarajan BI, Gupta S, Chaurasia AK, Kumar S, Dutta P, Gupta V, Sharma A, Tayo BO, Nischal K. The inheritance of juvenile onset primary open angle glaucoma. *Clin Genet* 2017;92(2):134-142.
- 14 Nag A, Lu H, Arno M, Iglesias AI, Bonnemaïjer P, Broer L, Uitterlinden AG, Klaver CC, van Duijn C, Hysi PG, Hammond CJ. Evaluation of the myocilin mutation Gln368Stop demonstrates reduced penetrance for glaucoma in European populations. *Ophthalmology* 2017;124(4):547-553.
- 15 Chen LJ, Rong SS, Pang CP. Genome-wide association study of age-related eye diseases in Chinese population. In: Prakash G, Iwata T. (eds) *Advances in Vision Research*, Volume I. 2017. Essentials in Ophthalmology. Springer, Tokyo.
- 16 Xie XB, Zhou X, Qu XY, Wen J, Tian YL, Zheng F. Two novel myocilin mutations in a Chinese family with primary open-angle glaucoma. *Mol Vis* 2008;14:1666-1672.
- 17 Liuska P, Kivelä T, Turunen J. Prevalence of risk variants for glaucoma in the MYOC gene in the general population. *Acta Ophthalmol* 2019;97.
- 18 Kee C, Ahn BH. TIGR gene in primary open-angle glaucoma and steroid-induced glaucoma. *Korean J Ophthalmol* 1997;11(2):75-78.
- 19 Yoon SJ, Kim HS, Moon JI, Lim JM, Joo CK. Mutations of the TIGR/MYOC gene in primary open-angle glaucoma in Korea. *Am J Hum Genet* 1999;64(6):1775-1778.
- 20 Park J, Kim M, Park CK, *et al.* Molecular analysis of myocilin and optineurin genes in Korean primary glaucoma patients. *Mol Med Rep* 2016;14(3):2439-2448.
- 21 Qin L, Li JM. Investigation on the mutation of MYOC gene in two family pedigrees with primary open-angle glaucoma in Shanxi. *Eye Sci* 2007;23(2):75-78.
- 22 Anderson RD, Patella VM. *Automated Static Perimetry*. 2nd ed. St. Louis: Mosby; 1999.
- 23 Foster PJ, Buhmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. *Br J Ophthalmol* 2002;86(2):238-242.
- 24 Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405-424.
- 25 Ruengkitpinyo W, Kongprawechnon W, Kondo T, Bunnun P, Kaneko H. Glaucoma screening using rim width based on ISNT rule. In: 2015 6th International Conference of Information and Communication Technology for Embedded Systems (IC-ICTES). IEEE. 2015:1-5.
- 26 Wei YT, Li YQ, Bai YJ, Wang M, Chen JH, Ge J, Zhuo YH. Pro370Leu myocilin mutation in a Chinese pedigree with juvenile-onset open angle glaucoma. *Mol Vis* 2011;17:1449-1456.
- 27 Rose R, Karthikeyan M, Anandan B, Jayaraman G. Myocilin mutations among primary open angle glaucoma patients of Kanyakumari district, South India. *Mol Vis* 2007;13:497-503.
- 28 Wang LN, Zhuo YH, Liu BQ, Huang SS, Hou F, Ge J. Pro370Leu mutant myocilin disturbs the endoplasm reticulum stress response and mitochondrial membrane potential in human trabecular meshwork cells. *Mol Vis* 2007;13:618-625.
- 29 Liu YH, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. *Hum Mol Genet* 2004;13(11):1193-1204.
- 30 Joe MK, Sohn S, Hur W, *et al.* Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. *Biochem Biophys Res Commun* 2003;312(3):592-600.
- 31 Aroca-Aguilar JD, Sánchez-Sánchez F, Ghosh S, Coca-Prados M, Escribano J. Myocilin mutations causing glaucoma inhibit the intracellular endoproteolytic cleavage of myocilin between amino acids Arg226 and Ile227. *J Biol Chem* 2005;280(22):21043-21051.
- 32 Ma F, Yaoting WU, Xu X. Correlation analysis of some physical chemistry properties among genetic codons and amino acids. *J Anhui Agric Coll* 2003;30(4):439-444.
- 33 Hill SE, Nguyen E, Donegan RK, *et al.* Structure and misfolding of the flexible tripartite coiled-coil domain of glaucoma-associated myocilin. *Structure* 2017;25(11):1697-1707.e5.
- 34 McMonnies CW. Glaucoma history and risk factors. *J Optom* 2017;10(2):71-78.