IGM Publication

Journal of Medical Science and Clinical Research
Volume||1||Issue||3||Pages141-147||2013
Website: www.jmscr.igmpublication.org ISSN (e): 2347-176X

Evaluation of The Association Between M98K and T34T Polymorphisms of the Optineurin (OPTN) Gene and Primary Open Angle Glaucoma in Indonesia

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ABSTRACT

PURPOSE: *Glaucoma is the leading cause of irreversible blindness in the world. Recent evidence indicates genetic susceptibility plays a role in primary open-angle glaucoma (POAG). The optineurin gene (OPTN) mutations have been identified to be associated with POAG. This study to analyze the association of M98K and T34T of the OPTN with POAG.*

METHODS: Primary open angle glaucoma patients were characterized by open angles on gonioscopy, intraocular pressure (IOP) > 21 mmHg or <21 mmHg and typical glaucomatous disc changes with corresponding visual field defects in the absence of any secondary cause. Sixty patients with POAG and 60 unrelated control subjects were identified for polymorphisms of M98K and T34T of the OPTN. Genotyping of M98K and T34T were performed by PCR-RFLP.

RESULTS: Polymorphism of M98K and T34T of the OPTN were identified in both POAG and control subjects. The M98K polymorphism of OPTN were observed in 35 POAG (35/60) and 18 in control subjects (18/60). The M98K polymorphism showed a significant association with POAG (P = 0.021, Chi-square test). The T34T polymorphism of the OPTN were observed in 18 POAG patients (18/60), and have no association with POAG (P = 0.183, Chi-square test).

CONCLUSIONS: There is evidence of a positive association only between M98K polymorphism of the OPTN and POAG, T34T polymorphism of the OPTN gene have no association with POAG susceptibility in Indonesian People.

KEY WORDS: *Polymorphism, M98K and T34T of the OPTN gene, primary open angle glaucoma.*

INTRODUCTION

Glaucoma is the second leading cause of blindness worldwide, characteristic by loss of retinal ganglion cells associated with optic nerve degeneration, elevated Intraocular Preassure (IOP)

and visual field loss. Well recognized risk factors for the development of POAG include IOP, positive family history of glaucoma, age, refractive error, and race [1-3].

Genetic factors play a major rele in POAG predisposition. HUGO (Human Genome Organization, Geneva, Switzerland), disease-associated sequence variants in three genes, myocilin (MYOC, GLC1A), optineurin (OPTN,GLC1E), and WD repeat domain 36 (WDR36, GLC1G), have been described in POAG. Recently, the optineurin (OPTN) gene on chromosome 10p14 was shown to be implicated in POAG [4,5].

The mechanistic role of OPTN in the pathogenesis of glaucoma remains unclear. It is widely expressed in both ocular and non-ocular tissues [6]. The OPTN protein interacts with different proteins that are involved in apoptosis. OPTN might play a neuroprotective role by reducing retinal ganglion cell susceptibility to apoptosis. Overexpression of OPTN blocks cytochrome c release from the mitochondria and protects the cell from hydrogen peroxide-induced cell death [7-10]. OPTN sequence variations have also been shown to be associated with POAG in Indian and Japanese populations [11,12] but less significant role in Caucasians [13].

The original report of OPTN involvement in glaucoma presented three likely disease-causing variants designated E50K, 691_692insAG, and R545Q, and one proposed risk factor M98K [9]. Further studies find association of some *OPTN* alleles with POAG, but others report no evidence of association of POAG with those same alleles [14].

Environment factor also play a role of gene mutation and make a diffrenet mutation in each country [15]. Previous studies in Western populations show a significant ethnic variation in glaucoma prevalence between white people and black people, largely related to variation in the prevalence of POAG [16-17].

M98K and T34T are the most common variants of OPTN in Asia [4,5]. That is the reason of choosing M98K of OPTN and analyze the association between polymorphisms of M98K and POAG**.** Until recently, similar data have been described in Asian populations [18-19]. Based on the survey of blindness in Indonesia (WHO criteria), the prevalence of blindness because primary open-angle glaucoma in the elderly Indonesian people was about 0,2% [20]. In this study, we aimed to evaluate the association of M98K and T34Tof the OPTN with POAG.

MATERIALS AND METHODS

A case and control study of unrelated 60 POAG patients and 60 non-glaucoma patients. Diagnosis of POAG was based on meeting all the following criteria: exclusion of secondary causes (e.g., trauma, uveitis, or steroid-induced glaucoma); anterior chamber angle open (grade III or IV gonioscopy); characteristic optic disc changes (e.g., vertical cup-to-disc ratio of >0.4, disc hemorrhage, or thin or notched neuroretinal rim); and characteristic visual field changes with reference to Anderson's criteria for minimal abnormality in glaucoma. Visual acuity was determined using the Snellen eye chart. Intraocular pressure more than 21 mmHg or <21 mmHg on applanation tonometry

measured by applanation tonometry and visual field testing was measured by perimeter (Automatic perimetry, Optopol). The patients with POAG in this study included those with low-tension glaucoma (LTG) or high-tension glaucoma (HTG), but patients with congenital glaucoma or with a family history of glaucoma were excluded. Unrelated control subjects were recruited from people who attended the clinic for conditions other than glaucoma, including post cataract surgery and refractive errors not more than 6 Diopter. Glaucoma and TIO >21 mmHg were ruled out in the control subjects by the same diagnostic criteria and the same ophthalmic examination as were used in the patients.

RESULTS

The patient group was composed of 60 sporadic patients with POAG: 19 males and 41 females, with age at diagnosis ranging from 40 to 72 years (mean ± SD: 56.8 ± 9.45). The IOP at diagnosis ranged from 13 to 58 mm Hg (mean ± SD: 27.3 ± 8.3). Among these 60 patients with POAG, 25 (41.7%) were LTG with IOP less than 22 mm Hg. There were 60 unrelated control subjects, 28 male and 32 female, with age ranging from 40 to 80 years (mean ± SD: 53.8 ± 8.9), and IOP less than 22 mm Hg **(tabel 1)**.

Tabel 1. Clinical characteristics of POAG and Control subjects

Clinical Characteristics	POAG n (%)	Control n (%)
Visual Acuity OD		
20/20-20/40	28 (46.7%)	37 (61.7%)
20/50-20/200	21 (35.0%)	23 (38.3%)
< 20/200	11 (18.3%)	0 (0.0%)
Visual Acuity OS		
20/20-20/40	31 (51.7%)	39 (65.0%)
20/50-20/200	19 (31.7%)	21 (35.0%)
< 20/200	10 (16.7%)	0 (0.0%)
IOP OD		
≤21 mmHg	29 (48.3%)	60 (100%)
>21 mmHg	31 (51.7%)	0 (0.0%)
IOP OS		
≤21 mmHg	25 (41.7%)	60 (100%)
>21 mmHg	35 (58.3%)	0 (0.0%)

IOP intraocular pressure, OD oculi dextra, OS oculi sinistra.

All study subjects were given a complete ocular examination.

Venous blood was obtained and stored at −20°C for less than 1 months before DNA extraction. The study protocol was approved by the Ethics Committee for Human Research of the Sumatera Utara University of Indonesia and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all study subjects after explanation of the nature and possible consequences of the study. Sequencing analysis was performed to confirmed the result of PCR-RFLP procedure from each genotype (homozygote wild type, heterozygote, and homozygote mutant) both groups

PCR and RFLP of M98K Polymorphism

Genomic DNA was extracted from 200 µL of whole blood by a commercial procedure (Genomic DNA Mini Kit, Geneaid, Germany). Genotyping of M98K was performed by PCR-RFLP. The sequence of OPTN exon 5, which encodes this variant, was deduced from cDNA sequence (Genebank accession number AF4203070). Amplification of a 157 bp fragment was carried out with the following primers: forward FAM-5'GGCCTGGACAGAGAAACAGA3' and primer reverse 5'GCTCACCTCAGAT GACCTTTC 3', using 25µL master mix PCR consist of 10µM of each primer, Kapa HiFi^{TM} HotStart ready Mix (Kapa Biosystems), and 50ng DNA genome. All coding sequences of OPTN, including intron–exon boundaries, were screened for sequence alterations using PCR followed by FLP by using polyacrilamide gel. PCR was performed on a thermal cycler (GeneAmp® PCR System 9700, Applied Biosystems), with an initial denaturation step of 5 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, a touchdown annealing temperature of 62°C minus 0.2°C per cycle for 1 minute, and 72°C for 60 seconds, and a final extension step of 72°C for 5 minutes. The c603T>A variant, which encodes the M98K amino acid variation, creates an Stu I restriction site; cleavage of the variant polymerase chain reaction (PCR) with Stu I at 37°C generated two fragments, of 80 and 77 bp, which were detected after migration on an automated sequencer.

PCR and RFLP of T34T Polymorphism

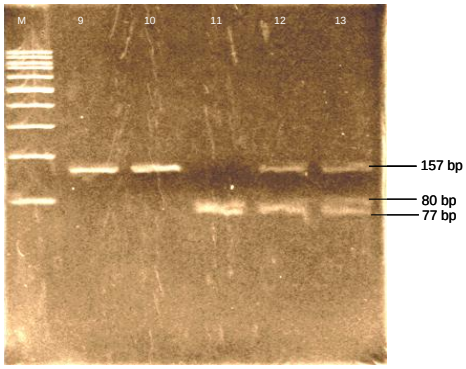
The same procedure for extracted DNA as M98K polymorphism. Amplification of a 319 bp fragment was carried out with the following primers: forward FAM- 5' CCAATGGGTTTGTGGGACTCC 3' and primer reverse 5TGCAAGAAAT GCCATCCCTTT 3'. RFLP by using agarose 2% gel. The c412G>A variant, which encodes the T34T amino acid variation, creates an Tai I restriction site; cleavage of the variant polymerase chain reaction (PCR) with Tai I at 37°C generated two fragments, of 193 and 126 bp were detected

Statistical Analysis

Statistical analyses were performed on computer (SAS ver. 8.2; SAS Institute, Cary, NC). The distribution frequency test was used to frequencies of M98K and T34T genotypes between patients and control subjects. Chi-Square Test was used to analyze the association of M98K polymorphism and POAG. $P < 0.05$ was considered significant (95% confidence interval). The confidence intervals for Odds Ratio (OR) were calculated by the logistic regression method. All the genotypes were set as categorical variables (homozygote, 2; heterozygote, 1; wild type, 0).

Polymorphism of M98K and T34T of the OPTN gene were identified in both POAG and control subjects. The M98K polymorphism of OPTN were observed in 35 POAG patients (35/60) and 18 patients in control subjects (18/60) contributing to 58,3% in POAG patients and 30% in control subjects. Genotype distribution of Polymorphism M98K in POAG patients were homozygote wild type found in 25 patients, heterozygote found in 30 patients and homozygote mutant found in 5 patients. In non-glaucoma

subjects homozygote wild type found in 42 patients, heterozygote found in 18 patients **(figure 1).**



M = Marker Ladder 100

Figure 1. Electrophoresis photo of the M98K polymorphism of OPTN gene in both groups of the study sample. Sample number 11 was in POAG patients (homozygote mutant), number 9 (homozygote wild type), 10 (homozygote wild type), 12 (heterozygote), and 13 (heterozygote)were in control subjects.

Polymorphism M98K in POAG patients were higher in patients with high IOP **(table 2).**

Tabel 2. The genotype distribution of the M98K Polymorphism of OPTN gene expression among POAG subjects.

Genotype Polimorphysme M98K of gene OPTN	Intraocular Preassure	
	≤ 21	> 21
Homozygote wild type	6	19
Heterozygote	7	23
Homozygote mutant	3	2

OPTN optineurin

The M98K polymorphism showed a significant association with POAG (P = 0.021, Chi-Square test),**(tabel 3).**

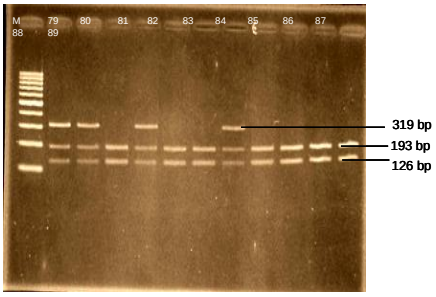
Tabel 3. The distribution of the M98K polymorphism of the OPTN gene expression among POAG and control subjects

Polymorfisme M98K of the OPTN gene	POAG	Control	P
Polymorfisme M98K (-) (Homozygote Wild Type)	25	42	
Polymorfisme M98K (+) (Heterozygote, Homozygote Mutant)	35	18	0,021*

Chi-Square Test p= 0,021* : significant

Odds Ratio (OR) were calculated by the logistic regression method. The risk factor for having POAG among the subjects with M98K Polymorphism was 3.3 times higher than the subjects that didn't have the M98K Polymorphism.

The T34T polymorphism of OPTN were observed in 18 POAG patients (18/60) and 25 patients in control subjects (25/60) contributing to 30% in POAG patients and 41,7% in control subjects. Genotype distribution of Polymorphism T34T in POAG patients were homozygote wild type found in 42 patients, heterozygote found in 18 patients and no homozygote mutan was found. In non-glaucoma subjects homozygote wild type found in 35 patients, heterozygote found in 25 patients **(figure 2).**



M = Marker Ladder 100

Figure 2. Electrophoresis photo results of the T34T polymorphism of OPTN gene in both groups of the study sample. Almost all the sample were POAG patients only number 82 wass control subject. Sample number 79, 80, 82, and 85 were heterozygote and sample number 81, 83, 8 , 86, 87, 88, and 89 were homozygote wild type.

There was no association between T34T polymorphism of the OPTN and POAG (P = 0.183, Chi-square test) **(tabel 4).**

Tabel 4. The distribution of the T34T polymorphism of the OPTN gene expression among POAG and control subjects

Polymorfisme T34T of the OPTN gene	POAG	Control	P
Polymorfisme T34T (-) (Homozygote Wild Type)	42	35	
Polymorfisme T34T (+) (Heterozygote)	18	25	0,183

Chi-Square Test p= 0,183 : not significant

DISCUSSION

Apart from T34T, M98K, and R545Q, which were previously identified in the study by Rezaie et al., 2002, M98K and T34Tpolymorphism of the OPTN also found in this study of Indonesian subjects were novel and this study showed that the M98K polymorphism have a significant association with POAG susceptibility in Indonesia.

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