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TWO VARIANTS OF MYOC GENES IN PRIMARY OPEN ANGLE GLAUCOMA

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ABSTRACT

Background

Glaucoma comprises a heterogeneous group of optic neuropathies with a complex genetic basis. It is the second leading cause of irreversible blindness in the world affecting more than 60 million people globally. Primary open angle glaucoma (POAG) is the most common type of glaucoma and accounts for half of all cases.

Purpose

This study investigates the association of MYOC gene polymorphisms with POAG in Iraqi population of Al- Najaf Al-Ashraf governorate and to detect the impact of these polymorphisms on intra ocular pressure and cup-disk ratio.

Methods

A case–control study was conducted to find the association of MYOC gene polymorphisms (rs2234926, rs2075648) with primary open angle glaucoma in Iraqi population. The study included 150 patients and 150 controls who attended the ophthalmology unit at Al-Sader medical city and Al-Hakeem hospital in Al- Najaf Al-Ashraf governorate. DNA was extracted from blood and genotyped by PCR-RFLP by using (BsmAI, AvaI) enzyme. To compare the proportion of genotypes and alleles the multinomial logistic regression was applied. The odd ratio was calculated with and without adjustment for age and sex to evaluate risk of developing of POAG.

Result

The results of analysis of the genotype and allele frequencies of MYOC gene polymorphisms (rs2234926, rs2075648) revealed that the homozygous genotype (AA) and heterozygous genotype (GA) were no significantly (P> 0.05) increased the risk of primary open angle glaucoma with respect to those of the wild type (GG) after adjustment for age and sex. The frequency of the A allele of (rs2234926, rs2075648) polymorphisms were no significantly difference between POAG (26%) and controls (24 %). The results also revealed no significant differences in clinical characteristics intra ocular pressure (IOP) and cup-disk ratio (C/D ratio) levels between wild genotype (TT) , heterozygous genotype (TC) and homozygous genotype (CC) in POAG patients (P= 0.6 and P= 0.3) respectively.

KEYWORDS: MYOC Gene Polymorphisms, POAG Patients & Primary Open Angle Glaucoma

INTRODUCTION

Glaucoma is a chronic and progressive group of optic neuropathies.(Quigley HA and Broman AT, 2006) It is associated with death of retinal ganglion cells resulting in characteristic cupping or degeneration of the optic nerve head

and loss of peripheral vision (Kwon YH et al.,2009)

Glaucoma is the second leading cause of blindness affecting more than60 million people globally. The number of people (aged 40-80 years) with glaucoma globally is about to grow to 76 million by 2020 and 112 million by 2040. (Barkana Y and Dorai raj S, 2015)

Primary open angle glaucoma (POAG) is one of the most common types of glaucoma which is clinically characterized by an open and normal anterior chamber angle with increased intraocular pressure (IOP) or normal IOP, the latter being referred to as normal-tension glaucoma (NTG).(Kwon YH et al.,2009)Risk factors associated with POAG include elevated IOP, age, family history, gender, ethnicity, central corneal thickness, and myopia (Kang, JH et al.,2015)

The inheritance pattern of POAG seems to be multifactorial resulting from the interaction of one or more genes and/or environmental stimuli. To date, there have been over 20 genetic loci and three genes, MYOC (myocilin), OPTN(optineurin), and WDR36, that have been associated with POAG (Jiao X et al., 2009) Shefield et al, identified the first locus associated with POAG in 1993 denominated as GLC1A. In 1997 Stone et al identified the gene to be Myocilin (MYOC). It is made up of three exons divided by two introns and a promoter region that has several regulatory elements.(Kirstein L*et al*.2000;Fingert JH*et al*.,1998).

More than 70 mutations in the MYOCgene that encodes a protein composed of only 504 amino acids have been found to contribute to the pathogenesis of POAG. over 90% of which occur within exon3, 11 mutations in exon 1 and none has been detected in exon 2. (Pradhan S *et al*., 2011; Hewitt AW *et al*., 2008)

Fingert et al was reported that MYOC gene mutation account for 3-5% of adult POAG patients worldwide. In 2007, Bhattacharjee et al was studied the Myocilin variants in Indian patients with POAG and Mengkegale et al in Japanese patients in 2008.

MATERIALS AND METHODS

This is a case–control study of 150 POAG (age, 61.96±9.5 years 77 women and 73 men) and 150 controls (age, 63.7±8.8years 91 women and 59 men) who attended the ophthalmology unit at Al-Sader medical city and Al-Hakeem hospital in Al- Najaf Al-Ashraf governorate from May 2014 to March 2015 were included in this study. All patients and age and sex matched controls underwent a complete ophthalmic examination in order to confirm the diagnosis of POAG by ophthalmologist.

Inclusion criteria for cases: Age ≥40 years, glaucomatous optic neuropathy with compatible visual field loss for POAG, open anterior chamber angles on gonioscopy and IOP consistently ≥22mmHg. While the exclusion criteria include age below 40 years, other types of primary glaucoma, secondary glaucoma due to preexisting ocular and extra ocular lesions and non-glaucomatous field losses and disc changes (high myopia).

Peripheral blood samples of POAG and control groups were collected in EDTA-anticoagulant tubes, and then DNA was extracted from whole blood samples using the Reliaprep genomic DNA extraction kit (Promega, U.S.A.). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (BioDrop, U.K.)

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for MYOC gene polymorphisms (rs2075648 , rs2234926) using thermocycler (Biometra, Germany). The primer sequences

for MYOC gene rs2234926, rs2075648 were used according to (Xie X et al., 2008) and (Kumar A et al., 2007).

Table 1: The Sequence and Annealing Temperature of Primers Used

Gene/Snp	Primer	Primer Sequences	Annealing
MYOC rs2075648	Forward Reverse	5'-CAGCCT CAC GTG GCC ACC TCT GTC-3' 5'-AGGCCC AAA GCT GCA GCA ACG TGC-3'	62 °C
MYOC rs2234926	Forward Reverse	5'-ACG TTG CTG CAG CTT TGG-3' 5'- GATGACTGACATGGCCTGG-3'	62 °C

Amplification was performed in a total volume of 25 µl which contained 12.5 µl of GoTaq Green Master Mix, (Promega Corporation, Madison. WI), 1.5 µl of each primer (1mM final concentration) (One Alpha, U.S.A.), 4.5 µl of nuclease free water and 5 µl of DNA template. PCR reaction program protocol for MYOC genepolymorphisms revealed in Table 2, 3.

Table 2: PCR Reaction Program Protocol for MYOC Gene Rs2234926 (Xie X Et Al, 2008)

Type of Cycle	Temperature	Time	No. of Cycles
Initial Denaturation	94 °C	5 min	1cycle
Denaturation	94°C	45s	35 cycles
Annealing	62 °C	45s	
Extension	72 °C	1min	
Final Extension	72 °C	10 min	1cycle

Table 3: PCR Reaction Program Protocol for MYOC Gene Rs2075648 (Kumar A *Et Al*, 2007)

Type of Cycle	Temperature	Time	No. of Cycles
Initial Denaturation	95 °C	2 min	1cycle
Denaturation	95°C	30s	35 cycles
Annealing	62 °C	30s	
Extension	72 °C	1min	
Final Extension	72 °C	5 min	1cycle

Amplification products of MYOC genepolymorphisms (rs2234926, rs2075648) were 189 bp and 196 bp respectively. The products were digested with 10 u of restriction enzyme BsmAI, AvaI (BioLab) and ran on 2% agarose gels.

STATISTICAL ANALYSIS

Student T tests and ANOVA test were used to compare phenotypic data between control and POAG groups using SPSS windows software (SPSS Inc., Chicago, IL). Genotype frequencies were tested for Hardy- Weinberg equilibrium by X^2 test using online software web-Assotest (www.ekstoem.com). Genotype and allele frequencies in POAG and control groups were tested by multinomial logistic regression analysis with and without adjustment for age and sex using SPSS.

RESULTS

General and clinical characteristics of study individuals are presented in Table 4.

Table 4: General and Clinical Characteristics of Study Individuals

Variable	POAG Groups	Control Groups	P Value
No.	150	150	
Sex(F/M)	77/73	91/59	0.104
Age (y)	63.7±8.8	61.96±9.5	0.101
No of subjects with family history	29 (19%)	0	0.000
IOP (mmHg)	21.4±10.4	16.2±3.5	0.000
C/D ratio	0.56±0.14	0.25±0.095	0.000

Results of digestion with restriction enzyme (BsmAI) for MYOC gene (rs2234926) included 189 bp band for wild (GG) genotype, three bands189, 96,93bp for the heterozygous genotype (GA) and two bands 96,93bp for homozygous genotype (AA) as shown in Figure 1.

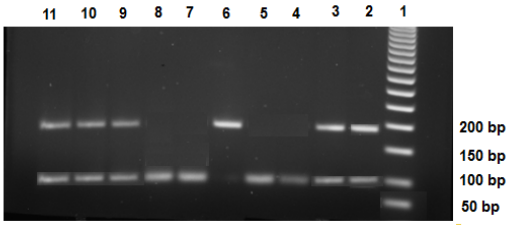

Figure 1: RFLP Pattern of MYOC Gene Polymorphism (rs2234926) on Agarosegel Electrophoresis. Lane 1: DNA Marker, Lane 6: GG Genotype 189bp, Lane 2, 3,9,10 and 11: GA Genotype 96 & 93bp, Lane 4, 5, 7 and 8: AA Genotype 96 & 93bp

The PCR product of MYOC gene polymorphism (rs2075648) was digested by AvaI restriction enzyme. The results of digestion products were analyzed by agarose gel electrophoresis revealed one band (196bp), two bands (120,76bp) and three bands (196,120,76bp) related to wild type (GG), homozygous (AA) and heterozygous (GA) genotypes respectively figure 2.

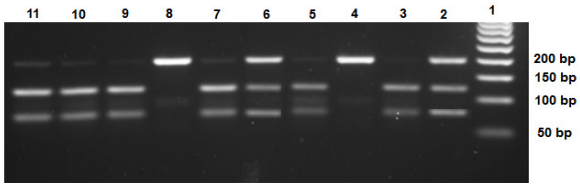

Figure 2: RFLP pattern of MYOC Gene Polymorphism (rs2075648) on Agarose gel Electrophoresis. Lane 1: DNA marker, Lane 4 and 8: GG genotype 196 bp, Lane 2 and 6: GA genotype 196, 120 & 76bp, Lane 3, 5, 9, 10 and 11: AA genotype 120 & 76bp

Genotype frequencies of (rs2234926, rs2075648) were consistent with Hardy- Weinberg equilibrium in both POAG patients (P=0.183, 0.696) and control individuals (P=0.266, 0.350) respectively. The results of analysis of the

genotype and allele frequencies of MYOC gene polymorphism (rs2234926, rs2075648) revealed that the homozygous genotype (AA) and heterozygous genotype (GA) were no significantly increased the risk of primary open angle glaucoma (P> 0.05) with respect to those of the wild type (GG) after adjustment for age and sex as shown in table 5,6.

Table 5: Genotype and Allele Frequency of MYOC Gene Polymorphism (Rs2234926) and Association of This Variant With Primary Open Angle Glaucomain Study Individuals

	Control N=150	POAG N=150	Unadjusted OR(95%CI)	P Value	Adjusted OR(95%CI)	P Value
Codominant						
GG(Reference)	85	79				
GA	57	64	1.208 (0.75-1.93)	0.43	1.215 (0.75-1.95)	0.42
AA	8	7	0.941 (0.33-2.72)	0.91	0.96 (0.33-2.80)	0.94
Frequency of A allele	73 (24.3%)	78 (26%)	1.09 (0.76-1.58)	0.64		

Table 6: Genotype and Allele Frequency Of MYOC Gene Polymorphism (Rs2075648) and Association of This Variant with Primary Open Angle Glaucomain Study Individuals

	Control N=150	POAG N=150	Unadjusted OR (95% CI)	P Value	Adjusted OR(95%CI)	P Value
Codominant						
GG(Reference)	83	78				
GA	60	64	1.14 (0.70-1.81)	0.60	1.13 (0.70-1.81)	0.62
AA	7	8	1.22 (0.42-3.51)	0.72	1.28 (0.44-3.74)	0.65
Frequency of A allele	74 (24.7%)	80 (26.7%)	1.11 (0.77-1.60)	0.58		

The results of MYOC gene polymorphisms (rs2234926, rs2075648) in present study revealed that no significant differences in intra ocular pressure (IOP) and cup-disk ratio (C/D ratio) levels between wild genotype (GG), heterozygous genotype (GA) and homozygous genotype (AA) in POAG patients (table 7, 8)

Table 7: Genotypes Correlation of MYOC Gene Polymorphism Rs2234926 with Clinical Characteristics in Primary Open Angle Glaucoma patients Group

Clinical Characteristics	GG (42)	GA (96)	AA (12)	P Value
IOP mmHg	22.38±11.65	22.03±11.15	25.58±12.48	0.596
C/D ratio	0.57±0.125	0.62±0.157	0.62±0.120	0.261

Table 8: Genotypes Correlation of MYOC Gene Polymorphism Rs2075648 with Clinical Characteristics in Primary Open Angle Glaucoma patients Group

Clinical Characteristics	GG (40)	GA (102)	AA (8)	P Value
IOP mmHg	22.18±11.90	22.19±11.12	26.50±12.40	0.582
C/D ratio	0.57±0.126	0. 61±0. 155	0. 65±0. 128	0.309

DISCUSSIONS

Glaucoma is regarded as the second leading cause of irreversible blindness in the world but it is a treatable disease when detected early. Primary open angle glaucoma usually develops slowly and a symptomatically until advanced retinal nerve fibers damage and visual field loss have occurred. This leads to high rate (> 50%) of undiagnosed glaucoma cases. (Balasubbu *et al*., 2012) This necessitates the provision of an accurate test to detect pre-symptomatic carriers at risk to prevent progression of glaucomatous damage into severe visual loss. (Williams *et al*., 2015; Tatham *et al*., 2014)

Information regarding the role of MYOC in Iraq POAG patients is scarce. So, the contribution of MYOC sequence variations to POAG in Iraq has not been analyzed so far. Therefore, the main purpose of this study was to analyze the contribution of MYOC sequence variations to adult-onset glaucoma in patients from this country. The first identified POAG gene is MYOC. It encodes myocillin protein which is expressed in most body tissues and in almost every ocular tissue. (Alward, 2000) Alward's and Fingert *et al*. set criteria for probable disease-causing mutations expected to alter the amino acid sequence of the corresponding protein, more commonly observed in patients with POAG not commonly observed in the general population present in less than 1% of the general population and absent from a glaucoma-free control group.

In the present study we have identified two MYOC gene polymorphisms were distributed along the promoter and coding region of the gene. (rs2234926) is the most common protein sequence polymorphism which usually occurred with the promoter polymorphismrs2075648in both patient and control groups. We observed that the allele frequencies of these two SNPs were identical in both groups and were not associated with POAG.

Our results suggest that these two SNPs of MYOC gene are not a risk factor for the development of POAG and is not associated with the phenotype and severity of glaucoma in our patients. The two SNPs would serve as a useful informative marker for investigating the cosegregation of MYOC with familial POAG prior to undertaking more labor-intensive mutation screening.(Mukhopadhyay A *et al*.,2002)

The current finding are in agreement with results of studies from Western countries (Fingert JH *et al.,*1999 ; Alward WL *et al*.,1998) , Japan (Suzuki R *et al*., 2000), Eastern India (Mukhopadhyay A *et al*.,2002), Hong Kong (Fan BJ *et al*., 2004) , Philippines (Wang DY *et al*., 2004).

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