BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348-0521, ISSN (E): 2454-4728
Vol. 4, Issue 4, Apr 2016, 147-154

TWO VARIANTS OF WDR36 GENE IN PRIMARY OPEN ANGLE GLAUCOMA

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ABSTRACT

Background

Glaucoma is the second leading cause of blindness affecting 67 million people worldwide. Primary open angle glaucoma (POAG) is the most common form of glaucoma. High intraocular pressure and a positive family history for glaucoma are commonly associated risk factors.

Purpose

To study the association of WDR36 gene polymorphisms (rs10038177, rs1971050) with primary open angle glaucoma (POAG) in Iraqi population and to detect the impact of these polymorphism on intra ocular pressure and cup-disk ratio.

Methods

A case–control study was conducted to find the association of WDR36 gene polymorphisms (rs10038177, rs1971050) 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 (AluI) 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.

Results

The results shown that homozygous (CC) significantly (OR= 3.57 CI95 %(1.49-8.57), P= 0.004) increased the risk of POAG by three fold with respect to those of the wild (TT) after adjustment for age and sex and heterozygous (TC) genotypes significantly (OR=2.04 (1.24-3.36) P= 0.005) raised the risk of POAG by two folds. The frequency of the C allele of rs10038177 (T/C) polymorphism was significantly higher (0.005) in POAG (33.3%) compared to controls (19.3%). While the results of genotype frequency of WDR36 gene polymorphism (rs1971050) shown that homozygous (CC) and heterozygous (TC) genotypes have no significant association with the risk of POAG disease (OR=1.28,CI 95% 0.67 -2.46, P= 0.45) and (OR=3.48,CI 95% 0.68 -17.78, P= 0.13) respectively.

Conclusions

The WDR36 gene polymorphism (rs10038177) is involved in the pathogenesis of POAG.

KEYWORDS: Glaucoma, WDR36 Gene Polymorphism, Primary Open Angle Glaucoma

INTRODUCTION

Glaucoma is a group of ocular disorders that varies clinically and genetically with different causes and associated with characteristic optic neuropathy including an excavation of the optic disc and progressive alteration of the visual field defect (Wiggs *et al.*, 2011; Melki *et al*., 2004). It is the second leading cause of blindness affecting 67 million people worldwide. (Balasubbu *et al*, 2012)

Primary open angle glaucoma (POAG) is the most common form of glaucoma. High intraocular pressure (IOP) above 21 mmHg and a positive family history for glaucoma are commonly associated risk factors. Genetically, most POAG cases follow a complex non-Mendelian pattern of inheritance, which manifests clinically in adulthood (>40 years) (Budde *et al*., 2000). To date, three genes, namely MYOC, OPTN, and WDR36 have been reportedly linked to POAG (Pasutto *et al*., 2008)

WDR36 is the POAG gene at the GLC1G locus and is composed of 23 exons that encode a 951 amino acid protein with multiple G beta winged domain 40 (WD40) repeats. (Monemi *et al*., 2005) The role of WDR36 in glaucoma remains unclear. It has been suggested that WDR36 may participate in T-cell activation (Mao *et al*., 2004). T-cell responses may be involved in optic nerve degeneration in glaucoma. These findings indicate that WDR36 may contribute to glaucoma by modifying optic nerve degeneration. (Bakalash *et al*., 2005)

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±8.8 years ; 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.)

Genotypic was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for WDR36 gene (rs10038177, rs1971050) using thermocycler ((Biometra, Germany). The primer sequences were obtained from Mookherjee *et al*. (2011)

Table 1: The Sequence and Annealing Temperature of Primers Used

Gene/SNP	Primer	Primer Sequences	Annealing Temperature
WDR36 rs1971050	Forward Reverse	5′GAGGTGAAGAGCAATTGGGTTTCTC- 3′ 5′-GCAGTGTCAGGAAAGACACTGTACC- 3′	60 °C
WDR36 rs10038177	Forward Reverse	5′-GCCTCTCATTTATTTTATTTCTCAAGG-3′ 5′-CCTCTGATACAGGGGACCAACTG-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, and 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 WDR36 gene polymorphisms revealed in Table 2

Table 2: PCR Reaction Program Protocol for WDR36 Gene Rs1971050 and Rs10038177 Polymorphisms (Mookherjee *Et Al*, 2011)

Type of Cycle	Temperature	Time	No. of Cycles
Initial Denaturation	95 °C	4 min	1cycle
Denaturation	95 °C	30s	35 cycles
Annealing	50–62 °C	30s	
Extension	72 °C	30s	
Final Extension	72 °C	4 min	1cycle

Amplification products of WDR36 gene polymorphisms (rs10038177, rs1971050) were 458 bp and 238 bp respectively. The products were digested with 10 u of restriction enzyme AIuI (Promega) and ran on 3% 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 3.

Table 3: 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 (AluI) for WDR36 gene (rs10038177) included 458 bp band for wild (TT) genotype, three bands 458,338,120 bp for the heterozygous genotype (TC) and two bands 338,120 bp for homozygous genotype (CC) as shown in Figure 1.

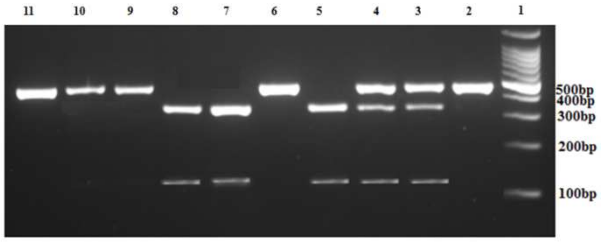

Figure 1: Genotyping Result for WDR36 Gene Rs10038177, Marker Lane 1, TT Genotype 458bp Lanes 1, 5, 9, 10, 11. TC Genotyping 458,338,120 Bp Lanes 3, 4. CC Genotyping 338,120 Lanes 5, 7, 8

Results of digestion with restriction enzyme (AluI) for WDR36 gene (rs1971050) included 238 bp band for wild (TT) genotype, three bands 238, 203, 35 bp for the heterozygous genotype (TC) and two bands 203,35 bp for homozygous genotype (CC) as shown in Figure 2.

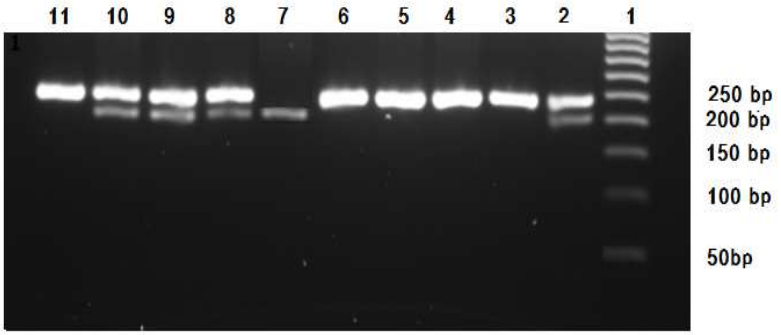

Figure 2: RFLP Pattern of WDR36 Gene Polymorphism (Rs1971050) On 3% Agarose Gel Electrophoresis. Lane 1: DNA Marker, Lane 3, 4, 5, 6 and 11: TT Genotype 238 Bp, Lane 2, 8, 9 And 10: TC Genotype 238, 203 & 35 Bp, Lane 7: CC Genotype 203 & 35 Bp.

Genotype frequencies of (rs10038177, rs1971050) were consistent with Hardy- Weinberg equilibrium in both POAG patients (P= 0.221) and control individuals (P= 0.210).

The results of genotype frequency shown that homozygous (CC) and heterozygous (TC) genotypes of WDR36 gene (rs10038177) significantly associated with POAG and the frequency of C allele was higher in POAG patients Table 4.

Table 4: Genotype and Minor Allele Frequency of WDR36 Gene (Rs10038177) Polymorphism and Association of This Variant with POAG in Study Individuals

	Control N=150	POAG N=150	Unadjusted OR(95% CI)	P value	Adjusted OR(95%CI)	P value
TT(Reference)	100	70				
TC	42	60	2.04 (1.24-3.36)	0.005	2.12(1.28-3.52)	0.004
CC	8	20	3.57 (1.49-8.57)	0.004	3.50(1.44-8.52)	0.006
Frequency of C allele	58 (19.3%)	100 (33.3%)	1.714 (1.18-2.50)	0.005		

While the results of genotype frequency of WDR36 gene polymorphism (rs1971050) shown that homozygous (CC) and heterozygous (TC) genotypes have no significant association with the risk of POAG disease (OR=1.28, CI 95% 0.67 -2.46, P= 0.45) and (OR=3.48,CI 95% 0.68 -17.78, P= 0.13) respectively (table 5)

Table 5: Genotype and Minor Allele Frequency of WDR36 Gene (Rs1971050) Polymorphism and Association of This Variant With POAG in Study Individuals

	Control N=150	POAG N=150	Unadjusted OR(95% CI)	P value	Adjusted OR(95%CI)	P value
TT (Reference)	128	120				
TC	20	24	1.280(0.67-2.44)	0.452	1.283(0.67-2.46)	0.453
CC	2	6	3.200(0.63-16.16)	0.159	3.478(0.68-17.78)	0.134
Frequency of C allele	24(8%)	36(12%)	1.568(0.91-2.70)	0.105		

The results of current study of WDR36 gene polymorphisms (rs10038177, rs1971050) showed that no significant differences in clinical characteristics intra ocular pressure (IOP) and cup-disk ratio (C/D ratio) between wild genotype

(GG), heterozygous genotype (GA) and homozygous genotype in primary open angle glaucoma patients (table 6 and 7 respectively).

Table 6: Genotypes Correlation of WDR36 Gene Polymorphism (Rs10038177) With Clinical Characteristics in POAG Patients Group

Clinical characteristics	**TT** (80)	**TC** (50)	**CC** (20)	**P value**
IOP mmHg	21.66±11.82	22.64±10.10	24.85±12.59	0.528
C/D ratio	0.58±0.141	0.60±0.140	0.67±0.170	0.065

Table 7: Genotypes Correlation of WDR36 Gene Polymorphism (Rs1971050) With Clinical Characteristics in POAG Patients Group

Clinical characteristics	**TT (120)**	**TC (24)**	**CC (6)**	**P value**
IOP mmHg	22.22±10.90	22.00±12.55	28.00±15.86	0.471
C/D ratio	0.60±0.150	0.58±0.125	0.68±0.156	0.328

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)

The silent onset of the disease has motivated the researchers to find new tests and markers for identifying individuals at risk before significant visual loss has developed. These tests include genetic screening tests. (Golubnitschaja and Flammer, 2007) Screening of the genes for the detection of mutation can give an idea about role of the genes in the development of particular disease and to determine and compare the difference in phenotype among patients having different polymorphism which provides batter view of molecular pathology of the disease. (Rose *et al*., 2011)

WDR36 is a novel POAG gene at the GLC1G locus. Mutations of WDR36 gene in patients with POAG vary in different ethnic groups with 3.7% in Chinese, 3.2% in Caucasian population from USA, 3.7%in Germany and 0.7% in Japanese population (Fan *et al*, 2009)

In current study the genotype frequencies of WDR36 gene (rs10038177, rs1971050) followed Hardy–Weinberg equilibrium in control groups. This result previously observed by (Jia *et al*., 2009; Mookherjee *et al*., 201; Fan *et al*., 2009). Results of allele and genotype frequencies of WDR36 gene polymorphism (rs10038177) demonstrated homozygous genotype (CC) carriers have two folds risk of development of primary open angle glaucoma (POAG) when compared with those of the reference type (TT) after adjustment for age, sex while the risk of heterozygous (TC) genotype carriers was more than three folds. Dominant and recessive models were demonstrated to raise the risk of POAG by more than two folds.

There are conflicting reports regarding the association of (rs10038177) with POAG. In USA Hauser *et al*.(2006), China Fan *et al*.(2009), India Mookherjee *et al*.(2011) revealed that this SNP was found to be associated with POAG. However, other studies did not reveal any association between this SNP and POAG in Caucasian populations Pasutto *et al*.(2008) and Weisschuh *et al*. (2007). Allele and genotype frequencies result of WDR36 gene polymorphism (rs1971050)

showed no association between this SNP and POAG in Iraqi population and the current findings are consistent with result of (Mookherjee *et al*., 2011) in East India population.

The results of Hauser *et al* in 2006 revealed that abnormalities in WDR36 alone are not sufficient to cause POAG and WDR36 may affect the disease severity of patients with POAG that is caused by mutations in other gene like MYOC. These results suggest that while defects in the WDR36 gene may contribute to the glaucomatous disease process,WDR36 most likely acts as a glaucoma modifier gene.

CONCLUSIONS

The WDR36 gene polymorphism (rs10038177) is involved in the pathogenesis of POAG. Carriers of the homozygous genotype (CC) have three folds risk to develop POAG while those of the TC genotype have two folds risk to develop the disease. WDR36 gene polymorphism, the rs10038177 is not involved in directing changes of the disease related phenotypes, including IOP and C/D ratio.

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