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ANALYSIS OF CDKN2B-AS1 SNP RS2157719 IN SAUDI PATIENTS WITH PRIMARY OPEN ANGLE GLAUCOMA

Khaled K. Abu-Amero*

Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Corresponding Author:- **Khaled K. Abu-Amero**
E-mail: abuamero@gmail.com

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ABSTRACT
Aims to investigate whether SNP rs2157719 in the CDKN2B-AS1 gene is associated with POAG or any of its clinical indices in a Saudi cohort. Eight-five unrelated POAG cases and 95 controls of Saudi origin were genotyped utilizing Taq-Man® assay. The association between genotypes and various clinical indices important for POAG was investigated. Comparison of the genotype and allelic frequency among cases and controls were not significantly different. The genotype frequencies did not deviate significantly from the HWE ($p>0.05$). There was no statistically significant difference between patients and controls for both heterozygous (A/G) and homozygous (G/G) genotypes, p values were 0.988 and 0.281 respectively. Similarly, the mutated allele (G) had similar frequencies in both groups. However, Statistically significant differences were observed between homozygous mutant genotype (G/G) and family history of glaucoma ($p= 0.018$), smoking ($p= 0.033$) and awareness of glaucoma ($p= 0.039$). SNP rs2157719 in the CDKN2B-AS1 is important risk factor for certain clinical indices associated with POAG.

INTRODUCTION

Primary open angle glaucoma (POAG) is the most prevalent form of Glaucoma and is the leading cause of blindness worldwide [1]. Although various physiological factors play a crucial role in the disease progression of POAG; it is a genetically complex disease with well-established associations with various known mutations and single nucleotide polymorphisms (SNPs) [2].

Transforming growth factor (TGF)-β may have possible involvement as a pathogenic factor causing structural damages in POAG patients as elevated level of TGF-β has been positively correlated with glaucoma [3, 4]. TGF-β highly induces the expression of tumor suppressor Cyclin-Dependent Kinase Inhibitor genes including CDKN2A, CDKN2B and CDKN2B antisense RNA 1 (CDKN2BAS-1) which is a long non-coding RNA located within the p15/CDKN2B-p16/CDKN2A-p14/ARF gene cluster in the antisense direction [5]. The actual function of CDKN2BAS-1 is largely unknown and needs further elucidation but it has been identified as an important genetic susceptibility locus associated with various diseases. [6, 7]. Mutations in CDKN2B-AS1 locus of 9p21 have also shown positive association with glaucoma in various studies making it a strong candidate gene for disease screening [8, 9].

Genome wide association studies (GWAS) conducted for POAG was done in GLAUGEN (Glaucoma Genes and Environment) and the NEIGHBOR (NEI Glaucoma Human genetics collaboration) populations along with a meta-analysis which found CDKN2B-AS1 SNP rs2157719 to be significantly associated with POAG [4]. More recently Li et al. performed a GWAS on 3504 POAG cases and 9746 controls replicating the positive significant findings in 9173 POAG cases and 26,780 controls across 18 different collections of Asian, African, and European populations providing strong evidence and confirming an association at the CDKN2B-AS1 locus (rs2157719, OR = 0.71, $p = 2.81 \times 10{-}33$) [10]. These studies which has been comprehensively done in various cohorts has established CDKN2B-AS1 locus of 9p21 as an important risk factor in the development of POAG

highlighting its possible association with disease pathogenesis [11].

The present study investigate the possible association of a CDKN2B-AS1 SNP rs2157719 with primary open angle glaucoma (POAG) and important clinical indices used to assess disease severity in a Saudi cohort.

MATERIALS AND METHODS

Study population

The study adhered to the tenets of the Declaration of Helsinki, and all participants signed an informed consent. The study was approved by the College of Medicine Ethical Committee (approval number # 08-657) at King Saud University. Study population was recruited at King Abdulaziz University Hospital in Riyadh, Saudi Arabia. Saudi POAG cases (n= 85) satisfied the following clinical criteria: i) thinning or notching of disc or retinal nerve fiber layer defect; ii) abnormalities in visual field (e.g. arcuate scotoma, nasal step, paracentral scotoma, generalized depression) in the absence of other causes or explanation; iii) age greater than 40 y and iv) open anterior chamber angles bilaterally on gonioscopy. The exclusion criteria included evidence of secondary glaucoma, e.g. pigmentary glaucoma, uveitic, pseudoexfoliation, etc. and history of long-term steroid use or ocular trauma. An ethically-matched healthy control group (n= 95) included individuals of age >20 y, normal IOP (< 21 mmHg without any medication), open angles on gonioscopy, normal optic disc and free from glaucoma on examination.

Genotyping

Genotyping rs2157719 of the CDKN2B-AS1 gene: rs2157719 (A>G) polymorphism in the intronic region of CDKN2B-AS1 gene (NC_000009.12) was genotyped using the TaqMan® SNP Genotyping assay ID: C_2618013_10 (Applied Biosystems Inc., Foster City, CA, USA) on ABI 7500 real-time PCR system (Applied Biosystems). Each PCR reaction was performed in a 96-well plate in a total volume of 25 µL consisting of 1X TaqMan® Genotyping Master Mix (Applied Biosystems), 1X SNP Genotyping Assay Mix, 20 ng DNA, and two no template (negative) controls. The cycling conditions for the real-time PCR on ABI 7500 included incubation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15s and annealing/ extension at 60°C for 1 min. The VIC® and 6-carboxy-fluorescein (FAM) fluorescence levels of the PCR products were measured at 60°C for 1 min. Automated 2-color allele discrimination software on ABI 7500 was used to identify both the genotypes of *CDKN2B-AS1* gene on a two-dimensional graph.

Statistical analysis

Data management, coding and storage were done using Microsoft Excel 2010® software (Microsoft Corporation; Redmond, WA, USA). Data were analyzed using SPSS® version 20.0 (IBM Inc., Chicago, Illinois, USA) and StatsDirect® statistical software, version 2.7.2 (StatsDirect Ltd., Cheshire, UK).

The categorical variables were presented as frequencies and percentages while continuous variables were presented as mean (± Standard Deviation, SD). Odds ratio was calculated and Chi2 test was used to detect any association between different characteristics and the genetic profiles (Fisher Exact test whenever indicated). Mann-Whitney U test was used to investigate whether there was any significant difference between the normal homozygous (A/A) and the mutated heterozygous (A/G) and homozygous (G/G) genotypes. The confidence interval level was set to 95% and a p value below 0.05 was considered statistically significant.

RESULTS:

Demographic characteristics

Both the recruited study groups showed an age interval of 20 years and above. As shown in Table-1, the mean age (SD) in the POAG cases was 60.9 and the ages ranged from (28-97 years), whereas for the controls, the mean age was 56.4 and the age range (20-70) and the difference was not statistically significant (p= 0.068). In cases, 53 (62.4%) were male and 32 (37.6%) were female whereas in controls, 70 (73.7%) were male and 25 (26.3%) were female. Both the groups were found to be similar in terms of age and gender (p= 0.068 and 0.321, respectively). Analysis of controls with cases in terms of clinical co-morbidity with systemic diseases showed that both the groups were similar in terms of diabetes mellitus (p= 0.097), smoking (p= 0.265), hypertension (p= 0.589), coronary artery disease (p= 0.361) and hypercholesterolemia (p= 0.076). However, family history of glaucoma (p= 0.014), and awareness to having glaucoma (p < 0.0001) were found to be statistically significant.

Genotype and allelic distribution

Table 2 shows the genotype and allelic frequency observed among cases and controls. The genotype frequencies did not deviate significantly from the HWE (p>0.05). There was no statically significant difference between patients and controls for both heterozygous (A/G) and homozygous (G/G) genotypes, p values were 0.988 and 0.281 respectively. Similarly, the mutated allele (G) had similar frequencies in both groups.

Genotype effect on systemic co-morbidities and specific glaucoma indices

Table 3 shows the comparison of demographic characteristics, systemic co-morbidities, and specific clinical indices among cases according to genotypes. Statistically significant differences were observed for family history of glaucoma (p= 0.018), smoking (p= 0.033) and awareness of glaucoma (p= 0.039).

Table 1. Demographic and glaucoma clinical indices at presentation

Characteristic	Cases (n= 85)	Controls (n= 95)	P value
Age [Mean (SD), Range].	60.9 (12.7), [28 – 97]	56.4 (15.8), [20 – 70]	0.068
Gender:			
Male [No. (%)]	53 (62.4)	70 (73.7)	0.321
Female [No. (%)]	32 (37.6)	25 (26.3)	
Family History of Glaucoma [No. (%)]	11 (12.9)	3 (3.2)	**0.014**
Diabetes Mellitus [No. (%)]	49 (57.6)	43 (45.3)	0.097
Smoking [No. (%)]	35 (41.2)	47 (49.5)	0.265
Hypertension [No. (%)]	44 (51.8)	53 (55.8)	0.589
Coronary Artery Disease [No. (%)]	6 (7.1)	3 (3.2)	0.361
Hypercholesterolemia [No. (%)]	14 (16.5)	8 (8.4)	0.076
Awareness to Glaucoma [No. (%)]	15 (17.6)	0 (0.0)	**<0.0001**

Table 2. Genetic profile by different allele combinations

Profile	Cases (n= 85) No. (%)	Control (n= 95) No. (%)	OR	95% CI	P value*
A/A	56 (65.9)	60 (63.2)			
A/G	27 (31.8)	29 (30.5)	0.9	[0.500 – 1.983]	0.988
G/G	2 (2.4)	6 (6.3)	0.4	[0.342 – 2.120]	0.281
A	139 (81.8)	149 (78.4)			
G	31 (18.2)	41 (21.6)	1.2	[0.733 – 2.077]	0.429

*p value is calculated considering the normal allele (T/T) as the reference group.

Table 3. Association of clinical glaucoma indices with different genotypes.

Characteristic	A/A (n= 56)	A/G (n= 27)	P value*	G/G (n= 2)	P value*
Age [Mean (SD), Range].	62.3 (11.9)	58.2 (14.0)	0.122	55.5 (16.3)	0.103
Gender:					
Male [No. (%)]	36 (64.3)	15 (55.6)	0.443	2 (100)	0.774
Female [No. (%)]	20 (35.7)	12 (44.4)		0 (0)	
Family History of Glaucoma [No. (%)]	7 (8.2)	2 (2.1)	0.747	2 (100)	0.018
Diabetes Mellitus [No. (%)]	34 (60.7)	13 (48.1)	0.279	2 (100)	0.701
Smoking [No. (%)]	23 (41.1)	11 (40.7)	0.971	1 (50)	0.033
Hypertension [No. (%)]	29 (51.8)	13 (48.1)	0.765	2 (100)	0.534
Coronary Artery Disease [No. (%)]	3 (3.5)	2 (2.1)	0.713	1 (50)	0.304
Hypercholesterolemia [No. (%)]	4 (4.7)	2 (2.1)	0.965	1 (50)	0.401
Awareness to Glaucoma [No. (%)]	9 (10.6)	4 (14.8)	0.883	2 (100)	0.039

*p value is calculated considering the normal allele (T/T) as the reference group.

DISCUSSION

Genome wide association studies (GWAS) searching for possible genetic risk factors for POAG had identified SNP rs2157719, at the CDKN2B-AS1 locus, as a strong risk factor for POAG. Further studies had confirmed the initial findings and confirmed this SNP as a POAG-risk factor in Asian, African, and European populations. As the exact function of CDKN2BAS-1 is largely unknown, this gene been identified as a risk factor for various diseases.

In our study, when we compared the genotype frequencies between patients and controls, we did not detect any significant difference. Similarly, the mutant allele was similar distributed in patients and controls. so, our study is largely negative.

When we studies the association of various clinical indices for POAG with different genotypes, the results were surprising. Homozygous mutant genotype (C/C) was associated with family history of glaucoma and awareness of glaucoma. More surprisingly, it was also associated with smoking status. Smoking was found to cause cell growth inhibition and disruption of cell cycle at multiple phases [12]. Cyclin-Dependent Kinase Inhibitor genes including CDKN2B is very important gene for cycle cycle and certainly smoking is going to disturb this protein and affect cell cycle. How that is link to glaucoma in particular is not known, but it will be interesting to study further.

CONCLUSION

In conclusion, our study had shown an association with various clinical indices important for glaucoma.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures performed in human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

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