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SNP rs2157719 in the CDKN2B-AS1 gene gene may influence cup-to-disc ratio in patients with primary open angle glaucoma

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Abstract

Background: Several studies have provided strong evidence that gene variants at the cyclin-dependent kinase inhibitor 2B antisense non-coding RNA (CDKN2B-AS1) locus of 9p21 is an important risk factor in the development of primary open angle glaucoma (POAG) making it a strong candidate for risk factor screening.

Objectives: The present study investigated the possible association of SNP rs2157719 in CDKN2B-AS1 with POAG and specific glaucoma indices used to assess disease severity in a Saudi cohort.

Materials and methods: Genotyping of SNP rs2157719 was performed in 85 unrelated POAG cases and 95 normal controls of Saudi origin using Taq-Man® real-time PCR assay and its association with the POAG and other clinical indices was evaluated.

Results: The genotype frequencies did not deviate significantly from the Hardy-Weinberg equilibrium (p>0.05). The minor 'G' allele frequency was observed to be 0.18 in cases and 0.21 in controls. Both the genotype and allele frequencies did not vary significantly between cases and controls. However, the homozygous mutant genotype (G/G) was found to be significantly associated with family history of glaucoma (p= 0.018), smoking (p= 0.033) and awareness of glaucoma (p= 0.039). In addition, carriers of the heterozygous (A/G) genotype had significantly higher cup/disc ratio (p= 0.028) as compared to the wild-type.

Conclusion: SNP rs2157719 in the CDKN2B-AS1 is not associated with POAG but may affect cup-to-disc ratio and thereby modulate optic nerve pathology.

Introduction

Primary open angle glaucoma (POAG) is a genetically complex disease exhibiting well-established associations with various known mutations and single nucleotide polymorphisms (SNPs) [1]. Genetic variants at the chromosome 9p21 locus has been shown to be strongly associated with POAG [2]. The associated locus includes cyclin-dependent kinase inhibitor-2A (*CDKN2A*), -2B (*CDKN2B*) and -2B antisense RNA 1 (*CDKN2B-AS1*) genes. *CDKN2BAS-1*, is a long non-coding RNA located within the *p15/CDKN2B-p16/CDKN2A-p14/ARF* gene cluster transcribed in the antisense direction [3]. Although the exact biological function of this non-coding gene is largely unknown. However, it has been shown to regulate transcription of *CDKN2A/2B* through epigenetic mechanisms and implicated in several other diseases [4-6]. In addition, the expression of *CDKN2B/AS1* is dramatically induced by transforming growth factor (TGF)-β which is known to play a critical role in POAG pathogenesis [7,8].

Several genome-wide association studies (GWASs) and candidate gene investigation among different ethnic groups have provided strong evidence that gene variants at the *CDKN2B-AS1* locus of 9p21 is an important risk factor in the development of POAG [9-14] highlighting its association with disease pathogenesis and making it a strong candidate for risk factor screening. A recent population-based GWAS performed by Li et al. across 18 collections of Asian, African and European descent provided strong evidence of association of SNP rs2157719 at the *CDKN2B-AS1* locus (odds ratio, OR = 0.71, P = 2.81 x 10^{-33}) [13]. The genetic contribution of this variants at the *CDKN2B-AS1* locus among Saudi POAG patients is not known. The present study investigated the possible association of SNP rs2157719 in *CDKN2B-AS1* with POAG and specific glaucoma indices used to assess disease severity in a Saudi cohort.

Material and methods

Study population

This case-control cross-sectional study was conducted between October 2015 and February 2016 at King Abdulaziz University Hospital,

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King Saud University, Riyadh, Saudi Arabia. The study adhered to the tenets of the Declaration of Helsinki for research involving humans and was approved by the Ethical Committee of College of Medicine, King Saud University (approval number # 08-657). All the participants signed an informed consent. POAG cases (n = 85) satisfied the following clinical criteria: (1) appearance of the disc or retinal nerve fibre layer showing, e.g. thinning or notching of disc rim, progressive changes, nerve fibre layer defect, (2) presence of characteristic abnormalities in the visual field (e.g. arcuate scotoma, nasal step, paracentral scotoma, generalized depression) in the absence of other causes or explanation, (4) ≥40 years of age at the time of recruitment, and (4) open anterior chamber angles bilaterally on gonioscopy. Secondary glaucoma, e.g. pigmentary dispersion syndrome, pseudoexfoliation, uveitis, history of steroid use, ocular trauma or non-participation were excluded. Inclusion criteria for ethnically matched healthy control subjects (n = 95) included: age ≥40 years, normal IOP (<21 mmHg), open angles and normal optic disc on examination.

DNA extraction

DNA was extracted from peripheral blood (7 mL) collected in EDTA tubes using the illustra blood genomicPrep Mini Spin kit (GE Healthcare, Buckinghamshire, UK). DNA was quantified using a NanoDrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and stored at –20°C in aliquots until further use.

Genotyping

Genotyping of intronic polymorphism, rs2157719 (A>G), of the *CDKN2B-AS1* gene (NC_000009.12) was performed 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 under cycling conditions recommended by the manufacturer [15]. Genotypes of *CDKN2B-AS1* rs2157719 SNP were identified using the automated 2-color allele discrimination software on ABI 7500 on a two-dimensional graph.

Statistical analysis

Data management, coding and storage were done using Microsoft Excel 2010® software (Microsoft Corporation; Redmond, WA, USA). The continuous variables were presented as mean (± Standard Deviation, SD) and analyzed by Student's *t*-test. Categorical variables were presented as frequencies and percentages. Hardy-Weinberg Equilibrium (HWE) deviation was tested by Pearson's Chi^2 test. Odds ratio was calculated and Chi^2 test was used to detect any association between different characteristics and the genetic profiles (Fisher Exact test where applicable). Yates' correction was applied for frequency less than 5. Mann-Whitney-U test was used to compare means across differnet allele groups to investigate association between genotypes and clinical variables. The confidence interval level was set to 95% and a *p* value below 0.05 was considered statistically significant. 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).

Results

Demographic and clinical characteristics

The POAG cases showed a mean age (±SD) of 60.9 (12.7) years as compared to 56.4 (15.8) in controls which was not significant (P = 0.068). Both the groups were found to be similar in terms of gender distribution (P = 0.321) with a pre-dominance of male subjects. As expected, family history of glaucoma (P = 0.014), and awareness to having glaucoma ($p < 0.0001$) were found to be statistically significantly different. However, smoking and presence of other systemic diseases such as diabetes, hypertension and hypercholesterolemia did not increase the risk of POAG (Table 1).

Genotype and Allele frequency distribution

Table 2 shows the genotype and allelic frequency observed among POAG cases and normal controls. The genotype frequencies did not deviate significantly from the HWE ($P > 0.05$). The overall genotype distribution as tested by 2x3 Fisher Exact probability test was found to be non-significant (P = 0.468). Besides, as compared to wild-type genotype (A/A), the heterozygous (A/G), the mutant homozygous (G/G) and the AG+GG (dominant effect) genotypes did not differ significantly between cases and controls. Similarly, the allele frequencies did not vary significantly between cases and controls. The minor 'G" allele frequency was found to be 0.18 and 0.21 among cases and controls, respectively.

Effect of Genotypes 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. Except for family history of glaucoma (P = 0.018), smoking (P = 0.033) and awareness of glaucoma (P = 0.039) no other clinical parameter analyzed was found to be associated only with mutant homozygous (G/G) genotypes. In addition, comparison of glaucoma specific indices such as IOP, cup/disc ratio and number of anti-glaucoma medications did not differ significantly, except that the patients carrying the heterozygous (A/G) genotype had significantly higher cup/disc ratio (P = 0.028) as compared to the wild-type (A/A) genotypes.

Discussion

Since the initial identification of association between *CDKN2B-AS1* locus with POAG in the Australian cohort [9], several other GWAS and candidate gene studies have replicated this association in the US Caucasians [10], African-Caribbean [16], Japanese [11], Asian, African and European population [12,13]. In this study, we report a lack of association between SNP rs215779 in *CDKN2B-AS1* and POAG in a Saudi cohort.

Table 1. Demographic and clinical co-morbidity in POAG patients and controls at presentation.

Characteristic	Cases (n= 85) No. (%)	Controls (n= 95) No. (%)	P value
Age in years, mean (±SD)	60.9 (12.7)	56.4 (15.8)	0.068
Male	53 (62.4)	70 (73.7)	0.321
Female	32 (37.6)	25 (26.3)	
Family history of glaucoma	11 (12.9)	3 (3.2)	**0.014**
Diabetes mellitus	49 (57.6)	43 (45.3)	0.097
Smoking	35 (41.2)	47 (49.5)	0.265
Hypertension	44 (51.8)	53 (55.8)	0.589
Coronary artery disease	6 (7.1)	3 (3.2)	0.361
Hypercholesterolemia	14 (16.5)	8 (8.4)	0.076
Awareness to glaucoma	15 (17.6)	0 (0.0)	**<0.001**

Significant P values are indicated in bold.

Table 2. Distribution of genotype and allele frequency in cases and controls.

Genotype/Allele	Cases (n= 85) No. (%)	Control (n= 95) No. (%)	Odds ratio	95% Confidence interval	P value*
A/A	56 (65.9)	60 (63.2)	1	-	-
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/G+G/G	29 (34.1)	35 (36.8)	0.88	0.48 – 1.63	0.88
A	139 (81.8)	149 (78.4)	1	-	-
G	31 (18.2)	41 (21.6)	1.2	0.733 – 2.07	0.429

**P value is calculated considering the normal allele (A/A) as the reference group.*

Table 3. Association of genotypes with demographic, systemic co-morbidities, and glaucoma specific indices among cases**.**

Characteristic	A/A (n= 56) No. (%)	A/G (n= 27) No. (%)	P value*	G/G (n= 2) No. (%)	P value*
Age in years, mean (±SD)	62.3 (11.9)	58.2 (14.0)	0.122	55.5 (16.3)	0.103
Male	36 (64.3)	15 (55.6)	0.443	2 (100)	0.774
Female	20 (35.7)	12 (44.4)	-	0 (0)	-
Family history of glaucoma	7 (8.2)	2 (2.1)	0.747	2 (100)	**0.018**
Diabetes mellitus	34 (60.7)	13 (48.1)	0.279	2 (100)	0.701
Smoking	23 (41.1)	11 (40.7)	0.971	1 (50)	**0.033**
Hypertension	29 (51.8)	13 (48.1)	0.765	2 (100)	0.534
Coronary artery disease	3 (3.5)	2 (2.1)	0.713	1 (50)	0.304
Hypercholesterolemia	9 (16.0)	4 (14.8)	0.882	1 (50)	0.768
Awareness to glaucoma	9 (16.0)	4 (14.8)	0.882	2 (100)	**0.039**
Intraocular pressure in mmHg, mean (±SD)	35.4 (7.6)	34.9 (6.7)	0.884	37.5 (9.1)	0.654
Cup/disc ratio, mean (±SD)	0.7 (0.2)	0.8 (0.2)	**0.028**	0.6 (0.1)	0.507
Number of anti-glaucoma medications, mean (±SD)	2.9 (90.7)	2.9 (0.5)	0.641	3.0 (1.4)	0.872

**P value is calculated considering the normal allele (A/A) as the reference group. Significant P values are indicated in bold.*

SNP rs215779 (G allele) has been reported to be associated with reduced risk of POAG in the Asian, African and Caucasian population [10,13]. The 'G' allele has a minor allele frequency (MAF) of 0.44 in Caucasians and almost absent in African population (dbSNP database - http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=215779). The MAF observed in our Saudi POAG subjects was 0.18 which is lower than the Caucasians but higher than the Africans. Perhaps, a very low frequency of this SNP may explain, atleast in part, the high risk of POAG in these ethnic groups. Both the genotype and allele frequency of rs215779 in the *CDKN2B-AS1* were not found to be a significantly associated with the risk of POAG in our Saudi cohort. However, it should be noted that consistent with the previous reports, our genotype analysis did indicate of a protective trend associated with the 'G' allele. However, this effect was found to be non-significant suggesting that due to small sample size the study lacks the power to detect this trend effectively.

Cup-to-disc ratio is an important clinical parameter for the diagnosis and monitoring of POAG disease process. Variants of *CDKN2B-AS1* are reported to be associated with cup-to-disc ratio in the general population [17,18] and POAG [10,19]. Interestingly, the variant (G) allele of rs215779 has been reported to be associated with smaller cup/disc ratio. In contrast, the patients carrying the heterozygous (A/G) genotype showed significant association with larger cup/disc ratio (p= 0.028) as compared to the wild-type (A/A) genotypes. This effect was absent in the homozygous mutant (G/G) POAG patients. The most likely reason for this discrepancy could be the reduced number of samples present in this group suggesting that a larger sample size with high power of study is required to detect any significant relative risk of this association.

Conclusion

Our study shows that SNP rs2157719 in the *CDKN2B-AS1* is not associated with POAG in Saudi cohort, but may affect cup-to-disc ratio. However, since the study is limited by the fact that it is performed in a specific ethnicity and evaluated in a relatively small number of patients this aspect would need further investigation in a much larger cohort.

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Availability of data and materials

The data supporting the conclusions of this article are all presented within the article.

Authors' contributions

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Competing interests

None to declare. Authors have no conflict of interests.

Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki and had received approval from the Institutional Review Board and Research Ethics Committee. Written, informed consent was obtained from all participants prior to their inclusion in this study.

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