

Lack of correlation between S1 RNA binding domain 1 SNP rs3213787/rs11884064 and normal-tension glaucoma in a population from the Republic of Korea

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Abstract

Previous studies have reported the association of the S1 RNA binding domain 1 (SRBD1) gene with open-angle glaucoma in various ethnic populations. However, in those studies, the definition of the patients differed, as did the results. Therefore, the relevance of the SRBD1 gene to normal tension glaucoma (NTG) appears uncertain at present. Thus, we investigated the relationship between the SRBD1 gene and NTG in a Korean NTG cohort.

In total, 159 unrelated Korean patients with NTG and 103 Korean control subjects were recruited. Thus, a total of 262 participants were analyzed for SRBD1 (rs3213787 and rs11884064) gene polymorphisms.

The minor allele frequency of rs3213787 was found to be 0.13 and 0.19 in NTG cases and controls, respectively. The genetic association analysis of SNP rs3213787 revealed no significant difference in genotype distribution between NTG cases and controls in allelic (odds ratio [OR]=0.634, $P=.063$), dominant (OR=0.589, $P=.066$) or recessive models (OR=0.639, $P=.7716$). The minor allele frequency of rs11884064 was found to be 0.24 and 0.25 in NTG cases and controls, respectively. For rs11884064, no significant difference in genotype distribution was observed between NTG cases and controls in allelic (OR=0.938, $P=.755$), dominant (OR=0.927, $P=.798$) or recessive models (OR=0.920, $P=1.000$).

The current study suggested that SRBD1 gene polymorphisms (rs3213787 and rs11884064) may not be associated with genetic susceptibility to NTG in a Korean cohort.

Abbreviations: CI = confidence interval, GWAS = genome-wide association study, MAFs = minor allele frequencies, NTG = normal tension glaucoma, OAG = open angle glaucoma, OR = odds ratio, PCR = polymerase chain reaction, ROS = reactive oxygen species, SNP = single nucleotide polymorphism, SRBD1 = S1 RNA binding domain 1.

Keywords: Genotyping, normal tension glaucoma, S1 RNA binding domain 1, single nucleotide polymorphism, South Korea

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The research was conducted in accordance with the tenets of the Declaration of Helsinki. This study was approved by the Institutional Review Board of the Uijeongbu St. Mary's Hospital of Korea (UC16SISE0060). The study details were explained to all participants before obtaining informed consent for the genetic analyses; those who refused to participate were excluded.

All data generated or analyzed during this study are included in this published article.

All authors have read and approved the manuscript.

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1. Introduction

Glaucoma is one of the leading causes of irreversible blindness worldwide.^[1] The pathogenesis of glaucoma is genetically heterogeneous and largely polygenic in nature.^[2] Normal-tension glaucoma (NTG) is the most common subtype of open-angle glaucoma (OAG) in the Republic of Korea.^[3] However, there have been few studies of NTG-related genes in Koreans. It is therefore important to study NTG-related genes rather than genes related to other types of glaucoma in the Korean population.

Meguro et al performed a genome-wide association study (GWAS) of more than 500,000 single nucleotide polymorphisms (SNPs) and showed that the most significant SNP with respect to risk of NTG was rs3213787, where for carriers of the major allele (A) the risk was increased 2.80-fold. This SNP is located in intron 17 of the S1 RNA binding domain 1 (SRBD1) gene.^[4] However, there have been conflicting results regarding the rs3213787 SNP in other ethnic NTG groups,^[5,6] following an initial Japanese study.^[4] In another GWAS study including NTG patients, Gibson et al found that rs11884064 within SRBD1 was associated with OAG.^[5] In the present study, we evaluated the possible associations of rs3213787 and rs11884064 with NTG to determine whether these SNPs were risk factors for NTG in our Korean cohort.

2. Methods

2.1. Patients

Clinically diagnosed NTG patients and healthy control subjects were selected from the eye clinic at Uijeongbu St. Mary's Hospital. Complete ophthalmic examinations were performed on all participants. The study was explained in detail to the patients and controls before obtaining informed consent for genetic screening; those refusing to participate in the study were excluded. The research was conducted in accordance with the tenets of the Declaration of Helsinki. This study was approved by the Institutional Review Board of the Uijeongbu St. Mary's Hospital of Korea. (UC16SISE0060)

Glaucoma was defined by glaucomatous visual field defects measured by a Humphrey automated field analyzer (Zeiss/Humphrey Systems, Dublin, CA), as confirmed by at least 2 VF tests and the presence of a glaucomatous optic disc showing neuroretinal rim thinning and/or retinal nerve fiber layer defects. To exclude patients with other types of glaucoma, the NTG patients included in this study satisfied the following criteria: an open-angle on slit-lamp and gonioscopic examinations, and intraocular pressure \leq 21 mm Hg without medication using repeated measurements. (159 patients; 60 men and 99 women; age 61.14 ± 11.94 years) Angle closure glaucoma, secondary cases of pigmentary glaucoma, uveitic, pseudoexfoliation, and a history of steroid use or ocular trauma were exclusionary criteria. Individuals ineligible for control group inclusion were ruled out using ophthalmic examinations. The control subjects $>$ 45 years of age showed the following characteristics at the time of recruitment: normal intraocular pressure (\leq 21 mm Hg), normal optic disc on fundus examination, and no family history of glaucoma. (103 individuals; 44 men and 59 women; age, 68.78 ± 9.82 years)

2.2. Sample preparation and allelic discrimination

Genomic DNA was extracted from peripheral blood using a MG Blood Genomic DNA Extraction SV kit (MGmed, Seoul,

Republic of Korea), according to the manufacturer's instructions. We evaluated 2 SNPs: rs3213787 and rs11884064 of SRBD1. Genotyping for rs3213787 polymorphism was conducted using the TaqMan SNP Genotyping Assay (assay ID: C__2773863_1; Applied Biosystems, Foster City, CA). Genotyping for the rs11884064 polymorphism was conducted using the TaqMan SNP Genotyping Assay (assay ID: C__1713255_20). SNP genotyping reactions were performed using the QuantStudio real-time polymerase chain reaction (PCR) system (Applied Biosystems) under the PCR conditions described by the manufacturer. After the PCR amplification, allelic discrimination was performed using the same instruments (QuantStudio).

2.3. Statistical analysis

The frequencies and ORs of the alleles and genotypes of all polymorphic SNPs were calculated with 95% confidence intervals, and the Chi-squared test and Fisher exact test were used to compare the results between NTG patients and controls. Three genetic models (dominant, recessive, and allelic) were used to test the SNP associations. Hardy-Weinberg equilibrium in the controls was tested for both SNPs. Statistical analyses were performed using PLINK^[7] and IBM SPSS version 25. *P*-values less than .05 were considered statistically significant.

3. Results

To evaluate the candidate NTG-associated genetic markers, we selected 2 SNPs (rs3213787 and rs11884064) from among the SRBD1 SNPs for a replication study in Koreans. General information of the 2 SNPs is summarized in Table S1, <http://links.lww.com/MD/E336>. We performed genotyping for the 2 SNPs in a total of 262 participants (159 NTG patients and 103 controls). The genotype frequencies were in Hardy-Weinberg equilibrium in the patients with NTG and the control subjects (Table S2, <http://links.lww.com/MD/E336>). The genotype and allele frequencies of the SRBD1 (rs3213787 and rs11884064) gene polymorphisms in patients with NTG and the control subjects are shown in Table 1.

The minor allele frequency (MAF) of rs11884064 in controls (0.248) was similar to a previous report (0.23)^[4] and to that of east Asians from the 1000 Genome Project (0.283; *P* = .173); meanwhile, the MAF of rs3213787 (0.189) in our controls was significantly higher than that of the east Asians from the 1000 Genome Project (0.136; *P* = .033), as well as the MAFs reported in other studies.^[4,6,8,9] In association analyses, neither rs11884064 nor rs3213787 were significantly associated with the risk of NTG (Table 1). For rs3213787 allelic (OR = 0.634; 95% CI = 0.393–1.023; *P* = .063), dominant (OR = 0.589; 95% CI = 0.340–1.020; *P* = .066), and recessive models (OR = 0.639; 95% CI = 0.156–2.613; *P* = .716); rs11884064 allelic (OR = 0.938; CI = 0.623–1.412; *P* = .755), dominant (OR = 0.927; CI = 0.561–1.532; *P* = .798), and recessive models (OR = 0.920; CI = 0.339–2.500; *P* = 1.000).

4. Discussion

NTG is the most common subtype of glaucoma in the Republic of Korea, similar to Japan.^[3] We investigated whether rs3213787 and rs11884064 were risk factors for NTG in a Korean cohort. These markers have previously been reported as risk factors for OAG^[4,5,8,9] but have not been studied in Koreans. To the best of

Table 1**Association results of rs3213787 and rs11884064 in different models.**

SNP	Allele		Genotype (case/control)			MAF (case/control)	Allelic model		Dominant model		Recessive model	
	1	2*	11	12	22		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs3213787	A	G	122/68	33/31	4/4	0.15 (0.13/0.19)	0.634 (0.393–1.023)	.063	0.589 (0.340–1.020)	.066	0.639 (0.156–2.613)	.716
rs11884064	T	C	94/59	55/37	10/7	0.24 (0.24/0.25)	0.938 (0.623–1.412)	.755	0.927 (0.561–1.532)	.798	0.920 (0.339–2.500)	1.000

CI=confidence interval, MAF=minor allele frequency, OR=odds ratio, SNP=single-nucleotide polymorphism.

*Minor allele.

our knowledge, this is the first report of the association of these SNPs of SRBD1 with OAG in Koreans. We found that both the genotype and allele frequencies of these 2 SNPs were not significantly different between patients and controls.

The S1 RNA-binding domain is found in a large number of RNA-associated proteins, such as polynucleotide phosphorylase,^[10] the overexpression of which generates reactive oxygen species; in turn, reactive oxygen species are responsible for activating the NF- κ B pathway and its downstream genes, especially those associated with proinflammatory cytokines^[11] and the induction of apoptosis.^[12,13]

The first GWAS on NTG included 305 Japanese NTG patients and 355 controls, and reported a strong association of intron SNP rs3213787, in the SRBD1 gene on chromosome 2p21, with the disease.^[4] Although the involvement of the SRBD1 gene in NTG pathogenesis remains to be determined, it has been reported that SRBD1 transcripts are expressed in retinal ganglion cells.^[4] In that report, it was suggested that enhanced SRBD1 expression was caused by rs3213787, which could indirectly lead to inhibition of apoptosis and homeostasis dysfunction, in turn resulting in the progressive loss of retinal ganglion cells.^[4] However, a second GWAS performed in an independent cohort in Hampshire (UK) showed different results: the most important of several significant SNPs within SRBD1 was rs11884064, and not rs3213787.^[5] Furthermore, another study using the multiplex Snapshot method reported no significant association between SNP rs3213787 and OAG in an Afro-Caribbean population in Barbados.^[6] The association of rs3213787 with NTG has not been reported in ethnic groups other than Japanese. In addition, there has been no case-control study on rs11884064 in other ethnic groups since it was first mentioned in the GWAS studies performed in Japan^[4] and the UK.^[5]

Our study did not reveal an association between 2 SNPs (rs3213787 and rs11884064) in SRBD1 and NTG in a Korean cohort. Studies including cohorts drawn from different ethnic groups showed conflicting results,^[4–6,8,9] and our Korean cohort study showed different results from a study including an East Asian Japanese cohort.^[4,8,9] These findings suggested that SRBD1 may not have an important role in NTG pathogenesis in Koreans. Another interpretation is that the conflicting observations among studies on different ethnic groups may be due to genetic differences among these groups.

Our study had some limitations. First, the sample size was not large. The MAF of rs3213787 in 1055 healthy Korean individuals was 14.2%, which was lower than that of the controls in this study (PMID: 28655895).^[14] Although increasing the number of control subjects would be unlikely to change the results of our study, further validation in a larger population is needed. Another limitation was that we only analyzed the 2 SNPs reported in previous GWAS studies. This may have led to other

potential causal SNPs of the SRBD1 gene being missed. Our study should be evaluated in the context of these limitations, and caution should be exercised when interpreting the relationship between SRBD1 and NTG; thus, a large-scale study replicating our results will be necessary.

5. Conclusion

Our study showed that SRBD1 gene polymorphisms (rs3213787 and rs11884064) may not be associated with the genetic susceptibility to NTG in Koreans. Further studies should be performed to evaluate whether the SRBD1 gene is associated with NTG in other ethnic populations.

Author contributions

Conceptualization: SHJ, HYS; Data collection: YCL, MYL, HYS; Formal analysis: SHJ, HYS; Methodology: SHJ, HYS; Interpretation of the data: SHJ, HYS; Funding acquisition: YCL, HYS; Supervision and Validation: SHJ, HYS; Writing – original draft: SHJ, HYS; Writing – review and editing: SHJ, HYS.

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