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Lack of association between SIX1/SIX6 locus polymorphisms and pseudoexfoliation syndrome in a population from the Republic of Korea

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Abstract

Previous studies have reported the association of the SIX1/SIX6 locus with open-angle glaucoma in various ethnic populations. However, the relevance of the SIX1/SIX6 locus to pseudoexfoliation syndrome (XFS) appears uncertain at present. Thus, we investigated the relationship between polymorphisms in the SIX1/SIX6 locus and XFS in a Korean XFS cohort. A total of 246 participants comprising 167 unrelated Korean patients with XFS and 79 ethnically matched control subjects were recruited. Four polymorphisms of the SIX1/SIX6 locus (rs33912345, rs12436579, rs2179970, and rs10483727) were genotyped using a TaqMan® allelic discrimination assay. Genotypic and allelic associations were analyzed using logistic regression. The minor allele frequency (MAF) of rs33912345 was found to be 0.287 and 0.247 in the XFS cases and controls, respectively, and the MAF of rs12436579 was found to be 0.383 and 0.361 in the XFS cases and control subjects, respectively. The MAF of rs2179970 was found to be 0.090 and 0.095 in the XFS cases and control subjects, respectively, and the MAF of rs10483727 was found to be 0.293 and 0.253 in the XFS cases and control subjects, respectively. Genetic association analysis of 4 SIX1/SIX6 locus single nucleotide polymorphisms (SNPs) revealed no significant difference in genotype distribution between the XFS cases and control subjects in the allelic, dominant, or recessive models (all, $P > .05$). The current study suggested that SIX1/SIX6 locus polymorphisms (rs33912345, rs12436579, rs2179970, and rs10483727) may not be associated with a genetic susceptibility to XFS in a Korean cohort.

Abbreviations: IOP = intraocular pressure, MAF = minor allele frequency, OAG = open angle glaucoma, OR = odds ratio, POAG = primary open angle glaucoma, PXG = pseudoexfoliative glaucoma, SIX = sine oculis homeobox homolog, SNP = single nucleotide polymorphism, XFS = pseudoexfoliation syndrome.

Keywords: genotyping, pseudoexfoliation syndrome, sine oculis homeobox homolog, single nucleotide polymorphism, South Korea

1. Introduction

Pseudoexfoliative syndrome (XFS) is an age-related disease of the extracellular matrix and it is characterized by the production and progressive accumulation of an abnormal extracellular fibrillary substance, most commonly on the iris, zonule, and anterior capsule of the lens.[1,2] XFS has been considered to be the most common overall identifiable cause of secondary open-angle glaucoma (OAG), which is pseudoexfoliative glaucoma (PXG). Numerous studies have reported that OAG is phenotypically and genetically heterogeneous.[3–6] Similarly, several studies in XFS patients reported that XFS was also genetically heterogeneous.[6–9]

Previous studies reported that deletions at 14q22–23 were associated with bilateral anophthalmia.[10–13] Gallardo, et al suggested that sine oculis homeobox homolog 6 (SIX6) haploinsufficiency was responsible for these developmental disorders.[13] SIX6 mRNA was reported to be strongly expressed in retinal ganglion cells.[13] And SIX homeobox 1/SIX homeobox 6 (SIX1/SIX6) has been studied extensively as a candidate gene for optic nerve parameters and RNFL thickness.[14,15] Subsequently, case-control genome-wide association studies on primary open angle glaucoma (POAG) found a significant association between rs10483727 and the risk of POAG.[16–18] In meta-analysis results published in 2019, Lu SY et al showed an association of rs10483727 and rs33912345 in SIX1/SIX6 with POAG.[19]

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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.(UC20SISI0142) The study details were explained to all participants before obtaining informed consent for the genetic analyses; those who refused to participate were excluded.

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Recent studies reported that OAG and PXG shared some genetic risk factors.[20–22] Hence, it is tempting to speculate that some XFS patients develop OAG because they have POAG-related genes. Therefore, the purpose of this study was to investigate whether known POAG-related SNPs of SIX1/SIX6 were associated with XFS in a Korean cohort. These markers were previously reported as risk factors of POAG but have not been studied in Korean patients with XFS.

2. Methods

2.1. Subjects and diagnostic criteria

All subjects are selected from the Eye clinic at Uijeongbu St. Mary's Hospital of Korea between November 2018 and August 2021. All patients and healthy controls were Korean. The purpose of the study and the blood collection procedures were explained in detail to all subjects and all participants provided written informed consent. The study protocol was approved by the Institutional Review Board of Uijeongbu St. Mary's Hospital. The research was performed in accordance with the tenets of the Declaration of Helsinki involving human subjects.

A total of 246 participants comprising 167 unrelated Korean patients with XFS and 79 ethnically matched control subjects were recruited for study participation. Before and after mydriatic examinations, a detailed high-magnification slit-lamp assessment was performed in all study participants. The lens capsule, endothelium, and pupillary margin were thoroughly checked for pseudoexfoliation material deposits. XFS patients were defined as those with clinical evidence of pseudoexfoliation material deposits in either eye (167 individuals; 86 males and 81 females; mean age, 77.02 ± 11.97 years). The unrelated control subjects met all of the following criteria: an intraocular pressure (IOP) consistently less than 21 mm Hg in both eyes without any anti-glaucoma medication, no evidence of glaucomatous optic disc damage, no pseudoexfoliation material deposits in either eye, and no known family history of glaucoma (79 individuals; 29 males and 50 females; mean age, 68.01 ± 12.15 years).

Genomic DNA was extracted from peripheral blood using an MG Genomic DNA purification SV kit (MGmed, Seoul, South Korea). We evaluated 4 SNPs of the SIX1/SIX6 locus: rs33912345, rs12436579, rs2179970, and rs10483727. Genotyping for rs33912345 polymorphism was conducted using the TaqMan® SNP Genotyping Assay (assay ID: C___2485201_10; Applied Biosystems, Foster City, CA). Genotyping for the rs12436579 polymorphism was conducted using the TaqMan® SNP Genotyping Assay (assay ID: C__11916555_30). Genotyping for the rs2179970 polymorphism was conducted using the TaqMan® SNP Genotyping Assay (assay ID: C__15840320_10). Genotyping for the rs10483727 polymorphism was conducted using the TaqMan® SNP Genotyping Assay (assay ID: C___2485225_20). SNP genotyping was performed on the QuantStudio™ real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The post-polymerase chain reaction product data were analyzed directly with allelic discrimination using the same instrument (QuantStudio; Applied Biosystems).

2.2. Statistical analysis

All SNPs were analyzed for deviation from Hardy-Weinberg equilibrium (HWE) using the Chi-squared test. Allele and genotype frequencies were compared between the XFS patient and control groups using the Chi-squared test or Fisher's exact test where appropriate. The allelic association was evaluated by logistic regression analysis. The odds ratio (OR) was calculated, and the confidence interval level was set to 95%. The 4 SNPs were tested for HWE using the Chi-squared test. Three genetic models (allelic, dominant, and recessive) were used to test the SNP associations. Statistical analyses were performed using R software. A P value of less than .0125 (=0.05/4) was considered statistically significant in the association analysis.

3. Results

A total of 246 unrelated participants comprised of 167 Korean patients with XFS and 79 ethnically matched control subjects were recruited in our study. To evaluate the candidate XFS-associated genetic markers, we selected 4 previously reported SNPs (rs33912345, rs12436579, rs2179970, and rs10483727) from among SIX1/SIX6 locus polymorphisms. General information on the 4 SNPs is summarized in Table 1. All 4 SNPs conformed to HWE in both the XFS cases and the controls. The genotype and minor allele frequencies (MAF) of the SIX1/SIX6 locus (rs33912345, rs12436579, rs2179970, and rs10483727) gene polymorphisms in the XFS cases and controls are shown in Table 2.

The MAF of rs33912345 was found to be 0.287 and 0.247 in the XFS cases and controls, respectively. The genetic association analysis of SNP rs33912345 revealed no significant difference in genotype distribution between the XFS cases and controls in the allelic (odds ratio [OR] = 1.231, P = .347), dominant (OR = 1.451, P = .178) or recessive models (OR = 0.868, P = .773). The MAF of rs12436579 was found to be 0.383 and 0.361 in the XFS cases and controls, respectively. For rs12436579, no significant difference in genotype distribution was observed between the XFS cases and controls in the allelic (OR = 1.101, P = .631), dominant (OR = 1.184, P = .544) or recessive models (OR = 1.038, P = .925). The MAF of rs2179970 was found to be 0.090 and 0.095 in the XFS cases and controls, respectively. The genetic association analysis of SNP rs2179970 revealed no significant difference in genotype distribution between the XFS cases and controls in allelic (OR = 0.941, P = .0.854), dominant (OR = 0.976, P = .945) or recessive models (OR = 0.470, P = .595). The MAF of rs10483727 was found to be 0.293 and 0.253 in the XFS cases and controls, respectively. For rs10483727, no significant difference in genotype distribution was observed between the XFS cases and controls in allelic (OR = 1.255, P = .354), dominant (OR = 1.417, P = .208) or recessive models (OR = 0.940, P = .893).

Table 1

General information and MAF from 1000 Genomes database the 4 SNPs.

SNP	Genotype (Major/Minor)	Position (GRCh38)	1000 genomes database (MAF)						KRGDB (MAF)
			All	AFR	AMR	EAS	EUR	SAS	
rs33912345	C/A	Chr14:60509819	0.342	0.032	0.660	0.213	0.597	0.403	0.271
rs12436579	C/A	Chr14:60516369	0.561	0.564	0.772	0.300	0.704	0.531	0.376
rs2179970	G/C	Chr14:60587507	0.025	0.010	0	0.085	0	0.028	0.103
rs10483727	T/C	Chr14:60606157	0.346	0.034	0.659	0.213	0.596	0.423	0.273

All, average of entire population; AFR = African, AMR = American, EAS = East Asian, EUR = European, SAS = South Asian, KRGDB = Korean Reference Genome Database, MAF = minor allele frequencies, SNP = single-nucleotide polymorphism.

Table 2

Association results of 4 SNPs in different models.

	Allele		Genotype (Case/Control)			MAF (Case/Control)	Allelic model		Dominant model		Recessive model	
SNP	1	2*	11	12	22		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs33912345	C	A	84/47	70/25	13/7	0.274 (0.287/0.247)	1.231 (0.799–1.896)	.347	1.451 (0.844–2.495)	.178	0.868 (0.332–2.269)	.773
rs12436579	C	A	63/33	80/35	24/11	0.376 (0.383/0.361)	1.101 (0.744–1.630)	.631	1.184 (0.686–2.044)	.544	1.038 (0.480–2.240)	.925
rs2179970	G	C	138/65	28/13	1/1	0.091 (0.090/0.095)	0.941 (0.491–1.804)	.854	0.976 (0.483–1.970)	.945	0.470 (0.029–7.611)	.595
rs10483727	T	C	85/47	66/24	16/8	0.28 (0.293/0.253)	1.225 (0.798–1.881)	.354	1.417 (0.824–2.436)	.208	0.940 (0.385–2.300)	.893

CI = confidence interval, MAF = minor allele frequency, N/A, not available, OR = odds ratio, SNP = single-nucleotide polymorphism.
*minor allele.

4. Discussion

XFS has been considered to cause secondary OAG, which is PXG. When PXG develops, it is well known to have more rapid disease progression and a poorer prognosis than POAG.[23] In patients with XFS, types of glaucoma other than OAG with high IOP are also observed. In some XFS patients, OAG with low IOP is sometimes observed.[24–26] Like OAG, PXG is thought to be phenotypically and genetically heterogeneous. We thought that it would be very important to examine the association of genetic risk factors that have already been reported to be associated with OAG in an XFS patient group, which is the most common cause of secondary glaucoma. To the best of our knowledge, this was the first study to investigate an association between 4 SIX1/SIX6 polymorphisms and XFS in a Korean population. These markers had been previously reported as risk factors for OAG but had not been studied in an East Asian XFS patient population. In the current study, we demonstrated that there was no association between the previously reported SIX1/SIX6 polymorphisms and XFS in a Korean population.

SIX1/SIX6 gene is on chromosome 14q22-23.[14] The rs10483727 SNP is located in the intergenic region between the SIX1 and SIX6 loci on chromosome 14,[14] and was significant in the discovery cohorts in genome-wide studies and was replicated consistently in other cohorts.[14,19,27,28] The rs33912345 SNP of the SIX6 gene is a common coding variant which is missense variant, (c.421C > A, p.His141Asn). It causes the loss of positive charge,[16] and was found to alter the protein function of SIX6.[29] It is also well-known to be significantly associated with POAG.[19,27,28,30] The rs12436579 SNP is downstream from the SIX6 coding region and the association of it with POAG was detected in East Asian and African ancestry.[19] The rs2179970 is intergenic SNP, which showed a marginal association with POAG in a Chinese population.[30] Although we chose 4 known SNPs in selecting the candidate regions, we found that there was no association of the previously reported POAG-related SIX1/SIX6 polymorphisms with XFS in a Korean population.

One study in 2014 on the association between SIX1/SIX6 and PXG found no association of rs10483727 with PXG in Pakistani cohorts, which are South Asian.[31] However, the authors also reported that there was no association between rs10483727 and POAG.[31] Contrary to the studies in Pakistani cohorts, a meta-analysis by Lu et al confirmed the association of rs10483727 and rs33912345 in SIX1/SIX6 with POAG in East Asian cohorts and there was an ethnic difference.[19] In South Asians (Indian and Pakistani), rs10483727 and rs33912345 SNPs were not associated with POAG, unlike in East Asian cohorts.[31–33] We also found that the 4 MAFs of the SIX1/SIX6 gene polymorphisms showed a large difference among various ethnic groups from the 1000 Genomes Project and the MAF of rs10483727 in East Asians (0.213) was not similar to that of South Asians (0.423)(Table 1) In addition, in large Korean population-based cohorts, a recent study reported that rs10483727 in SIX1/SIX6 was associated with the risk of POAG.[34] Therefore, we tried to determine whether there was an association between the 4 SIX1/SIX6 polymorphisms and XFS in a Korean population. But we were unable to demonstrate a genetic association between the 4 SNPs of SIX1/SIX6 and PXG, unlike POAG patients in a Korean cohort.

Our study had some limitations. First, we analyzed only 4 SNPs among the SNPs reported in previous studies. Therefore, this could result in missing other potential causal SNPs at the SIX1/SIX6 locus. Second, the sample size was not large, especially in the control group. However, the MAFs of the 4 SNPs of the control group were similar to those in the Korean Reference Genome Database (Table 1) Third, the recruitment of all patients and control subjects was done at Uijeongbu St. Mary's Hospital, which may be confounded with potential selection bias. Further studies in a larger Korean population are required to validate our results. Lastly, we showed that the 4 MAFs of the SIX1/SIX6 gene polymorphisms showed a large difference among various ethnic groups from the 1000 Genomes Project. Therefore, it is difficult to rule out the possibility that results in other populations will be different from our findings. Our findings cannot be generalized because of several limitations and should be interpreted with caution in the context of these limitations.

In conclusion, the results of the present study suggest that SIX1/SIX6 locus polymorphisms (rs10483727, rs33912345, rs12436579, and rs2179970) may not be associated with a genetic susceptibility to XFS in Korean populations. Further replication studies are needed to evaluate whether the SIX1/SIX6 locus is associated with XFS in other ethnic populations.

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