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Original Article

A pilot exome-wide association study of age-related cataract in Koreans

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Abstract

Age-related cataract (ARC) is the most common cause of visual impairment and blindness worldwide. A previous study reported that genetic factors could explain approximately 50% of the heritability of cataract. However, a genetic predisposition to ARC and the contributing factors have not yet been elucidated in the Korean population. In this study, we assessed the influence of genetic polymorphisms on the risk of ARC in Koreans, including 156 cataract cases and 138 healthy adults. We conducted an exome-wide association study using Illumina Human Exome-12v1.2 platform to screen 244,770 single nucleotide polymorphisms (SNPs). No SNPs reached exome-wide significance level of association ($P < 1\times10^{-6}$). *B3GNT4* rs7136356 showed the most significant association with ARC ($P = 6.54\times10^{-5}$). Two loci (*MUC16* and *P2RY2*) among the top 20 ARC-associated SNPs were recognized as probably linked to cataractogenesis. Functions of these genes were potentially related to regulating dehydration or homeostasis of the eyes, and showed a potential association with dry eye disease. This finding suggests that mucin- and dry eye disease-related genes may play a significant role in cataractogenesis. Our study provides insight into the genetic predisposition of ARC in Koreans. Additional studies with larger sample sizes are required to confirm the results of this study.

Keywords: age-related cataract, exome-wide association study, single nucleotide polymorphism, genetic predisposition, dry eye disease

Introduction

Age-related cataract (ARC) is the primary cause of blindness worldwide[1]. Global prevalence of cataract is estimated to rise continuously due to increasing aging population[1-2]. Numerous studies have identified potential risk factors for ARC, such as increasing age, use of tobacco and alcohol, low socioeconomic status, diabetes, hypertension, and exposure to sunlight[1-4].

Genetic factors are also associated with the pathogenesis of ARC. Twin studies have shown that genetic factors could explain approximately 50% of heritability

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of cataract, and those factors have a larger contribution to the variation of ARC compared to environmental factors[5-6]. Several studies have evaluated genetic predisposition for ARC using a candidate gene approach, but the results are inconsistent; thus, the causal genetic factors remain inconclusive at present[6-7].

Recently, Liao *et al.* conducted the first genome-wide association study for age-related nuclear cataract in a multi-ethnic Asian population, and identified two susceptibility loci that were suggested to be located in *KCNAB1* on chromosome 3q25.31 and in the proximity of *CRYAA* on chromosome 21[8]. However, they also found significant heterogeneity in the associations across ethnicities, even among Asians. These inter-ethnic differences in genetic predisposition or gene-environment interactions in ARC may contribute to the observed differences in the prevalence or age at onset for ARC among studies and populations[9-10].

Prevalence of ARC in Korean population is higher than that in any other ethnic group, and approximately 90% of individuals aged 70 years and older have a high possibility for developing ARC[11]. This suggests a strong influence of a genetic factor or a gene-environment interaction effect for ARC in general, and that these effects might be particularly important in the Korean population. However, genetic predisposition for ARC in Koreans has not been elucidated to date. Hence, in this pilot study, we assessed the influence of genetic polymorphisms on the risk of ARC in a Korean population using an exome-wide screening method.

Materials and methods

Study subjects

The study subjects included 294 residents in rural villages or Cheongju City in Chungbuk Province, Korea. Trained interviewers filled out a questionnaire including items on demographic factors, working history, smoking habit, alcohol drinking, and history of major systemic diseases, eye diseases, and cataract surgery. All of the subjects provided informed consent and underwent an assessment of corrected and uncorrected visual acuity and reflective error measurement with an auto-reflector (model ARK-530A, Nidek, Japan). Cataracts were identified by slit-lamp examination with a portable slit lamp (model XL-1, Shin-Nippon, Japan). Subjects with cataracts or cataract extraction upon slit-lamp examination were classified as cataract-prevalent cases. Finally, 156 cataract cases and 138 healthy controls were included in this study. Peripheral blood samples were collected from all the subjects for genetic analysis. The study followed the tenets of the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of Chungbuk National University Hospital (CBNUH-2015-06-019). All subjects provided informed consent.

Exome-wide association screening and quality control

Genomic DNA was isolated from peripheral blood using a DNA purification kit (DNA Extractor WB, Wako, Osaka, Japan) according to the manufacturer's protocol. All DNA samples were electrophoresed on 1% agarose gel, and samples with intact genomic DNA showing no smearing on agarose gel electrophoresis were selected for further analysis. Exome-wide association screening was conducted using Human Exome Chip v1.2 platform (Illumina, San Diego, CA, USA) in which 244,770 single nucleotide polymorphisms (SNPs) could be simultaneously analyzed. SNP chip data were checked for quality using the call rate and Hardy-Weinberg equilibrium test.

Statistical analysis

Associations between ARC and SNPs were estimated by unconditional logistic regression analysis with an additive genetic model. To maximize the opportunity to detect an association between SNPs and the risk of ARC, we identified a subgroup with an extreme phenotype, designated as "super-cases" (early onset cases; age at diagnosis <65 years), which were compared to corresponding “super-controls” (healthy elderly controls; age ⩾65 years), and further conducted subgroup analysis. We used Bonferroni correction for multiple tests (n = 32,865 tests) and set the statistical significance and suggestive threshold to a P-value less than 1.0×10^{-6} and 1.0×10^{-4}, respectively. All genetic association analyses were performed using PLINK v 1.07 software. Manhattan plot of results of exome-wide association study was generated using Haploview 4.2 software. *In silico* analysis was performed using Polyphen-2 and SIFT program to predict the potential effect of each SNP on protein function[12-13].

Results

The average call rate of all samples was greater than 99.92%. Monomorphic SNPs (n = 199,391), 54 SNPs not in Hardy-Weinberg equilibrium (P < 0.001), or SNPs with call rates less than 95% (n = 5,719) were excluded. The 32,865 SNPs located on autosomal chromosomes that satisfied the criterion of a minor allele frequency >1% were selected for final analysis.

The Manhattan plot of P-values (in –log-10 scales) derived from the association analysis between ARC and SNPs using unconditional logistic regression analysis with an additive genetic model is shown in ***Fig. 1***. The peak signal was observed at the rs7136356 locus in

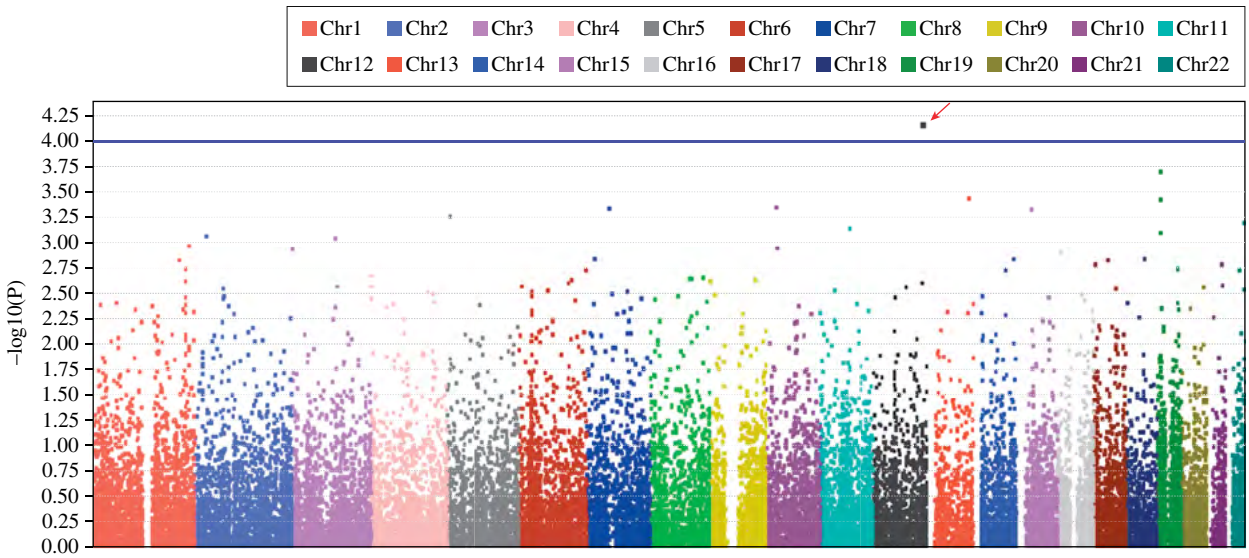

***Fig. 1* Manhattan plot for the exome-wide association study of age-related cataract.** *P*-values in –log10 scale are plotted against their chromosomal locations. The blue horizontal line indicates the suggestive association level ($P = 1.00\times10^{-4}$). The arrow indicates *B3GNT4* rs7136356, which showed the strongest association with age-related cataract ($P = 6.54\times10^{-5}$).

exon 2 of the UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 4 (*B3GNT4*) gene on chromosome 12. *B3GNT4* rs7136356 showed the strongest association with ARC and achieved the suggestive association level applied in this study ($P = 6.54\times10^{-5}$).

The top 20 most significantly associated SNPs in the analysis of 156 cataract cases and 138 healthy controls are presented in ***Table 1***. Among them, four nonsynonymous SNPs (rs2547065, rs60106152, rs1108380, and rs17000957) of the mucin 16, cell surface-associated (*MUC16*) gene and rs663263 of the chemokine (C-C motif) ligand 25 (*CCL25*) gene were located on chromosome 19. There were also four other SNPs (rs663263, rs2505323, rs2505327, and rs2429485) in patched domain containing 3 (*PTCHD3*) on chromosome 10 that showed significant associations with ARC.

In the subgroup analysis comparing super-cases and super-controls, there were no significant SNPs associated with ARC after adjustment for multiple testing. Furthermore, there were no shared SNPs among the top 20 most significantly associated SNPs in the total analysis and subgroup analysis. The intergenic SNP rs10240278 between ADP-ribosylation factor-like GTPase 4A (*ARL4A*) and ribosomal protein L26 pseudogene 21 (*RPL26P21*), located on chromosome 7, showed a suggestive association with ARC (odds ratio = 0.37, $P = 9.00\times10^{-5}$) (data not shown).

Discussion

To the best of our knowledge, this pilot study presents the first potential evidence of an association between genetic variants and ARC in Korean population. Although no SNPs reached the threshold for statistical significance of an exome-wide association study ($P < 1.0\times10^{-6}$), our study nevertheless provides some insight into the genetic predisposition of ARC in Koreans.

In our study, *B3GNT4* rs7136356 was suggestively associated with ARC. *B3GNT4* rs7136356 is a nonsynonymous SNP that results in the substitution from proline to alanine at position 8. *In silico* analysis using PolyPhen-2 estimated that this amino acid change is "possibly damaging" (score: 0.679), and may have a regulatory role of exon splicing enhancement or silencing. However, the roles of this SNP in cataract, as well as in eye diseases in general or other diseases, remain unknown. B3GNT4 is a member of the beta-1,3-*N*-acetylglucosaminyl transferase protein family and is able to catalyze the initiation and elongation of poly-*N*-acetyllactosamine sugar chains[14].

Interestingly, 13 of the genes included in the list of the 20 most highly associated SNPs in this study (e.g., *B3GNT4, MUC16, HHLA2, FAM118A, ALK, PTCHD3,* P2RY2, and SLC10A2) functionally clustered as glycosylation-associated genes or transmembrane proteins.

MUC16 is a membrane-associated mucin protein that is expressed on the human ocular surface[15]. MUC16 is also well known as an ovarian tumor cell antigen (CA125)[16], and has been detected in human tears as well as an important component of the glycocalyx barrier at the ocular surface[15]. Therefore, MUC16 might play a significant role in this barrier as a defense molecule. Blalock *et al.* demonstrated that MUC16 plays a pivotal role in preventing bacterial adherence[17]. MUC16 has also been reported to be involved in the

Table 1 **Top 20 SNPs most significantly associated with age-related cataract.**

SNP ID	Chr.	Position	Nearest gene	SNP type	Major allele	Minor allele	MAF Case	MAF Control	OR (95% CI)	*P*-value
rs7136356	12	122689181	*B3GNT4*	nonsynonymous	G	C	0.23	0.38	0.46 (0.32–0.67)	6.54×10^{-5}
rs2547065	19	9080462	*MUC16*	nonsynonymous	G	C	0.11	0.23	0.41 (0.26–0.65)	1.93×10^{-4}
rs4771450	13	103969491	*SLC10A2*	intergenic	G	A	0.37	0.53	0.55 (0.39–0.76)	3.52×10^{-4}
rs60106152	19	9083791	*MUC16*	nonsynonymous	G	A	0.11	0.22	0.42 (0.26–0.68)	3.63×10^{-4}
rs1108380	19	9085958	*MUC16*	nonsynonymous	A	G	0.11	0.22	0.42 (0.26–0.68)	3.63×10^{-4}
rs17000957	19	9090182	*MUC16*	nonsynonymous	T	C	0.11	0.22	0.42 (0.26–0.68)	3.63×10^{-4}
rs3748220	10	24884829	*ARHGAP21*	synonymous/splicing	A	G	0.01	0.08	0.11 (0.03–0.38)	4.34×10^{-4}
rs1533956	7	57460667	*MIR3147*	intergenic	A	G	0.34	0.48	0.53 (0.38–0.76)	4.48×10^{-4}
rs2222299	15	37561268	*MEIS2*	intergenic	C	T	0.48	0.33	1.81 (1.30–2.52)	4.50×10^{-4}
rs4701732	5	6454662	*UBE2QL1*	intronic	G	A	0.41	0.55	0.55 (0.39–0.77)	5.29×10^{-4}
rs6007594	22	45728370	*FAM118A*	nonsynonymous	G	A	0.57	0.42	1.75 (1.27–2.42)	6.08×10^{-4}
rs7111814	11	72935825	*P2RY2*	intronic	T	C	0.22	0.35	0.52 (0.35–0.76)	7.02×10^{-4}
rs1129763	19	8121369	*CCL25*	nonsynonymous/splicing	C	T	0.12	0.04	3.51 (1.69–7.29)	7.71×10^{-4}
rs2631941	2	29981286	*ALK*	intronic	A	G	0.35	0.22	1.89 (1.30–2.75)	8.40×10^{-4}
rs6779254	3	108072298	*HHLA2*	nonsynonymous	T	C	0.14	0.25	0.47 (0.30–0.73)	8.69×10^{-4}
rs3738531	1	236175327	*NID1*	nonsynonymous	C	A	0.22	0.34	0.52 (0.35–0.77)	1.03×10^{-3}
rs663263	10	27679816	*PTCHD3*	intergenic	C	A	0.24	0.37	0.55 (0.39–0.79)	1.11×10^{-3}
rs2505323	10	27687225	*PTCHD3*	stop loss	A	G	0.24	0.37	0.55 (0.39–0.79)	1.11×10^{-3}
rs2505327	10	27687965	*PTCHD3*	nonsynonymous	A	G	0.24	0.37	0.55 (0.39–0.79)	1.11×10^{-3}
rs2429485	10	27688109	*PTCHD3*	nonsynonymous/splicing	T	C	0.24	0.37	0.55 (0.39–0.79)	1.11×10^{-3}

Chr.: chromosome; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval.

maintenance of hydration and lubrication of the epithelial surface[18]. Moreover, MUC16 expression was significantly decreased in patients with an unstable tear film or aqueous deficiency, and alteration of MUC16 has been associated with dry eye syndrome[15,19].

Furthermore, purinergic receptor P2Y, G-protein coupled, 2 (*P2RY2*), which harbored the 12th ranked associated SNP (rs7111814) in this study, is also linked to dry eye syndrome through its role in regulation of mucin secretion at the ocular surface[20-21]. Recently, an agonist of P2Y2 receptor (diquafosol) was developed as a new pharmacologic agent for dry eye disease[20]. In addition, *B3GNT4*, which was the most strongly associated locus in the present study, shows glycosyl transferase activity that helps to regulate the formation of mucin by *O*-glycosylation[22].

These facts suggest that mucin- and dry eye disease-related genes might play a significant role in cataractogenesis. Although there is no direct evidence that dry eye disease causes cataract, both of these conditions commonly occur in the elderly population, and share similar risk factors such as smoking and inflammation[2,23]. In particular, corticosteroid use, which is one of the treatments for dry eye disease, is a risk factor for cataract[2,23]. Dry eye disease increases oxidative stress in ocular tissues, because human tears contain various nonenzymatic and enzymatic antioxidants such as ascorbic acid, uric acid, glutathione, L-cysteine and L-tyrosine, and superoxide dismutase[24]. Oxidative stress is directly associated with the development of ARC through damage to lens proteins and lipids[25]. The human tear film is composed of 99% water, and primarily functions to absorb ultraviolet radiation from sunlight. Consequently, an individual with dry eyes might have a higher level of exposure to ultraviolet radiation in the cornea and lens, due to loss of the tear film water[26-27]. Therefore, SNPs in dry eye disease-related genes may contribute to genetic susceptibility for ARC by influencing the dehydration condition in the eyes.

In subgroup analysis, the intergenic SNP rs10240278 between *ARL4A* and *RPL26P21* showed a suggestive association with ARC. *RPL26P21* is a pseudogene that might be unexpressed and functionless. *ARL4A* is a member of the ADP-ribosylation factor family of GTP-binding proteins[28], and there is little information about this gene with respect to human cataractogenesis. However, one study showed that ethyl pyruvate inhibited *ARL4A* expression in human corneal keratocytes, which protects cataract formation by decreasing oxidative stress, in a rodent model[29].

This pilot study has limited statistical power due to the small sample size and uneven distribution of age in the cases and controls. We also cannot exclude the possibility of outcome heterogeneity. There are three main

subtypes of cataract, which included cortical, nuclear, and posterior subcapsular, but we did not classify the cases according to cataract subtypes[2]. As different subtypes have different etiologies and risk factors, a genetic association study among subjects with a homogenous cataract type is needed to identify a more specific genetic susceptibility marker[3]. This heterogeneity in outcome might bias the observed association toward a null result. Therefore, our results should be interpreted with caution and require further investigation for confirmation.

In conclusion, this pilot exome-wide association study has identified potentially plausible genes linked to cataractogenesis. Functions of these genes are associated with mechanisms of the regulation of dehydration or homeostasis of the eyes, indicating a connection with dry eye syndrome. Additional studies with larger sample sizes are needed to detect a significant association between the candidate SNPs and risk of ARC.

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