• Investigation •

The impact of *GJA3* SNPs on susceptibility to age-related cataract

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Abstract

• AIM: To determine the association of *gap junction protein alpha 3* (*GJA3*) gene tag single-nucleotide polymorphisms (SNPs) with susceptibility to age-related cataract (ARC).

• METHODS: In total, 486 ARC patients were matched with 500 healthy controls. All the participants underwent complete ophthalmic examinations. Haplotype-tagging SNPs of *GJA3* gene were selected from the HapMap Beijing Han Chinese population. Genomic DNA was extracted from the peripheral blood leukocytes of all the subjects. Under three different genetic models: dominant, recessive, and additive, the association between SNPs and ARC was examined. After adjusting for age and sex, the genetic effects of the *GJA3* SNPs were evaluated with logistic regression analysis.

• RESULTS: Four tag *GJA3* SNPs (rs6490519, rs9506430, rs9509053, and rs9552089) were included in the present study. None of the SNPs showed a significant relationship with an altered risk of total ARC under the dominant, recessive, or additive models. In the subgroup analysis, rs9506430 had a significant effect on the formation of a posterior subcapsular cataract (*P*=0.002, OR: 0.227, 95%CI: 0.088-0.590) under the recessive model.

• CONCLUSION: Our study indicates that *GJA3* variants may influence the development of posterior subcapsular cataracts. Further studies need to be designed to confirm this possibility.

• KEYWORDS: *gap junction protein alpha 3*; single-nucleotide polymorphisms; age-related cataract

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INTRODUCTION

Age-related cataract (ARC) is one of the leading causes of avoidable vision impairment worldwide, accounting for about 80% of senile blindness[1-2]. Although substantial progress has been achieved in cataract surgery technology, advanced cataract surgery with less complications, and that is more effective and stable (such as phacoemulsification and intraocular lens implantation), still caused economical burden to patients in developing countries[3-4]. With the global increase in the elderly population, ARC has become a severe public health challenge around the world, especially in developing countries. It is therefore critical to investigate the potential factors influencing the development of ARC.

The precise etiology of ARC is not currently fully understood. It is reportedly related to multiple risk factors, including diabetes, myopia, blood pressure, smoking, drinking, and high myopia, among others. Notwithstanding, genetic variations are also considered integral to the complex cataractogenesis process. Some studies have suggested that broad-sense heritability is 48% for the nuclear subtype of ARC and 58% for the cortical subtype of ARC[5-6]. Thus far, several genes, such as *glutathione S transferase* (*GST*), *xeroderma pigmentosum complementation group D* (*XPD*), and *X-ray cross-complementing group 1* (*XRCC1*), are thought to be related to ARC susceptibility[7].

The *gap junction protein alpha 3* (*GJA3*) gene encodes the CX46 protein, which consists of gap junctions, and is critical to maintain the ionic and water balance and transparency and optical properties of the lens. At present, most previous *GJA3*-related studies have focused on congenital cataracts. The only study that focused on ARC patients detected two variations in *GJA3* (c.-39C>G and c.415G>A), but according to Polyphen, none of them had potential pathogenicity[8]. We therefore conducted this study to fully screen *GJA3* tag single-nucleotide polymorphisms (SNPs) in ARC patients and to determine correlations between the genotype and phenotype in ARC patients with *GJA3* tag SNPs.

SUBJECTS AND METHODS

Ethical Approval This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China. In addition, informed consent was obtained from each participant.

Study Participants In this study, 986 unrelated participants, with 486 ARC patients and 500 healthy controls, were included. All the participants were Han Chinese and were recruited from the Eye Center of the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China.

Complete ophthalmic examinations were underwent in all the ARC patients, including best corrected visual acuity measurements, fundus photography, and lens examinations using a slit lamp biomicroscope after mydriasis. The lens opacities classification system II is the base of all clinical diagnoses and classifications[9]. According to the degenerative regions of the lens, four subtypes of ARC were classified: cortical cataract (CC), posterior subcapsular cataract (PSC), nuclear cataract (NC) and mixed cataract (MC), which means more than one cataract subtype in an eye[10]. However, if patients suffered with cataracts caused by uveitis, trauma, high myopia, diabetes or other nongenetic causes were excluded.

The control subjects had received routine health examinations at the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China as healthy individuals. All the control subjects also underwent complete ophthalmic evaluations to confirm lens transparency.

Single-nucleotide Polymorphism Selection Haplotype-tagging SNPs in the *GJA3* gene were selected from the HapMap Beijing Han Chinese population (HapMap Genome Browser release #27, accessed on April 29, 2014; available at http://hapmap.ncbi.nlm.nih.gov/). Four SNPs in *GJA3* were selected, based on the tagger-pairwise method, with a minor allele frequency (MAF) >0.10 and an R square (r^2) >0.8. Genomic DNA was extracted *via* the Simgen Blood DNA mini kit (Simgen, Hangzhou, China), from the peripheral blood leukocytes of all the subjects.

Statistical Analysis The Hardy-Weinberg equilibrium (HWE) of each SNP was assessed with the χ^2 test using PLINK (v1.07; available at http://pngu.mgh.harvard.edu/~purcell/plink/). The continuous variables of the subjects' characteristics were presented as the mean±standard deviation (SD). Additionally, the association between ARC and the SNPs was tested under three different genetic models: recessive, dominant and additive. The allelic distributions of the control subjects and the ARC patients were compared *via* the χ^2 test. Then, in order to evaluate the genetic effects of the *GJA3* SNPs after adjusting for sex and age, the logistic regression analysis was operated. Moreover, the Bonferroni correction for multiple testing was also used to reduce the rate of type I errors. All the other statistical analyses were performed by SPSS software (version 11.0, USA). Two-tailed *P* value <0.05 was considered as a statistical significance, unless indicated.

Table 1 The general demographic characteristics of the participants involved in the present study

Group	*n*	Gender		Age (y)	
		Male (%)	Female (%)	Mean±SD	Range
Control	500	57.40	42.60	63.39±6.57	49-87
ARC	486	41.98	58.02	69.45±9.72	38-91
CC	130	34.62	65.38	67.78±8.41	43-88
NC	126	38.10	61.90	68.26±9.86	45-87
PSC	73	45.21	54.79	66.48±10.27	45-90
MC	157	49.68	50.32	73.20±9.24	38-91

ARC: Age-related cataract; CC: Cortical cataract; PSC: Posterior subcapsular cataract; NC: Nuclear cataract; MC: Mixed cataract.

Table 2 The four involved *GJA3* SNPs bioinformatics characteristics

SNPs	Minor allele	Call rate	MAF	Test for HWE (*P*)	Control (MAF)	ARC (MAF)
rs6490519	G	0.99696	0.319	0.3362	0.303	0.334
rs9506430	T	0.99696	0.439	0.6244	0.457	0.420
rs9509053	T	0.99696	0.221	0.2835	0.217	0.224
rs9552089	G	0.99696	0.122	0.7045	0.121	0.122

SNPs: Single-nucleotide polymorphisms; *GJA3*: *Gap junction protein alpha 3*; MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium; ARC: Age-related cataract.

RESULTS

The General Demographic Characteristics of the Involved Participants Overall, 486 ARC patients (PSC=73, NC=126, CC=130, MC=157) and 500 healthy controls were included in this study. The general demographic characteristics of the 986 participants are summarized in Table 1. Statistically significant differences were detected between the two groups in terms of age and sex (P<0.05).

The Bioinformatics Characteristics of *GJA3* Tag SNPs Four tag SNPs in *GJA3* were selected for genotyping in accordance with the screening technique described in the Materials and Methods section of this paper, and their bioinformatics characteristics are summarized in Table 2. No SNPs showed departure from the HWE.

Association Between the SNPs and the Risk of ARC As indicated in Table 3, none of the SNPs showed a significant relationship with an altered risk of ARC under the dominant, recessive, or additive models using the logistic model. In the subgroup analysis, rs9506430 had a significant effect on the formation of PSC (P=0.002, OR: 0.227, 95%CI: 0.088-0.590) under the recessive model, and the result remained significant after the Bonferroni correction.

Table 3 The relationship between *GJA3* tag SNPs and the risk of ARC under three different genetic models

SNPs	Subtype	Genetic model	χ^2 test	Logistic regression	
			(*P*)	*P*	OR (95%CI)
rs6490519	CC	Dominant	0.467	-	-
		Recessive	0.146	-	-
		Additive	0.333	-	-
	MC	Dominant	0.353	-	-
		Recessive	0.151	-	-
		Additive	0.313	-	-
	NC	Dominant	0.664	-	-
		Recessive	0.230	-	-
		Additive	0.350	-	-
	PSC	Dominant	0.116	-	-
		Recessive	0.556	-	-
		Additive	0.288	-	-
rs9506430	CC	Dominant	0.268	-	-
		Recessive	0.318	-	-
		Additive	0.437	-	-
	MC	Dominant	0.268	-	-
		Recessive	0.388	-	-
		Additive	0.475	-	-
	NC	Dominant	0.364	-	-
		Recessive	0.950	-	-
		Additive	0.612	-	-
	PSC	Dominant	0.167	-	-
		Recessive	0.003	0.002	0.227 (0.088, 0.590)
		Additive	0.011	-	-
rs9509053	CC	Dominant	0.960	-	-
		Recessive	0.900	-	-
		Additive	0.992	-	-
	MC	Dominant	0.730	-	-
		Recessive	0.630	-	-
		Additive	0.794	-	-
	NC	Dominant	0.858	-	-
		Recessive	0.550	-	-
		Additive	0.790	-	-
	PSC	Dominant	0.184	-	-
		Recessive	0.439	-	-
		Additive	0.371	-	-
rs9552089	CC	Dominant	0.639	-	-
		Recessive	0.275	-	-
		Additive	0.407	-	-
	MC	Dominant	0.917	-	-
		Recessive	0.767	-	-
		Additive	0.956	-	-
	NC	Dominant	0.377	-	-
		Recessive	0.555	-	-
		Additive	0.476	-	-
	PSC	Dominant	0.162	-	-
		Recessive	0.491	-	-
		Additive	0.352	-	-

SNPs: Single-nucleotide polymorphisms; *GJA3*: *Gap junction protein alpha 3*; ARC: Age-related cataract; CC: Cortical cataract; MC: Mixed cataract; NC: Nuclear cataract; PSC: Posterior subcapsular cataract.

DISCUSSION

Multiple genes have been implicated in influencing ARC formation[11]. In the present study, we focused on *GJA3*, which is a major component of gap junction channels and hemi-channels in the lens. *GJA3* also plays a vital role in lens homeostasis and lens transparency maintenance[12-13]. Up to now, there is no study has thoroughly explored the relationship between *GJA3*-tagged SNPs and ARC. Therefore, this study investigated the association between *GJA8*-tagged SNPs and ARC in a Chinese population. None of the SNPs were significantly associated with an altered risk of ARC under the dominant, recessive, or additive models. However, rs9506430 played a significant role in PSC formation in ARC, which has not been reported in previous studies.

GJA3 belongs to the connexin gene family, which is the main component of gap junctions[14]. More than 30 *GJA3* variants are reported to associate with congenital cataracts, which makes *GJA3* one of the most frequently mutated genes related to lens opacity[15-17]. To date, only one study has focused on the relationship between *GJA3* and ARC. Two variations (c.-39C>G and c.415G>A) identified in the study were found not to be pathogenic[14]. In the present study, we identified one novel variation (rs9506430) that has not been previously reported. This variation conferred a 4.4-fold decreased risk for PSC under the recessive model.

Generally, connexin, like the GJA3 protein, consists of four conserved domains, namely, two extracellular loop domains, one intracellular loop domain, and cytoplasmic NH2- and COOH-terminal domains[18]. It is worth noting that the majority of the variants located in the extracellular loop domains of the GJA3 protein are phenotypically the NC subtype, whereas the variants located in the COOH-terminal domain are the NC or cortical subtype[15]. These findings point to a possible correlation between the genotype and phenotype of *GJA3* in cataracts. In our study, rs9506430 was associated with an altered risk of the PSC subtype.

Overall, those variants in lens proteins, causing proteins to aggregate rapidly and directly, tend to more likely lead to congenital cataracts. By way of contrast, variants that merely increase susceptibility to environmental risk factors usually contribute to the development of ARC[19]. It is interesting that *GJA3* variants are associated with both congenital cataracts and ARC. Importantly, in this study, the rs9506430 variation played a significant role in PSC formation in ARC. To date, many *GJA3* variants have been reported to be associated with an increased risk of cataracts[19-20]. Su *et al*[21] found that downregulation of *GJA3* in lens epithelial cells (LEC) was associated with ARC genesis through interference with H_2O_2-induced LEC apoptosis[22], and LEC apoptosis was reported to be the molecular basis for the initiation and subsequent

progression of cataract formation[21]. Thus, we speculate that rs9506430 may enhance the activity of the GJA3 protein to suppress the apoptosis process in LEC. Further fundamental research is needed to confirm our hypothesis.

In summary, the results of the current study demonstrate a new inheritance pattern of *GJA3* gene variants in ARC. However, as the single population and limited sample size in our study, further studies with larger and ethical diverse populations are warranted. Furthermore, the relationship between *GJA3* and ARC needs to be addressed by elucidating the interactive molecules in detail in future studies.

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