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The link between apolipoprotein E, presenilin 1, and kinesin light chain 1 gene polymorphisms and age-related cortical cataracts in the Chinese population

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Purpose: To study whether presenilin 1 (*PSEN1*), apolipoprotein E (*APOE*), and kinesin light chain 1 (*KLC1*) genotypes are associated with the risk of developing age-related cortical cataracts in the Han Chinese population.
Methods: We collected and analyzed the blood samples of 227 cortical cataract patients and 263 controls. Genotyping was performed by direct sequencing after PCR amplification, and allele frequencies were tested for the Hardy–Weinberg equilibrium.
Results: The G allele and GG genotype of *KLC1 rs8702* were significantly over-represented among cataract patients, as compared to healthy controls (allele P[χ2]=0.001 and genotype P[χ2]=0.008, respectively) and are associated with an odds ratio for cataract development of 1.54 (95% confidence interval of 1.19–2.01). More specifically, carrying the rs8702 C allele was associated with a decreased cortical cataract risk among individuals devoid of the *APOE4* allele (OR=0.55; P[χ2]=0.003), whereas it has no significant effect among *APOE4* carriers (OR=0.57; P[χ2]=0.36).
Conclusions: The *KLC1* and *APOE* genes may be novel susceptibility genes for age-related cataracts.

Degenerative diseases are becoming common, as the population continues to age [1]. Alzheimer disease (AD) appears to be the most common cause of dementia in the elderly [2], which is characterized by extracellular amyloid plaque and intracellular neurofibrillary tangles in the brain [3]. Mutations in the kinesin [4], presenilin (*PSEN1* and *PSEN2*) [5], and apolipoprotein E (*APOE*) genes [6] have been identified as causative of AD.

Cataracts are also a degenerative disease characterized by pathological protein aggregation, which is similar to AD. It has been suggested that cataracts and AD may share the same etiological mechanisms, as amyloid precursor protein (APP), presenilin (*PSEN*), and β-amyloid (Aβ), which is influenced by the inheritance of the *APOE*4 allele [7], were found in the cataractous lens [8,9]. However, Zetterberg et al. [7] found no association between any of the *APOE* polymorphisms and cataracts. Moreover, no association study has been published supporting any functionality of *PSEN* gene polymorphisms in cataracts.

Kinesin is composed of two subunits, including the heavy chain protein and the light chain protein. The heavy chain protein contains the ATP- and microtubule-binding motifs, which are essential for the transport of vesicles [10]. The light chain protein encoded by the kinesin light chain 1 gene [11] (*KLC*, previously designated *KNS2*) binds to the heavy chain, and it contains the cargo-binding domain of the complex [12]. Andersson et al. [13] found that the *KLC1* gene (rs8702, G>C) might be a susceptibility gene for age-related cataracts. Similarly, a weak correlation between the *KLC1* gene (rs8702) and age-related cataracts was reported by Otter et al.'s study [14].

The purpose of this study was to investigate *PSEN1* (rs165932, rs7523), *KLC* (rs8702), and *APOE* (rs7412, rs429358), as well as to explore their relationship with cataracts. This is the first study to report their actual presence in a large population sample, as well as their association with age-related cataracts in the Chinese population.

METHODS

Study subjects: We included 227 unrelated Chinese patients with cataracts and 263 unrelated control subjects from the Research Institute of Field Surgery, Da Ping Hospital (The Third Military Medical University, Chongqing, PR China) from June 2012 to April 2013. All participants were ethnic Han Chinese living in Chongqing city. The research was approved by the local hospital ethics committees acting in accordance with the principles of the Declaration of Helsinki. The type of cataract (nuclear, cortical, posterior subcapsular, and mixed cataracts) was determined using a biomicroscope

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and an ophthalmoscope before surgery. A lens examination was performed according to the Lens Opacities Classification System III (LOCSIII), including grading of nuclear opalescence and color (N; 0.1–6.9), cortical opacity (C; 0.1–5.9), and subcapsular opacity (P; 0.1–5.9) [15]. Only patients with pure cortical opacity with grades of more than LOCS 2 and patients without nuclear or posterior capsule opacity were included in this study [16]. For the control subjects, all parameters of the LOCSIII grading system were lower than LOCS 2. All subjects gave their informed consent to be included in the study, which was approved by the ethics committees of the participating institutions.

Genotyping: The genomic DNA was extracted from 5 to 10 ml of peripheral whole blood from all participants, as well as purified from lymphocyte pellets according to standard procedures using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). The primer sequences used in the PCR analysis are shown in Table 1. PCR was performed in a 25-µl reaction volume containing 2.5 µl 10 × GC buffer (Tiangen, Beijing, and China), 200 µmol/l of dNTP, 0.2 µmol/l of each primer, 1.0 unit of Tag DNA polymerase (Tiangen), and 60 ng of genomic DNA. The conditions, after initial denaturation at 95 °C for 15 min, were 35 cycles of 30 s at 94 °C, 1 min at 50–62 °C, and 1 min at 72 °C, followed by a final extension at 72 °C for 7 min. The products of PCR remained at 4 °C and they were purified using a MultiScreen-PCR plate (Millipore). The purified PCR products were bi-directionally sequenced using the ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA).

Statistics: Allele frequencies of all single nucleotide polymorphisms (SNPs) detected were assessed for Hardy–Weinberg equilibrium (HWE) using a chi-square ($\chi 2$) test with a one degree of freedom. The distributions of allele and genotype frequencies in patients were compared with those of the control subjects using the $\chi 2$ test or Fisher's exact test. As well, a p value of <0.05 was considered statistically significant based on previous reports. The strength of the association of the genotypes with cataracts (expressed as the odds ratio [OR] and 95% confidence interval [CI]) was obtained from the 2 × 2 contingency tables. Bonferroni's correction was applied to adjust the significance level in multiple comparisons, and a p value of less than 0.017 (equal to 0.05/3) was considered statistically significant [17]. All calculations were performed with the SPSS software, version 15.0 (SPSS Science, Chicago, IL).

RESULTS

We included 227 unrelated Chinese patients with cataracts (mean age, 65.6±6.0 years; 55% females) and 263 unrelated control subjects (mean age, 64.5±5.6 years; 50% females). There was no significant difference in age and gender between the two groups.

Allele frequencies and genotypes in patients and control subjects were listed in Table 2. The genotype distribution was in agreement with the HWE both in the control subjects and the patients (p>0.05, data shown in Table 2). Based on the corrected p value at p=0.017, there is no statistically significant difference among allele frequencies and genotypes for *PSEN1* rs7523 (allele P[$\chi 2$]=0.40, genotype P[$\chi 2$]=0.54; OR=1.16), rs165932 (allele P[$\chi 2$]=0.28; genotype P[$\chi 2$]=0.53; OR=0.85), and *APOE4* (allele P[$\chi 2$]=0.03; genotype P[$\chi 2$]=0.11; OR=1.74) polymorphisms between the 227 cataract cases and the 263 control subjects. However, the allele frequency and genotype of *KLC1* rs8702 showed a significant difference between the cataract and control

Table 1. The primer sequence and the product length in the PCR analysis.

Gene	Fragment name	Primer sequence (5′-3′)	Product size (bp)
KLC1	rs8702	F:CACAGGTCAGTCGGAGTGAGT	466
		R:CTGTGAACTGGCCATTCCTT	
APOE	rs7412	F:ATGCCGATGACCTGCAGA	684
		R:ACTGGCGCTGCATGTCTT	
	rs429358	F:TCGGAACTGGAGGAACAACT	260
		R:TACACTGCCAGGCGCTTCT	
PSEN1	rs165932	F:CAAAGAAGGCCAAGCTACAG	544
		R:TGGCCTTTACCACCACTTTAC	
	rs7523	F:GATGCCTCCTCTGTCCTCATT	154
		R:GCTGACAGCACCGATTCAT	

Table 2. KLC1, PSEN1 and APOE genotype and allele distribution.

SNP	*Genotype (%)*			HWE	*P value*	*Allele frequency (%)*		*P value*	*OR (95%CI)*
rs8702	GG	CG	CC		0.008	G	C	0.001	1.54 (1.19 −2.01)
Cases (n=227)	111 (0.49)	90 (0.40)	26 (0.11)	0.24		312 (0.69)	142 (0.31)		
Controls (n=263)	95 (0.36)	119 (0.45)	49 (0.19)	0.28		309 (0.59)	217 (0.41)		
rs165932	AA	AT	TT		0.53	A	T	0.28	0.85 (1.62 −1.14)
Cases (n=227)	128 (0.56)	85 (0.38)	14 (0.06)	0.98		341 (0.75)	113 (0.25)		
Controls (n=263)	135 (0.51)	109 (0.42)	19 (0.07)	0.64		379 (0.72)	147 (0.28)		
rs7523	GG	GA	AA		0.54	G	A	0.40	1.16 (0.82 −1.63)
Cases (n=227)	158 (0.70)	60 (0.26)	9 (0.04)	0.28		376 (0.83)	78 (0.17)		
Controls (n=263)	189 (0.72)	68 (0.24)	6 (0.02)	0.97		446 (0.85)	80 (0.15)		
APOE	E3/E3	E3/E4	E4/E4		0.11	E3	E4	0.03	1.74 (1.05 – 2.90)
Cases (n=227)	191 (0.84)	33 (0.15)	3 (0.01)	0.26		415 (0.91)	39 (0.09)		
Controls (n=263)	238 (0.90)	23 (0.09)	2 (0.01)	0.10		501 (0.95)	27 (0.05)		

SNP: single nucleotide polymorphism. HWE: Hardy–Weinberg equilibrium. OR: odds ratio. CI: confidence interval. APOE: apolipoprotein E.

subjects after Bonferroni's correction (allele P[χ2]=0.001; genotype P[χ2]=0.008; OR=1.54).

Stratification by *APOE* showed a possible association between the *KLC1* (rs8702) polymorphism and cataracts, depending on the *APOE* genotype (Table 3). Thus, among individuals devoid of the *APOE4* allele, the rs8072 C allele was less common in cases (48% C allele carriers) than in controls (62%), and the association was significant after Bonferroni's correction (genotype P[χ2]=0.003; OR=0.55). Among *APOE4* carriers, although the rs8702 C allele was less common in the cataract cases (70%) compared to the controls (80%), the association was not significant (genotype P[χ2]=0.36; OR=0.57). These results indicate that the *KLC1* (rs8702) C allele reduces cortical cataract risk among individuals devoid of the *APOE4* allele, whereas it has no significant effect on *APOE4* carriers.

DISCUSSION

Cataracts and AD were supposed to be associated [18]. This study analyzed the relationship between cataracts and the *PSEN1*, *KLC1*, and *APOE* genes in the Han Chinese population in Northeastern China. Our statistical analyses showed that these three genes were not associated with cataracts in that population.

Multiple studies suggest that genetic variations in the coding and promoter regions of *PSEN* may affect AD susceptibility [19] or the rate of cognitive impairment progression [20]. Presenilins are key components of the secretase multiprotein complexes that are responsible for Alzheimer precursor protein (AβPP) processing, as well as the generation of the cytotoxic β-amyloid polypeptide (Aβ). Both *PSEN1* and *PSEN2* are known to be expressed in the lens, where the Aβ-polypeptide has been shown to accumulate in the lens

Table 3. KLC1 (rs8702) association with cataract in the sample stratified by APOE genotype.

Cases/Controls	*rs8702 (%)*			**OR (95% CI)*	*P value*
	GG	**GC**	**CC**		
Without APOE4					
Cases (n=191)	100 (0.52)	70 (0.37)	21 (0.11)	0.55 (0.38–0.81	0.003
Control (n=238)	90 (0.38)	107 (0.45)	41 (0.17)		
With APOE4					
Cases (n=36)	11 (0.30)	20 (0.56)	5 (0.14)	0.57 (0.17–1.90)	0.36
Controls (n=25)	5 (0.20)	12 (0.48)	8 (0.32)		

*OR with 95% CI, for the individuals carrying one or two copies of the rs8702 C allele (any C) compared with GG genotype.

epithelia of both AD and non-AD patients [8,21]. However, to our knowledge, an association between *PSEN1* and cataracts has not been previously reported. The present study, focusing on the two *PSEN1* polymorphisms (rs165932 and rs7523), showed that no differences in the distribution of the T (rs165932) and A (rs7523) alleles and genotypes could be seen between the controls and cataract patients.

Kinesin molecular motor proteins generate the movement of vesicles containing a wide variety of materials in neuronal and other cells, and they are [22] also expressed in the lens [23]. Further, although much more speculative, some biochemical and epidemiological data support an association between cataracts and AD [18]. It is reported that the GG genotype of rs8702 in *KLC1* represents a high risk for cataracts in the Estonian population [13]. The *KLC1* rs8702 allele and genotype frequencies found in this study are different from those reported in other European populations [13,14]. Moreover, our results point to a possible relation to cataracts in an *APOE*-dependent manner, with evidence suggesting that the rs8702 polymorphism influences the risk of developing cataracts without *APOE4* carriers. Thus, more studies are necessary to validate this result, as well as to determine the mechanisms responsible for the association in the future.

Zetterberg et al. performed genotyping of *APOE* on 502 patients with senile cataracts, as well as on 187 individuals in a control group, without finding any significant difference for any *APOE* alleles [7]. This indicates that if there is a common pathogenic mechanism between cataracts and AD, it does not involve the *APOE* polymorphism. However, Utheim et al. tested their results further by examining 88 healthy individuals for cataracts, half of whom were *APOE4* carriers, and a weak negative association between *APOE4* and cataracts was disclosed [24]. In the present study, the *APOE4* allele was more frequent in cases than in controls (p=0.03), but the difference was not significant after Bonferroni's correction.

Altogether, these results do not support *PSEN1* as a major susceptibility gene for cataracts. A small influence of *APOE* or *KLC1* (rs8702) on the risk of cataracts cannot be excluded, but it must be confirmed by additional replication studies as well as experimental studies showing a functional effect of these SNPs.

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