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Association of S18Y isomer in UCHL1 gene polymorphism with age-related cataract pathogenesis

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UCHL1 基因多态性中的 S18Y 异构体与年龄相关性白内障发病机制的相关性

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摘要

目的:研究泛素羧基末端酯酶 L1(UCHL1)的基因多态性异构体 S18Y 在年龄相关性白内障发病机制中的作用。

方法:采用病例对照研究的方法,使用聚合酶链式反应(PCR)分别扩增白内障组(242 例)和正常人组(144 例)血液中 UCHL1 基因,测序后分析 S18Y 异构体的基因频率。

结果:UCHL1-S18Y 的基因多态性在等位基因(P=0.746)和基因型频率(P=0.813)上没有统计学差异;二元 Logistic 回归模型分析显示年龄在两组间具有统计学意义(P<0.001),将年龄和性别作为混杂因子校正后计算 UCHL1 的 S18Y 等位基因中(腺嘌呤)A-携带者的 *OR* 比值比和 95%置信区间(95% *CI*),结果显示(腺嘌呤)A-携带者在两组间无统计学差异(P=0.818)。

结论:本研究提示 UCHL1-S18Y 的基因多态性在年龄相关性白内障发生发展过程中没有起到显著作用或者保护作用。

关键词:白内障;泛素羧基末端酯酶 L1;泛素;S18Y;异构体

Abstract

• AIM: To explore the possible effects of genetic variant S18Y of ubiquitin carboxyl-terminal esterase L1 (UCHL1) gene on age-related cataract formation.

• METHODS: We have investigated the frequency of this variant among the Han-Chinese population in a case-control study. A total of 242 cortical cataract patients and 144 controls were genotyped using polymerase chain reaction (PCR) and genomic sequencing.

• RESULTS: There is no statistical difference between cataract patients and healthy controls for the frequency of alleles (P=0.746) and genotypes (P=0.813). Since there was in our sample a significant difference between the groups in mean age (P<0.001), *OR* and *CI* for UCHL1 allele A positivity were computed by a binary Logistic regression model, no association was obtained for a positive UCHL1 allele A carrier status (P=0.818).

• CONCLUSION: We did not find any difference between the cases and the controls at allelic and genotypic level. Our results do not support a role for this variant in cataracts.

• KEYWORDS: cataract; UCHL1; ubiquitin; S18Y; variant
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INTRODUCTION

Cataract is one of the leading causes of blindness in the world, and it is characterized by partial or complete opacity of the lens resulting from the aggregation of lens proteins, or disturbance of the regular alignment, or packing of the fiber cells. There are three types of cataracts, congenital, infantile and age-related lens opacities[1]. Congenital and infantile cataracts are rare, and age-related cataracts are common in older individuals over the age of 40. Age-related cataracts are multi-factor disorder, involving complex interaction between genetic and environmental risk factors[2]. Therefore, the pathophysiology behind the age-related cataracts hasn't to be fully understood yet. It is suggested that the ubiquitin-proteasome system (UPS), a cellular pathway responsible for the degradation of misfolded and damaged proteins, play a pivotal role in preventing the formation of protein aggregates by proteolytic degradation[3],

and oxidation is a very early or initial event in the overall process in the sequence of events which lead to cataracts[4].

The ubiquitin carboxyl-terminal esterase L1 (UCHL1), also known as neuron-specific protein gene product 9.5 (PGP 9.5), is a member of the ubiquitin C-terminal hydrolase family of de-ubiquitinating enzymes, which catalyze the hydrolysis of peptide-ubiquitin bonds and processing of ubiquitin precursors[5-6], thus being a component of the ubiquitin proteasome system. In addition to a de-ubiquitination function, UCHL1 also possesses dimerization-dependent ubiquitin ligase activity[7]. UCHL1 protein is especially abundant in the brain, but it is also reported to be expressed in other tissues, such as retina and lens epithelial cells[8]. Two genetic variants in the UCHL1 gene, I93M and S18Y (c.53C >A, rs id 5030732), have been examined in association with Parkinson's disease (PD), although some studies found S18Y has no association with the disease[9-10]. The I93M variant leads to a 50% reduced activity and the S18Y variant exhibits increased hydrolytic activity and decreased ligase activity[11]. Moreover, it has also been demonstrated that the S18Y variant of UCHL1 confers an antioxidant function that is not present in the wild type form[12]. The increased hydrolytic activity and antioxidant function of S18Y variant may underlie the protective effects of this variant in certain diseases, such as PD disease. In fact, some studies also reported that S18Y also had a protective role for Huntington's[13] and Alzheimer's disease (AD)[14], although conflicting data exist[15].

Recently, a study observed a significant association of UCHL1 S18Y variant with cataracts[16]. However, the study does not support a protective role of S18Y in cataracts development, but instead suggests a disease-promoting effect. To investigate the role of UCHL1 18Y in cataracts, we performed an association study of UCHL1 S18Y in a sample of age-related cataracts and control subjects coming from China.

SUBJECTS AND METHODS

Patients and Controls An informed consent form was obtained from all of the subjects before participating the study. All of subjects are volunteers from Chinese Han and were recruited from the Second Affiliated Hospital of Soochow University in Suzhou, and all of the participants have no stipend from the study. The study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Soochow University. The research was conducted according to the principles described in the Declaration of Helsinki. All of the patients had a comprehensive ophthalmologic examination including dilated indirect contact lens and slit-lamp biomicroscopy and the diagnosis of age-related cataract was made according to the LOCS II. Exclusion criteria included high myopia and associated underlying systemic diseases. The case group included 242 age-related cataract patients. For control subjects, only individuals above the age of 40 years were included, yielding 144 individuals with a mean age (±SD) of 61.9±5.8 (range: 40-74) years. Mean age in the cataract group was 65.0±6.0 (range: 41-82) years.

Table 1 UCHL1 genotype and allele frequencies in cataract patients and controls *n* (%)

UCHL1	Control	Cataract	*P*
Genotype frequencies			
AA	43 (29.9)	79 (32.6)	0.813
AC	73 (50.7)	115 (47.5)	
CC	28 (19.4)	48 (19.8)	
Allele frequency			
A	159 (55.2)	273 (56.4)	0.746
C	129 (44.8)	211 (43.6)	

AA: Adenine adenine; AC: Adenine cytosine; CC: Cytosine cytosine; A: Adenine; C: Cytosine.

Genomic Sequencing Genomic DNA was extracted from whole blood samples using standard methods. The PCR were carried out with Pfu DNA polymerase (Tiangen) in a final volume of 40 μL, containing 12 μL of template DNA, about 50 ng. PCR primers used to amplify regions spanning S18Y polymorphism of the UCHL1 gene were as follows: the forward primer 5'-GGACTGGGGCTCCTCCCAGG-3' and the reverse primer 5'-GGGCCGTGAGGGGAAACAGC-3'. Optimal conditions were: 20 μL 2xPFU, 1 μL of the 10 mM forward primer and 1 μL of the 10 mM reverse primer, 4 μL DMSO and 2 μL ddH_2O. The cycling profile were: 5min at 94℃, then 33 cycles: 30s at 94℃, 30s at 60℃, 30s at 72℃ and finally 5min at 72℃. We sent the PCR products to BioSune Company to identify UCHL1 alleles.

Statistical Analysis Student's *t*-test was used to assess differences in age and sex between the cataract patients and control subjects. The frequencies of alleles and genotypes in the patients and controls were analyzed using the Pearson's Chi-square test. Odds ratios (*OR*) and relative 95% confidence intervals (95% *CI*) were estimated for UCHL1 allele A-carrier status and adjusted for sex and age using a binary Logistic regression model. All these analyses were performed using SPSS 20.0 (SPSS Inc, Chicago, II).

RESULTS

Considering age-related cataracts usually begin after age of 40, all the study population are at or over 40. The S18Y variant was genotyped in 144 Chinese controls and 242 Chinese age-related cataract patients to determine the distribution of the S18Y variant in UCHL1. The distributions of the UCHL1 genotypes were in Hardy-Weinberg equilibrium for the control group ($P=0.764$) as well as for the cataract group ($P=0.600$). In the cataract group, the mean age was 65.0±6.0 (range: 41-82) years and 115 (47.5%) were females. In the control group, the mean age was 60.9±5.8 (range: 40-74) years and 78 (54.2%) were females. There is a significant difference between the groups in mean age ($P<0.001$), but no significant difference in sex ($P>0.228$).

Table 2 Binary Logistic regression of cataract versus control group for age, sex and UCHL1 allele A-carrier status

Parameters	B	SE	Adjusted *OR*	95%*CI*	*P*
Age	0.124	0.022	1.132	1.085-1.182	<0.001
Gender	-0.198	0.224	0.821	0.529-1.272	0.377
UCHL1, allele A-carrier	0.064	0.280	1.067	0.616-1.848	0.818

B: Regression coefficient; SE: Standard error; *OR*: Odds ratio; *CI*: Confidence interval.

Allele and genotype frequencies of UCHL1 S18Y gene polymorphism in both groups are shown in Table 1. There was no statistical difference between cataract patients and healthy controls for the frequency of alleles and genotypes. The allele frequencies found in this study were similar with previous reports on Chinese population[17], but not Caucasian population[16].

Since there was a significant difference between the groups in mean age, *OR* and *CI* for UCHL1 allele A positivity were computed by a binary Logistic regression model to correct for age and gender. No association was obtained for a positive UCHL1 allele A carrier status (Table 2).

DISCUSSION

UCHL1 is a component of the ubiquitin proteasome system, and its main function is to hydrolyse ubiquitin from the C-terminal end of substrates. A number of reports demonstrated that UCHL1 S18Y had a protective role for some neurodegenerative diseases, such as PD, AD, Huntington's disease and even cancer[18-19], although conflicting data exist[20-21]. The mechanism for this protective effect is not known, but it may be at least partially explained by the increased hydrolytic activity of UCHL1 S18Y that more effectively prevents the formation of protein aggregates by proteolytic degradation.

Age-related cataracts are responsible for nearly half of all blindness worldwide. However, the biochemical mechanisms that result in cataracts formation have remained unclearly. As aggregation of protein, especially crystallins in lens, can cause cataracts, it has been proposed that failure of the ubiquitin-proteasome pathway could contribute to age-related cataracts. In addition, oxidative damage is a major cause or consequence of cortical and nuclear cataracts, the most common types of age-related cataracts[1]. Considering the increased hydrolytic activity and antioxidant function of UCHL1 S18Y variant, it is probably that S18Y plays a protective role for cataracts. There are few reports on association between UCHL1 S18Y and cataracts. Up to now, there is only one report on UCHL1 S18Y in cataracts, suggesting a disease-promoting effect of UCHL1 S18Y, instead of a protective effect[16]. However, the association found in the study was quite weak and argues against any pivotal role for the UCHL1 S18Y variant in cataracts. In our study, we did not find an overall difference in the frequency of the S18Y polymorphism in the UCHL1 gene between cases and controls, also indicating that UCHL1 S18Y is not important for cataracts.

There may be several explanations for these results contradicting to expectation. Firstly, UCHL1 is expressed mostly in neural tissues, indicating UCHL1 plays important role in brain. In other words, the lower expression of UCHL1 in lens makes it less effect on lens related diseases. Secondly, there may be varying patterns of association according to the ethnic/geographic origin of patients and controls. In fact, comparing to Caucasian and Swedish population[16], the frequency of the S18Y polymorphism in this study was higher, in accordance with previous reports on Chinese populations[14,17]. Thirdly, nuclear cataracts result from the aggregation and insolubilization of lens proteins, while cortical cataracts involve the disruption of fiber cell membranes, followed by disintegration of the cytoplasmic contents of the damaged fiber cells. In addition, a causal role of oxidation is strong for nuclear, but substantially lower for cortical and posterior subcapsular cataracts[1]. Thus, if some types of cataracts associate with S18Y, it might be nuclear cataracts. However, most of the patients we collected in this study are cortical cataract patients.

In summary, the present study in our Chinese sample suggests that the UCHL1 S18Y does not influence, at least, on cortical cataract risk. Nevertheless, it is also possible that the UCHL1 S18Y plays a minor role in the development of cataracts. Further studies in other populations could help to elucidate the effect of UCHL1 S18Y on cataracts.

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