Original Article

Senile cataract and genetic polymorphisms of APE1, XRCC1 and OGG1

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Abstract: Polymorphisms of DNA repair enzymes which may influence their repair efficiency lead to diseases, for example, senile cataract. In this study, we aimed to analyze the association of single nucleotide polymorphisms in AP endonuclease-1 (APE1), 8-oxoguanine glycosylase-1 (OGG1) and X-ray repair cross-complementing-1 (XRCC1) genes with the risk of age-related cataract in a Chinese population. Genotyping was carried out by the polymerase chain reaction and DNA sequencing on 402 cataract patients and 813 controls in this study. Differences in the frequencies were estimated by the chi-square test, and risk was estimated using unconditional logistic regression after adjusting for age and gender. Our results demonstrated there was a significant difference between the case and control groups in the APE1-141 G/G genotype (P=0.002). This difference still existed after adjusting for age and gender (P*=0.003). The APE1-141 T/T genotype and T allele frequencies were significantly higher in cataract patients, while the G/G genotype and G allele frequencies in patients were significantly lower than in controls (P < 0.05). The APE1-141 G/G genotype (OR, 0.49; 95% CI, 0.31-0.77) seems to have a protective role against cataract, and the T allele seems to have a deleterious role in the development of cataract. In OGG1 Ser326Cys and XRCC1 Arg399Gln polymorphisms, there were no significant differences in frequencies of the variant homozygous in patients compared with controls.

Keywords: Genetic polymorphisms, OGG1 gene, APE1 gene, XRCC1 gene, senile cataract

Introduction

Aging is marked by changes that affect organs and resident stem cell function. In aging process, DNA damage, oxidative stress and other factors cause the eventual demise of cells. Besides senile cataract in this study and other ophthalmic diseases like AMD, heart disease, stroke, cognitive impairment, and hearing loss, etc. are all diseases that occur mostly during aging in human [1, 2].

Age-related cataract is the leading cause of blindness in the world. According to WHO, there is an estimated 180 million visually disabled people worldwide, and 40-45 million of these persons are judged to be without useful vision-they are unable to walk about unaided. An estimated 46% of these cases are the result of cataracts. Although the pathophysiology of cataract is still not fully understood, as a multifactorial disease caused by interaction between genetic and environmental factors, epidemiological investigations prompt many risk factors such as diabetes, gender, sunlight or ultraviolet radiation, smoking and nutritional deficiencies, etc. may relate to cataract formation [3].

It has been well accepted that oxidative stress plays a critical role in the pathogenesis of senile cataract. The lens contains only a single layer of epithelial cells on its anterior surface and this single layer of lens epithelial cells is essential for maintaining metabolic homeostasis and transparency of the entire lens. Under normal physiological conditions, most of these cells have a relatively long life span. However, if such conditions are altered or disturbed by factors such as oxidative stress, the viability of the lens epithelial cells may be jeopardized [4]. Many studies found out that in the progress of aging, oxidative damage in the lens increased gradu-
Genetic polymorphisms and senile cataract

As the balance between oxidative and anti-oxidative system was broken, proteins, lipids and DNA damage caused by endogenous reactive oxygen species begun to accumulate [5, 6]. An association between the development of lens opacities and oxidative stress-induced DNA damage in the lens epithelium has been proved [4, 7-9]. In addition, ultraviolet light—one of the risk factors that lead to cataract, has also been shown to cause DNA damage in lens epithelial cells [10-12].

DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotide residues generated by exposure to carcinogens and cytotoxic compounds [13]. Studies have confirmed that polymorphisms of DNA repair genes decreased their ability to repair DNA damage, leaving human body a greatly increased susceptibility to cancer or age-related diseases [14-16]. Base excision repair (BER) is one of the most crucial DNA repair pathways. As the key enzymes of the BER pathway, association between 8-oxoguanine glycosylase-1 (OGG1), AP endonuclease-1 (APE1) and X-ray repair cross-complementing-1 (XRCC1) genes polymorphisms and age-related macular degeneration, pterygium and onset primary open-angle glaucoma have been studied frequently [17-19]. In addition to ophthalmic disorders, malignancies, diabetes and neurological disorders such as Huntington's disease are also focuses of SNP researches [20-23]. In OGG1 and XRCC1 genes, OGG1 Ser326Cys (rs1052133), XRCC1 Arg399Gln (rs25487) and APE1 -141T/G (rs1760944) and senile cataract, thus making an exploration and supplement to the pathogenesis of senile cataract.

Materials and methods

Cataract patients and controls

Case group: During October 2013 to August 2014, a total of 402 patients (172 males and 230 females) with age-related cataracts were enrolled in the study at the Department of Ophthalmology, TONGJI Hospital affiliated to Tongji Medical College, Huazhong University of science &technology in China. Control group: Eight hundred and thirteen volunteers (405 males and 408 females) without age-related cataract or other age-related ocular diseases were enrolled as the control group. All subjects were recruited from the Han Chinese population and underwent basic physical and ophthalmic examinations. Visual acuities were determined using the Snellen chart. Cataract status was determined by lens examination using a slit lamp biomicroscope. Patients with secondary cataract caused by trauma, diabetes, and other known causes were excluded. The mean ages for the cataract patients and the controls were 67.97 ± 8.60 years and 67.45 ± 7.01 years.

Blood samples and DNA isolation

After informed consent, five milliliters of venous blood were collected into ethylene diamine tetraacetic acid tubes from all patients and controls. Immediately after collection, whole blood was stored in aliquots at -80°C until use. Genomic DNA was extracted from leukocytes using TIANamp Blood DNA kit (TIANGEN BIOTECH (BEIJING) CO., LTD) according to the manufacturer’s instructions. The use of blood

| Table 1. Demographic information of the patients and controls |
|-----------------|-----------------|
| **Cataract Group** | **Control group** |
| No. of patients | 402 | 813 |
| Age (yrs) |
| Mean ± standard deviation | 67.97 ± 8.60 | 67.45 ± 7.01 |
| Gender |
| Male, n (%) | 172 (42.8%) | 405 (49.8%) |
| Female, n (%) | 230 (57.2%) | 408 (50.2%) |
samples in this experiment is in accord with the Helsinki Declaration.

**Genotyping of OGG1 codon 326**

OGG1 genotypes were detected using a polymerase chain reaction-DNA sequencing method. A fragment of 461 bp containing codon 326 in exon 7 was amplified using primers given in Table 2. The PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. PCR product was subjected to direct sequencing; CG mutation on codon 326 of OGG1 gene could result in a Cys → Ser change.

**Genotyping of XRCC1 codon 399**

XRCC1 genotypes were detected using a polymerase chain reaction-DNA sequencing method. A fragment of 278 bp containing codon 399 in exon 10 was amplified using primers given in Table 2. The PCR conditions were 94°C for 4 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. PCR product was subjected to direct sequencing; AG mutation on codon 399 of XRCC1 gene could result in an Arg → Gln change.

**Genotyping of APE1-141T/G**

APE1 genotypes were detected using a polymerase chain reaction-DNA sequencing method. A fragment of 352 bp was amplified using primers given in Table 2. The PCR conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. PCR product was subjected to direct sequencing.

**Statistical analyses**

Statistical analyses were performed with SPSS for Windows version 17.0 (SPSS, Inc., Chicago,
Genetic polymorphisms and senile cataract

Figure 2. DNA sequencing diagrams are represented as above. Genotypes of OGG1 Ser326Cys are C/C (A1), C/G (A2) and G/G (A3) separately; genotypes of XRCC1 Ar399Gln are separately G/G (B1), G/A (B2) and A/A (B3); genotypes of APE1 -141T/G are separately T/T (C1), T/G (C2) and G/G (C3).
Genetic polymorphisms and senile cataract

IL). Differences between the means of 2 continuous variables were evaluated by Student’s t test. The chi-square test ($\chi^2$ test) was used to compare the gender distribution, to test the association between the genotypes and alleles in relation to the cases and controls and to test for deviation of genotype distribution from the Hardy-Weinberg equilibrium (HWE). The odds ratio (OR) and their 95% confidence intervals (CI) were calculated to estimate the strength of the association between polymorphism genotypes and alleles in patients and controls. $P < 0.05$ was used as the criterion of significance.

**Results**

As shown in Table 1, the study included 402 cases with age-related cataract and 813 healthy controls. Although the groups were statistically different with respect to sex ($P < 0.05$), all results were adjusted for sex by binary logistic regression.

The results of PCR and DNA sequencing are shown separately in Figures 1 and 2. The frequencies of the genotypes and alleles of OGG1 Ser326Cys, XRCC1 Arg399Gln and APE1-141T/G polymorphisms in the case and control groups are shown in Table 3. For all polymorphisms, the more common allele was considered as the reference genotype, whereas the less common allele was examined as the variant. The distributions of OGG1 Ser326Cys, XRCC1 Arg399Gln and APE1-141T/G genotypes were in accordance with the Hardy-Weinberg equilibrium among the patients ($P=0.71$ $P=0.30$ $P=0.88$, respectively) and the controls ($P=0.42$ $P=0.055$ $P=0.37$, respectively).

The analysis of the polymorphisms located at APE1-141T/G in cataract group showed that 156 (38.8%) were homozygous for the T/T genotype, 56 (13.9%) were variant homozygous for the G/G genotype, and 190 (47.3%) were heterozygous for the T/G genotype. There was a significant difference between the case and control groups in the APE1-141 G/G genotype ($P=0.002$). This difference still existed after adjusting for age and gender ($P*=0.003$). The APE1-141 T/T genotype frequency was sig-

### Table 3. Polymorphisms in DNA repair genes OGG1 Ser326Cys, XRCC1 Arg399Gln and APE1-141T/G and risk of cataract development

<table>
<thead>
<tr>
<th>Genotype/Allele</th>
<th>Control, n (%)</th>
<th>Patients, n (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P*</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGG1 Ser326</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>813</td>
<td>402</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/G</td>
<td>326 (40.0)</td>
<td>60 (15.0)</td>
<td>0.67</td>
<td>0.91 (0.62-1.31)</td>
<td>0.44</td>
<td>0.93 (0.63-1.35)</td>
</tr>
<tr>
<td>C allele</td>
<td>1142 (0.53)</td>
<td>510 (0.41)</td>
<td>0.29</td>
<td>1.10 (0.89-1.36)</td>
<td>0.31</td>
<td>1.10 (0.88-1.35)</td>
</tr>
<tr>
<td>XRCC1 Arg399</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>432 (53.1)</td>
<td>222 (55.2)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>360 (43.6)</td>
<td>144 (35.6)</td>
<td>0.64</td>
<td>0.94 (0.73-1.21)</td>
<td>0.52</td>
<td>0.92 (0.71-1.19)</td>
</tr>
<tr>
<td>A allele</td>
<td>1008 (0.42)</td>
<td>484 (12.0)</td>
<td>0.29</td>
<td>1.10 (0.89-1.36)</td>
<td>0.31</td>
<td>1.10 (0.88-1.35)</td>
</tr>
<tr>
<td>APE1-141</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>228 (28.0)</td>
<td>156 (38.8)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/G</td>
<td>417 (51.3)</td>
<td>190 (47.3)</td>
<td>0.023</td>
<td>0.67 (0.47-0.95)</td>
<td>0.028</td>
<td>0.68 (0.47-0.96)</td>
</tr>
<tr>
<td>G allele</td>
<td>1073 (0.42)</td>
<td>494 (12.4)</td>
<td>0.29</td>
<td>1.10 (0.89-1.36)</td>
<td>0.31</td>
<td>1.10 (0.88-1.35)</td>
</tr>
</tbody>
</table>

Ref: Reference; *: Adjusted for age by binary logistic regression.
Genetic polymorphisms and senile cataract

significantly higher in cataract patients, while the G/G genotype and G allele frequency in patients was significantly lower than that in controls (P < 0.05). The statistical analysis revealed a possible protective effect of the APE1-141 G/G genotype (OR, 0.49; 95% CI, 0.31-0.77) in controls and a possible deleterious effect of APE1-141 T-allele in the development of cataract. In OGG1 Ser326Cys and XRCC1 Arg399Gln polymorphisms, no significant differences were found in frequencies of the variant homozygous in patients compared with controls.

Discussion

We analyzed the association of genetic polymorphisms in 8-oxoguanine glycosylase-1 (OGG1), X-ray repair cross-complementing-1 (XRCC1) and AP endonuclease-1 (APE1) genes with the risk of age related cataract in our study and found significant difference between the case and control groups in the APE1-141 G/G genotype (P=0.002). Meanwhile, the frequency of APE1-141 T-allele was significantly higher in cataract patients, while the G-allele frequency in patients was significantly lower in controls. The APE1-141 G/G genotype (OR, 0.49; 95% CI, 0.31-0.77) seems to have a protective role against cataract and the T-allele seems to have a deleterious role in the development of cataract.

ROS-induced cell damage in lens including oxidation of protein, DNA damage and lipid peroxidation may all relate to the formation of cataracts [24, 25]. ROS can cause DNA strand breaking, base modifications and oxidation of guanine residues into 8-hydroxy 2-deoxyguanosine (8-OHdG), which serves as a sensitive biomarker of oxidative DNA damage [26]. Studies found that the level of 8-OHdG in leukocyte DNA of the patients with cataract was higher than that of the control group. Moreover, there was a correlation between the levels of 8-OHdG in leukocyte DNA and plasmic malondialdehyde (MDA)-an indicator of lipid peroxides [27]. Some researches also found that DNA damage in lens epithelial cells (LECs) and peripheral blood lymphocytes increased in ARC [28].

Genome instability caused by the great variety of DNA-damaging agents would be an overwhelming problem for cells and organisms if it were not for DNA repair [13]. Base excision repair (BER) is of great importance in DNA excision repair pathway, and OGG1, APE1 and XRCC1 are the key enzymes in base excision repair pathway [29]. The OGG1 gene is located on chromosome 3p26.2; it is responsible for the excision of 8-oxoguanine in human body, a major base lesion produced by reactive oxygen species. Oh8G can cause a G:C to T:A conversion in DNA and further contribute to the developing of diseases [30]. XRCC1 gene locates on chromosome 19q13.2. The protein encoded by this gene is involved in the efficient repair of DNA single-strand breaks induced by exposure to ionizing radiation and alkylating agents. It interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase to participate in the base excision repair pathway. Apurinic/apyrimidinic (AP) sites occur frequently in DNA molecules by spontaneous hydrolysis, by DNA damaging agents or by DNA glycosylases that remove specific abnormal bases. Abasic sites are common DNA lesions with cytotoxic and mutagenic potential offset mainly by base excision repair (BER), with AP site removal initiated by AP endonucleases [31]. The APE1 gene, which encodes a majority of AP endonucleases in human body, is located on chromosome 14q11.2. The protein fills a vital role in handling endogenous DNA damage that, when left unrepaired, triggers apoptotic cell death [32].

In XRCC1 and OGG1 genes, XRCC1 Arg399Gln and OGG1 Ser326Cys are the most commonly and frequently studied SNPs. Prior to this, there have been some researches exploring the relationship between polymorphism of XRCC1 gene and risk of senile cataract, but the conclusion is not consistent. Luo YF found there was a significant difference between the case and control groups in the XRCC1G399A genotype. Moreover, individuals who carried at least one A-allele (G/A or A/A) had a 1.68-fold increased risk of developing age-related cataract compared with those who carried the G/G wild type genotype [33]. Yet similar studies of populations in India and Turkey did not find any genotypic differences in XRCC1G399A between cases and controls [34, 35]. APE1 -141T/G is a commonly studied SNP of APE1 located in the promoter region. Besides its role in DNA repair, APE1/Ref-1 is also known as a transcriptional coactivator for numerous transcription factors, such as AP-1, NF-κB, and p53, which are...
involved in cancer promotion and progression by regulating the expression of their target genes. Lu j discovered that this genetic polymorphism may alter APE1/Ref-1 expression by affecting the binding affinity of Oct-1 to the promoter, thus playing a causal role in disease susceptibility [36]. Association between neoplastic disease and APE1-141T/G has been studied frequently, showing that APE1 -141 G/G genotype and G allele may reduce the risk of cancer, presenting a protective effect on resisting diseases [37, 38]. To the best of our knowledge, there is no study focusing on the association between APE1-141T/G and senile cataract before.

Conclusively, we found that APE1-141 G/G genotype seemed to play a role in blocking the formation of cataracts, which was in line with previous findings on cancer diseases that APE1-141 G-allele may have a protective effect in preventing diseases. The present data support the significances of DNA repair gene polymorphisms and DNA repair genes as potential pharmacologic targets to promote DNA repair and to maintain genome stability. Studies of different ethnics and nationalities, however, are still needed to confirm the achieved findings and to examine more completely the possible relationship between DNA repair gene polymorphisms and cataract.

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Disclosure of Conflict of Interest

None.

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Genetic polymorphisms and senile cataract
