AGE (2013) 35:2435–2444
DOI 10.1007/s11357-013-9512-4

# Polymorphisms of the WRN gene and DNA damage of peripheral lymphocytes in age-related cataract in a Han Chinese population

**Shengqun Jiang · Nan Hu · Jing Zhou · Junfang Zhang · Ruifang Gao · Jianyan Hu · Huaijin Guan**

Received: 7 July 2012 / Accepted: 9 January 2013 / Published online: 20 January 2013


**Abstract** Werner syndrome is caused by mutations in the DNA repair Werner helicase (WRN) gene and characterized by accelerated aging including cataracts. Age-related cataract (ARC) cases ($N$=504) and controls ($N$=244) were recruited from a population-based study to evaluate the association of single-nucleotide polymorphisms (SNPs) of WRN and another DNA repair gene (human 8-oxoguanine DNA $N$-glycosylase 1) with ARC. Among the five SNPs tested, only WRN rs1346044 was found to be significantly associated between cases and controls before multiple-testing adjustment. The minor C allele of rs1346044 was associated with ARC with an odds ratio (OR) of 0.66, suggesting a protective role of the C allele for developing ARC. The stratification analysis on the subtypes of ARC showed that rs1346044 was significantly associated with cortical cataract, but not with nuclear, posterior subcapsular, and mixed types after multiple-testing adjustment (OR=0.51, $p$<0.01). The genetic model analysis showed that the results fit the dominant model (OR=0.44, $p$<0.001). The comet assay used to assess the extent of DNA damage in peripheral lymphocytes of ARC cases found that the DNA damage in lymphocytes from patients with CC genotype was significantly less than that in patients with TT genotype. We concluded that the C allele of rs1346044, a non-synonymous SNP resulting in the conversion of Cys to Arg at amino acid position 1367 of WRN, alters susceptibility to ARC, especially the cortical type of the disease, in the Han Chinese. The underlying mechanism of its protective role might be related to the improved DNA repair function.

**Keywords** Age-related cataract (ARC) · WRN · Single-nucleotide polymorphism · Comet assay · DNA damage · Cortical cataract

S. Jiang · N. Hu · J. Zhou · J. Zhang · R. Gao · J. Hu · H. Guan (✉)
Department of Ophthalmology, Affiliated Hospital of Nantong University,
20 Xisi Road,
Nantong, Jiangsu, China
e-mail: gtnantongeye@gmail.com

## Introduction

Age-related cataract (ARC) is one of the dominant causes of visual impairment in the elderly (Di Stefano 2001). ARC is a complex disease with multiple genetic and environmental risk components, but its etiology is not fully understood (Shiels and Hejtmancik 2007; Hammond et al. 2000). The disease is subtyped cortical, nuclear, posterior subcapsular, or mixed type according to the lesion site within the lens (Klein et al. 1992). It has been suggested that the subtypes of cataract are associated with different risk factors (Lewis et al. 2004). Studies on mono- and dizygotic twins have demonstrated that genetic factors play an important role in the etiology of ARC (Hammond et al. 2001, 2000). Multiple genetic loci have been reported to be associated with ARC (Hejtmancik and Kantorow 2004).

Involvement of oxidative stress appears to be one of the critical events in ARC pathogenesis (Ates et al. 2010). DNA–DNA cross-linking and DNA breaks can be formed in cells treated with both UV-B irradiation and $H_2O_2$ (Kleiman et al. 1990b). The percentage of DNA single-strand breaks in lens epithelial cells from patients undergoing cataract surgery were substantially higher than those from non-cataractous human eye bank lenses of similar ages (Kleiman and Spector 1993). DNA copy number variation within glutathione S-transferase, an enzyme functioning in phase II biotransformation of oxidized toxic compounds, was shown to be linked with ARC, particularly in the Asian population (Guven et al. 2007; Sun et al. 2010; Zhou et al. 2010). Recently, studies have reported that single-nucleotide polymorphism (SNPs) and expression of genes involved in oxidative DNA damage repair are associated with ARC (Padma et al. 2011; Luo et al. 2011).

Oxidative stress induces various types of DNA damage that contribute significantly to aging and age-related disorders (Martins et al. 1991). Thus, the lens must have systems in place to efficiently repair oxidative DNA damage. There are numerous DNA repair genes, including CSB (Mahmoud et al. 2002), XPD (Takayama et al. 1996), Werner helicase (WRN) (Singh et al. 2009), and human 8-oxoguanine DNA *N*-glycosylase 1 (hOGG1) (Yang et al. 2006). The WRN gene plays an important role in aging and is known to function extensively in the DNA repair process (Singh et al. 2009). WRN gene encodes a nuclear protein of the RecQ family. The protein is of 1,432 amino acids and possesses both 3′→5′ DNA helicase and 3′→5′ DNA exonuclease activities. These biochemical functions are known to have roles in DNA replication, repair of DNA damage, gene transcription, and telomere maintenance (Singh et al. 2009). WRN disruption causes Werner's syndrome (WS), an autosomal recessive segmental progeroid syndrome that results in accelerated aging and affects multiple organs and tissues (Gee et al. 2002). Apart from systematic symptoms such as neuropathy, skin atrophy, regional loss of subcutaneous fat, loss and graying of hair, and development of multiple cancers, most WS patients develop bilateral ocular cataract when they are 20 years old (Epstein et al. 1966; Goto 1997).

Human 8-hydroxyguanine (8-oxo-Gua) DNA glycosylase is the primary enzyme for the repair of 8-oxo-dG in human cells (Yang et al. 2006). The presence of 8-oxo-dG in DNA may lead to mutagenesis, and the level of 8-oxo-dG in cell and body fluid is commonly used as a biomarker of oxidative DNA damage (Boiteux and Radicella 2000). The amount of 8-oxo-dG in DNA was reported to increase significantly with age in the lenses of rats (Zhang et al. 2010).

Because ARC occurs in the elderly and mutations in the WRN gene lead to accelerated aging and lens opacity, we hypothesized that polymorphisms in the WRN gene might confer ARC susceptibility. We also included the hOGG1 gene due to its central role in the repair of oxidatively damaged DNA. In this study, we first tested the association of the polymorphisms of the above genes with ARC. We then examined the correlation between DNA repair capability and WRN genotypes.

## Materials and methods

### Study participants

The research was approved by the Ethics Committee of Affiliated Hospital of Nantong University and followed the tenets of the Declaration of Helsinki. Each participant was fully informed of the purpose of and procedures involved in the study and signed informed consent form. The study participants were recruited from a population-based survey in Nantong, Jiangsu, China. The covered area has a stable and ethnically homogenous population. The participants were unrelated and self-identified Han Chinese (at least all four grandparents were ethnically Han Chinese). Only the ARC cases who met the including criteria were included in this study. The control group was from the same population.

ARC was defined as the appearance of the clinical sign of cataract in one or both eyes in a person older than 50 years. The clinical sign was characterized as opacity of the lens resulting in visual acuity of 20/40 or worse in the cataract eye, as measured by the ETDRS chart. All patients with ARC and controls were subjected to a full ophthalmic examination, including visual acuity measurement, lens examination in transient, and side illumination using a slit-lamp biomicroscope after mydriasis and ophthalmoscopic examination. The opacity of the lens was classified using the lens opacities classification system II (Chylack et al. 1989). We only included the cases in

≥ NII of nuclear cataract, in ≥ CII of cortical cataract, and in ≥ PII of posterior subcapsular cataract and excluded the cases with different cataract types between two eyes and with aphakia. The patients with systemic diseases, a history of ocular trauma, secondary cataract due to glaucoma, age-related macular degeneration, uveitis, diabetes, or other known causes were excluded. The control included 244 unrelated individuals with transparent lenses and a visual acuity better than 20/25 in both eyes. The individuals with other major eye diseases such as dislocated lenses, glaucoma, age-related macular degeneration, diabetic retinopathy, uveitis, or systemic diseases such as diabetes, kidney diseases, and cancers were excluded from the control group. The demographic information for the study participants is summarized in Table 1.

## SNP selection and genotyping

We focused on non-synonymous SNPs by searching NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp) and found only four in WRN (rs1346044, rs1801195, rs2230009, and rs3087414) and one in hOGG1 (rs1052133) with the minor allele frequency larger than 5 %. Even though there are different opinions about the importance of rare and common alleles in disease susceptibility, we adopted the notion that "common alleles cause common diseases." We deselected rs3087414 from WRN because it is within the same domain as rs1801195. We added hOGG1 rs125701 based on a silicon analysis which indicates that the SNP is located in a transcription factor-binding site (SNP function prediction, http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm). All of the SNPs examined as well as their associated genes are listed in Table 2.

DNA was extracted from leucocytes in venous blood by proteinase K digestion followed by phenol–chloroform separation. The DNA samples were genotyped by the pre-designed TaqMan SNP assays (Applied Biosystems, Foster City, CA, USA) using a 7500 real-time PCR system.

## Comet assay

Comet assay (also known as the single-cell gel electrophoresis assay) is a sensitive technique for the detection of DNA damage at the level of an individual cell. We randomly performed the assay on 114 cortical ARC cases.

The peripheral lymphocytes from whole blood in EDTA anticoagulation tube were isolated and suspended in phosphate-buffered saline (PBS) at $1\times10^4$ cell/mL. The freshly prepared cell suspension (250 cells in 100 μL of 0.75 % low-melting-point agarose in PBS) was spread onto microscope slides precoated with 0.5 % normal-melting-point agarose. The cells were then lysed for 2 h at 4 °C in a lysis buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1 %Triton X-100, and 10 mM Tris, pH10, followed by electrophoresis (20 V, 200 mA) in the buffer consisting of 300 mM NaOH and 1 Mm EDTA for 20 min. The slides were then washed in deionized water and stained with 2 μg/mL of ethidium bromide. To prevent additional DNA damage, all the steps described above were performed under dimmed light or in the dark (Wozniak et al. 2009).

The comet images were observed at ×400 magnification under a fluorescence microscope (Leica) controlled by the image analysis system CASP, a program available on the web (www.casp.of.pl).

**Table 1** Demographics of study participants

| Group | *N* | Gender | | Age (years) | |
|---|---|---|---|---|---|
| | | Male (*N*, %) | Female (*N*, %) | Means | Range |
| Control | 244 | 115 (47.1) | 129 (52.9) | 60.2±5.7 | 50–82 |
| ARC case | 504 | 188 (37.3)* | 316 (62.7)* | 70.9±8.2* | 50–90 |
| Cortical | 229 | 80 (34.9) | 149 (65.1) | 69.9±7.5 | 50–90 |
| Nuclear | 102 | 43 (42.2) | 59 (57.8) | 71.6±7.8 | 51–87 |
| Posterior subcapsular | 46 | 14 (30.4) | 32 (69.6) | 71.8±9.1 | 51–87 |
| Mixed | 127 | 50 (39.4) | 77 (60.6) | 71.8±8.1 | 50–89 |

*$p<0.05$, in the comparisons between the cases and the controls

**Table 2** SNPs being tested and assay information from Applied Biosystems

| Pathway | Gene | SNP ID | SNP type |
|---|---|---|---|
| DSBR | WRN | rs1346044 | C/T, exon_34 R1367C |
| | | rs1801195 | T/G,, exon_26 F1074L |
| | | rs2230009 | A/G, exon_4 I114V |
| BER | hOGG1 | rs125701 | A/G, 5′-UTR (TFBS) |
| | | rs1052133 | A/G, exon_7 S326C |

Twenty images were randomly selected from each sample. The percentage of DNA in the tail of comets and the olive tail moment (OTM) were measured. Amount of DNA in the comet tail was counted by the sum of intensities of pixels in the tail. OTM was calculated by (in percent of DNA in the tail) × (tail length). Higher percentage of DNA in the tail of comets and OTM indicate severer DNA damage.

### Statistical analysis

The chi-square test was performed using Stata 8.0 (Stata Corp., College Station, TX, USA) to compare the genotype distribution of SNPs between cases and controls and to estimates odds ratio (OR) and 95 % confidence interval (95 % CI). Hardy–Weinberg equilibrium (HWE) tests were performed separately for controls and case groups by using chi-square analysis. Logistic regression was carried out to adjust for age and gender, and Bonferroni correction was performed for any association found in the initial analysis. The values of the comet assay in this study were expressed as mean ± SD. The ANOVA was used to compare the differences of the comet assay parameters between the genotypes. $p<0.05$ was considered to be statistically significant.

## Results

### Data quality

The call rates of all SNP genotyping were >98 %. The call accuracy (consistency of duplicate wells) of 10 % DNA samples was 100 %. Significant deviation from HWE was only found in the ARC group in WRN rs1346044 and rs1801195 (Table 3).

### Genetic analysis

Among the five SNPs tested, only the distribution of WRN rs1346044 was found to be significantly different between cases and controls before adjustment (Table 3). The minor C allele of rs1346044 was associated with ARC with an OR of 0.66 and 95 % CI of 0.47–0.92, suggesting that the minor allele might play a protective role for developing ARC. Although the association of combined types of ARC with rs1346044 did not meet the significant level after Bonferroni correction, stratification analysis on the subtypes of ARC showed that rs1346044 was significantly associated only with cortical cataract after Bonferroni correction (OR=0.51, 95 % CI 0.33–0.80, $p<0.01$) (Table 4). The genetic model analysis showed that the results fit the dominant model. As such, individuals carrying at least one C allele had a reduced risk of developing cortical ARC (OR=0.44, 95 % CI 0.27–0.71, $p<0.001$) (Table 4). No association was detected between WRN rs1346044 and the other three subtypes of ARC (Table 4).

Although there was no association between WRN rs2230009 and combined types of ARC (Table 3), the analysis on the subtypes of ARC showed a suggestive association of WRN rs2230009 with the posterior subcapsular type (Table 5). The A allele of rs2230009 imposed a risk to the development of this subtype with OR of 4.8 ($p<0.05$ before Bonferroni correction). We did not detect any association between ARC and control in the two SNPs of hOGG1 (Table 3).

### Supplemental analysis

Because there was a 10-year difference in average ages between cases and controls, and logistical regression alone might be insufficient to account for this confounding factor, we performed a supplemental analysis on rs1346044. We equilibrated the average ages of cases and controls and selected the lower age quartile in the cortical ARC group ($N=164$) and the higher age quartile in the control group ($N=157$) for the analysis, which brought an average age to 64.8±5.8 in cases and 63.7±3.5 years in controls ($p>0.05$). The results showed that the rs1346044 association with cortical ARC followed a dominant model (OR=0.34, 95 % CI 0.16–0.75, $p<0.01$) and was even stronger than the result with all ages included (OR=

**Table 3** SNP allele/genotype distribution in ARC and control groups

| Gene/SNP | allele | Case *N* (%) | Control *N* (%) | *p*/Pa | OR (95 % CI) | *p* values of HWE (Control/case) |
|---|---|---|---|---|---|---|
| WRN/rs1346044 | T | 917 (91.0) | 424 (86.9) | | | 0.2/0.0079 |
| | C | 91 (9.0) | 64 (13.1) | 0.018/0.09 | 0.66 (0.47–0.92) | |
| | TT | 422 (83.7) | 185 (75.8) | | | |
| | CT | 73 (14.5) | 54 (22.1) | | | |
| | CC | 9 (1.8) | 5 (2.1) | 0.78[a] | 0.87 (0.29–2.62) | |
| | CT+CC | 82 (16.3) | 59 (24.2) | 0.013[b]/0.065 | 0.61 (0.42–0.89) | |
| WRN/rs1801195 | G | 368 (36.5) | 184 (37.7) | | | 0.13/0.04 |
| | T | 640 (63.5) | 304 (62.3) | 0.69 | 1.05 (0.84–1.32) | |
| | GG | 78 (15.5) | 36 (14.8) | | | |
| | GT | 212 (42.1) | 112 (45.9) | | | |
| | TT | 214 (42.4) | 96 (39.3) | 0.43[a] | 1.14 (0.83–1.55) | |
| | GT+TT | 426 | 208 | 0.83[b] | 0.95 (0.62–1.45) | |
| WRN/rs2230009 | G | 987 (97.9) | 482 (98.8) | | | 0.85/0.63 |
| | A | 21 (2.1) | 6 (1.2) | 0.30 | 1.71 (0.69–4.26) | |
| | GG | 483 (95.8) | 238 (97.5) | | | |
| | GA | 21 (4.2) | 6 (2.5) | | | |
| | AA | 0 (0.0) | 0 (0.0) | | | |
| | GA+AA | 21 | 6 | 0.30[b] | 1.73 (0.69–4.33) | |
| hOGG1/rs1052133 | C | 366 (36.3) | 183 (37.5) | | | 2.41/0.28 |
| | G | 642 (63.7) | 305 (62.5) | 0.69 | 1.05 (0.84–1.32) | |
| | CC | 72 (14.3) | 40 (16.4) | | | |
| | CG | 222 (44.0) | 103 (42.2) | | | |
| | GG | 210 (41.7) | 101 (41.4) | 1.000[a] | 1.01 (0.74–1.38) | |
| | CG+GG | 432 | 204 | 0.45[b] | 0.18 (0.77–1.79) | |
| hOGG1/rs125701 | G | 968 (96.0) | 466 (95.5) | | | 0.54/0.81 |
| | A | 40 (4.0) | 22 (4.5) | 0.68 | 0.88 (0.51–1.49) | |
| | GG | 465 (92.3) | 222 (91.0) | | | |
| | GA | 38 (7.5) | 22 (9.0) | | | |
| | AA | 1 (0.2) | 0 | 1.000[a] | 1.46 (0.06–35.92) | |
| | GA+AA | 39 | 22 | 0.57[b] | 0.85 (0.49–1.46) | |

*Pa p* value after Bonferroni correction

[a] In comparison with the other two genotypes in combined

[b] In comparison with the wild-type genotype

0.44, 95 % CI 0.27–0.71, $p<0.001$) (Table 6). The association remained significant after Bonferroni correction (Table 6).

## Genotype–phenotype correlation analysis

To further analyze the impact of the genotypes on the phenotypic appearance of ARC, we did correlation analysis of the level of cortical opacity and rs1346044 genotypes. The results show that TT carriers only had a slightly higher level of cortical opacity than the individuals with at least one C allele, but the difference did not reach the statistical significance ($p>0.05$) (data not shown).

## Comet assay on DNA damage

Individuals homozygous for the C allele of rs1346044 had less DNA damage than TT carriers in their peripheral lymphocytes as shown in tail of comet (tail

**Table 4** Association between WRN rs1346044 and the subtypes of age-related cataract

| Allele | Control | Subtypes of ARC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* (%) | Cortical | | | Nuclear | | | Posterior subcapsular | | | Mixed | | |
| | *N*=244 | *N* (%) | *p*/Pa | OR | *N* (%) | *p* | OR | *N* (%) | *p* | OR | *N* (%) | *p* | OR |
| T | 424 (86.9) | 425 (92.8) | | | 188 (92.2) | | | 82 (89.1) | | | 222 (87.4) | | |
| C | 64 (13.1) | 33 (7.2) | 0.004/0.02 | 0.51 (0.33–0.80) | 16 (7.8) | 0.05 | 0.56 (0.32–1.00) | 10 (10.9) | 0.61 | 0.81 (0.40–1.64) | 32 (12.6) | 0.91 | 0.96 (0.61–1.50) |
| TT | 185 (75.8) | 201 (87.8) | | | 87 (85.3) | | | 38 (82.6) | | | 96 (75.6) | | |
| TC+CC | 59 (24.2) | 28 (12.2) | 0.0009/0.005 | 0.44 (0.27–0.71) | 15 (14.7) | 0.06 | 0.54 (0.29–1.01) | 8 (17.4) | 0.45 | 0.66 (0.29–1.49) | 31 (24.4) | 1.000 | 1.01 (0.61–1.67) |

The association of C allele with the cortical type of ARC was significant after logistic regression adjustment against age and gender, $p<0.01$

*Pa p* value after Bonferroni correction

**Table 5** Association between WRN rs2230009 and the subtypes of age-related cataract

| Allele | | Subtypes of ARC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Cortical | | | Nuclear | | | Posterior subcapsular | | | Mixed | | |
| | *N* (%) | *N* (%) | *p* | OR | *N* (%) | *p* | OR | *N* (%) | *p*/Pa | OR | *N* (%) | *p* | OR |
| G | 482 (98.8) | 450 (98.3) | | | 200 (98.0) | | | 87 (94.6) | | | 250 (98.4) | | |
| A | 6 (1.2) | 8 (1.7) | 0.60 | 1.43 (0.49–4.15) | 4 (2.0) | 0.49 | 1.61 (0.45–5.76) | 5 (5.4) | 0.019/0.095 | 4.62 (1.38–15.47) | 4 (1.6) | 0.74 | 1.29 (0.36–4.60) |
| GG | 238 (97.5) | 221 (96.5) | | | 98 (96.1) | | | 41 (89.1) | | | 123 (96.9) | | |
| GA+AA | 6 (2.5) | 8 (3.5) | 0.59 | 1.44 (0.49–4.21) | 4 (3.9) | 0.49 | 1.62 (0.45–5.87) | 5 (10.9) | 0.018/0.09 | 4.84 (1.41–16.59) | 4 (3.1) | 0.74 | 1.29 (0.36–4.66) |

*Pa p* value after Bonferroni correction

**Table 6** Association between WRN rs1346044 and cortical ARC in age-matched cases and control

| Allele | Control (%) | Subtypes of ARC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cortical | | | Nuclear | | | Posterior subcapsular | | | Mixed | | |
| | *N*=157 | *N*=91 | *p*/Pa | OR | *N*=36 | *p* | OR | *N*=20 | *p* | OR | *N*=17 | *p* | OR |
| T | 272 (86.6) | 172 (94.5) | | | 68 (94.4) | | | 36 (90.0) | | | 28 (82.4) | | |
| C | 42 (13.4) | 10 (5.5) | 0.006/0.03 | 0.38 (0.18–0.77) | 4 (5.6) | 0.07 | 0.38 (0.13–1.10) | 4 (10.0) | 0.80 | 0.72 (0.24–2.13) | 6 (17.6) | 0.44 | 1.39 (0.54–3.55) |
| TT | 119 (75.8) | 82 (90.1) | | | 32 (88.9) | | | 16 (80.0) | | | 11 (64.7) | | |
| TC+CC | 38 | 9 | 0.007/0.035 | 0.34 (0.16–0.75) | 4 (11.1) | 0.12 | 0.39 (0.13–1.18) | 4 (20.0) | 0.79 | 0.78 (0.25–2.49) | 6 | 0.38 | 1.71 (0.59–4.93) |

*Pa p* value after Bonferroni correction

DNA%) and OTM ($p<0.01$) (Table 7). We did the analysis of the comet assay results according to the dominant model, e.g., combination of TC and CC vs TT. Even though TC+CC had less DNA damage than that of TT, the difference was not statistically significant. This result indicated that the functional consequence of an impact allele does not always follow the pattern of genetic model.

## Discussion

We examined possible associations of five polymorphisms in two candidate DNA repair genes with prevalence of ARC in a Han Chinese population. The results showed that WRN rs1346044 is associated with ARC and that the C allele is protective against ARC, particularly against the cortical type of the disease. The model analysis showed the association to be present in a dominant mode. The carriers of homozygous protective allele(s) had milder DNA damage in their peripheral lymphocytes. This suggests that the SNP might play a role in enhanced DNA repair functionality.

Previous epidemiological studies have reported association of others diseases, but not ARC, with WRN rs1346044, which is a non-synonymous variation that causes a conversion of Cys to Arg at amino acid position 1367 (Bohr et al. 2004; Hirai et al. 2005; Payao et al. 2004; Smith et al. 2005; Ye et al. 1997; Castro et al. 2000, 1999; Kuningas et al. 2006; Morita et al. 1999; Ogata et al. 2001). The association of WRN rs1346044 with myocardial infarction was first reported in a Japanese population (Ye et al. 1997). The authors found that patients homozygous for Cys were at a nearly threefold higher risk of myocardial infarction than the general population and suggested a

**Table 7** The correlation of WRN rs1346044 genotypes and DNA damage measured using comet assay in peripheral lymphocytes from cortical ARC patients

| WRN rs1346044 | *N* | Age (mean ± SD) | Tail DNA% ± SD | OTM ± SD |
|---|---|---|---|---|
| TT | 99 | 69.2±7.6 | 23.73±0.34 | 7.34±0.18 |
| TC | 12 | 72.1±8.9 | 22.32±0.73 | 7.00±0.51 |
| CC | 3 | 73.7±7.0 | 17.74±0.51* | 5.15±0.18* |

*$p<0.01$, in comparison of TT and TC combined

protective role for the Arg variation. Other Japanese studies found that the minor allele of rs1346044 may be associated with a lower risk of type 2 diabetes mellitus and bone and soft tissue sarcomas in a dominant model (Hirai et al. 2005; Nakayama et al. 2008).

We noticed that an Israeli study reported the lack of association of WRN rs1346044 with senile cataract (Ehrenberg et al. 2010). However, the study only included 81 cases and potentially had insufficient power to produce conclusive results. It might also suggest that the association of rs1346044 with ARC susceptibility is ethnic group-dependent. We have not found a report of ocular expression of WRN in the human eye by PubMed search. However, UniGene database (http://www.ncbi.nlm.nih.gov/unigene) lists WRN to be expressed in the human eye, and WRN expression has been detected in ARPE19 cells, a retinal pigment epithelium cell line, and is believed to play a role in mitochondria function of ARPE19 (Miceli and Jazwinski 2005). Additionally, WRN is one of the key components for maintaining homeostasis and DNA repair in fibroblasts (Rossi et al. 2010).

The WRN Cys1367Arg is located only three amino acids away from the nuclear localization signal motif which contains multiple Arg and Lys residues. An additional Arg residue near this domain could therefore enhance the strength of translocation of WRN from the cytoplasm to the nucleus (Matsumoto et al. 1998, 1997), thereby enhancing DNA repair capacity. The protein with 1367Arg also exhibits higher helicase/exonuclease activities relative to the wild-type WRN (Kamath-Loeb et al. 2004).

Oxidative stress induces various types of DNA damage in lens which causes cataract (Ehrenberg et al. 2010; Kleiman et al. 1990a). The WRN gene is known to function extensively in repair of damaged DNA, playing a vital role in repairing double-strand breaks (Yan et al. 2005). It is known that WRN with the C allele had stronger DNA repair capacity (Matsumoto et al. 1998, 1997; Kamath-Loeb et al. 2004). Our results indicated that the tail DNA% and OTM values in patients with homozygous C allele were smaller than those in patients with T allele. Those results were in line with a study that found elevated levels of 8-oxo-dG, a marker of oxidative DNA damage, and MDA, a marker for lipid peroxidation, in the leukocytes of patients with cataract (Ates et al. 2010).

The stronger association with cortical ARC than with other types of ARC might come from different genetic contributions among subtypes. Heritability for cortical cataract in female twins was estimated to be as high as 58 %, while only 11 % of total attribution was accounted for by age (Heiba et al. 1995). In segregation analysis in the Beaver Dam Eye Study, a single major gene accounted for 58 % of the variability of age- and gender-adjusted measures of cortical cataract (Hammond et al. 2001). After adjustment for confounding factors, cortical, but not posterior subcapsular, cataract showed significant familial aggregation in an older population (Congdon et al. 2005).

In conclusion, we found that a functional variation of the gene responsible for Werner syndrome confers a protective role in the development of ARC, particularly in the cortical type of the disease in Han Chinese. The underlying mechanism of its protective role might be related to the improved cellular DNA repair functionality.

**Acknowledgments** This research was supported by the National Natural Science Foundation of China (no. 81070718), the 333 Project of Jiangsu Province (no. BRA2010173), and the Nantong Municipal Special Project of Major Scientific and Technologic Innovation (no. XA2009001-8).

## References

Ates O, Alp HH, Kocer I, Baykal O, Salman IA (2010) Oxidative DNA damage in patients with cataract. Acta Ophthalmol 88(8):891–895

Bohr VA, Metter EJ, Harrigan JA, von Kobbe C, Liu JL, Gray MD, Majumdar A, Wilson DM 3rd, Seidman MM (2004) Werner syndrome protein 1367 variants and disposition towards coronary artery disease in Caucasian patients. Mech Ageing Dev 125(7):491–496

Boiteux S, Radicella JP (2000) The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 377(1):1–8

Castro E, Ogburn CE, Hunt KE, Tilvis R, Louhija J, Penttinen R, Erkkola R, Panduro A, Riestra R, Piussan C, Deeb SS, Wang L, Edland SD, Martin GM, Oshima J (1999) Polymorphisms at the Werner locus: I. Newly identified polymorphisms, ethnic variability of 1367Cys/Arg, and its stability in a population of Finnish centenarians. Am J Med Genet 82(5):399–403

Castro E, Edland SD, Lee L, Ogburn CE, Deeb SS, Brown G, Panduro A, Riestra R, Tilvis R, Louhija J, Penttinen R, Erkkola R, Wang L, Martin GM, Oshima J (2000) Polymorphisms at the Werner locus: II. 1074Leu/Phe, 1367Cys/Arg, longevity, and atherosclerosis. Am J Med Genet 95 (4):374–380

Chylack LT Jr, Leske MC, McCarthy D, Khu P, Kashiwagi T, Sperduto R (1989) Lens opacities classification system II (LOCS II). Arch Ophthalmol 107(7):991–997

Congdon N, Broman KW, Lai H, Munoz B, Bowie H, Gilbert D, Wojciechowski R, West SK (2005) Cortical, but not posterior subcapsular, cataract shows significant familial aggregation in an older population after adjustment for possible shared environmental factors. Ophthalmology 112(1):73–77

Di Stefano AF (2001) VISION 2020: the right to sight. A global initiative for the elimination of avoidable blindness. Optometry 72(10):619–622

Ehrenberg M, Dratviman-Storobinsky O, Avraham-Lubin BR, Goldenberg-Cohen N (2010) Lack of association of the WRN C1367T polymorphism with senile cataract in the Israeli population. Mol Vis 16:1771–1775

Epstein CJ, Martin GM, Schultz AL, Motulsky AG (1966) Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Med (Baltimore) 45(3):177–221

Gee J, Ding Q, Keller JN (2002) Analysis of Werner's expression within the brain and primary neuronal culture. Brain Res 940(1–2):44–48

Goto M (1997) Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. Mech Ageing Dev 98(3):239–254

Guven M, Unal M, Sarici A, Ozaydin A, Batar B, Devranoglu K (2007) Glutathione-S-transferase M1 and T1 genetic polymorphisms and the risk of cataract development: a study in the Turkish population. Curr Eye Res 32(5):447–454

Hammond CJ, Snieder H, Spector TD, Gilbert CE (2000) Genetic and environmental factors in age-related nuclear cataracts in monozygotic and dizygotic twins. N Engl J Med 342(24):1786–1790

Hammond CJ, Duncan DD, Snieder H, de Lange M, West SK, Spector TD, Gilbert CE (2001) The heritability of age-related cortical cataract: the twin eye study. Invest Ophthalmol Vis Sci 42(3):601–605

Heiba IM, Elston RC, Klein BE, Klein R (1995) Evidence for a major gene for cortical cataract. Invest Ophthalmol Vis Sci 36(1):227–235

Hejtmancik JF, Kantorow M (2004) Molecular genetics of age-related cataract. Exp Eye Res 79(1):3–9

Hirai M, Suzuki S, Hinokio Y, Yamada T, Yoshizumi S, Suzuki C, Satoh J, Oka Y (2005) WRN gene 1367 Arg allele protects against development of type 2 diabetes mellitus. Diabetes Res Clin Pract 69(3):287–292

Kamath-Loeb AS, Welcsh P, Waite M, Adman ET, Loeb LA (2004) The enzymatic activities of the Werner syndrome protein are disabled by the amino acid polymorphism R834C. J Biol Chem 279(53):55499–55505

Kleiman NJ, Spector A (1993) DNA single strand breaks in human lens epithelial cells from patients with cataract. Curr Eye Res 12(5):423–431

Kleiman NJ, Wang RR, Spector A (1990a) Hydrogen peroxide-induced DNA damage in bovine lens epithelial cells. Mutat Res 240(1):35–45

Kleiman NJ, Wang RR, Spector A (1990b) Ultraviolet light induced DNA damage and repair in bovine lens epithelial cells. Curr Eye Res 9(12):1185–1193

Klein BE, Klein R, Linton KL (1992) Prevalence of age-related lens opacities in a population. The Beaver Dam Eye Study. Ophthalmology 99(4):546–552

Kuningas M, Slagboom PE, Westendorp RG, van Heemst D (2006) Impact of genetic variations in the WRN gene on age related pathologies and mortality. Mech Ageing Dev 127(3):307–313

Lewis A, Congdon N, Munoz B, Bowie H, Lai H, Chen P, West SK (2004) Cataract surgery and subtype in a defined, older population: the SEECAT Project. Br J Ophthalmol 88(12):1512–1517

Luo YF, Wang BB, Zhou Z, Ding XC, Hu SS, Zhou GK, Ma X, Qi YH (2011) Polymorphisms of the DNA repair genes XPD and XRCC1 and the risk of age-related cataract development in Han Chinese. Curr Eye Res 36(7):632–636

Mahmoud AA, Yousef GM, Al-Hifzi I, Diamandis EP (2002) Cockayne syndrome in three sisters with varying clinical presentation. Am J Med Genet 111(1):81–85

Martins EA, Chubatsu LS, Meneghini R (1991) Role of antioxidants in protecting cellular DNA from damage by oxidative stress. Mutat Res 250(1–2):95–101

Matsumoto T, Shimamoto A, Goto M, Furuichi Y (1997) Impaired nuclear localization of defective DNA helicases in Werner's syndrome. Nat Genet 16(4):335–336

Matsumoto T, Imamura O, Goto M, Furuichi Y (1998) Characterization of the nuclear localization signal in the DNA helicase involved in Werner's syndrome. Int J Mol Med 1(1):71–76

Miceli MV, Jazwinski SM (2005) Common and cell type-specific responses of human cells to mitochondrial dysfunction. Exp Cell Res 302(2):270–280

Morita H, Kurihara H, Sugiyama T, Hamada C, Yazaki Y (1999) A polymorphic variant C1367R of the Werner helicase gene and atherosclerotic diseases in the Japanese population. Thromb Haemost 82(1):160–161

Nakayama R, Sato Y, Masutani M, Ogino H, Nakatani F, Chuman H, Beppu Y, Morioka H, Yabe H, Hirose H, Sugimura H, Sakamoto H, Ohta T, Toyama Y, Yoshida T, Kawai A (2008) Association of a missense single nucleotide polymorphism, Cys1367Arg of the WRN gene, with the risk of bone and soft tissue sarcomas in Japan. Cancer Sci 99(2):333–339

Ogata N, Shiraki M, Hosoi T, Koshizuka Y, Nakamura K, Kawaguchi H (2001) A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J Bone Miner Metab 19(5):296–301

Padma G, Mamata M, Reddy KR, Padma T (2011) Polymorphisms in two DNA repair genes (XPD and XRCC1)—association with age related cataracts. Mol Vis 17:127–133

Payao SL, de Labio RW, Gatti LL, Rigolin VO, Bertolucci PH, Smith Mde A (2004) Werner helicase polymorphism is not associated with Alzheimer's disease. J Alzheimers Dis 6(6):591–594

Rossi ML, Ghosh AK, Bohr VA (2010) Roles of Werner syndrome protein in protection of genome integrity. DNA Repair (Amst) 9(3):331–344

Shiels A, Hejtmancik JF (2007) Genetic origins of cataract. Arch Ophthalmol 125(2):165–173

Singh DK, Ahn B, Bohr VA (2009) Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. Biogerontology 10(3):235–252

Smith MA, Silva MD, Araujo LQ, Ramos LR, Labio RW, Burbano RR, Peres CA, Andreoli SB, Payao SL, Cendoroglo MS (2005) Frequency of Werner helicase 1367 polymorphism and age-related morbidity in an elderly Brazilian population. Braz J Med Biol Res 38(7):1053–1059

Sun L, Xi B, Yu L, Gao XC, Shi DJ, Yan YK, Xu DJ, Han Q, Wang C (2010) Association of glutathione S-transferases polymorphisms (GSTM1 and GSTT1) with senile cataract: a meta-analysis. Invest Ophthalmol Vis Sci 51(12):6381–6386

Takayama K, Salazar EP, Broughton BC, Lehmann AR, Sarasin A, Thompson LH, Weber CA (1996) Defects in the DNA repair and transcription gene ERCC2(XPD) in trichothiodystrophy. Am J Hum Genet 58(2):263–270

Wozniak K, Szaflik JP, Zaras M, Sklodowska A, Janik-Papis K, Poplawski TR, Blasiak J, Szaflik J (2009) DNA damage/repair and polymorphism of the hOGG1 gene in lymphocytes of AMD patients. J Biomed Biotechnol 2009:827562

Yan H, McCane J, Toczylowski T, Chen C (2005) Analysis of the Xenopus Werner syndrome protein in DNA double-strand break repair. J Cell Biol 171(2):217–227

Yang N, Chaudhry MA, Wallace SS (2006) Base excision repair by hNTH1 and hOGG1: a two edged sword in the processing of DNA damage in gamma-irradiated human cells. DNA Repair (Amst) 5(1):43–51

Ye L, Miki T, Nakura J, Oshima J, Kamino K, Rakugi H, Ikegami H, Higaki J, Edland SD, Martin GM, Ogihara T (1997) Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. Am J Med Genet 68(4):494–498

Zhang Y, Zhang L, Bai J, Ge H, Liu P (2010) Expression changes in DNA repair enzymes and mitochondrial DNA damage in aging rat lens. Mol Vis 16:1754–1763

Zhou J, Hu J, Guan H (2010) The association between copy number variations in glutathione S-transferase M1 and T1 and age-related cataract in a Han Chinese population. Invest Ophthalmol Vis Sci 51(8):3924–3928