The Associations between Single Nucleotide Polymorphisms of DNA Repair Genes, DNA Damage, and Age-Related Cataract: Jiangsu Eye Study

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Purpose. Age-related cataract (ARC) is one of the most common causes of severe visual impairment among the elderly worldwide with four subtypes, such as cortical, nuclear, subcapsular, and mixed types. DNA damage and malfunction of DNA repair are believed to contribute to the pathogenesis of ARC. This study examined the associations of 18 single nucleotide polymorphisms (SNPs) in four DNA repair genes (*BLM*, *WRN*, *ERCC6*, and *OGG1*) with ARC in Han Chinese from the Jiangsu Eye Study, a population-based epidemiologic study. We also determined the possible functional consequence of the SNPs to DNA damage.

Methods. Eighteen SNPs in four DNA repair genes were genotyped in 789 ARC patients and 531 normal controls from the Jiangsu Eye Study. The Comet assay was to assess the extent of DNA damage in peripheral lymphocytes of selected subjects.

Results. The results show that *WRN*-rs11574311 was initially associated with ARC in general, cortical, and mixed cataracts ($P = 0.003$, odds ratio [OR] = 1.49; $P = 0.001$, OR = 1.68; and $P < 0.0001$, OR = 2.08), *BLM*-rs1063147 with nuclear cataract ($P = 0.03$, OR = 1.31), *WRN*-rs2725383 with cortical cataract ($P = 0.01$, OR = 1.49), and *WRN*-rs4733220 and *WRN*-rs2725338 with mixed cataract ($P = 0.04$, OR = 0.74; $P = 0.003$, OR = 0.60). However, the significances of some of the above-cited associations disappeared after multiple testing corrections. *WRN*-rs11574311 remains associated with cortical and mixed cataract and *WRN*-rs2725338 with mixed cataract after multiple testing correction. We did not find any correlation between DNA damage of peripheral lymphocytes and SNP types.

Conclusions. We concluded that *WRN* genes might be involved in ARC pathogenesis in the Han Chinese population. The associations were ARC subtype specific. These findings stress the importance of detailed phenotyping in ARC subtypes, which may be associated with different risk factors and disease mechanisms. (*Invest Ophthalmol Vis Sci.* 2013;54:1201–1207) DOI:10.1167/iovs.12-10940

Age-related cataract (ARC) is one of the most common causes of severe visual impairment among the elderly worldwide, rendering the disease a major public health issue.[1,2] ARC is a progressive opacification of the ocular lens, leading to visual impairment and blindness. According to the degenerative region of lens, ARC can be classified as nuclear (N), cortical (C), posterior subcapsular (PSC), and mixed type (M).[3]

ARC has a multifactorial etiology. Age, sex, smoking, exposure to sunlight, chronic ocular inflammation, estrogen sufficiency or deficiency, and cardiovascular factors may influence ARC predisposition. The strongest evidence came from twin studies demonstrating a heritability of 48% for nuclear cataract[4] and 59% for cortical cataract.[5] The importance of genetic risk factors for ARC was highlighted in several recent studies, which have reported that *OGG1*, *EPHA2*, and glutathione S-transferase (*GST*) polymorphisms are associated with the C type of ARC in the Chinese population.[6–9]

Although the pathophysiology of ARC is far from clearly understood, it is well accepted that oxidative stress plays an important role in the disease pathogenesis. Reactive oxygen species (ROS) are generated within the mitochondria in the lens epithelium and the superficial fiber cells. When ROS production exceeds the capacity of detoxification, they may cause oxidative damage to mitochondrial DNA and nuclear DNA. In a normal physiologic condition, most oxidative DNA lesions are rapidly repaired by base excision repair (BER), nucleotide excision repair (NER), and double-strand break repair (DSBR) pathways.[10,11] In BER, an altered base is removed by a DNA glycosylase enzyme, followed by excision of the resulting sugar phosphate. The small gap left in the DNA helix is filled in by the sequential action of DNA polymerase and DNA ligase. NER recognizes a wide range of substrates, including damage caused by ultraviolet (UV) irradiation and chemicals. The process removes a small DNA helix surrounding the damage and has the small gap to be filled by the sequential action of DNA polymerase and DNA ligase. DSBR is via homologous and nonhomologous mechanisms to reform a continuous DNA helix. The BER, NER, and double-strand end resection (DSER) pathways involve a highly coordinated process catalyzed by the sequential actions of DNA repair enzymes and play an important role in maintaining genome stability and integrity. It has been hypothesized that polymorphisms in DNA repair genes modify their capacity to repair DNA damage and thereby lead to a greater susceptibility to cancer or age-related diseases.[12,13] Recent studies have

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reported the association of DNA repair gene polymorphisms with age-related macular degeneration (AMD) and glaucoma.[14,15]

BLM and *WRN* are the key enzymes of the DSER pathway, and *ERCC6* is the key enzyme of the NER. Mutations in *BLM*, *WRN*, and *ERCC6* engender three rare premature aging syndromes: Bloom (BS), Werner (WS), and Cockayne (CS). Patients suffering from the syndrome(s) appear much older than their chronological age, and exhibit many of the clinical signs and symptoms of normal aging at an early stage in life, such as cataract and cancer. *OGG1* is the key enzyme of the BER pathway. A recent study in Han Chinese has reported that the *OGG1*-Ser326Cys polymorphism may be associated with increased risk of ARC, although in *XRCC*1, *APE1*, and *XPD* polymorphisms, there were no significant differences in frequencies of the variant homozygous in ARC patients compared with controls.[16] Here we selected 18 common single nucleotide polymorphisms (SNPs) of *BLM*, *WRN*, *ERCC6*, and *OGG1* to test whether these gene variations are related to the occurrence of ARC.

Methods

Study Design and Participants

The research followed the tenets of the Declaration of Helsinki. All participants signed the respective informed consent forms. The study was approved by the Ethics Committee of Affiliated Hospital of Nantong University.

This study was a part of the Jiangsu Eye Study, a population-based epidemiologic study. Jiangsu Province is located in the Yangtze River Delta. Southern and Northern Jiangsu represent different levels of socioeconomic development. One rural district/county within southern and northern Jiangsu was selected as the sampling area, which are Binhu District and Funing County, respectively. The surveys were carried out by randomly selecting individuals within each district/county, which was similar to the method we described previously.[2] The sampling frame was constructed using geographically defined clusters based on village register data. Cluster boundaries were defined such that each cluster would have a population of approximately 1000 individuals (all ages). Sample size was based on estimating an anticipated 4% prevalence for visual impairment <20/200 within an error bound (precision) of 20% with 95% confidence interval. Assuming an examination response rate of 85%, and a design effect of 1.5 to account for inefficiencies associated with the cluster sampling design, a sample of 4068 persons ≥50 years of age was required for each district/county.[2] Depending on the percentage of population ≥50 years of age, 28 to 30 clusters were randomly selected (with equal probability) by the Chinese Ophthalmological Society from the sampling frame for each district/county. Geographically defined cluster sampling included 6722 individuals ≥50 years of age in Binhu District from January to December 2010. Actually, 6106 subjects were examined with the response rate of 90.8%. The same sampling was used in 6145 randomly selected individuals ≥50 years of age in Funing county from September 2010 to May 2011 and actually 5947 subjects were examined with the response rate of 96.8%. The covered area of the Jiangsu Eye Study has a stable and ethnically homogeneous population. All participants were unrelated self-identified Han Chinese (at least all four grandparents were ethnically Han Chinese). Participants were brought to the village clinics or village offices for general physical and full ophthalmic examinations. The cataract was defined as opacification of ocular lens and best-corrected visual acuity (BCVA) <20/40.[2] Lens opacities were graded according to the Lens Opacities Classification System III (LOCSIII)[17] in 0.1 unit steps for each opacity up to a maximum of 6.9 for nuclear opacities, and 5.9 for cortical and PSC. The presence of more than one cataract type in at least one eye, or different pure types in both eyes were classified into the mixed type.[18] We identified a total of 2208 cataract patients from Binhu District and Funing County. The prevalence of cataract was 18.3%. In this study, we selected ARC patients as research subjects. The inclusion criteria for ARC included: (1) opacification of the ocular lens; (2) ≥50 years of age; (3) BCVA <20/40; and (4) without other clear reasons for cataract. The exclusion criteria: (1) the complicated cataract due to glaucoma, high myopia, uveitis, diabetes, ocular trauma, or other known causes; and (2) patients who were pseudophakic or aphakic in either eye. According to the above criteria, a total of 1144 cataract patients were excluded. Consequently, 1064 ARC patients (C = 335, N = 470, PSC = 42, M = 217) were included. The further exclusion removed 163 participants, which included ARC patients with systemic diseases such as diabetes, kidney diseases, cancers, and so on; ARC patients with macular diseases and other retinal diseases; and 67 ARC patients with the worse eye with LOCSIII grade <2 of all types. As a result, there were in all 834 ARC patients illegible in this study. There were 45 cases failed in DNA extracting and genotyping. We finally examined 789 ARC patients. The details of study participants are summarized in Table 1. Normal controls were also selected from the Binhu District and Funing County. There was no biogenetic relation between normal controls, and between normal controls and ARC patients. Inclusion criteria for normal controls: (1) individuals with transparent lenses; (2) the BCVA better than 20/25 in both eyes. Exclusion criteria: (1) individuals with other major eye diseases such as dislocated lens, glaucoma, myopia, macular diseases, diabetic retinopathy, and uveitis; and (2) individuals with systemic diseases such as diabetes, kidney diseases, and cancer. After matching for age and sex, 531 individuals were included as normal controls.

Table 1. Demographic Information of Study Participants

Variable	Control	ARC
n	531	789
Age, y, mean ± SD	69.66 ± 4.51	70.38 ± 7.72
Fasting blood sugar, mean ± SD	5.19 ± 0.88	5.28 ± 0.85
Female, *n* (%)	291 (54.8)	474 (60.1)
Male, *n* (%)	240 (45.2)	315 (39.9)
Cortical, *n* (%)	0	257 (32.6)
Nuclear, *n* (%)	0	366 (46.4)
PSC, *n* (%)	0	34 (4.3)
Mixed, *n* (%)	0	132 (16.7)

Selection of SNPs and Genotyping

Eighteen SNPs were selected that capture common variations in *BLM*, *WRN*, *ERCC6*, and *OGG1*. We selected haplotype-tagging SNP by searching Han Chinese data from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) using the Tagger program. The SNPs with a minor allele frequency ≥4% in the HapMap CHB population were included. A further basic selection criterion was an r^2 value ≤0.8, excluding strong linkage disequilibrium between adjacent variants.

Peripheral venous blood was collected in an EDTA anticoagulation tube. Genomic DNA was isolated from leukocytes by the phenol–chloroform method. Genotyping of all SNPs were conducted with a commercial gene expression assay (TaqMan Assay; Applied Biosystems, Foster City, CA), as described in our previous publications.[19,20]

Comet Assay

The 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 performed the assay on 69 ARC patients (N = 22, C = 32, M = 15) and 28 age- and sex-matched normal controls;

the average age is 69.45 ± 4.52 years in ARC patients and 68.39 ± 3.42 years in controls ($P > 0.05$).

The peripheral lymphocytes from whole blood in an EDTA anticoagulation tube were isolated and suspended in PBS at 1×10^4 cells/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 hours at 4°C in a lysis buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10, followed by electrophoresis (20 V, 200 mA) in the buffer consisting of 300 mM NaOH and 1 mM EDTA for 20 minutes. 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 earlier were performed under dimmed light or in the dark.[21]

The Comet images were observed at a magnification of ×400 under a fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany) controlled by the image analysis system CASP, a program available on the web (www.casp.of.pl). Twenty images were randomly selected from each sample. The percentage of DNA in the tail of Comets (tail DNA%) and Olive Tail Moment (OTM) were measured. OTM, defined as the product of the distance between the barycenters of the head and tail by the percentage of DNA in the tail of the Comet-like images, was used to evaluate the extent of DNA damage (DNA breaks) in individual cells.

Statistical Analysis

Statistical analyses were performed with a commercial statistical software program (Stata 8.0; Stata Corp., College Station, TX). The χ^2 test was used to test the association between the alleles frequencies of all ARC patients and normal controls and various subtypes of ARC, and to estimate odds ratios (OR) and 95% confidence interval (CI). Hardy-Weinberg Equilibrium (HWE) of genotype distributions were also tested by the χ^2 test. If any positive association was found in the initial allele analysis, Bonferroni correction was performed. Various genetic model analyses were performed to characterize the association as dominant (heterozygote and homozygote versus wild type), recessive model (homozygote versus wild type and heterozygote), additive model (homozygote versus heterozygote versus wild type), and heterozygote advantage model (heterozygote versus homozygote and wild type). We only present the most significant model in the results. 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 as statistically significant.

Results

The demographic information for the study participants was generalized in Table 1. The fasting blood sugar values of all subjects recruited in our study are all <6.5 mmol/L. We used *WRN*-rs11574311 data as a reference for power analysis. Based on a predefined two-sided alpha of 0.05, there was greater than 90% power to detect a ±3% departure from an rs11574311 allele frequency of 15.2% in our sample sets. All of the tested SNPs are in HWE in the control population, except *BLM*-rs8027126 that was excluded from further analysis. We compared the allele frequency between ARC patients and normal controls and found that only *WRN*-rs11574311 was associated with ARC ($P = 0.003$, OR = 1.49, 95% CI: 1.17–1.90) (Table 2), but the significance was lost after Bonferroni correction (Pa = 0.054).

We then analyzed the distribution of allele frequencies after stratifying ARC by the subtypes. *BLM*-rs1063147 was observed to be associated with the N type of ARC ($P = 0.03$, OR = 1.31) (Table 3), suggesting a hazard role for the minor T allele in development of the N type of ARC. Two SNPs of the *WRN* gene, rs11574311 and rs2725383, were also found to be associated with the C type of ARC ($P = 0.001$, OR = 1.68; $P = 0.01$, OR = 1.49) (Table 4), suggesting a risk role for the minor C allele in development of the C type of ARC. Moreover, *WRN*-rs4733220 and *WRN*-rs2725338 may be associated with the M type of ARC ($P = 0.04$, OR = 0.74; $P = 0.003$, OR = 0.60) (Table 5), suggesting a protective role for the minor A allele in development of the M type of ARC; and *WRN*-rs11574311 may be associated with the M type of ARC ($P < 0.0001$, OR = 2.08) (Table 5), suggesting a risk role for the minor C allele in development of the M type of ARC. The genetic model analysis found that more than five SNPs were suggestively associated with the relevant ARC types in the dominant model. Among those, the associations between *WRN*-rs11574311 and the C and M types of ARC, and between *WRN*-rs2725338 and the M type of ARC remained significant after Bonferroni correction ($P < 0.05$) (Tables 4, 5). The significances of the remaining three SNPs were lost after Bonferroni correction.

Table 2. Distribution of Minor Allele of Tested 18 SNPs and Their Association with ARC

Gene Name	SNPs Minor/Major	Control Minor/Major (%)	All ARC Minor/Major (%)	*P*	OR (95% CI)
BLM	rs1063147 T/C	164/898 (15.4)	266/1312 (16.9)	0.36	1.11 (0.90–1.37)
	rs7183308 G/A	52/1010 (4.9)	89/1489 (5.6)	0.41	1.16 (0.82–1.65)
	rs17273206 A/G	233/829 (21.9)	1206/372 (23.6)	0.33	1.10 (0.91–1.32)
	rs8027126 T/G	84/978 (7.9)	133/1445 (8.4)	0.63	1.07 (0.81–1.43)
	rs7175811 A/G	296/766 (27.9)	462/1116 (29.3)	0.43	1.07 (0.90–1.27)
	rs3815003 C/T	300/762 (28.2)	477/1101 (30.2)	0.27	1.10 (0.93–1.31)
	rs6496724 C/A	324/738 (30.5)	519/1059 (32.9)	0.20	1.12 (0.94–1.32)
WRN	rs4733220 A/G	442/620 (41.6)	623/955 (39.5)	0.27	0.92 (0.78–1.07)
	rs2725361 A/G	369/693 (34.7)	590/988 (37.4)	0.17	1.12 (0.95–1.32)
	rs2725338 A/G	283/779 (26.6)	391/1187 (24.8)	0.30	0.91 (0.76–1.08)
	rs1801195 G/T	376/686 (35.4)	593/985 (37.6)	0.26	1.10 (0.93–1.29)
	rs2725383 C/G	117/945 (11.0)	215/1363 (13.6)	0.06	1.27 (1.00–1.62)
	rs1863280 G/T	231/831 (21.7)	349/1229 (22.1)	0.82	1.02 (0.85–1.23)
	rs11574311 C/T	112/950 (10.5)	236/1342 (15.0)	0.003/0.054 (Pa)	1.49 (1.17–1.90)
ERCC6	rs4838519 C/A	523/539 (49.2)	777/801 (49.2)	0.99	1.00 (0.86–1.17)
	rs4253038 G/A	332/730 (31.3)	521/1057 (33.0)	0.35	1.08 (0.92–1.28)
OGG1	rs2072668 C/G	426/636 (40.1)	600/978 (38.0)	0.30	0.92 (0.78–1.07)
	rs2304277 G/A	446/616 (42.0)	618/960 (39.2)	0.14	0.89 (0.76–1.04)

All HWE ($P > 0.05$) except *BLM*-rs8027126 ($P < 0.05$). Pa, *P* value after Bonferroni correction.

Table 3. Association between *BLM*-rs1063147 and the N Type of ARC

Allele	Control, *n* (%)	N Type of ARC, *n* (%)	*P*/Pa	OR (95% CI)
C	898 (84.6)	591 (80.7)	0.03/0.54	1.31 (1.02–1.67)
T	164 (15.4)	141 (19.3)		
CC	379 (71.4)	238 (65.0)	0.04/0.72	1.34 (1.01–1.78)
TC + TT	152 (28.6)	128 (35.0)		

Table 4. Association between *WRN*-rs11574311, rs2725383, and the C Type of ARC

Gene/SNP	Allele	Control, *n* (%)	C Type of ARC, *n* (%)	*P*/Pa	OR (95% CI)
WRN/rs11574311	T	950 (89.5)	429 (83.5)	0.001/0.018	1.68 (1.24–2.28)
	C	112 (10.5)	85 (16.5)		
	TT	428 (80.6)	175 (68.1)	0.0001/0.0018	1.95 (1.39–2.73)
	CT + CC	103 (19.4)	82 (31.9)		
WRN/rs2725383	G	945 (89.0)	434 (84.4)	0.01/0.18	1.49 (1.10–2.02)
	C	117 (11.0)	80 (15.6)		
	GG	422 (79.5)	183 (71.2)	0.01/0.18	1.57 (1.11–2.21)
	CG + CC	109 (20.5)	74 (28.8)		

Comet assay showed the percentage of DNA damage in the tail of the Comets and the value of the OTMs were: 8.64 ± 3.46 and 1.97 ± 1.02 for the control subjects; 22.13 ± 2.13 and 6.39 ± 0.93 for homozygous C allele of rs1063147, 19.57 ± 3.74, and 5.55 ± 1.02; for T allele carriers of rs1063147 for the N type of ARC; 22.84 ± 4.25 and 6.66 ± 1.62 for homozygous T allele of rs11574311, 21.68 ± 1.75 and 6.18 ± 0.88 for C allele carriers of rs11574311; 22.06 ± 2.87 and 6.40 ± 1.21 for homozygous G allele of rs2725383; 23.16 ± 4.77 and 6.68 ± 1.78 for C allele carriers of rs2725383 for the C type of ARC. 18.67 ± 2.58 and 5.36 ± 1.04 for homozygous G allele of rs4733220; 19.07 ± 4.12 and 5.56 ± 1.26 for A allele carriers of rs4733220; 18.31 ± 2.60 and 5.28 ± 1.03 for homozygous G allele of rs2725338; 19.55 ± 4.21 and 5.74 ± 1.25 for A allele carriers of rs2725338; 18.74 ± 2.44 and 5.24 ± 0.88 for homozygous T allele of rs11574311; and 19.01 ± 4.20 and 5.67 ± 1.34 for C allele carriers of rs11574311 for the M type of ARC. The differences were significant ($P < 0.001$) between ARC patients and normal controls, although we did not found correlation of DNA damage of peripheral lymphocytes with the genotypes of the five SNPs (Figs. 1–3). We also analyzed the DNA damage extent of 69 ARC patients between male and female, and no difference between sexes was found ($P > 0.05$, data not shown).

Discussion

This study presented the evidences of involvement of DNA repair mechanism in the susceptibility of ARC. Strengths of our study included: (1) the large, well-characterized patients; (2) classification of ARC by standardized ophthalmologic examinations; (3) population-based design that avoided possible bias in comparison with hospital-sourced case-control study; and (4) unrelated controls from the same geographic region as the ARC patients. To the best of our knowledge, this was the first report on the role of the DNA repair genes *BLM*, *WRN*, and *ERCC6* polymorphisms on ARC susceptibility in the Chinese population. *OGG1* Ser326Cys has been studied in the Chinese population but the two SNPs of *OGG1* tested in this study were the first time to be studied.

The SNPs tested in this study may alter DNA repair capacity and subsequently may lead to synergistic effects on ARC induced by oxidative damage. Oxidative stress has long been recognized as a vital mediator of apoptosis in lens epithelial cells and also plays an important role in the pathogenesis of cataract.[22,23] The selected genes coding for DNA repair enzymes play a vital role in the DSER, NER, and BER pathways.[24–26] The *BLM* gene is thought to arise in S/G_2 phases of cell cycle as a result of crossing over during

Table 5. Association between *WRN*-rs4733220, rs2725338, rs11574311, and the M Type of ARC

Gene/SNP	Allele	Control, *n* (%)	M Type of ARC, *n* (%)	*P*/Pa	OR (95% CI)
WRN/rs4733220	G	620 (58.4)	173 (65.5)	0.04/0.72	0.74 (0.56–0.98)
	A	442 (41.6)	91 (34.5)		
	GG	190 (35.8)	60 (45.5)	0.04/0.72	0.67 (0.45–0.98)
	AG + AA	341 (64.2)	72 (54.5)		
WRN/rs2725338	G	779 (73.4)	217 (82.2)	0.003/0.054	0.60 (0.42–0.84)
	A	283 (26.6)	47 (17.8)		
	GG	290 (54.6)	92 (69.7)	0.002/0.036	0.52 (0.35–0.79)
	AG + AA	241 (45.4)	40 (30.3)		
WRN/rs11574311	T	950 (89.5)	212 (80.3)	<0.0001/<0.0018	2.08 (1.45–2.99)
	C	112 (10.5)	52 (19.7)		
	TT	428 (80.6)	86 (65.2)	0.0001/ 0.0018	2.22 (1.46–3.38)
	CT + CC	103 (19.4)	46 (34.8)		

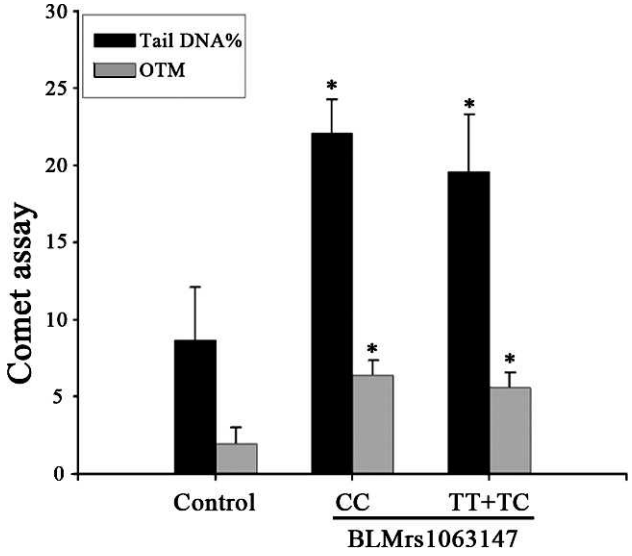

Figure 1. The differences of the Comet assay parameters between the *BLM*-rs1063147 genotypes, and between ARC patients and controls. *$P < 0.05$, compared with control; nuclear cataract patients ($n = 22$) with homozygous C allele and T allele carriers of *BLM*-rs1063147 had more DNA damage than age-matched controls ($n = 28$). No difference was found in DNA damage between C allele homozygous and T allele carriers of *BLM*-rs1063147 ($P > 0.05$).

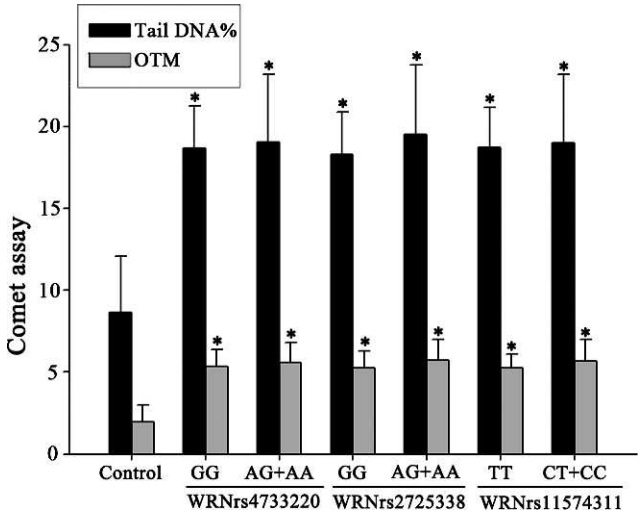

Figure 3. The differences of the Comet assay parameters between the *WRN*-rs4733220, *WRN*-rs2725338, and *WRN*-rs11574311 genotypes, and between ARC patients and controls. *$P < 0.05$, compared with control; mixed cataract patients ($n = 15$) with homozygous G allele and A allele carriers of *WRN*-rs4733220 and *WRN*-rs2725338 and homozygous T allele and C allele carriers of *WRN*-rs11574311 had more DNA damage than that of age-matched controls ($n = 28$). No difference was found in DNA damage between G allele homozygous and A allele carriers of *WRN*-rs4733220 and *WRN*-rs2725338 and between T allele homozygous and C allele carriers of *WRN*-rs11574311 ($P > 0.05$).

homologous recombination-mediated DNA repair events.[27] *BLM* ensures genome integrity by faithful chromosome segregation. Mutations causing Bloom syndrome delete or alter helicase motifs and may disable the 3′–5′ helicase activity. Furthermore, a recent work provides the direct evidence that

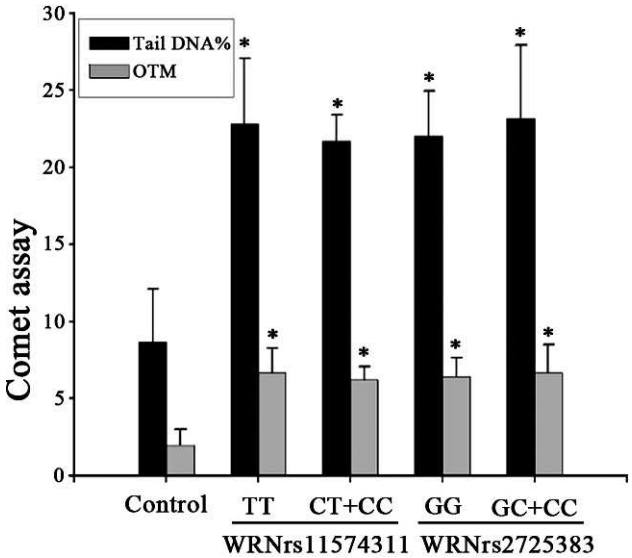

Figure 2. The differences of the Comet assay parameters between the *WRN*-rs11574311 and *WRN*-rs2725383 genotypes, and between ARC patients and controls. *$P < 0.05$, compared with control; cortical cataract patients ($n = 32$) with homozygous T allele and C allele carriers of *WRN*-rs11574311 and cortical cataract patients with homozygous G allele and C allele carriers of *WRN*-rs2725383 had more DNA damage than age-matched controls ($n = 28$). No difference was found in DNA damage between T allele homozygous and C allele carriers of *WRN*-rs11574311, and no difference in DNA damage between G allele homozygous and C allele carriers of *WRN*-rs2725383 ($P > 0.05$).

BLM influences the DSB repair pathway choice in human chromosomes.[28] Previous work reported that polymorphisms in *BLM* were associated with colorectal cancer[26] and breast cancer.[29] Although disease pathogenesis of cancers differs from ARC, they might share the same pathway in terms of aging and genome instability.

The *WRN* gene is responsible for caretaking the genome, and serves as an important link between repair of defective DNA and processes related to aging.[30] Previous studies have investigated associations between *WRN* polymorphisms and age-related diseases, including myocardial infarction,[31] type 2 diabetes mellitus,[32] and breast cancer.[33] Recently, a study reported that cataract formation in the elderly is not linked to the *WRN* C1367T (rs1346044) polymorphism in the Israeli population.[34] However, our work shows that *WRN*-rs11574311 is associated with the C and M types of ARC, whereas *WRN*-rs2725338 is associated with the M type of ARC. It is interesting that rs11574311 is in strong linkage disequilibrium with rs1346044. The inconsistency can be caused by genetic heterogeneity between populations and the limited number of participants in the Israeli study.

Molecular genetics studies found that mutations in the *ERCC6* gene may lead to Cockayne syndrome, which often presents severe cataracts.[35] Two studies suggested that *ERCC6* polymorphisms are risk factors for AMD.[36,37] Both ARC and AMD, as common age-related eye diseases, may be affected by long-term UV radiation, oxidative damage, aging, and similar genetic factors. However, in our study, we find no association between *ERCC6* and ARC.

In the BER pathway, *OGG1* is responsible for the removal of 8-oxoguanine, which arises through the incorporation of 8-oxo-dGTP from oxidation of dGTP by ROS during DNA replication. In our study, we tested two new common SNPs, rs2304277 and rs2072668, and found no association of them with ARC.

In our study, the *WRN* gene had an association with the C and M types of ARC. Because genetic polymorphisms may alter the function of the enzyme, this influence may lead to different subtypes of cataract. Because of the absence of cell nuclei in the lens nucleus, the functional contribution might originate from lens epithelial cells. The aberrant metabolism of lens epithelial cells can easily cause dysfunction in the lens fibers.

Oxidative stress induces various types of DNA damage in the lens, which causes cataract.[34,38,39] The extent of DNA damage in peripheral lymphocytes assessed by Comet assay was significantly higher in ARC than that in age-matched normal controls. This result was in line with a study that found elevated levels of 8-OH-Gua, a marker of oxidative DNA damage, in the leukocytes of patients with cataract.[40] However, we did not find the correction of the DNA damage with the genotypes. This may be due to the nature of the local lesion (lens) in ARC. Variants in the *WRN* gene might mainly act in ocular tissue, which affects the phenotype of ARC. The measurement of DNA damage in ocular tissue and the characterization of its correlation with the SNP genotypes are warranted in our future studies.

In conclusion, we found that *WRN* genetic variants are associated with the risk of ARC in the Chinese population. These associations are often related to the disease subtype. These findings stress the importance of detailed phenotyping in ARC subtypes, which may be associated with different risk factors and disease mechanisms. To further confirm the role of *WRN* in the pathogenesis of ARC, the current results from this population-based study will serve as the baseline for prospective observation of the role of genetic factors in the development of ARC.

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