# Copy Number Variations of DNA Repair Genes and the Age-Related Cataract: Jiangsu Eye Study

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**Purpose.** DNA damage is critical in the pathogenesis of age-related cataract (ARC). This study examined the association of copy number variations (CNVs) of DNA repair genes with susceptibility to ARC in the Han Chinese.

**Methods.** Study participants were from the population-based Jiangsu Eye Study, which includes 780 ARC patients and 525 controls. DNA was extracted from blood for copy number (CN) assays using RT-PCR. The Comet assay was to assess DNA damage in peripheral lymphocytes.

**Results.** Novel CNV was detected in *WRN*. Initial analyses found that CN = 3+ for *WRN* had an increased risk of ARC (odds ratio [OR] = 1.88, $P = 0.02$); CN = 1 for *HSF4* had an increased risk of ARC (OR = 4.09, $P = 0.004$). CN = 3+ for *WRN* was associated with nuclear and posterior subcapsular cataract (OR = 2.06, $P = 0.02$; OR = 3.72, $P = 0.02$). CN = 1 for *HSF4* was associated with nuclear and posterior subcapsular cataract (OR = 5.73, $P = 0.001$; OR = 6.80, $P = 0.01$). The combination *WRN* and *HSF4* CNVs markedly increased the risk of ARC; the OR was increased from 2.63 by *HSF4* alone to 6.80 by combined *WRN* and *HSF4* CNVs. However, after multiple testing correction, only *HSF4* CNV was associated with ARC overall and with nuclear and posterior subcapsular cataract as well. The DNA damage in lymphocytes from ARC patients was significantly higher when compared to normal controls.

**Conclusions.** *HSF4* and *WRN* CNVs might be involved in ARC pathogenesis in the Han Chinese. These findings suggest the importance of DNA repair in ARC susceptibility and distinct risk factors in ARC subtypes. (*Invest Ophthalmol Vis Sci.* 2013; 54:932–938) DOI:10.1167/iovs.12-10948

Age-related cataract (ARC) is one of the leading causes of visual impairment and blindness in the world.[1] It is reported that cataract affects approximately 37 million people and accounts for 48% of blindness worldwide.[2] The global burden of blindness due to ARC is increasing as a result of a growing elderly population.[3] ARC can be classified as cortical (C), nuclear (N), posterior subcapsular (PSC), and mixed type (M) according to the location of the opacity within the lens.[4]

Although the pathogenesis of ARC is still far from completely understood, many studies suggest that oxidative stress–induced DNA damage and genetic defects are the critical risk factors in the pathogenesis of ARC.[5] Oxidative stress has long been recognized as an important mediator of apoptosis in lens epithelial cells (LECs) and also plays a vital role in the pathogenesis of cataract.[6,7] Recent studies have reported the association between reactive oxygen species (ROS)-induced DNA damage of LECs and the development of cataract.[7–9] Under normal growth conditions, ROS leads to a low level of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) damage that is rapidly repaired. Oxidative DNA lesions are repaired by nucleotide excision repair, double-strand break (DSB) repair, and base excision repair.[10,11] It has been hypothesized in many studies that single nucleotide polymorphisms (SNPs) in DNA repair genes alter their capacity to repair DNA damage and thereby change the susceptibility to diseases.[12,13] Based on their major functions, complementation group 6 (*ERCC6*), Werner syndrome helicase (*WRN*), and 8-oxoguanine glycosylase-1 (*OGG1*) operate in nucleotide excision repair, DSB, and base excision repair, respectively.[10] DNA repair gene polymorphisms have previously been associated with age-related macular degeneration and ARC.[14–16]

Previous studies have demonstrated several genes to be associated with ARC, such as heat shock factor protein 4 (*HSF4*), eph-receptor tyrosinekinase-type A2 (*EPHA2*), and glutathione S-transferase (*GST*). HSF4 regulates the expression of several heat shock protein (HSP) genes.[17–19] The proper protein organization is essential for lens transparency. HSPs play important roles in maintenance of the supramolecular organization of lens protein. It has been reported that *HSF4* mutations account for a small fraction of ARC in the Han Chinese population.[18,19] Additionally, a recent study found evidence that *HSF4* contributes to the repair of DNA DSB.[20]

Much of the previous work on genetic factors has focused on SNPs. Copy number variation (CNV) is increasingly recognized as a source of interindividual differences in genome sequence and has been proposed as a driving force for genome evolution and phenotypic variation.[21] Our previous studies reported an association between *GSTT1* CNV and ARC in a Han Chinese population.[22]

In the present study, we selected ARC patients and normal controls from a population-based epidemiological study, the Jiangsu Eye Study. Our aim was to characterize the possible association between CNVs of several DNA repair genes and ARC in the Han Chinese population. The correlation between DNA damage of peripheral lymphocytes and CNV in DNA repair genes was also examined.

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**Table 1.** Demographic Data of the ARC Patients and Normal Controls

| | ARC ($n = 780$) | Control ($n = 525$) | *P* |
|---|---|---|---|
| Sex | | | 0.053 |
| Male, *n* (%) | 310 (39.7) | 237 (45.1) | |
| Female, *n* (%) | 470 (60.3) | 288 (54.9) | |
| Age, y; mean ± SD | 70.55 ± 7.64 | 69.88 ± 4.37 | 0.068 |

## Materials and Methods

### Ethics Statement

This research adhered to the tenets of the Declaration of Helsinki. Each participant signed the respective informed consent forms. The study was approved by the Ethics Committee of Affiliated Hospital of Nantong University.

### Jiangsu Eye Study and Study Participants

This study was a part of the Jiangsu Eye Study, a population-based epidemiological study. Jiangsu province is located in the Yangtze River Delta. According to the different levels of economic development, Jiangsu province is divided into southern and northern areas. To estimate the prevalence of blindness and low vision among older adults aged ≥50 years, one district or county was selected as the sampling area from both southern and northern Jiangsu: Binhu district and Funing county, respectively. The surveys were carried out by randomly selecting individuals within each district or county, which was similar to the method we described previously.[23] The sampling frame was constructed using geographically defined clusters based on village register data. Cluster boundaries were defined so 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. 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 or county.[23] 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. A total of 12,867 persons aged ≥50 years were enumerated in Binhu district and Funing county. Geographically defined cluster sampling initially included 6722 individuals aged ≥50 years in Binhu district from January to December 2010. Actually 6106 persons were examined with the response rate of 90.8%. The same sampling was initially applied to 6145 individuals aged ≥50 years in Funing county from September 2010 to May 2011 and 5947 individuals were examined for a response rate of 96.8%. The covered area of Jiangsu Eye Study has a stable and ethnically homogenous population. All participants were unrelated self-identified Han Chinese (all four grandparents were ethnically Han Chinese). Participants were brought to village clinics or offices for general physical and full ophthalmic examinations. Cataract was defined as opacification of the ocular lens and best corrected visual acuity of <20/40.[23] Lens opacities were determined according to the Lens Opacities Classification System III (LOCS III) in 0.1-unit steps for each opacity up to a maximum of 6.9 for N, 5.9 for C, and PSC subtypes.[24] Presence of more than one cataract type in at least one eye or different pure types in both eyes was classified as mixed type.[25] 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) age ≥50 years, (3) best corrected visual acuity <20/40, (4) no other clear reasons to cause cataract. The exclusion criteria were (1) complicated cataract due to glaucoma, high myopia, uveitis, diabetes, ocular trauma, or other known causes, (2) either eye being pseudophakic or aphakic, and (3) previous treatment with radiation therapy or steroids. Based on these criteria, 1144 patients were excluded, and 1064 ARC patients (C = 335, N = 470, PSC = 42, M = 217) were included. A further 163 participants were excluded; specifically, ARC patients with systemic diseases such as diabetes, kidney diseases, and cancers and ARC patients with macular diseases and other retinal diseases. Another 67 ARC patients of all subtypes were excluded, with their worse eye having LOCSIII grade <2. As a result, there were total 834 ARC patients eligible to participate in this study. Of these 834 ARC patients, DNA extraction failed in 29 and 25 could not be genotyped. We finally examined 780 ARC patients, among which there were 257 with C type, 368 with N type, 34 with PSC type, and 121 with M type of ARC.

Unrelated normal controls were selected from the same epidemiological study. Inclusion criteria for normal controls were (1) individuals who with transparent lenses and (2) a best corrected visual acuity better than 20/25 in both eyes. Exclusion criteria were (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, 525 individuals were included as normal controls. The demographic information for the study participants is listed in Table 1.

### DNA Extraction and Quantification of Copy Numbers

Peripheral blood was collected in EDTA tubes from each participant and immediately kept at −70°C until use. Genomic DNA was extracted from blood by the phenol extraction method. DNA concentrations were measured by UV absorbance using Gene Quant 100 (Applied Biosystems, Foster City, CA) and diluted to 10 ng/μL.

The quantitation of CN was performed using duplex RT-PCR–based copy number analysis (TaqMan Copy Number Assays; Applied Biosystems) for four DNA repair genes (Table 2). The same method was previously used to determine CNVs of *GSTT1* and *GSTM1* in our laboratory.[22] In these DNA repair genes, CNV within *WRN* has not been reported in the Database of Genomic Variants (http://projects.tcag.ca/variation/), a centralized summary of structural variations in the human genome. For each 10-μL single-well reaction containing 10 ng of genomic DNA and 5 μL of TaqMan Universal PCR Master Mix II, No AmpErase UNG, a 0.5-μL TaqMan Copy Number Assay, which contained forward primer, reverse primer, and FAM dye–labeled MGB probe specific for the gene of interest, was run simultaneously with a 0.5-μL TaqMan Copy Number Reference Assay, which contained forward primer, reverse primer, and a VIC dye–labeled TAMRA probe specific for RNase P according to the manufacturer's instructions.

**Table 2.** TaqMan Copy Number Assay Information

| Gene | Target Variation ID | TaqMan Assay ID | Assay Location | Assay Cytoband | Assay Gene Location |
|---|---|---|---|---|---|
| *ERCC6* | Variation_60013 | Hs01920803_cn | Chr10:50681070 | 10q11.23a | Intron 14–exon 15 |
| *WRN* | — | Hs06237156_cn | Chr8:30954001 | 8p12d | Intron 16 |
| *OGG1* | Variation_115655 | Hs01955678_cn | Chr3:9803327 | 3p25.3c | Intron 6–exon 6 |
| *HSF4* | Variation_114213 | Hs02989737_cn | Chr.16:6719837 | 16q22.1a | Intron 2–exon 3 |

**Table 3.** Copy Number Frequencies in ARC Patients and Normal Controls

| CN | ARC *n* (%), *n* = 780 | Control *n* (%), *n* = 525 | OR (95% CI) | *P*/Pc |
|---|---|---|---|---|
| *ERCC6* | | | | |
| 0 | 0 (0) | 0 (0) | — | — |
| 1 | 7 (0.9) | 7 (1.3) | 0.68 (0.24–1.96) | 0.48 |
| 2 | 742 (95.1) | 506 (96.4) | 1.0 (reference) | — |
| 3+ | 31 (4.0) | 12 (2.3) | 1.76 (0.90–3.46) | 0.10 |
| *WRN* | | | | |
| 0 | 1 (0.1) | 1 (0.2) | 0.69 (0.04–11.08) | 0.79 |
| 1 | 4 (0.5) | 4 (0.8) | 0.69 (0.17–2.78) | 0.60 |
| 2 | 726 (93.1) | 502 (95.6) | 1.0 (reference) | — |
| 3+ | 49 (6.3) | 18 (3.4) | 1.88 (1.08–3.27) | 0.02/0.08 |
| *OGG1* | | | | |
| 0 | 0 (0) | 0 (0) | — | — |
| 1 | 10 (1.3) | 5 (1.0) | 1.35 (0.46–3.98) | 0.58 |
| 2 | 760 (97.4) | 514 (97.9) | 1.0 (reference) | — |
| 3+ | 10 (1.3) | 6 (1.1) | 1.13 (0.41–3.12) | 0.82 |
| *HSF4* | | | | |
| 0 | 0 (0) | 0 (0) | — | — |
| 1 | 29 (3.7) | 5 (1.0) | 4.09 (1.57–10.63) | 0.004/0.016 |
| 2 | 724 (92.8) | 510 (97.1) | 1.0 (reference) | — |
| 3+ | 27 (3.5) | 10 (1.9) | 1.90 (0.91–3.96) | 0.09 |

The reactions were run on an Applied Biosystems 7500 real-time PCR system using absolute quantitation settings. Thermal cycling conditions were adjusted as follows: initial denaturation step for 10 minutes at 95°C; 40 cycles including denaturation for 15 seconds at 95°C; and annealing/extension for 1 minute for 60°C. Each sample was assayed in triplicate on the same plate and the data were collected using SDS 2.0 software (Applied Biosystems). CNVs were estimated using the ΔΔCt relative quantification method, and the calculation was carried out by a maximum likelihood algorithm built in the CopyCaller Software version 2.0 (Applied Biosystems). This analytical method calculated the relative CN of a target gene normalized to RNase P, a reference known to exist in two copies in a diploid genome. Quantitative CN was defined as an integer number of copy determined by the algorithm (CN = 0, 1, 2, or 3+). CN = 2 category was selected as the reference for statistical analysis because humans (diploids) are expected to have two copies of most genes. Copy number (CN) gain is defined as CN higher than 2. CN loss is defined as CN less than 2.

## 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 performed the assay on 67 ARC patients (N = 28, C = 28, PSC = 2, M = 9) and 40 age- and sex-matched normal controls; the average age was 70.51 ± 6.72 years in ARC patients and 68.80 ± 4.87 years in normal controls ($P > 0.05$).

The peripheral lymphocytes from whole blood in EDTA anticoagulation tube were isolated and suspended in PBS at $1 \times 10^4$ 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, and 10 mM Tris, pH 10, followed by electrophoresis (20 V, 200 mA) in a 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 above were performed under dimmed light or in the dark.[26]

The Comet images were observed at 400× magnification under a fluorescence microscope (Leica, Solms, 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) was measured.

## Statistical Analysis

All statistical analyses were performed using Stata software (version 10.0; StataCorp Lakeway, College Station, TX). Chi-square was used to compare the frequencies of each CN category (CN = 0, 1, 2, or 3+) of each gene, respectively, between ARC patients and normal controls. The odds ratios (OR) and their 95% confidence intervals (CI) were calculated to estimate the genotype distributions of CNVs between ARC patients and normal controls, and $P < 0.05$ was considered statistically significant. If an initial analyses reached significant, the *P* values were corrected (Pc) by Bonferroni correction with the number of analyses performed. The ANOVA was used to compare the differences of the Comet assay parameters between ARC patients and normal controls.

We estimated our statistical power based on a predefined two-sided alpha of 0.05, there was greater than 90% power to detect a ±3% departure from an HSF4 CNV frequency of 2.9% in our sample set. Study power remained greater than 85% after stratifying the cases by cataract subtypes and sex.

# Results

The CNV distribution of *ERCC6*, *WRN*, *OGG1*, and *HSF4* in ARC patients and normal controls are listed in Table 3. Novel CNV was detected within *WRN* in both groups. ARC patients with CN = 3+ for *WRN* had an increased risk of ARC (OR = 1.88; CI, 1.08–3.27; $P = 0.02$). ARC Patients with CN = 1 for *HSF4* had an increased risk of ARC (OR = 4.09; CI, 1.57–10.63; $P = 0.004$). However, after Bonferroni correction, CN = 3+ for *WRN* lost significance.

Table 4 shows the distribution of CN frequencies of *ERCC6*, *WRN*, *OGG1*, and *HSF4* in ARC patients and normal controls after stratifying by the cataract subtypes. When comparing with normal controls, the CN frequencies of CN = 3+ for *WRN* was significantly different in N and PSC type of ARC (OR = 2.06; CI, 1.11–3.83; $P = 0.02$; and OR = 3.72; CI, 1.18–11.68; $P = 0.02$). However, after Bonferroni correction, the significance was lost. The CN frequencies of CN = 1 for *HSF4* was also significantly different in N and PSC type of ARC (OR = 5.73; CI, 2.12–15.50; Pc = 0.004; and OR = 6.80; CI, 1.27–36.52; Pc = 0.04). No statistically significant difference was found for the CNVs in *ERCC6* and *OGG1* between ARC patients and normal controls or after stratifying ARC by the subtypes.

The stratification based on sex revealed a statistically significant association between CN = 3+ for *WRN* and ARC for women (OR = 2.31; CI, 1.04–5.12; $P = 0.03$); additionally, CN = 1 for *HSF4* was associated with ARC for women (OR = 4.33; CI, 1.27–14.70; $P = 0.01$) (Table 5). However, after Bonferroni correction, only CN = 1 for *HSF4* remained significant for women (Pc = 0.04). We also analyzed CNV frequencies of *WRN* and *HSF4* between male and female patients and between male and female controls, and no statistically significant difference was found ($P > 0.05$).

We examined the combined effects of CNVs of *ERCC6*, *WRN*, *OGG1*, and *HSF4* in ARC patients and normal controls (Table 6). CN gain and loss for *WRN* was not associated with ARC risk. However, CN gain and loss for *HSF4* was associated with risk for ARC (OR = 2.63; CI, 1.47–4.70; $P = 0.001$). We

**Table 4.** Distribution of CNVs of *ERCC6*, *WRN*, *OGG1*, and *HSF4* in Normal Controls and in Various ARC Subtypes

| | | Subtypes of ARC | | | |
|---|---|---|---|---|---|
| **CN** | **Control *n* (%)** | **Cortical *n* (%), *n* = 257** | **Nuclear *n* (%), *n* = 368** | **PSC *n* (%), *n* = 34** | **Mixed *n* (%), *n* = 121** |
| *ERCC6* | | | | | |
| 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 1 | 7 (1.3) | 2 (0.8) | 5 (1.4) | 0 (0) | 0 (0) |
| 2 | 506 (96.4) | 245 (95.3) | 350 (95.1) | 33 (97.1) | 114 (94.2) |
| 3+ | 12 (2.3) | 10 (3.9) | 13 (3.5) | 1 (2.9) | 7 (5.8) |
| *WRN* | | | | | |
| 0 | 1 (0.2) | 0 (0) | 1 (0.3) | 0 (0) | 0 (0) |
| 1 | 4 (0.8) | 1 (0.4) | 3 (0.8) | 0 (0) | 0 (0) |
| 2 | 502 (95.6) | 244 (95.0) | 339 (92.1) | 30 (88.2) | 113 (93.4) |
| 3+ | 18 (3.4) | 12 (4.7) | 25 (6.8)* | 4 (11.8)* | 8 (6.6) |
| *OGG1* | | | | | |
| 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 1 | 5 (1.0) | 2 (0.8) | 5 (1.4) | 1 (2.9) | 2 (1.7) |
| 2 | 514 (97.9) | 251 (97.7) | 358 (97.3) | 32 (94.1) | 119 (98.3) |
| 3+ | 6 (1.1) | 4 (1.6) | 5 (1.4) | 1 (2.9) | 0 (0) |
| *HSF4* | | | | | |
| 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 1 | 5 (1.0) | 6 (2.3) | 19 (5.2)† | 2 (5.9)† | 2 (1.7) |
| 2 | 510 (97.1) | 240 (93.4) | 338 (91.8) | 30 (88.2) | 116 (95.9) |
| 3+ | 10 (1.9) | 11 (4.3) | 11 (3.0) | 2 (5.9) | 3 (2.5) |

* $P < 0.05$, Pc $> 0.05$.
† $P < 0.05$, Pc $< 0.05$.

found that the combination of *WRN* CNVs and *HSF4* CNVs markedly increased the risk of ARC. The OR was increased from 2.63 by *HSF4* alone to 6.80 by combined *WRN* and *HSF4* CNVs. The combined effects of CNVs were not evident in other combinations.

Coincidently, all the samples selected for the Comet assay were from individuals with CN = 2 of four DNA repair genes. Therefore, the data could not be used for the analysis on the correlation between DNA damage of peripheral lymphocytes and CNVs. But the DNA damage in lymphocytes (the Tail DNA% and OTM values in Comet assay) from ARC patients was significantly higher when compared to normal controls (Table 7). The stratification on sex did not unveil statistically significant differences of the Tail DNA% and OTM values between men and women either in ARC patients or in normal controls (Table 8). The statistically significant differences remained between patients and controls in both male and female groups ($P < 0.01$).

## Discussion

This study, to the best of our knowledge, is the first population-based study to investigate the association of CNVs in DNA repair genes with ARC. The design of a population-based study can minimize sample selection bias often existing in hospital-based case–control study.

CNVs are segments of DNA that are 1 kb or larger and present at a variable CN in comparison with a reference genome. CNVs in general are stable and can be inherited. Beside SNPs, CNVs are now considered an important form of genetic variation. Findings in the past few years have indicated a strong association of CNVs with several complex and common disorders. The genomic rearrangements responsible for CNVs can lead to pathogenic phenotypes by modulating gene dosage, interrupting a gene, creating a fusion gene, exerting position effects, or unmasking a deleterious recessive mutation.[27–30] Therefore, higher CN does not always mean gain-of-function, it could also result in loss-of-function.

**Table 5.** Copy Number Frequencies of *WRN* and *HSF4* in ARC Patients and Normal Controls, Stratified by Sex

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| **CN** | **ARC *n* (%)** | **Control *n* (%)** | **OR (95% CI)** | ***P*/Pc** | **ARC *n* (%)** | **Control *n* (%)** | **OR (95% CI)** | ***P*/Pc** |
| *WRN* | | | | | | | | |
| 0 | 0 (0) | 1 (0.4) | 0.26 (0.01–6.37) | 0.26 | 1 (0.2) | 0 (0) | 1.91 (0.08–47.14) | 0.43 |
| 1 | 2 (0.6) | 3 (1.3) | 0.52 (0.09–3.12) | 0.46 | 2 (0.4) | 1 (0.3) | 1.27 (0.11–14.12) | 0.84 |
| 2 | 288 (92.9) | 223 (94.1) | 1.0 (reference) | — | 438 (93.2) | 279 (96.9) | 1.0 (reference) | — |
| 3+ | 20 (6.5) | 10 (4.2) | 1.55 (0.71–3.38) | 0.27 | 29 (6.2) | 8 (2.8) | 2.31 (1.04–5.12) | 0.03/0.12 |
| *HSF4* | | | | | | | | |
| 0 | 0 (0) | 0 (0) | — | — | 0 (0) | 0 (0) | — | — |
| 1 | 9 (2.9) | 2 (0.8) | 3.54 (0.76–16.56) | 0.09 | 20 (4.3) | 3 (1.0) | 4.33 (1.27–14.70) | 0.01/0.04 |
| 2 | 291 (93.9) | 229 (96.6) | 1.0 (reference) | — | 433 (92.1) | 281 (97.6) | 1.0 (reference) | — |
| 3+ | 10 (3.2) | 6 (2.5) | 1.31 (0.47–3.66) | 0.60 | 17 (3.6) | 4 (1.4) | 2.76 (0.92–8.28) | 0.06 |

**Table 6.** Combined Effects of CNVs of *ERCC6*, *WRN*, *OGG1*, and *HSF4* in ARC Patients and Normal Controls

| | CN Gain and Loss | | | |
|---|---|---|---|---|
| Gene | ARC, *n* (%) | Control, *n* (%) | OR (95% CI) | *P*/Pc |
| *ERCC6* | 38 (4.87) | 19 (3.62) | 1.36 (0.78–2.39) | 0.28 |
| *WRN* | 54 (6.92) | 23 (4.38) | 1.62 (0.98–2.68) | 0.06 |
| *OGG1* | 20 (2.56) | 11 (2.10) | 1.23 (0.58–2.59) | 0.59 |
| *HSF4* | 56 (7.18) | 15 (2.86) | 2.63 (1.47–4.70) | 0.001/0.004 |
| *ERCC6+WRN* | 10 (1.28) | 7 (1.33) | 0.96 (0.36–2.54) | 0.94 |
| *ERCC6+0GG1* | 2 (0.26) | 5 (0.95) | 0.27 (0.05–1.38) | 0.09 |
| *ERCC6+HSF4* | 3 (0.38) | 3 (0.57) | 0.67 (0.14–3.34) | 0.60 |
| *WRN+OGG1* | 4 (0.51) | 2 (0.38) | 1.35 (0.25–7.39) | 0.73 |
| *WRN+HSF4* | 10 (1.28) | 1 (0.19) | 6.80 (0.87–53.35) | 0.03/0.12 |
| *OGG1+HSF4* | 5 (0.64) | 3 (0.57) | 1.12 (0.27–4.72) | 0.87 |

**Table 7.** DNA Damage in Lymphocytes from ARC Patients and Normal Controls

| | ARC ($n = 67$) | Control ($n = 40$) | *P* |
|---|---|---|---|
| Age, y; mean ± SD | 70.51 ± 6.72 | 68.80 ± 4.87 | 0.11 |
| Sex | | | 0.78 |
| Male, *n* (%) | 30 (44.8) | 19 (47.5) | |
| Female, *n* (%) | 37 (55.2) | 21 (52.5) | |
| Tail DNA% | 22.00 ± 4.78 | 10.21 ± 6.09 | <0.01 |
| OTM | 6.42 ± 1.77 | 2.45 ± 1.81 | <0.01 |

Defects in DNA repair pathways are connected to many different types of diseases. The *WRN* gene plays an important role in aging and is known to function extensively in the DNA repair process.[31] To date, researchers have investigated the possible relationship of many mutations, deletions, and polymorphisms of the *WRN* gene with age-related diseases such as cardiovascular disease, hypertension, diabetes mellitus, dementia, osteoporosis, and some cancers.[32–37] The CNVs in *WRN* identified in our study have not been documented in CNV databases such as Database of Genomic Variants, and the findings suggested that CN = 3+ for *WRN* appeared to be a likely risk factor for ARC. Although the function of *WRN* has been intensively investigated in primary fibroblast and fibroblast cell lines, little is known about the normal expression pattern of the protein in the eye.[15] Therefore, the functional consequences of CNV in *WRN* and its impact on ARC require further evaluation.

HSF4 is expressed exclusively in the ocular lens and plays a critical role in lens formation and differentiation. Mutations in the *HSF4* gene lead to congenital and senile cataract. A recent study found evidence that *HSF4* contributes to the repair of DNA DSB.[20] In addition, *HSF4* regulates a group of specific lens structural proteins that play essential roles in lens differentiation and maintenance of the lens' normal functions. *HSF4* mutations disrupt the *HSF4* heat shock elements binding abilities and reduce the expression of lens structural proteins.[38,39] It has been reported that *HSF4* mutations account for a small fraction of ARC in the Han Chinese population.[18,19] In our present study, we found that CN = 1 for *HSF4* appeared to be a risk factor for ARC, and the association was again observed mainly with N and PSC type of ARC. The interference of DNA repair and decreased expression of lens structural proteins may account for the association between the *HSF4* CNV and ARC.

The stronger association with N and PSC type of ARC might come from different oxidative stresses and genetic contribution to the different types of cataracts. The evidence for a causal role of oxidation damage is strong for the N type, but substantially lower for the C and PSC types of ARC. Moreover, earlier studies found that family history was a risk factor for ARC,[40–43] while the strongest evidence came from twin studies demonstrating a heritability of 48% for nuclear cataract.[44] Even though we tried our best to enlarge the sample size, our PSC cases remained few in number. Because our study was population based, the number of cases was fixed at the time point of the survey. We could confirm the results for PSC cases in the follow-up if additional cases are included.

Many studies worldwide have reported a higher prevalence of cataract among women.[45–48] Our results indicate that women with CN = 1 for *HSF4* were more likely to suffer from ARC. This susceptibility might be due to intrinsic differences such as hormonal factors.[46]

Studies have reported that a SNP (rs3793784) in the *ERCC6* gene is associated with a risk of age-related macular degeneration development, and *OGG1* Cys/Cys genotype may be associated with increased risk of ARC.[16,26,49,50] But we did not find statistically significant difference of CNVs in *ERCC6* and *OGG1* between ARC patients and normal controls. The low CNV frequencies in *ERCC6* and *OGG1* other than CN = 2 might be one reason an association could not be identified.

A recent study found evidence that a combination of *GSTM1* positive and *GSTT1* null genotypes was significantly associated with cortical ARC development.[17] Our results showed that the combination *WRN* and *HSF4* CNVs markedly increased the risk of ARC. It is conceivable that the development of ARC may not depend upon a single CNV but a combination of various CNVs and other genetic variations such as SNPs.[27]

The extent of DNA damage can be assessed by Comet assay. In the present study, DNA damage in lymphocytes in ARC patients was higher than that of the normal controls. This result was in line with a study that found elevated levels of 8-OH-Gua, marker of oxidative DNA damage, in the leukocytes of patients with cataract.[51] Additional studies are needed to further investigate possible links between DNA damage of peripheral lymphocytes and CNVs in DNA repair genes.

In summary, this is the first population-based study to evaluate the possible association between CNVs in four DNA repair genes and ARC in the Han Chinese population. The data support the notion of DNA repair mechanism in the pathogenesis of ARC. However, the functional consequences of CNVs and the underlying mechanisms of the heterogeneous expression levels need to be investigated in the future.

**Table 8.** DNA Damage Extent of ARC Patients and Normal Controls, Stratified by Sex

| | ARC | | | Control | | |
|---|---|---|---|---|---|---|
| DNA Damage | Male | Female | *P* | Male | Female | *P* |
| Tail DNA% | 21.88 ± 4.36 | 22.05 ± 5.06 | >0.05 | 8.16 ± 3.47 | 12.73 ± 7.63 | >0.05 |
| OTM | 6.39 ± 1.38 | 6.44 ± 1.96 | >0.05 | 1.83 ± 1.08 | 3.22 ± 2.23 | >0.05 |

Manipulating these targets may provide a strategy to prevent or slow the progression of ARC.

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