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RESEARCH ARTICLE

Predictive impact of genetic polymorphisms in DNA repair genes on susceptibility and therapeutic outcomes to colorectal cancer patients

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Abstract Several hereditary syndromes characterized by defective DNA repair are associated with high risk of colorectal cancer (CRC). To explore whether common polymorphisms in DNA repair genes affect risk and prognosis of CRC, we evaluated the association between single nucleotide polymorphisms (SNPs) in *XPG*, *XPC*, and *WRN* gene and susceptibility of CRC, and clinical outcomes in a population-based case–control study. A total of 890 CRC cases and 910 controls recruited into the study provided a biologic sample. Individuals with variant genotypes of *XPC* Ala499Val appeared to be associated with the increased risk of CRC. *WRN* Cys1367Arg variants carriers showed an increased susceptibility for CRC. More importantly, the risk of CRC increased further in a combined analysis of multiple polymorphisms. Furthermore, stratified analyses revealed that *XPG* Arg1104His polymorphism was associated with tumor differentiation of CRC patients (P=0.043). Log-rank test and adjusted multivariate Cox regression analysis verified that *XPG* Arg1104His variants were associated with a longer disease-free survival (DFS) [CG genotype: adjusted HR (95 % confidence interval (CI))= 0.163 (0.107–0.248), P<0.001; CC genotype: adjusted HR (95 % CI)=0.333 (0.235–0.470), P<0.001; CG/CC genotype: adjusted HR (95 % CI)=0.333 (0.235–0.470)] in patients with oxaliplatin-based chemotherapy (N=718). Moreover, *XPC* Ala499Val CT genotype showed a significant impact on DFS [CC genotype: adjusted HR (95 % CI)=0.691 (0.528–0.904), P=0.007; CT/CC genotype: adjusted HR (95 % CI)= 0.602 (0.389–0.934), P=0.024]. However, no correlation was found between *WRN* Cys1367Arg polymorphism and prognosis in CRC patients. Our findings will add to the literature on the impact of genetic variation in DNA repair genes involved in susceptibility for CRC and therapeutic outcomes in response to oxaliplatin-based chemotherapy.

Keywords *XPG* · *XPC* · *WRN* · Genetic polymorphisms · Colorectal cancer · Susceptibility · Prognosis

Abbreviations

XPG	Xeroderma pigmentosum complementation group G
XPC	Xeroderma pigmentosum complementation group C
WRN	Werner syndrome, RecQ helicase-like
OR	Odds ratio
CI	Confidence interval
LD	Linkage disequilibrium
PCR–RFLP	Polymerase chain reaction-restriction fragment length polymorphism
HWE	Hardy–Weinberg equilibrium
Folfox	Oxaliplatin, leucovorin plus 5-FU
Xelox	Oxaliplatin plus capecitabine
Lv5-Fu2	Leucovorin plus 5-FU
FUP	(Fluorouracil plus cisplatin

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Introduction

Colorectal cancer (CRC) is the third most common gastrointestinal tract malignancy and the fourth leading cause of cancer mortality worldwide. Nowadays, CRC is becoming one of major challenge to public health in China because of lifestyle changes and the process of westernization [1]. Accumulating evidence supports that genetics play an important role in the etiology of CRC [2]. CRCs frequently occur in some hereditary cancer syndromes with germline mutations of DNA repair genes; however, genetic factors responsible for sporadic CRCs have not been largely determined.

DNA repair has an essential role in protecting the genome from damage by endogenous and environmental agents [3]. Further, repair of DNA damage is a complex process in maintaining genome integrity, which is conducted by a series of DNA repair pathways, consisting of more than 130 genes. Nucleotide excision repair (NER) is one of major pathway, which deal with the UV radiation or chemical agents induced DNA damage, is responsible for removal of a wide variety of structural unrelated bulky DNA adducts and helix-distorting lesions [4, 5]. Previous studies provided evidence that those polymorphisms of the NER pathway genes may alter the DNA repair capacity and thus could play an important role in carcinogenesis or response to chemotherapy [6–9].

Xeroderma pigmentosum group G (*XPG*) is one of the NER genes and responsible for 1,186 amino acid structure-specific endonuclease activity, thereby playing a key role in NER of helix-distorting DNA damage [5]. Previous studies investigated the role of common polymorphism Asp1104His (G-to-C transition in 13q33 exon15, rs17655) of *XPG* in the etiology of various cancers [10–13]. Only two studies demonstrated the association of Asp1104His polymorphism of *XPG* with CRC risk. Liu et al. observed that *XPG* Asp1104His (GC or CC genotype) had an increased risk for CRC [14]. However, another study did not found any association of Asp1104His with CRC risk and found no significant results either [15]. Taken together, the two results remain conflicting rather than conclusive and need further investigations for solving these discrepancies.

Xeroderma pigmentosum group C (*XPC*) is located at chromosome 3p25, which is also one of the eight core genes in the NER pathway, particular plays an important role in the early steps (damage recognition, open complex formation and reparation) of genome NER [5]. Recent studies have showed that polymorphisms of *XPC* gene may alter the DNA repair capacity (DRC) and modulate the susceptibility to cancer. The Ala499Val (C/T) in exon 9 of *XPC* gene has been previously identified in several tumors. However, very little information is available about the association of Ala499Val polymorphism with CRC risk. Only Wu et al. reported that individuals carrying the Ala499Val CT + TT genotypes showed a significantly decreased risk of rectal cancer in a small size [16].

The DNA repair gene *WRN* is located at chromosome 8p11-12. WRN protein is an important member of the RecQ helicase family, which is involved in multiple DNA repair pathways, protecting the genome from incorrect recombination during mitosis and maintaining its stability. Several studies have shown a relationship between WRN expression and malignancy and have indicated that epigenetic inactivation of WRN is of importance in carcinogenesis [17–20]. Since mutations in the *WRN* gene can accelerate aging, it has been reasoned that *WRN* polymorphisms may also be associated with age-related pathologies, including the cancer susceptibility. Cys1367Arg (rs1346044), one of the common SNPs in *WRN* gene, usually manifests T to C variation and leads to a protein variation of 1367 Cys to Arg. In previous studies, WRN Cys1367Arg had been found to be associated with many diseases, including chronic kidney disease, glioma, and familial breast cancer [21–23]. However, the association between the Cys1367Arg and CRC susceptibility and prognosis has not been explored yet.

Therefore, in the present study, we analyzed the common genetic polymorphisms in the DNA repair genes *XPG*, *XPC*, and *WRN* in a large sample case–control study from 890 CRCs and 910 healthy controls, and attempted to elucidate the association between these polymorphisms and CRC susceptibility, clinicopathological features, and clinical outcomes with oxaliplatin-based chemotherapy.

Patients and methods

Subjects

This study included 890 patients with CRC who were admitted to the General Hospital of CNPC in Jilin between 2006 and 2012. Approximately, 89 % of contacted patients consented to enrollment in the study. Finally, 890 patients were included in the study. The principal clinical characteristics were obtained from the interviewer-administered health risk questionnaires and medical records. Tumor differentiation or pathological grade of these CRC patients was performed according to the World Health Organization criteria and DUKE's criteria, respectively. The patients received several chemotherapy regimens, including oxaliplatin-based chemotherapy (Folfox regimen: oxaliplatin, leucovorin plus 5-FU; Xelox regimen: oxaliplatin plus capecitabine), LV5–FU2 (leucovorin plus 5-FU), or FU alone (fluorouracil).

We also included 910 unrelated age- and gender–matched healthy controls. The population had no known medical illness or hereditary disorders and was not taking any medications. Before its commencement, this study was approved by the Research Ethics Committee of the First Affiliated Hospital of Dalian Medical University, and informed consent was obtained from each participant.

Genotyping assay

Genomic DNA was isolated from a leukocyte cell pellet of each blood sample, using the TaKaRa DNA Blood Mini Kit (TaKaRa Biotechnology (Dalian) Co., Ltd, Dalian, China). The *XPG*, *XPC*, and *WRN* Cys1367Arg genetic polymorphisms were genotyped by PCR–RFLP assay. PCR was performed using 50~100 ng of genomic DNA, 300 nM of each primer, 200 nM dNTPs, and 0.5 U Taq polymerase in PCR buffer (TaKaRa Biotechnology (Dalian) Co., Ltd, Dalian, China) in a total volume of 25 μl. For *XPG* Arg1104His, the 271-base pair (bp) PCR products were digested with Hsp92II overnight at 37 °C. The T allele was uncut, and the polymorphic allele (C) produced a 227-bp and 44-bp fragment. The PCR products of *XPC* Ala499Val were digested with SacII (Promega Corporation, U.S.A.) at 37 °C overnight. The T allele was uncut, and the C allele had a SacII restriction site, 2 bands were generated (131-bp and 21-bp fragment), while the T allele had a single fragment with a size of 152-bp. For *WRN* Cys1367Arg, the 193-bp PCR products were digested with HSP92II (Promega Corporation, U.S.A.) at 37 °C overnight. The C allele was cut into 118-bp, 53-bp, and the T allele was cut into 90-bp and 53-bp bands. Samples were coded for case–control status, and at least 10 % of the samples were randomly selected and subjected to repeat analysis as quality control for verification of genotyping procedures. Two researchers independently performed RFLP and reviewed all genotyping results.

Statistical analysis

The SPSS software package version 16.0 (SPSS Inc, Chicago, USA) was used for statistical analyses. The association between the distributions of demographic, epidemiologic, and clinical parameters was assessed by using the chi-square (Pearson χ^2 test) or Fisher exact test. The associations between genotypes and CRC risk were calculated by odd ratios (OR) and 95 % confidence interval (CI). The population genetic analysis program SNPAlyze 2.2 (Dynacom Co. Ltd, Yokohama, Japan) was used for linkage disequilibrium (LD) analysis, and the Hardy–Weinberg equilibrium test. The disease-free survival (DFS) was defined as the time between the diagnosis and an occurrence of relapse, death, or last known follow-up. The overall survival (OS) was defined as the time between the diagnosis and death, or last known follow-up. The DFS or OS curves were plotted by using the Kaplan–Meier method, and the statistical differences in survival among groups were compared by log-rank test. The independent prognostic values of different polymorphism in *XPG*, *XPC*, and *WRN* gene and their association with survival time of CRC patients were analyzed by univariate or multivariate Cox hazards regression model. The Cox hazard ratio and its 95 % CI were obtained accordingly. All statistical significance was set at $P<0.05$, and all tests were two-sided.

Results

The baseline characteristics and clinical features of 890 CRC patients and 910 controls are summarized in Table 1. There were no significant differences in the distributions of gender and age between CRC patients and controls ($P=0.646$ and $P=0.625$, respectively). The mean age at diagnosis (SD) was 58.2(10.3) and at the range of 23~79 years old. All the cases included in this study, 57.2 % of CRC patients were male, and 84.6 % of them were in the status of ever smoking. Among 890 patients, the vast majority patients were in grade 2 (G2, moderate, 82.4 %), while only 8.7 % of them were in grade 1 (G1, well). Most of CRC patients (80.7 %) received oxaliplatin-based chemotherapy, whereas 11.3 % of them underwent fluorouracil chemotherapy.

We carried out a standard allelic association analysis and genotype distribution in *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms between CRC patients and controls, outlined in Table 2. The observed genotype frequency among individuals in control group fit well to Hardy–Weinberg equilibrium ($P=0.498$ for *XPG* Arg1104His, $P=0.067$ for *XPC* Ala499Val, $P=0.630$ for *WRN* Cys1367Arg). A significant increased in the frequency of CC genotype in *XPC* Ala499Val polymorphism was observed [CC vs TT, $P<0.001$; adjusted OR (95 % CI)=2.194 (1.598–3.011)]. Moreover, there was a significant difference in the CT/CC genotypes or C allele of the *XPC* Ala499Val between CRC patients and controls and appeared to be associated with an 1.212-, 1.294-fold increased risk of CRC [$P=0.049$, adjusted OR (95 % CI)=1.212 (0.966–1.476); $P<0.001$, adjusted OR (95 % CI)=1.294 (1.132–1.479), respectively] in recessive model. For *WRN* Cys1367Arg polymorphism, the heterozygous variant CT genotype or homozygous variant TT genotype showed a significantly increased risk of CRC in comparison to the CC genotype [CT vs CC, $P<0.001$; adjusted OR (95 % CI)=3.371 (1.752–6.487); TT vs CC, $P<0.001$; adjusted OR (95 % CI)=3.131 (1.663–5.895)]. Additionally, compared with the corresponding homozygous CC genotypes, the variant genotypes CT/TT of *WRN* Cys1367Arg had a significant increased risk of developing CRC after adjusted for age and gender in the recessive model [$P<0.001$; adjusted OR (95 % CI)=3.183(1.694–5.981)]. No significant difference was detected for CRC risk and *WRN* Cys1367Arg polymorphism in the dominant model. However, there were no significant associations observed between *XPG* Arg1104His polymorphism and the risk of CRC (Table 2).

Table 1 Clinical variables of the CRC patients in the study (*N*=890)

Variables	Cases (*N*=890)	%	Controls (*N*=910)	%	*P*[a]
Gender					
Male	509	57.2	516	56.7	0.646
Female	381	42.8	394	43.3	
Age (years)					
≤58	462	51.9	404	44.4	0.625
>58	428	48.1	506	55.6	
Mean age in years (SD)	58.2(10.3)		58.5(10.7)		
Smoking					
Ever	753	84.6	792	87.0	0.123
Never[a]	137	15.4	118	13.0	
First-degree family history of CRC					
No	777	87.3	–	–	
Yes	113	12.7	–	–	
Tumor size (cm)					
≤4.0	214	24	–	–	
>4.0	676	76	–	–	
Pathological grade					
Dukes A	162	18.2	–	–	
Dukes B	346	38.9	–	–	
Dukes C	302	33.9	–	–	
Dukes D	80	9	–	–	
Tumor differentiation					
Grade 1 (well)	77	8.7	–	–	
Grade 2 (moderate)	733	82.4	–	–	
Grade 3 (poor)	80	9	–	–	
Lymph node metastases					
No	340	38.2	–	–	
Yes	550	61.8	–	–	
Prime cancer					
Rectum	474	53.3	–	–	
Colon	416	46.7	–	–	
Therapeutic regimen					
Oxaliplatin-based chemotherapy[b]	718	80.7	–	–	
Fluorouracil chemothrapy[c]	101	11.3	–	–	
Other chemotherapies or treatments	71	8	–	–	

[a] Defined as <100 cigarettes in lifetime

[b] Oxaliplatin-based chemotherapy contains the following: Folfox (oxaliplatin, leucovorin plus 5-FU); Xelox (oxaliplatin plus capecitabine)

[c] Fluorouracil chemotherapy: Lv5-Fu2 (leucovorin plus 5-FU); FU (5-fluorouracil)

To further test our hypothesis that multiple SNPs in the DNA repair genes may have a joint effect on CRC risk, we estimated the combined effect based on two different SNPs. In this study, *XPG* Arg1104His C allele, *XPC* Ala499Val C allele, and *WRN* Cys1367Arg T allele were considered as favorable high-risk alleles. Table 3 showed the risk of CRC increased significantly with the number of putative risk genotypes for the combined *XPG* Arg1104His and *XPC* Ala499Val [two risk genotypes: *P*=0.016; adjusted OR (95 % CI)=1.523 (1.079–2.512); >2 risk genotype: *P*=0.005, adjusted OR (95 % CI)=1.718 (1.176–2.512), respectively] and adjusted OR=1.380, 95 % CI=1.046–1.821, *P*=0.022 for combined *XPC* Ala499Val and *WRN* Cys1367Arg. However, when *XPG* Arg1104His and *WRN* Cys1367Arg were considered together, no apparent increase in CRC risk was observed as the number of risk genotypes increased, as illustrated in Table 3.

We further investigated the correlation of *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg

Table 2 Frequency distribution of *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms and the associations with CRC risk

Genotypes	Cases No. (%)	Controls[a] No. (%)	Adjusted OR (95 % CI)[b]	P^b
All patients	890 (100.0)	910 (100.0)		
XPG Arg1104His				
GG	216 (24.3)	227 (24.9)	1 (reference)	
CG	476 (53.5)	497 (54.6)	1.007 (0.804–1.260)	0.954
CC	198 (22.2)	186 (20.4)	1.119 (0.851–1.471)	0.421
Dominant model				
GG	216 (24.3)	227 (24.9)	1 (reference)	
CG/CC	674 (75.7)	683 (75.0)	1.037(0.837–1.285)	0.739
Recessive model				
CC	198 (22.2)	186 (20.4)	1 (reference)	
CG/GG	692 (77.8)	724 (79.6)	0.898 (0.717–1.125)	0.349
Allele frequency				
G allele	908 (51.0)	951 (52.3)	1 (reference)	
C allele	872 (49.0)	869 (47.7)	1.051 (0.922–1.198)	0.456
XPC Ala499Val				
TT	149 (16.7)	79 (8.7)	1 (reference)	
CT	465 (52.2)	510 (56.0)	1.060 (0.865–1.301)	0.573
CC	276 (31.0)	321 (35.3)	2.194 (1.598–3.011)	*<0.001*
Dominant model				
CC	276 (31.0)	321 (35.3)	1 (reference)	
CT/TT	614 (69.0)	589 (64.7)	0.473 (0.354–0.632)	*<0.001*
Recessive model				
TT	149 (16.7)	79 (8.7)	1 (reference)	
CT/CC	741 (83.2)	831 (91.3)	1.212 (0.996–1.476)	*0.049*
Allele frequency				
T allele	763 (42.9)	668 (36.7)	1 (reference)	
C allele	1017 (57.1)	1152 (63.3)	1.294 (1.132–1.479)	*<0.001*
WRN Cys1367Arg				
C	13 (1.5)	41 (4.5)	1 (reference)	
CT	202 (22.7)	189 (20.8)	3.371 (1.752–6.487)	*<0.001*
TT	675 (75.8)	680 (74.7)	3.131 (1.663–5.895)	*<0.001*
Dominant model				
TT	675 (75.8)	680 (74.7)	1 (reference)	
CT/CC	215 (24.2)	230 (25.3)	0.942 (0.760–1.167)	0.582
Recessive model				
CC	13 (1.5)	41 (4.5)	1 (reference)	
CT/TT	877 (98.5)	869 (95.5)	3.183 (1.694–5.981)	*<0.001*
Allele frequency				
C allele	228 (12.8)	271 (14.9)	1 (reference)	
T allele	1552 (87.2)	1549 (85.1)	1.191 (0.985–1.440)	0.070

The significance levels are $P<0.05$ for all the italic values

OR, odds ratio, *CI* confidence interval

[a] The observed genotype frequency among individuals in the control group was in agreement with Hardy–Weinberg equilibrium ($P=0.498$ for *XPG* Arg1104His, $P=0.067$ for *XPC* Ala499Val, $P=0.630$ for *WRN* Cys1367Arg)

[b] *P* values and adjusted OR (95 % CI) were calculated by unconditional logistic regression adjusted for age and gender

polymorphisms with clinical variables of 890 CRC patients (Table 4). We only observed that the distribution frequency of the *XPG* Arg1104His polymorphism was associated with tumor differentiation (grade 1, 2, 3) of patients. The frequency

Table 3 The combined effect analysis between DNA repair genes polymorphisms and CRC susceptibility

No. of high-risk genotypes[a]	Cases No. (%)	Controls No. (%)	Adjusted OR (95 % CI)[b]	P^b
XPG Arg1104His and *XPC* Ala499Val				
0	65 (7.3)	96 (10.5)	1 (reference)	
1	241 (27.1)	268 (29.5)	1.328 (0.927–1.903)	0.121
2	399 (21.1)	387 (42.5)	1.523 (1.079–2.149)	*0.016*
>2	185 (9.8)	159 (17.5)	1.718 (1.176–2.512)	*0.005*
XPG Arg1104His and *WRN* Cys1367Arg				
0	171 (19.2)	166 (18.2)	1 (reference)	
1	398 (44.7)	418 (45.9)	0.924 (0.717–1.191)	0.543
2	267 (30.0)	266 (29.2)	0.974 (0.742–1.280)	0.852
>2	54 (6.1)	60 (6.6)	0.874 (0.571–1.337)	0.533
XPC Ala499Val and *WRN* Cys1367Arg				
0	216 (24.3)	233 (25.6)	1 (reference)	
1	415 (46.6)	468 (51.4)	0.957 (0.762–1.201)	0.701
2	206 (23.1)	161 (17.7)	1.380 (1.046–1.821)	*0.022*
>2	53 (6.0)	48 (5.3)	1.191 (0.773–1.835)	0.427

The significance levels are $P<0.05$ for all the italic values

OR, odds ratio, *CI* confidence interval

[a] The combined effect was group according to the numbers of favorable high-risk alleles (*XPG* Arg1104His C allele, *XPC* Ala499Val C allele, and *WRN* Cys1367Arg T allele were considered as favorable high-risk alleles)

[b] *P* values and adjusted OR (95 % CI) were calculated by unconditional logistic regression adjusted for age and gender

(32.5 %) of *XPG* Arg1104His GG genotype in the patients with grade 3 was significantly higher than that (16.9 %) in patients with grade 1 ($P=0.043$) (Table 4). However, no significant correlation of genotype distributions of *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms were found with the clinicopathological characteristics.

We further evaluated the correlation of *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms with the prognosis of CRC patients with oxaliplatin-based chemotherapy ($N=718$).

We found that *XPG* Arg1104His polymorphism have a significant impact on DFS (log-rank test: $P<0.001$; Fig. 1, A1) but not OS for CRC patients. The estimated median DFS time for *XPG* Arg1104His CG or CC genotype carriers were longer [136 months (127–142), 85 months (75–95), respectively] compared with the GG genotype carriers [44 months (40–48)]. The multivariate Cox regression analysis also established *XPG* Arg1104His as an independent prognostic factor [CG genotype, adjusted HR (95 % CI)=0.163 (0.107–0.248), $P<0.001$; CC genotype, adjusted HR (95 % CI)=0.333 (0.235–0.470), $P<0.001$; CG/CC genotype, adjusted HR (95 % CI)=0.333 (0.235–0.470)]. Furthermore, the *XPC* Ala499Val polymorphism was associated with a longer DFS (log-rank test, $P=0.018$; Fig. 1 B1). The estimated median PFS was 83 months for patients with CT genotype (95 % CI=64–101) and 59 months for patients with TT genotype (95 % CI=51–77). Moreover, the multivariate COX regression analysis also verified that *XPC* Ala499Val CT genotypes showed a better survival [CC genotype, adjusted HR (95 % CI)=0.691 (0.528–0.904), $P=0.007$; CT/CC genotype, adjusted HR (95 % CI)=0.602 (0.389–0.934), $P=0.024$], outlined in Table 5.

In addition, no correlation of *WRN* Cys1367Arg polymorphism was found with DFS (log-rank test, $P=0.437$; Fig. 1 C1) along with a tendency toward longer OS (log-rank test, $P=0.059$; Fig. 1, C2) for patients with postoperative oxaliplatin-based chemotherapy.

Discussion

DNA repair has an essential role in protecting the genome from damage by endogenous and environmental agents. Multiple genetic alterations contribute to CRC susceptibility. Further, increasing studies provided evidence that epigenetic modifications such as germline polymorphisms in DNA repair pathway genes can either change protein coding or alter the levels of transcription or translation, thereby reducing DNA repair capacity and inducing genetic instability or carcinogenesis [24]. In present molecular epidemiological case–control study, we sought to identify genetic polymorphisms of DNA repair genes *XPG*, *XPC*, and *WRN* that confer individual susceptibility to CRC and as predictors of response of patients to chemotherapy and survival.

Table 4 Association between *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms and clinicopathological features in CRC patients

Characteristics	*XPG* Arg1104His			*XPC* Ala499Val			*WRN* Cys1367Arg		
	GG No. (%)	CG/CC No. (%)	P^a	TT No. (%)	CT/CC No. (%)	P^a	CC No. (%)	CT/TT No. (%)	P^a
Sex			0.584			0.368			0.339
Men	127 (25.0)	382 (75.0)		164 (32.2)	345 (67.8)		380 (74.7)	129 (25.3)	
Women	89 (23.4)	292 (76.6)		112(29.4)	269 (70.6)				
Age (years)			0.984			0.329			0.233
≤58	112 (24.2)	350 (75.8)		150 (32.5)	312 (67.5)		358 (77.5)	104 (22.5)	
>58	104 (24.3)	324 (75.7)		126 (29.4)	302 (70.6)		317 (74.1)	111 (25.9)	
Smoking			0.144			0.918			0.269
Ever	176 (23.4)	577 (76.6)		233 (30.9)	520 (69.1)		566 (75.2)	187 (24.8)	
Never	40 (29.2)	97 (70.8)		43 (31.4)	94 (68.6)		109 (79.6)	28 (20.4)	
First-degree family history of CRC			0.921			0.835			0.438
No	189 (24.3)	588 (75.7)		240 (30.9)	537 (69.1)		586 (75.4)	191 (24.6)	
Yes	27 (23.9)	86 (76.1)		36 (31.9)	77 (68.1)		89 (78.8)	24 (21.2)	
Tumor size (cm)			0.471			0.339			0.128
<4	48 (22.4)	166 (77.6)		72 (33.6)	142 (66.4)		154 (72.0)	60 (28.0)	
≥4	168 (24.9)	508 (75.1)		204 (30.2)	472 (69.8)		521 (77.1)	155 (22.9)	
Tumor differentiation			*0.043*			0.328			0.616
Grade 1	13 (16.9)	64 (83.1)		21 (27.3)	56 (72.7)		61 (79.2)	16 (20.8)	
Grade 2	177 (24.1)	556 (75.9)		235 (32.1)	498 (67.9)		556 (75.9)	177 (24.1)	
Grade 3	26 (32.5)	54 (67.5)		20 (25.0)	60 (75.0)		58 (72.5)	22 (27.5)	
Pathological grade			0.518			0.287			0.654
Dukes A	39 (24.1)	123 (75.9)		50 (30.9)	112 (69.1)		126 (77.8)	36 (22.2)	
Dukes B	87 (25.1)	259 (74.9)		116 (33.5)	230 (66.5)		266 (76.9)	80 (23.1)	
Dukes C	76 (25.2)	226 (74.8)		92 (30.5)	210 (69.5)		226 (74.8)	76 (25.2)	
Dukes D	14 (17.5)	66 (82.5)		18 (22.5)	62 (77.5)		57 (71.2)	23 (28.8)	
Lymph node metastases			0.934			0.830			0.205
No	82 (24.1)	258 (75.9)		104 (30.6)	236 (69.4)		250 (73.5)	90 (26.5)	
Yes	134 (24.4)	416 (75.6)		172 (31.3)	378 (68.7)		425 (77.3)	125 (22.7)	
Prime cancer			0.350			0.664			0.307
Rectum	121 (25.5)	353 (74.5)		144 (30.4)	330 (69.6)		366 (77.2)	108 (22.8)	
Colon	95 (22.8)	321 (77.2)		132 (31.7)	284 (68.3)		309(74.3)	107 (25.7)	

[a] *P* was obtained from two-sided chi-square test

Our results obtained by analyzing 890 CRC patients and 910 controls, and a significant association between *XPC* Ala499Val polymorphism and CRC risk were observed. To date, there have been only three studies exploring relationship between the *XPC* Ala499Val variation and CRC risk [16, 25, 26].Similarly, Wu et al. found that Ala499Val variant individuals were at an increased risk for developing rectal cancer in a Chinese population, but in relative small samples. In contrast, the TT genotype carriers showed case-cohort studies, no association was found between Ala499Val genotypes and CRC risk. Previously, the associations between Ala499Val and risk of other variety of cancers have been studied, such as nasopharyngeal cancer, lung cancer, bladder cancer, and squamous cell cancer [27–30]. However, those findings were also inconsistent. For example, the individuals with heterozygous CT genotype or homozygous variant TT genotype had a lower risk for oral premalignant lesions cancer and gastric cancer [31, 32]. In contrast, the TT genotype showed an increased risk of nasopharyngeal cancer, lung cancer, bladder cancer, and squamous cell cancer. Thus, it might be stated that the role of *XPC* Ala499Val polymorphisms in cancer risk may vary with different diseases or race/ethnicity, and sample size, a possibility that warrants further investigation. Further functional assays for the expressional influence of the polymorphism will be needed.

Regarding *XPG* Arg1104His polymorphism, we found no association between the remaining variants of *XPG* and CRC

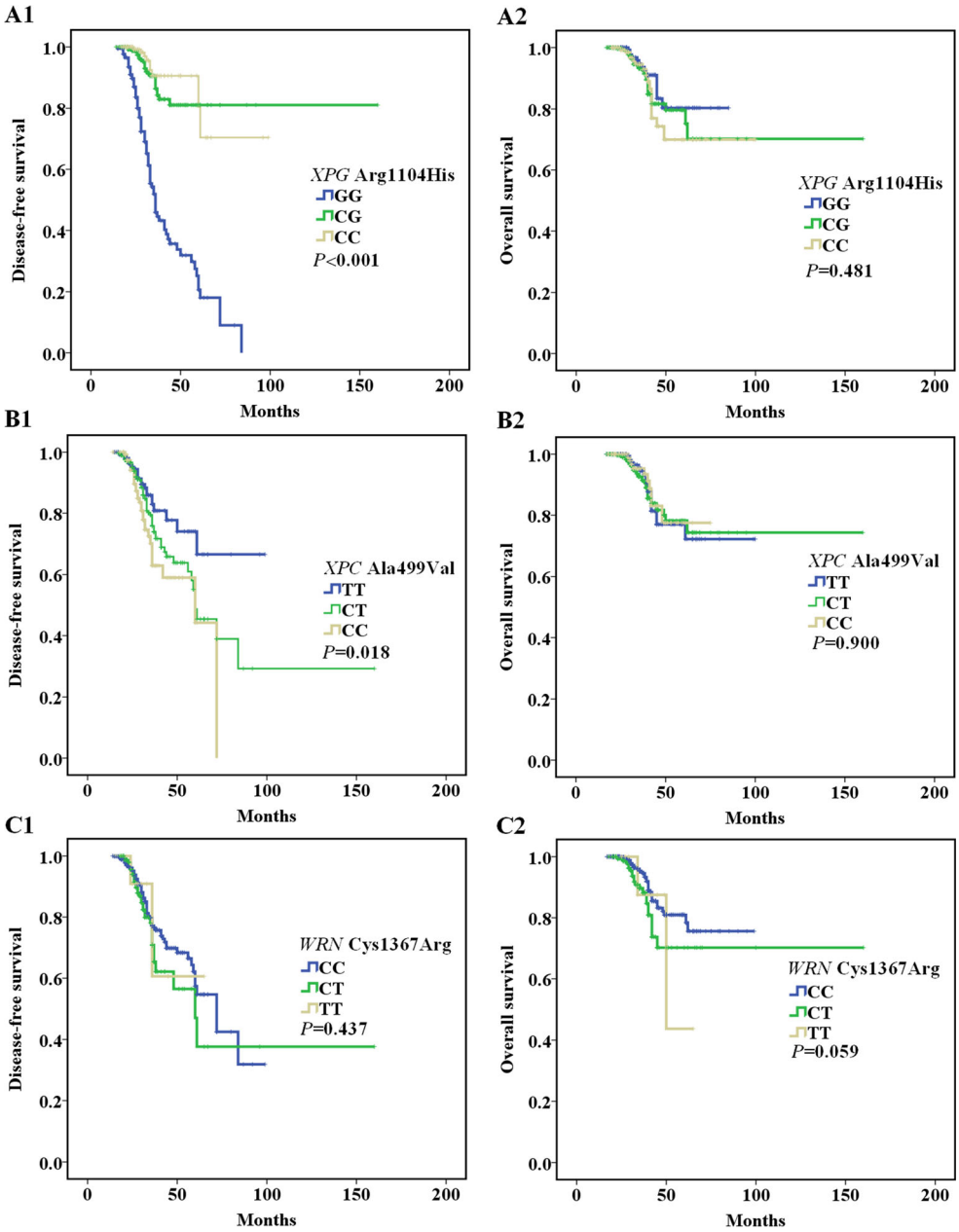

Fig. 1 Kaplan–Meier survival curves illustrating the DFS and OS in CRC patients with *XPG* Arg1104His, *XPC* Ala499Val, and *WRN* Cys1367Arg polymorphisms after postoperative oxaliplatin-based chemotherapy. *A1, B1, C1* disease-free survival (DFS). *A2, B2, C2* overall survival (OS)

risk. Up to now, only two reports have identified the correlation between CRC risk and the investigated Arg1104His genotypes. Nevertheless, consistent with our finding, Gil et al. [15] found no association between *XPG* Arg1104His and CRC susceptibility. In another case-cohort study, an increased CRC risk among individuals with the variant allele of Arg1104His was observed [14]. Taken together, these data suggests that further work is needed to extend these findings by carrying out extended haplotype analyses or related genes and to replicate the observations in other studies.

To the best of our knowledge, this is the first study to examine the polymorphism *WRN* Cys1367Arg and CRC risk and prognosis in a relatively large sample of Chinese population. Previous studies examined the role of *WRN* Cys1367Arg polymorphism in modulating cancer susceptibility, except CRC. Our findings suggest that *WRN* Cys1367Arg heterozygous variant or homozygous variant carriers were at a significantly increased risk of CRC, which was consistent with previous study on breast cancer [23] and chronic kidney disease (CKD) [21]. Wirtenberger et al. revealed a significant association of the *WRN* Cys1367Arg polymorphism with high risk of familial breast cancer on German familial breast cancer patients [23]. Moreover, Yoshida et al. found that T→C (Cys1367Arg) polymorphism of *WRN* was significantly associated with the prevalence of chronic kidney disease (CKD) in a Japanese population [21]. While inconsistent with our results, Nakayama et al. found that *WRN* Cys1367Arg SNP was to be a protective factor of bone and soft tissue sarcomas

Table 5 Cox regression analysis of potential factors for DFS and OS in CRC patients after oxaliplatin-based chemotherapy

Variables	Disease-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95 % CI)[a]	P^a	Adjusted HR (95 % CI)[b]	P^b	HR (95 % CI)[a]	P^a	Adjusted HR (95 % CI)[b]	P^b
XPG Arg1104His								
GG	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
CG	0.168 (0.111–0.254)	*<0.001*	0.163 (0.107–0.248)	*<0.001*	1.377 (0.733–2.589)	0.320	1.273 (0.672–2.411)	0.459
CC	0.340 (0.241–0.480)	*<0.001*	0.333 (0.235–0.470)	*<0.001*	1.237 (0.858–1.784)	0.255	1.210 (0.829–1.767)	0.322
CG/CC	0.153 (0.105–0.223)	*<0.001*	0.149 (0.102–0.219)	*<0.001*	1.415 (0.772–2.594)	0.262	1.332 (0.721–2.459)	0.360
XPC Ala499Val								
TT	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
CT	0.654 (0.414–1.032)	0.068	0.647 (0.409–1.022)	0.062	1.034 (0.596–1.794)	0.906	0.998 (0.572–1.740)	0.994
CC	0.691 (0.528–0.904)	*0.007*	0.712 (0.541–0.936)	*0.015*	0.939 (0.639–1.378)	0.746	0.930 (0.628–1.377)	0.716
CT/CC	0.602 (0.389–0.934)	*0.024*	0.598 (0.385–0.929)	*0.022*	1.00 (0.587–1.702)	0.999	0.985 (0.576–1.683)	0.955
WRN Cys1367Arg								
CC	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
CT	1.295 (0.868–1.931)	0.206	1.281 (0.858–1.914)	0.226	0.544 (0.323–0.917)	*0.022*	0.547 (0.323–0.925)	*0.025*
TT	0.981 (0.486–1.977)	0.956	1.018 (0.505–2.054)	0.960	0.791 (0.389–1.608)	0.517	0.832 (0.405–1.709)	0.616
CT/TT	1.267 (0.857–1.874)	0.235	1.261 (0.852–1.867)	0.247	0.550 (0.332–0.911)	*0.020*	0.556 (0.334–0.924)	*0.024*
Gender								
Men	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Women	0.847 (0.589–1.220)	0.373	0.824 (0.571–1.190)	0.302	0.957 (0.587–1.559)	0.859	0.985 (0.603–1.611)	0.953
Age at diagnosis								
≤58	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
>58	1.229 (0.864–1.749)	0.252	1.267 (0.889–1.805)	0.190	1.180 (0.731–1.907)	0.498	1.116 (0.687–1.816)	0.657
First-degree family history of CRC								
No	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Yes	0.721 (0.405–1.282)	0.265	0.679 (0.380–1.211)	0.190	1.228 (0.627–2.405)	0.549	1.300 (0.656–2.578)	0.452
Smoking								
Never	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Ever	1.317 (0.854–2.030)	0.213	1.437 (0.926–2.232)	0.106	0.652 (0.311–1.365)	0.256	0.632 (0.297–1.342)	0.232
Prime cancer								
Rectum	1.00 (reference)		1.00 (reference)		1.00 (reference)		1.00 (reference)	
Colon	0.715 (0.497–1.028)	0.070	0.737 (0.512–1.062)	0.102	0.851 (0.524–1.383)	0.516	0.847 (0.520–1.380)	0.505

The significance levels are $P<0.05$ for all the italic values

HR hazard ratio

[a] *P* value, HR (95 % CI) was assessed using univariate Cox regression analysis

[b] *P* value, adjusted HR (95 % CI) were calculated by using multivariate Cox regression analysis adjusted by gender, age, first-degree family history of CRC, and smoking status

(BSTSs) in Japan [18]. In another Brazilian population-based case control study, found that *WRN* Cys1367Arg SNP was not involved either in susceptibility to developing gliomas or in patient survival [22].

Although increasing studies investigated the relevance between the polymorphisms of DNA repair pathway genes and CRC risk or prognosis, the roles of these genes in carcinogenesis remain complex and need to be further elucidated. Oxaliplatin (the third-generation platinum derivative compound) is one of the effective chemotherapy for CRC, and the genetic polymorphisms involved in the DNA repair pathway plays an important role on changing the efficacy of chemotherapy. In the present pharmacogenetic study, we further assessed the association between *XPG*, *XPC*, and *WRN* polymorphisms and prognosis of CRC patients receiving oxaliplatin-based chemotherapy. Our results suggest that

XPG Arg1104His variant carriers had a longer DFS in comparison to the CC genotype carriers in the CRC patients receiving oxaliplatin-based chemotherapy. Similarly, Liu et al. found a significantly longer progression-free survival (PFS) after oxaliplatin-based chemotherapy in patients with Asp1104His polymorphism. Moreover, the multivariate COX regression analysis also verified that carrying *XPC* Ala499Val CT genotype has a significant impact on prognosis. Taken together, our study provides significant information on prognostic value of *XPG* and *XPC* for customized chemotherapy to improve oxaliplatin treatment efficacy. Nevertheless, this study was performed only in Chinese population, and need to be replicated in other race/ethnicities or geographic areas.

In conclusion, this case–control study indicates that genetic polymorphisms of *XPC* Ala499Val, *WRN* Cys1367Arg were alone and combined significantly associated with the CRC risk. Furthermore, our study identified that *XPC* Ala499Val, *XPG* Arg1104His genetic variation had a significant impact on survival in CRC patients with oxaliplatin-based chemotherapy, which indicate that these genetic polymorphisms could be used as candidate molecular prognostic and predictive markers of CRC patients toward individualizing treatment strategies.

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Conflicts of interest None

References

1. Pourhoseingholi MA. Increased burden of colorectal cancer in Asia. World J Gastrointest Oncol. 2012;4(4):68–70.
2. George B, Kopetz S. Predictive and prognostic markers in colorectal cancer. Curr Oncol Rep. 2011;13(3):206–15.
3. Friedberg EC. How nucleotide excision repair protects against cancer. Nat Rev Cancer. 2001;1(1):22–33.
4. Sugasawa K, Shimizu Y, Iwai S, Hanaoka F. A molecular mechanism for DNA damage recognition by the xeroderma pigmentosum group C protein complex. DNA Repair (Amst). 2002;1(1):95–107.
5. Janićijević A, Sugasawa K, Shimizu Y, Hanaoka F, Wijgers N, Djurica M, et al. DNA bending by the human damage recognition complex XPC-HR23B. DNA Repair (Amst). 2003;2(3):325–36.
6. Peng Q, Lao X, Li R, Qin X, Li S. Methodological remarks concerning a recent meta-analysis on *XPG* Asp1104His and *XPF* Arg415Gln polymorphisms and breast cancer risk. Breast Cancer Res Treat. 2013;139(2):617–8.
7. Zhu ML, Wang M, Cao ZG, He J, Shi TY, Xia KQ, et al. Association between the *ERCC5* Asp1104His polymorphism and cancer risk: a meta-analysis. PLoS One. 2012;7(7):e36293.
8. Biason P, Hattinger CM, Innocenti F, Talamini R, Alberghini M, Scotlandi K, et al. Nucleotide excision repair gene variants and association with survival in osteosarcoma patients treated with neoadjuvant chemotherapy. Pharmacogenomics J. 2012;12(6):476–83.
9. Ding DP, He XF, Zhang Y. Lack of association between *XPG* Asp1104His and XPF Arg415Gln polymorphism and breast cancer risk: a meta-analysis of case–control studies. Breast Cancer Res Treat. 2011;129(1):203–9.
10. Rouissi K, Ouerhani S, Hamrita B, Bougatef K, Marrakchi R, Cherif M, et al. Smoking and polymorphisms in xenobiotic metabolism and DNA repair genes are additive risk factors affecting bladder cancer in Northern Tunisia. Pathol Oncol Res. 2011;17(4):879–86.
11. Doherty JA, Weiss NS, Fish S, Fan W, Loomis MM, Sakoda LC, et al. Polymorphisms in nucleotide excision repair genes and endometrial cancer risk. Cancer Epidemiol Biomarkers Prev. 2011;20(9):1873–82.
12. Rajaraman P, Bhatti P, Doody MM, Simon SL, Weinstock RM, Linet MS, et al. Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists. Int J Cancer. 2008;123(11):2713–6.
13. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, et al. Polymorphism of xeroderma pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer. 2006;118(3):714–20.
14. Liu D, Wu HZ, Zhang YN, Kang H, Sun MJ, Wang EH, et al. DNA repair genes *XPC*, *XPG* polymorphisms: relation to the risk of colorectal carcinoma and therapeutic outcome with oxaliplatin-based adjuvant chemotherapy. Mol Carcinog. 2012;51 Suppl 1:E83–93.
15. Gil J, Ramsey D, Stembalska A, Karpinski P, Pesz KA, Laczmanska I, et al. The C/A polymorphism in intron 11 of the *XPC* gene plays a crucial role in the modulation of an individual's susceptibility to sporadic colorectal cancer. Mol Biol Rep. 2012;39(1):527–34.
16. Wu Y, Jin M, Liu B, Liang X, Yu Y, Li Q, et al. The association of *XPC* polymorphisms and tea drinking with colorectal cancer risk in a Chinese population. Mol Carcinog. 2011;50(3):189–98.
17. Innocenti F, Mirkov S, Nagasubramanian R, Ramírez J, Liu W, Bleibel WK, et al. The Werner's syndrome 4330T>C (Cys1367Arg) gene variant does not affect the in vitro cytotoxicity of topoisomerase inhibitors and platinum compounds. Cancer Chemother Pharmacol. 2009;63(5):881–7.
18. Nakayama R, Sato Y, Masutani M, Ogino H, Nakatani F, Chuman H, et al. 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. 2008;99(2):333–9.
19. Shen M, Zheng T, Lan Q, Zhang Y, Zahm SH, Wang SS, et al. Polymorphisms in DNA repair genes and risk of non-Hodgkin lymphoma among women in Connecticut. Hum Genet. 2006;119(6):659–68.
20. Aggarwal M, Banerjee T, Sommers JA, Iannascoli C, Pichierri P, Shoemaker RH, et al. Werner syndrome helicase has a critical role in DNA damage responses in the absence of a functional fanconi anemia pathway. Cancer Res. 2013;73(17):5497–507.
21. Yoshida T, Kato K, Yokoi K, Watanabe S, Metoki N, Satoh K, et al. Association of candidate gene polymorphisms with chronic kidney disease in Japanese individuals with hypertension. Hypertens Res. 2009;32(5):411–8.
22. Pinto GR, Yoshioka FK, Clara CA, Santos MJ, Almeida JR, Burbano RR, et al. *WRN* Cys1367Arg SNP is not associated with risk and prognosis of gliomas in Southeast Brazil. J Neurooncol. 2008;90(3):253–8.
23. Wirtenberger M, Frank B, Hemminki K, Klaes R, Schmutzler RK, Wappenschmidt B, et al. Interaction of Werner and Bloom syndrome genes with *p53* in familial breast cancer. Carcinogenesis. 2006;27(8):1655–60.
24. He J, Shi TY, Zhu ML, Wang MY, Li QX, Wei QY. Associations of Lys939Gln and Ala499Val polymorphisms of the XPC gene with cancer susceptibility: a meta-analysis. Int J Cancer. 2013;133(8):1765–75.
25. Berndt SI, Platz EA, Fallin MD, Thuita LW, Hoffman SC, Helzlsouer KJ. Genetic variation in the nucleotide excision repair pathway and

colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2006;15(11):2263–9.
26. Huang WY, Berndt SI, Kang D, Chatterjee N, Chanock SJ, Yeager M, et al. Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: *XPC* polymorphisms modify smoking-related risk. Cancer Epidemiol Biomarkers Prev. 2006;15(2):306–11.
27. Yang ZH, Liang WB, Jia J, Wei YS, Zhou B, Zhang L. The xeroderma pigmentosum group C gene polymorphisms and genetic susceptibility of nasopharyngeal carcinoma. Acta Oncol. 2008;47(3): 379–84.
28. Hu Z, Wang Y, Wang X, Liang G, Miao X, Xu Y, et al. DNA repair gene *XPC* genotypes/haplotypes and risk of lung cancer in a Chinese population. Int J Cancer. 2005;115(3):478–83.
29. Sak SC, Barrett JH, Paul AB, Bishop DT, Kiltie AE. Comprehensive analysis of 22 *XPC* polymorphisms and bladder cancer risk. Cancer Epidemiol Biomarkers Prev. 2006;15(12):2537–41.
30. An J, Liu Z, Hu Z, Li G, Wang LE, Sturgis EM, et al. Potentially functional single nucleotide polymorphisms in the core nucleotide excision repair genes and risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev. 2007;16(8):1633–8.
31. Wang Y, Spitz MR, Lee JJ, Huang M, Lippman SM, Wu X. Nucleotide excision repair pathway genes and oral premalignant lesions. Clin Cancer Res. 2007;13(12):3753–8.
32. Dong Z, Guo W, Zhou R, Wan L, Li Y, Wang N, et al. Polymorphisms of the DNA repair gene *XPA* and *XPC* and its correlation with gastric cardiac adenocarcinoma in a high incidence population in North China. J Clin Gastroenterol. 2008;42(8):910–5.