Lack of association between XRCC1 SNPs and acute radiation-induced injury or prognosis in patients with nasopharyngeal carcinoma

YUHONG ZHENG[1], JINGFENG ZONG[2], YANSONG CHEN[1], JUNYING GUO[1], TIANZHU LU[2], XIAOQIN XIN[1] and YAN CHEN[1]

Departments of [1]Clinical Laboratory and [2]Radiation Oncology, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian 350014, P.R. China

Received February 16, 2023; Accepted September 5, 2023

DOI: 10.3892/ol.2023.14130

Abstract. The response to radiation therapy (RT) is closely associated with DNA damage repair. X-ray repair cross-complementing group-1 (XRCC1) is a key gene in the DNA damage repair pathway, and SNPs in this gene alter the expression and activity of its effector protein, which may in turn affect sensitivity to RT. Therefore, the course of tumor treatment and local control rate can be influenced. In the present study, a group of 158 patients with nasopharyngeal carcinoma (NPC) who received intensity-modulated RT at Fujian Cancer Hospital (Fuzhou, China) between July 2012 and October 2013 were included in retrospective chart review and followed up. Plasma was collected before treatment for genotype analysis of the three SNPs of XRCC1, namely Arg194Trp, Arg280His and Arg399Gln. Acute radiation-induced injuries sustained during treatment was graded according to the Radiation Therapy Oncology Group scoring criteria. Post-treatment follow-up was performed until August 2020. In the 158 cases of NPC, no statistically significant association was observed between the three SNPs of the XRCC1 gene and the severity of acute radiation-induced injury or prognosis. However, the AA genotype of XRCC1-Arg399Gln tended to be associated with worse progression-free survival (PFS) compared with the GA + GG genotype, although this was not significant (P=0.069). In addition, multivariate logistic analysis showed that nodal stage was significantly associated with the occurrence of acute severe radiation-induced oral mucositis (P=0.018), and there was also a trend towards an association between nodal stage and the incidence of acute severe radiation-induced pharyngitis; however, this was not statistically significant (P=0.061). Furthermore, multivariate Cox regression analysis showed that older age, distant metastasis and higher clinical stage were independent risk factors for PFS in patients with NPC. In conclusion, relying solely on the aforementioned SNPs of the XRCC1 gene may not provide a robust enough basis to predict the response to RT or prognosis in patients with NPC.

Correspondence to: Dr Yan Chen, Department of Clinical Laboratory, Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, 420 Fuma Road, Fuzhou, Fujian 350014, P.R. China
E-mail: yanc979@sina.com

Abbreviations: EBV, Epstein-Barr virus; HR, hazard ratio; IMRT, intensity-modulated radiation therapy; NPC, nasopharyngeal carcinoma; OR, odds ratio; PFS, progression-free survival; RT, radiation therapy; XRCC1, X-ray repair cross-complementing group-1

Key words: NPC, acute radiation-induced injury, prognosis, XRCC1, SNP

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck malignancies in Southeast Asia (1), and radiation therapy (RT) is the main treatment approach (2). Intensity-modulated RT (IMRT) is a conformal RT technique that has been widely used in patients with NPC (3). IMRT accurately controls the radiation dose locally in the lesion, reduces damage to surrounding normal tissues, decreases the incidence of complications (such as temporal lobe necrosis, cranial neuropathy and/or hypothyroidism), and improves the local area control rate of the tumor, while increasing the 5-year overall survival rate of patients with NPC to ~80% (4-6). However, ~85% of patients treated with IMRT experience different degrees of acute and late radiation-induced injuries such as oral mucositis, dermatitis, pharyngitis, temporal lobe neuropathy, late xerostomia and trismus (7,8). More specifically, ~20% of these patients suffer from acute severe radiation-induced injuries, which often cause uncontrollable pain, resulting in treatment interruptions (8,9). These interruptions eventually lead to failure of tumor control, thereby shortening the overall survival time (10,11). However, the lack of sensitive and specific biomarkers makes it difficult to predict the occurrence of acute severe radiation-induced injury in patients.

To understand the mechanism by which radiation-induced injury occurs and to find useful markers, numerous risk factors have been analyzed over the past few decades, including

age, sex, smoking status, radiation dose, RT technique, chemotherapy and genetics-related factors such as genetic variants and epigenetics (12,13). With the rapid advancement of radio-genomics, numerous studies have presented associations between genetic variants of candidate genes and the toxicity and efficacy of radiotherapy in patients with NPC (14,15).

Since radiation exposure destroys cells by inducing DNA damage, the ability for DNA damage repair is a central factor that influences tissue radiation sensitivity or damage (16). X-ray repair cross-complementing group-1 (XRCC1) is a pivotal DNA repair gene. The XRCC1 protein encoded by this gene serves a critical role in repairing base excision and single-strand breaks induced by radiation (17,18). Prior research has indicated that minor sequence variations within these DNA repair genes, such as single-nucleotide polymorphisms (SNPs), have the potential to disrupt the function of these genes, subsequently altering protein function and the ability of individuals to effectively repair damaged DNA (19). Ultimately, these genetic variations can influence susceptibility to radiation-induced injuries (20).

Through the analysis of the human XRCC1 gene sequence, ~16 SNP sites have been found to be located in exons or promoter regions, with the three most important functional SNP sites being Arg194Trp (rs1799782), Arg280His (rs25489) and Arg399Gln (rs25487) (19). A previous meta-analysis of the existing literature showed that, in breast, prostate and other cancer types, the Arg399Gln SNP was notably associated with the risk of acute adverse reactions induced by RT (21). These results suggest that this SNP in the XRCC1 gene is likely to be a predictor of individual response to radiation.

However, in NPC, most of the studies on XRCC1 SNPs investigated their relationship with tumor susceptibility (22,23), whereas the associations with RT response and prognosis have not been extensively analyzed (24,25). Therefore, relevant observational studies are urgently needed to extend the current understanding of the relationship between SNPs in the XRCC1 gene and the therapeutic effect of RT in NPC. The purpose of the present study was to investigate whether the aforementioned three major SNPs in the XRCC1 gene are associated with the severity of acute radiation-induced injury and prognosis of patients with NPC treated with IMRT.

Materials and methods

Patient selection. All patients with primary NPC who were first diagnosed and treated with IMRT at Fujian Cancer Hospital (Fuzhou, China) between July 2012 and October 2013 were considered for inclusion in the present retrospective study.

Patients with NPC who met any of the following criteria on admission were excluded: i) A Karnofsky Performance Status (26) score <80; ii) severe dysfunction of the heart, lungs, liver and/or kidneys; iii) history of any other malignancies; and iv) prior clinical interventions such as surgery, radiotherapy and chemotherapy.

The following inclusion criteria were applied: i) Availability of comprehensive diagnostic information, including general clinical characteristics, pathology reports, radiological findings, as well as routine laboratory test results, with a particular focus on plasma EBV-DNA concentration; ii) peripheral blood was collected before treatment, genotype analysis of XRCC1 SNP was performed, and relevant results were recorded; iii) definitive post-admission IMRT treatment was received, radiation-induced injuries were examined and the severity of damage was recorded; and iv) after the end of treatment, prognostic follow-up examinations of the patients were regularly performed, and complete case data, including recurrence or death, were recorded.

The research protocol used in the present study was approved by the Ethics Committee of Fujian Cancer Hospital (approval no. K2021-046-01; Fuzhou, China). Written informed consent was obtained from all adult participants, as well as from the parents or guardians of minors included in the study.

SNP genotyping. EDTA-K2 anticoagulated peripheral blood (10 ml) was collected from patients before treatment. Subsequently, 2 ml whole blood was utilized for the genotype analysis of three SNP loci XRCC1-Arg194Trp, -Arg280His and -Arg399Gln, which was completed within 3 months. The remaining blood samples were kept at -80˚C for re-examination.

For SNP genotyping, genomic DNA was extracted from whole-blood samples with the DNeasy Blood and Tissue Kit (Qiagen, Inc.) following the manufacturer's instructions. Relevant segments of DNA were amplified by PCR using HotStar Taq DNA polymerase (Qiagen, Inc.) under the following conditions: An initial denaturation step at 95˚C for 2 min, followed by 45 cycles of 15 sec at 94˚C and 35 sec at 60˚C, concluding with a final cooling step at 25˚C for 1 min. The primer sequences for the SNP sites were as follows: Arg194Trp forward, 5'-GCCAGGGCCCCTCCTTCAA-3' and reverse, 5'-TACCCTCAGACCCACGAGT-3'; Arg280His forward, 5'-CCAGTGGTGCTAACCTAATC-3' and reverse, 5'-CAC TCAGCACCACTACCACA-3'; and Arg399Gln forward, 5'-TTGTGCTTTCTCTGTGTCCA-3' and reverse, 5'-TCC TCCAGCCTTTTCTGATA-3'. The quality of the PCR product was tested by running 5 μl PCR product on a 1% agarose gel containing SYBR Green I Dye (Biosharp Life Sciences). Next, the PCR products underwent Sanger sequencing, employing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The sequencing was performed on the ABI 3100 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sequencing results were aligned to the corresponding reference sequence (NG_033799.1), and the SNPs were genotyped using SeqManII sequence analysis software (version 6.3; DNASTAR, Inc.). Finally, the variants of the three candidate SNPs in the XRCC1 gene were classified into wild-type and polymorphic variants: Arg194Trp, CC and CT/TT; Arg280His, GG and GA/AA; and Arg399Gln, GG and GA/AA, respectively.

Grading of acute radiation-induced injuries. The detailed RT regimen was as previously described (27). Briefly, patients were examined by their radiation therapists weekly during IMRT and at 4-6 weeks post-treatment for radiation-induced injury. The severity of radiation-induced injury was assessed using the radiation toxicity grading criteria of the Radiation Therapy Oncology Group (28). Using this scale, grades 0-4 were designated, where 0 represented the absence of change over the baseline and 4 indicated ulceration, hemorrhage or necrosis

of the mucosa or skin. All scores were confirmed by the same senior consultant physician to eliminate observer bias.

Follow-up frequency and content. The first post-treatment visit of all patients was scheduled at 4 weeks post-IMRT treatment. The follow-up frequency was as follows: Once a month for the first 3 months, every 2 months for the next 6 months, every 3 months for the next 2 years, and every 6 months thereafter. The clinical follow-up included medical history collection, physical examination, direct laryngoscopy and routine laboratory tests such as liver function, complete blood count and plasma EBV-DNA concentration measurement, as well as MRI of the nasopharynx, neck and skull base every 6-12 months. Additional chest CT, abdominal ultrasound and bone scans were performed if necessary to detect local or distant recurrence. Outpatient visits, sending text messages, telephone inquiries and reviewing medical records were implemented as follow-up methods. The follow-up period ended in August 2020.

Data collection. Basic information and general clinicopathological characteristics such as name, age, sex, pathological type, tumor size, lymph node status and distant metastasis were collected from patient admission to the time of diagnosis. Clinical staging of patients was performed according to the American Joint Commission on Cancer guidelines (7th edition; 2010) (29). The grade of the most serious acute radiation-induced injuries that occurred in patients during the observation period was recorded. Acute radiation-induced injuries of less than grade 2 were generally considered to represent an acceptable level of injury that did not further affect RT outcomes. Therefore, grades 0-1 were defined as none or mild, and grades 2+ were defined as moderate to severe. Throughout the follow-up period, the progression-free survival (PFS), instances of disease recurrence, metastasis, development of second primary tumor and various survival endpoints were documented. If follow-up continued to the end of the study in August 2020, the survival time was calculated from the beginning of treatment to the end of the study in August 2020. Incomplete data from patients who were lost to follow-up were treated as censored data.

Finally, the basic patient information and general clinicopathological characteristics, tumor RT regimen and dose, chemotherapy information, SNP genotyping, laboratory test results, type and severity of acute radiation-induced injury, as well as clinical follow-up time and outcomes were documented.

Statistical analysis. The data are presented as the mean or as number (%). Every SNP was tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the Pearson χ^2 test with two degrees of freedom.

The following univariate tests were employed to compare the frequency of oral mucositis, dermatitis and pharyngitis among patients with different genotypes, adhering to the following criteria: When the sample size (n) was ≥40 and all cells had a theoretical frequency (T) of ≥5, Pearson χ^2 test was utilized; when n≥40 but at least one cell had a T of 1-5, the Yates's correction for continuity was applied; and when n<40 or at least one cell had T<1, Fisher's exact test was used (30,31). Subsequently, multivariate logistic regression analysis was performed to evaluate the association between every factor and the occurrence of acute severe radiation-induced injuries by computing the odds ratio (OR) and the corresponding 95% CI.

Survival rates were estimated using a univariate Kaplan-Meier survival curve, which determined whether the risk of PFS varied depending on the patient genotype. A log-rank test was implemented to compare the differences in survival time. In addition, multivariate Cox regression analysis was performed, incorporating other variables that had previously been reported as significant in the literature, to identify the parameters having an independent, significant influence on PFS, and to calculate hazard ratios (HRs).

All statistical analyses were performed using SPSS (version 22.0; IBM Corp.), and P≤0.05 was considered to indicate a statistically significant difference. Graphs were created with GraphPad Prism (version 8.3.0; Dotmatics).

Results

Clinicopathological characteristics of patients with NPC. A total of 158 patients with NPC were included in the present study, and their general clinicopathological characteristics are presented in Table I. The majority of patients with NPC were male, and the mean age was 43.6 years (range, 11-74 years). The predominant pathological type was non-keratinizing undifferentiated NPC. Before treatment, 72 cases (46.5%) were plasma EBV-DNA-positive, whereas 83 cases (53.5%) were negative; no results were obtained for 3 cases. In general, 140 cases (88.6%) received the intended radiation dose ranging from 66 to 70.95 Gy for the gross tumor volume of the primary focus. Additionally, 10 cases (6.3%) received a dose <66 Gy, while 8 cases (5.1%) received a dose >70.95 Gy. The total incidence of acute radiation-induced injuries, including oral mucositis, dermatitis and pharyngitis, in the patient cohort was 98.1, 84.2 and 88.0%, respectively.

Allele frequencies and genotype distribution. The representative gel images and Sanger sequencing traces of PCR products containing XRCC1 gene SNPs are shown in Fig. S1, and the characteristics of the three candidate SNPs of the XRCC1 gene are presented in Table II. Overall, all the genotype distributions were in HWE (P>0.05 based on χ^2 test for each allele).

Association between candidate SNPs and the severity of acute radiation-induced injuries. Acute radiation-induced injuries that are most frequently observed in patients with NPC during IMRT treatment include oral mucositis, dermatitis and pharyngitis or dysphagia (3). In the present study, 24.7% (n=39) and 75.3% (n=119) of patients experienced grade 0-1 and 2+ radioactive oral mucositis, respectively. A total of 84.2% (n=133) and 15.8% (n=25) of patients experienced grade 0-1 and 2+ radioactive dermatitis, respectively. A total of 88.6% (n=140) and 11.4% (n=18) of the patients developed grade 0-1 and 2+ radioactive pharyngitis, respectively. No grade 4 adverse reactions were observed in the current study (Table III).

The data indicated no statistically significant association between Arg194Trp, Arg280His and Arg399Gln SNPs in the XRCC1 gene and the severity of acute radiation-induced injuries in patients with NPC treated with IMRT (P>0.05).

Table I. Clinicopathological characteristics of 158 patients with nasopharyngeal carcinoma.

Clinicopathological characteristics	No. (%)
Sex	
Male	113 (71.5)
Female	45 (28.5)
Age at diagnosis, years	
<60	145 (91.8)
≥60	13 (8.2)
Pathological types	
NKU	150 (94.9)
Others	8 (5.1)
T stage	
T1-T2	69 (43.7)
T3-T4	89 (56.3)
N stage	
N0-N1	45 (28.5)
N2-N3	113 (71.5)
Distant metastasis	
M0	152 (96.2)
M1	6 (3.8)
Clinical stage	
I-II	15 (9.5)
III-IV	143 (90.5)
EBV-DNA (n=155)[a]	
Positive	72 (46.5)
Negative	83 (53.5)
Radiation dose, Gy	
<66	10 (6.3)
66-70.95	140 (88.6)
>70.95	8 (5.1)
Acute radiation toxicity[b]	
Oral mucositis	155 (98.1)
Dermatitis	133 (84.2)
Pharyngitis	139 (88.0)

[a]Among the 158 patients, there were 3 individuals for whom no EBV-DNA test results were available. [b]Some patients may simultaneously exhibit more than one type of radiation-induced injury. NKU, non-keratinising undifferentiated nasopharyngeal carcinoma; IMRT, intensity-modulated radiation therapy; EBV, Epstein-Barr virus.

Risk factors for moderate to severe radiation-induced injuries. Multivariate logistic regression analysis was used to evaluate the effects of risk factors that could have affected the occurrence of acute severe oral mucositis, dermatitis and pharyngitis. Sex, age, pathological type, tumor stage, nodal stage, distant metastasis, clinical stage, plasma EBV-DNA concentration, radiation dose, and XRCC1-Arg194Trp, XRCC1-Arg280His and XRCC1-Arg399Gln were included as independent variables (Table IV). Of these, only nodal stage was significantly associated with the occurrence of acute severe radiation-induced oral mucositis (OR, 2.213; 95% CI, 1.149-4.263; P=0.018). Nodal stage also showed a trend towards an association with moderate to the incidence of acute severe radiation-induced pharyngitis, although this was not statistically significant (OR, 4.796; 95% CI, 0.930-24.736; P=0.061). No association was observed between acute severe radiation-induced injuries and the three candidate SNPs (P>0.05).

Associations between candidate SNPs and PFS. The present study followed up patients from July 2012 to August 2020. During the follow-up period, primary lymph node recurrence occurred in 6 patients, a local relapse in 7 patients and metastasis in 29 patients. Additionally, a second primary tumor developed in 6 patients. The mean PFS time was 69.8 months (range, 0-97 months).

Patients with the AA genotype at the XRCC1-Arg399Gln SNP had a worse PFS compared with patients with the GA + GG genotype; however, this was not statistically significant (P=0.069; Fig. 1F). None of the other SNPs examined in the present study was associated with PFS (all P>0.05; Fig. 1A-E).

Survival analysis. Multivariate Cox regression analysis was performed to identify the factors associated with PFS. The results suggested that age (HR, 1.033; 95% CI, 1.002-1.066; P=0.039), distant metastasis (HR, 36.641; 95% CI, 6.908-194.349; P<0.001) and clinical stage (HR, 2.315; 95% CI, 1.073-4.995; P=0.032) were independent risk factors for PFS. No statistically significant association was observed between the investigated SNPs and PFS (P>0.05; Fig. 2).

Discussion

In the present study, the relationship between SNPs in the XRCC1 gene, which is a key factor in the DNA damage repair pathway (17,18), and the severity of acute radiation-induced injuries as well as prognosis in patients with NPC who received IMRT treatment, were retrospectively analyzed. The analyses of the current study aimed to explore biological indicators that predict the response of patients with NPC to RT, which would provide guidance to clinicians in the choice of personalized treatment plans based on the specific tissue damage and tumor progression risk.

The results of the present study indicated no significant association between the three candidate SNPs in the XRCC1 gene and acute radiation-induced injuries during IMRT treatment in patients with NPC. This is consistent with the research findings of Wang *et al* (32) in that the SNPs of two loci in the XRCC1 gene, Arg280His and Arg399Gln, were not associated with the severity of acute radiation mucositis and dermatitis in patients with NPC who received IMRT combined with chemotherapy. Similarly, Zhai *et al* (33) did not observe an association between XRCC1-Arg399Gln and acute radiation-induced injuries to the skin, mucous membranes and salivary glands of 60 patients with stage III-IVA NPC. Chen *et al* (34) found no notable difference in the severity of acute radiation-induced oral mucositis among patients with NPC treated with IMRT with different genotypes of XRCC1-Arg399Gln; however, the risk of acute radiation-induced dermatitis of grade 2 or more in patients with the GG genotype was notably higher than that of patients with the other two genotypes. Li *et al* (35)

Table II. Genotype distribution of the three candidate SNPs in the X-ray repair cross-complementing group-1 gene.

SNP (genotypes)	NCBI dbSNP ID	Alleles (amino acids)	Genotype distribution[a], n	HWE (P-value)
Arg194Trp (CC/CT/TT)	rs1799782	C>T (Arg>Trp)	80/67/11	0.547
Arg280His (GG/GA/AA)	rs25489	G>A (Arg>His)	133/22/3	0.083
Arg399Gln (GG/GA/AA)	rs25487	G>A (Arg>Gln)	85/63/10	0.712

[a]Genotype distribution is shown as wild-type homozygotes/wild-type heterozygotes/mutant homozygotes. NCBI, National Center for Biotechnology Information; ID, identification; HWE, Hardy-Weinberg equilibrium; dbSNP, Single Nucleotide Polymorphism Database.

Table III. Relationship between candidate SNPs and the severity of acute radiation-induced injuries.

	Oral mucositis grade, n (%)			Dermatitis grade, n (%)			Pharyngitis grade, n (%)		
SNP/genotypes	G_{0-1} (n=39)	G_{2+} (n=119)	P-value	G_{0-1} (n=133)	G_{2+} (n=25)	P-value	G_{0-1} (n=140)	G_{2+} (n=18)	P-value
XRCC1-Arg194Trp									
CC-wild (n=80)	17 (10.8)	63 (39.9)	0.311	67 (42.4)	13 (8.2)	0.882	69 (43.7)	11 (7.0)	0.345
CT + TT (n=78)	22 (13.9)	56 (35.4)		66 (41.8)	12 (7.6)		71 (44.9)	7 (4.4)	
TT-mutant (n=11)	4 (2.5)	7 (4.4)	0.569	10 (6.3)	1 (0.6)	0.837	11 (6.9)	0 (0.0)	0.616
CT + CC (n=147)	35 (22.2)	112 (70.9)		123 (77.9)	24 (15.2)		129 (81.7)	18 (11.4)	
XRCC1- Arg280His									
GG-wild (n=133)	32 (20.3)	101 (63.9)	0.675	113 (71.5)	20 (12.7)	0.533	119 (75.3)	14 (8.9)	0.655
GA + AA (n=25)	7 (4.4)	18 (11.4)		20 (12.7)	5 (3.1)		21 (13.3)	4 (2.5)	
AA-mutant (n=3)	0 (0.0)	3 (1.9)	>0.999	3 (1.9)	0 (0.0)	>0.999	3 (1.9)	0 (0.0)	>0.999
GA + GG (n=155)	39 (24.7)	116 (73.4)		130 (82.3)	25 (15.8)		137 (86.7)	18 (11.4)	
XRCC1- Arg399Gln									
GG-wild (n=85)	21 (13.3)	64 (40.5)	0.994	73 (46.2)	12 (7.6)	0.526	78 (49.4)	7 (4.4)	0.178
GA + AA (n=73)	18 (11.4)	55 (34.8)		60 (38.0)	13 (8.2)		62 (39.2)	11 (7.0)	
AA-mutant (n=10)	1 (0.6)	9 (5.7)	0.463	8 (5.1)	2 (1.3)	>0.999	8 (5.1)	2 (1.3)	0.711
GA + GG (n=148)	38 (24.1)	110 (69.6)		125 (79.1)	23 (14.5)		132 (83.5)	16 (10.1)	

All toxicities have been graded according to the Radiation Therapy Oncology Group morbidity criteria. Wild, wild-type homozygote; mutant, mutant homozygote; XRCC1, X-ray repair cross-complementing group-1.

also observed that the GA genotype of XRCC1-Arg399Gln was significantly associated with the occurrence of grade 3 dermatitis (P=0.037) and also showed a trend towards an association with the incidence of grade 3 mucositis (P=0.065) in patients with NPC. The discrepancies between the findings of Li *et al* (35) and the present study may be due to analysis biases in their cases, which used not only IMRT but also three-dimensional (3D) conformal RT.

The multivariate logistic regression analysis performed in the present study demonstrated a significant association between nodal stage and the occurrence of acute severe radiation-induced oral mucositis, and there was a trend towards an association with the incidence of acute severe radiation-induced pharyngitis; however, this was not statistically significant. Similar results were also obtained in the study by Chen *et al* (34), which established that nodal stage was significantly associated with grade 2 or greater acute radiation-induced dermatitis (P<0.001). These findings might suggest that for improved tumor control, as the nodal stage increases, the local irradiation dose and area of the oropharynx and/or neck near the lymph nodes should be accordingly increased, resulting in intensification of related radiation damage. Chen *et al* (34) also demonstrated that there was no significant association between the severity of acute radiation-induced injuries and radiation dose in patients with NPC treated with IMRT, which is consistent with the findings in the studies by Wang *et al* (32) and Li *et al* (35). This lack of association might have been caused by higher uniformity of the irradiation dose administered to local lesions, as IMRT can be programmed with a target volume consistent with the lesion parameters by 3D conformal technology, thereby concentrating the effective dose on the lesion.

Prognostic analysis in the present study revealed that the AA genotype of the XRCC1-Arg399Gln SNP tended to be associated with a worse PFS compared with the GA + GG genotype (P=0.069); however, this was not statistically

Table IV. Multivariate logistic regression analyses of the risk factors for acute severe radiation injuries.

	Oral mucositis (G_{2+})			Dermatitis (G_{2+})			Pharyngitis (G_{2+})		
Clinicopathological characteristics	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value	Adjusted OR	95% CI	P-value
Sex	0.60	0.257-1.413	0.244	0.60	0.185-1.963	0.400	0.67	0.164-2.715	0.573
Age	0.99	0.951-1.021	0.415	1.02	0.979-1.069	0.314	1.04	0.982-1.107	0.168
Pathological type	2.44	0.269-22.207	0.428	0.00[a]	<0.001->999.999[a]	0.999[a]	0.00[a]	<0.001->999.999[a]	0.999[a]
Tumor stage	1.40	0.826-2.352	0.214	1.08	0.600-1.934	0.802	1.04	0.531-2.062	0.896
Nodal stage	2.21	1.149-4.263	0.018	1.18	0.517-2.694	0.695	4.80	0.930-24.736	0.061
Distant metastasis	>999.99[a]	<0.001->999.999[a]	0.999[a]	2.76	0.375-20.216	0.319	1.81	0.140-23.407	0.651
Clinical stage	0.71	0.295-1.727	0.454	1.09	0.357-3.320	0.881	0.31	0.053-1.824	0.195
Plasma EBV-DNA concentration	0.99	0.999-1.000	0.176	0.99	0.992-1.003	0.441	0.99	0.968-1.009	0.267
Radiation dose	0.97	0.876-1.079	0.595	1.06	0.910-1.225	0.475	0.98	0.915-1.039	0.433
XRCC1-Arg194Trp									
CC vs. CT + TT	0.68	0.288-1.596	0.374	1.25	0.452-3.443	0.669	1.44	0.414-4.989	0.568
TT vs. CT + CC	2.39	0.602-9.453	0.216	2.07	0.237-18.063	0.511	>999.99[a]	<0.001->999.999[a]	0.999[a]
XRCC1-Arg280His									
GG vs. GA + AA	0.57	0.203-1.626	0.296	1.53	0.471-4.966	0.479	2.20	0.576-8.417	0.249
AA vs. GA + GG	0.00[a]	<0.001->999.999[a]	0.999[a]	>999.99[a]	<0.001->999.999[a]	0.999[a]	>999.99[a]	<0.001->999.999[a]	0.999[a]
XRCC1-Arg399Gln									
GG vs. GA + AA	0.59	0.246-1.414	0.237	1.28	0.457-3.587	0.638	2.28	0.650-8.024	0.198
AA vs. GA + GG	0.52	0.058-4.777	0.567	0.82	0.133-5.058	0.830	0.83	0.132-5.164	0.837

[a]Sparse data bias typically arises when the sample size for a specific variable or one of its subgroups is too small, possibly even zero. As a result, it leads to exceptionally large OR or zero OR and exceptionally wide 95% CI. In such instances, the statistical power is limited, rendering the data inconclusive. OR, odds ratio; CI, confidence intervals; EBV, Epstein-Barr virus; XRCC1, X-ray repair cross-complementing group-1.

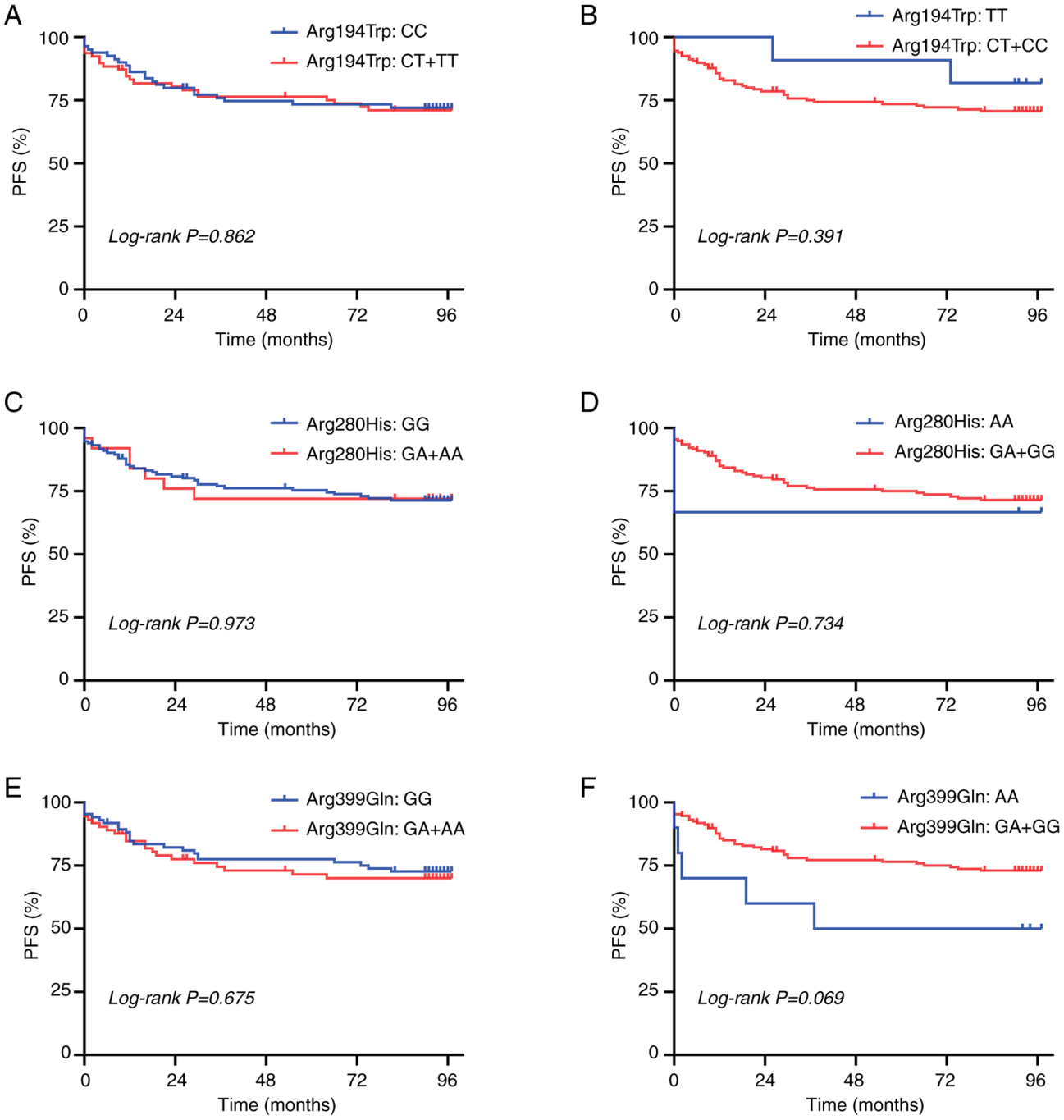

Figure 1. Kaplan-Meier estimates of the probability of PFS according to the candidate SNPs of the X-ray repair cross-complementing group-1 gene. The log-rank test was used to assess survival differences between two groups. (A) Arg194Trp, CC vs. CT + TT combined. (B) Arg194Trp, TT vs. CT + CC combined. (C) Arg280His, GG vs. GA + AA combined. (D) Arg280His, AA vs. GA + GG combined. (E) Arg399Gln, GG vs. GA + AA combined. (F) Arg399Gln, AA vs. GA + GG combined. The PFS rate in patients with the Arg399Gln AA genotype appeared to be worse than that in patients with the Arg399Gln GA and GG genotypes; however, there was no significant difference (P=0.069). PFS, progression-free survival.

significant, and there was no evidence that other candidate SNPs in the XRCC1 gene were associated with poor prognosis in patients with NPC. These results are consistent with the findings in the study by Zhai *et al* (33). However, the study by Jin *et al* (36) showed that heavy smokers (>20 packs/year) with the XRCC1-Arg399Gln GG genotype had significantly higher PFS times than smokers with other genotypes (P=0.047). This discrepancy could potentially be attributed to their stratification of patients based on smoking status, as well as their detection of XRCC1 Arg399Gln from paraffin-embedded biopsy specimens. Wang *et al* (32) found that the GG genotype of XRCC1-Arg280His was positively associated with primary tumor response at the end of RT in patients with NPC, which is inconsistent with the findings of the present study. Nevertheless, the main difference in the methodologies was that the study by Wang *et al* (32) only explored short-term effects 3 months after RT, which is not comparable with long-term effects at the 7- to 8-year follow-up assessed in the current study.

The multivariate Cox regression analysis conducted in the present study showed that older age, the presence of distant

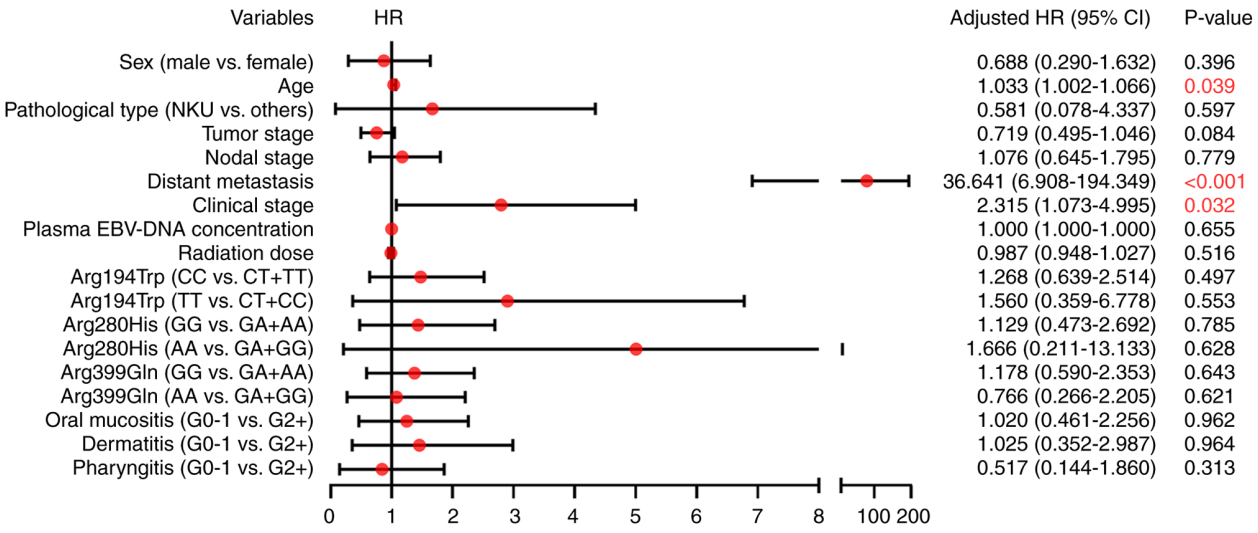

Figure 2. Multivariate Cox regression analysis of the risk factors for progression-free survival. NKU, non-keratinizing undifferentiated nasopharyngeal carcinoma EBV, Epstein-Barr virus; HR, hazard ratio.

metastasis and a higher clinical stage were independent risk factors for poor PFS in patients with NPC, which is consistent with previously published results (37). However, the findings of the current study showed that PFS time in patients with NPC was not associated with pretreatment plasma EBV-DNA results, which is inconsistent with the results of other studies (38,39). We hypothesized that the observed discrepancy may have been caused by differences in primer fragments used at the Clinical Laboratory of Fujian Cancer Hospital (Fuzhou, China) to detect plasma EBV-DNA between 2012 and 2013, and those used after 2016 (40,41).

The main strengths of the current study include the following: i) To the best of our knowledge, the study was the first to simultaneously analyze the three most important functional SNPs in the XRCC1 gene in patients with NPC, which provides a more comprehensive understanding of the impact that the XRCC1 gene exerts in these patients; ii) prognostic follow-up examinations were carried out over a period of 7-8 years, which is notably longer than the follow-up duration adopted in a number of previous studies (36,42), thus the present study provided strong evidence for clarifying the relationship between these SNPs and prognosis; and iii) the choice of radiotherapy modality and the specific types of acute radiation-induced injuries were deliberately restricted to minimize potential sources of bias in the study design.

Nonetheless, several limitations of the present study should be acknowledged: i) The study was a single-center, retrospective study, and selection biases could not be avoided; ii) the size of the patient cohort and the total number of included events are relatively small. This limitation is particularly noticeable within specific subgroups, such as patients with distant metastases, where the total number of cases is limited. This may result in 'sparse data bias' in some subgroups, consequently limiting statistical power (43,44); iii) in view of the limited clinical data collected ≥10 years ago, not all patients had complete records of relevant clinicopathological, including the absence of descriptions of smoking status, drinking habits and oral hygiene (45), which might have influenced the accuracy of the multivariate analysis results; and iv) due to the limited number of cases, the present study did not stratify patients with cancer who received RT alone from those undergoing combined radiochemotherapy regimens. The use of chemotherapeutic drugs often exacerbates the severity of acute radiation-induced injuries (46,47), which also might have introduced bias into the analysis.

Previous studies have demonstrated that, in addition to the SNPs in the DNA damage repair gene mentioned in the present study, numerous other gene SNPs are also involved in the process of radiation damage and repair, including SNPs in genes associated with angiogenesis (48), autophagy (49), the Wnt/β-catenin pathway (50), and the cell cycle and NF-κB pathway (51), suggesting that the mechanism of acute radiation-induced injury may be influenced by the combined effect of multiple SNP pathways. In radiogenomics, an essential focal point revolves around the creation of risk scores based on combinations of SNPs within genes associated with radiation-induced signaling pathways. These risk scores are primarily aimed at predicting the likelihood of experiencing acute or delayed radiation-induced injuries, serving as valuable guidance for informing clinical decision-making (52). Within these risk scores, each SNP is assigned individualized weights, which are determined by their respective significance. Therefore, an in-depth elucidation of the relationship between each SNP and radiation damage is important for overall risk assessment.

In summary, the current study suggested that the Arg194Trp, Arg280His and Arg399Gln SNPs in the XRCC1 gene of the DNA damage repair pathway cannot independently predict the severity of acute radiation-induced injury and prognosis in patients with NPC treated with IMRT. It may be necessary to expand the study across multiple medical centers, and increase the sample size through a prospective cohort study, or combine data with that of other related gene SNPs to build a prediction model, so as to explore the relationship between gene-related factors and RT efficacy.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Fujian Provincial Health Technology Project (grant no. 2020GGA016) and the National Natural Science Foundation of China (grant no. 81972717).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YHZ and YC conceived the study idea and designed the experiments. YHZ, JYG and XQX performed the experiments. JFZ, YSC and TZL collected and analyzed the data. YHZ wrote the first draft of the manuscript. YC supervised the study and commented on previous versions of the manuscript. YHZ and YC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in the present study involving biological samples/medical record information/data were approved by the Ethics Committee of Fujian Cancer Hospital (approval no. K2021-046-01; Fuzhou, China). Written informed consent was obtained from all adult participants, as well as from the parents or guardians of minors included in the study. All methods were performed in accordance with the relevant guidelines and regulations (Declaration of Helsinki).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. World Health Organization: 4-Nasopharynx-fact-sheet. Global Cancer Observatory, 2020.
2. Lee AW, Ma BB, Ng WT and Chan AT: Management of nasopharyngeal carcinoma: Current practice and future perspective. J Clin Oncol 33: 3356-3364, 2015.
3. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y and Ma J: Nasopharyngeal carcinoma. Lancet 394: 64-80, 2019.
4. Ma Z, Umezawa R, Yamamoto T, Ishikawa Y, Takahashi N, Takeda K, Suzuki Y, Tang L, Ito K, Kadoya N and Jingu K: IMRT improves local control in patients with NPC compared with conventional radiotherapy: Propensity score-matched analysis. Jpn J Clin Oncol 51: 1444-1451, 2021.
5. Yeh SA, Hwang TZ, Wang CC, Yang CC, Lien CF, Wang CC, Hsu TY, Hsu RF, Shih YC, Huang YC, *et al*: Outcomes of patients with nasopharyngeal carcinoma treated with intensity-modulated radiotherapy. J Radiat Res 62: 438-447, 2021.
6. Chen L, Zhang Y, Lai SZ, Li WF, Hu WH, Sun R, Liu LZ, Zhang F, Peng H, Du XJ, *et al*: 10-Year results of therapeutic ratio by Intensity-Modulated radiotherapy versus Two-Dimensional radiotherapy in patients with Nasopharyngeal Carcinoma. Oncologist 24: e38-e45, 2019.
7. Kouloulias V, Thalassinou S, Platoni K, Zygogianni A, Kouvaris J, Antypas C, Efstathopoulos E and Nikolaos K: The treatment outcome and radiation-induced toxicity for patients with head and neck carcinoma in the IMRT era: A systematic review with dosimetric and clinical parameters. Biomed Res Int 2013: 401261, 2013.
8. Yao Z and Cheng B: Morbidity in patients with nasopharyngeal carcinoma and Radiation-induced skin lesions: Cause, risk factors, and dermatitis evolution and severity. Adv Skin Wound Care 34: 1-8, 2021.
9. Bossi P, Cossu Rocca M, Corvo R, Depenni R, Guardamagna V, Marinangeli F, Micciche F and Trippa F: The vicious circle of treatment-induced toxicities in locally advanced head and neck cancer and the impact on treatment intensity. Crit Rev Oncol Hematol 116: 82-88, 2017.
10. Yang XL, Zhou GQ, Lin L, Zhang LL, Chen FP, Lv JW, Kou J, Wen DW, Ma J, Sun Y, *et al*: Prognostic value of radiation interruption in different periods for nasopharyngeal carcinoma patients in the intensity-modulated RT era. Cancer Med 10: 143-155, 2021.
11. Yao JJ, Zhang F, Gao TS, Zhang WJ, Lawrence WR, Zhu BT, Zhou GQ, Ma J, Wang SY and Sun Y: Survival impact of radiotherapy interruption in nasopharyngeal carcinoma in the intensity-modulated radiotherapy era: A big-data intelligence platform-based analysis. Radiother Oncol 132: 178-187, 2019.
12. Applegate KE, Ruhm W, Wojcik A, Bourguignon M, Brenner A, Hamasaki K, Imai T, Imaizumi M, Imaoka T, Kakinuma S, *et al*: Individual response of humans to ionising radiation: Governing factors and importance for radiological protection. Radiat Environ Biophys 59: 185-209, 2020.
13. Wardill HR, Sonis ST, Blijlevens NMA, Van Sebille YZA, Ciorba MA, Loeffen EAH, Cheng KKF, Bossi P, Porcello L, Castillo DA, *et al*: Prediction of mucositis risk secondary to cancer therapy: A systematic review of current evidence and call to action. Support Care Cancer 28: 5059-5073, 2020.
14. Yang DW, Wang TM, Zhang JB, Li XZ, He YQ, Xiao R, Xue WQ, Zheng XH, Zhang PF, Zhang SD, *et al*: Genome-wide association study identifies genetic susceptibility loci and pathways of radiation-induced acute oral mucositis. J Transl Med 18: 224, 2020.
15. Brothwell MRS, West CM, Dunning AM, Burnet NG and Barnett GC: Radiogenomics in the era of advanced radiotherapy. Clin Oncol (R Coll Radiol) 31: 319-325, 2019.
16. Liu J, Bi K, Yang R, Li H, Nikitaki Z and Chang L: Role of DNA damage and repair in radiation cancer therapy: A current update and a look to the future. Int J Radiat Biol 96: 1329-1338, 2020.
17. London RE: XRCC1-Strategies for coordinating and assembling a versatile DNA damage response. DNA Repair (Amst) 93: 102917, 2020.
18. Guo Z, Shu Y, Zhou H, Zhang W and Wang H: Radiogenomics helps to achieve personalized therapy by evaluating patient responses to radiation treatment. Carcinogenesis 36: 307-317, 2015.
19. Xi T, Jones IM and Mohrenweiser HW: Many amino acid substitution variants identified in DNA repair genes during human population screenings are predicted to impact protein function. Genomics 83: 970-979, 2004.
20. Rosen EM, Fan S, Goldberg ID and Rockwell S: Biological basis of radiation sensitivity. Part 2: Cellular and molecular determinants of radiosensitivity. Oncology 14: 741-757, 2000.
21. Zhao J, Zhi Z, Zhang M, Li Q, Li J, Wang X and Ma C: Predictive value of single nucleotide polymorphisms in XRCC1 for radiation-induced normal tissue toxicity. Onco Targets Ther 11:3901-3918. 2018.
22. Lin J, Ye Q, Wang Y, Wang Y and Zeng Y: Association between XRCC1 single-nucleotide polymorphisms and susceptibility to nasopharyngeal carcinoma: An update meta-analysis. Medicine (Baltimore) 97: e11852, 2018.
23. Yang J, Li L, Yin X, Wu F, Shen J, Peng Y, Liu Y, Sun Y, Lu H and Zhang Y: The association between gene polymorphisms and risk of nasopharyngeal carcinoma. Med Oncol 32: 398, 2015.
24. Zhang SQ, Pan SM, Liang SX, Han YS, Chen HB and Li JC: Research status and prospects of biomarkers for nasopharyngeal carcinoma in the era of highthroughput omics (Review). Int J Oncol 58: 9, 2021.
25. Siak PY, Khoo AS, Leong CO, Hoh BP and Cheah SC: Current status and future perspectives about molecular biomarkers of nasopharyngeal carcinoma. Cancers (Basel) 13: 3490, 2021.
26. Mor V, Laliberte L, Morris JN and Wiemann M: The karnofsky performance status scale. An examination of its reliability and validity in a research setting. Cancer 53: 2002-2007, 1984.

27. Lin S, Pan J, Han L, Guo Q, Hu C, Zong J, Zhang X and Lu JJ: Update report of nasopharyngeal carcinoma treated with reduced-volume intensity-modulated RT and hypothesis of the optimal margin. Radiother Oncol 110: 385-389, 2014.
28. Cox JD, Stetz J and Pajak TF: Toxicity criteria of the radiation therapy oncology group (RTOG) and the european organization for research and treatment of cancer (EORTC). Int J Radiat Oncol Biol Phys 31: 1341-1346, 1995.
29. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A: AJCC Cancer Staging Manual. Seventh Edition. New York, Springer, 2010.
30. Jiqian Fang: Health Statistics. People's Medical Publishing House, Beijing, pp150-171, 2012.
31. Prescott RJ: Two-tailed significance tests for 2x2 contingency tables: What is the alternative? Stat Med 38: 4264-4269, 2019.
32. Wang J, Guo C, Gong X, Ao F, Huang Y, Huang L, Tang Y, Jiang C, Xie X, Dong Q, *et al*: The impacts of genetic polymorphisms in genes of base excision repair pathway on the efficacy and acute toxicities of (chemo)radiotherapy in patients with nasopharyngeal carcinoma. Oncotarget 8: 78633-78641, 2017.
33. Zhai XM, Hu QC, Gu K, Wang JP, Zhang JN and Wu YW: Significance of XRCC1 Codon399 polymorphisms in Chinese patients with locally advanced nasopharyngeal carcinoma treated with radiation therapy. Asia Pac J Clin Oncol 12: e125-e132, 2016.
34. Chen H, Wu M, Li G, Hua L, Chen S and Huang H: Association between XRCC1 single-nucleotide polymorphism and acute radiation reaction in patients with nasopharyngeal carcinoma: A cohort study. Medicine (Baltimore) 96: e8202, 2017.
35. Li H, You Y, Lin C, Zheng M, Hong C, Chen J, Li D, Au WW and Chen Z: XRCC1 codon 399Gln polymorphism is associated with radiotherapy-induced acute dermatitis and mucositis in nasopharyngeal carcinoma patients. Radiat Oncol 8: 31, 2013.
36. Jin H, Xie X, Wang H, Hu J, Liu F, Liu Z, Zhou J, Zhang Y, Xi X, Hu B, *et al*: ERCC1 Cys8092Ala and XRCC1 Arg399Gln polymorphisms predict progression-free survival after curative radiotherapy for nasopharyngeal carcinoma. PLoS One 9: e101256, 2014.
37. Chiang CL, Guo Q, Ng WT, Lin S, Ma TSW, Xu Z, Xiao Y, Li J, Lu T, Choi HCW, *et al*: Prognostic factors for overall survival in nasopharyngeal cancer and implication for TNM staging by UICC: A systematic review of the literature. Front Oncol 11: 703995, 2021.
38. Alami IE, Gihbid A, Charoute H, Khaali W, Brahim SM, Tawfiq N, Cadi R, Belghmi K, El Mzibri M and Khyatti M: Prognostic value of Epstein-Barr virus DNA load in nasopharyngeal carcinoma: A meta-analysis. Pan Afr Med J 41: 6, 2022.
39. Qu H, Huang Y, Zhao S, Zhou Y and Lv W: Prognostic value of Epstein-Barr virus DNA level for nasopharyngeal carcinoma: A meta-analysis of 8128 cases. Eur Arch Otorhinolaryngol 277: 9-18, 2020.
40. Zong J, Zheng Y, Lin C, Chen Y, Chen C, Pan J and Lin S: The value of plasma EBV DNA in monitoring the therapeutic effect of nasopharyngeal carcinoma. Chin J Radiat Oncol 28: 881-885, 2019.
41. Zong JF, Zheng YH, Weng YI, Chen LS, Yun XU, Chen Y, Pan JJ and Lin SJ: Prognostic Value of Pre-treatment plasma EBV DNA in nasopharyngeal carcinoma patients treated with intensity-modulated radiation therapy. J Chin Oncol 22: 810-815, 2016.
42. Nanda SS, Gandhi AK, Rastogi M, Khurana R, Hadi R, Sahni K, Mishra SP, Srivastava AK, Bhatt MLB and Parmar D: Evaluation of XRCC1 gene polymorphism as a biomarker in head and neck cancer patients undergoing chemoradiation therapy. Int J Radiat Oncol Biol Phys 101: 593-601, 2018.
43. Leopold SS and Porcher R: Editorial: Sparse-data Bias-What the savvy reader needs to know. Clin Orthop Relat Res 476: 657-659, 2018.
44. Greenland S, Mansournia MA and Altman DG: Sparse-data bias: A problem hiding in plain sight. BMJ 352: i1981, 2016.
45. Vera-Llonch M, Oster G, Hagiwara M and Sonis S: Oral mucositis in patients undergoing radiation treatment for head and neck carcinoma. Cancer 106: 329-336, 2006.
46. Wang C, Wang F, Min X, Zhang Q, Shen LJ, Jiang Y and Yan J: Toxicities of chemoradiotherapy and radiotherapy in nasopharyngeal carcinoma: An updated meta-analysis. J Int Med Res 47: 2832-2847, 2019.
47. Xu YC, Chen KH, Liang ZG and Zhu XD: A systematic review and Meta-Analysis of studies comparing concurrent chemoradiotherapy with radiotherapy alone in the treatment of stage II Nasopharyngeal carcinoma. Front Oncol 12: 843675, 2022.
48. Ma WL, Liu R, Huang LH, Zou C, Huang J, Wang J, Chen SJ, Meng XG, Yang JK, Li H, *et al*: Impact of polymorphisms in angiogenesis-related genes on clinical outcomes of radiotherapy in patients with nasopharyngeal carcinoma. Clin Exp Pharmacol Physiol 44: 539-548, 2017.
49. Yang Z and Liu Z: Potentially functional variants of autophagy-related genes are associated with the efficacy and toxicity of radiotherapy in patients with Nasopharyngeal carcinoma. Mol Genet Genomic Med 7: e1030, 2019.
50. Yu J, Huang Y, Liu L, Wang J, Yin J, Huang L, Chen S, Li J, Yuan H, Yang G, *et al*: Genetic polymorphisms of Wnt/β-catenin pathway genes are associated with the efficacy and toxicities of radiotherapy in patients with nasopharyngeal carcinoma. Oncotarget 7: 82528-82537, 2016.
51. Guo C, Huang Y, Yu J, Liu L, Gong X, Huang M, Jiang C, Liao Y, Huang L, Yang G and Li J: The impacts of single nucleotide polymorphisms in genes of cell cycle and NF-kB pathways on the efficacy and acute toxicities of radiotherapy in patients with nasopharyngeal carcinoma. Oncotarget 8: 25334-25344, 2017.
52. Andreassen CN, Schack LM, Laursen LV and Alsner J: Radiogenomics-current status, challenges and future directions. Cancer Lett 382: 127-136, 2016.