RESEARCH ARTICLE

Predictive Value of Xrcc1 Gene Polymorphisms for Side Effects in Patients undergoing Whole Breast Radiotherapy: a Meta-analysis

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Abstract

Radiation-induced side effects on normal tissue are determined largely by the capacity of cells to repair radiation-induced DNA damage. X-ray repair cross-complementing group 1 (XRCC1) plays an important role in the repair of DNA single-strand breaks. Studies have shown conflicting results regarding the association between XRCC1 gene polymorphisms (Arg399Gln, Arg194Trp, -77T>C and Arg280His) and radiation-induced side effects in patients undergoing whole breast radiotherapy. Therefore, we conducted a meta-analysis to determine the predictive value of XRCC1 gene polymorphisms in this regard. Analysis of the 11 eligible studies comprising 2,199 cases showed that carriers of the XRCC1 399 Gln allele had a higher risk of radiation-induced toxicity than those with the 399 ArgArg genotype in studies based on high-quality genotyping methods [Gln vs. ArgArg: OR, 1.85; 95% CI, 1.20-2.86] or in studies with mixed treatment regimens of radiotherapy alone and in combination with chemotherapy [Gln vs. ArgArg: OR, 1.60; 95% CI, 1.09-2.23]. The XRCC1 Arg399Gln variant allele was associated with mixed acute and late adverse reactions when studies on late toxicity only were excluded [Gln allele vs. Arg allele: OR, 1.22; 95% CI, 1.00-1.49]. In contrast, the XRCC1 Arg280His variant allele was protective against radiation-induced toxicity in studies including patients treated by radiotherapy alone [His allele vs. Arg allele: OR, 0.58; 95% CI, 0.35-0.96]. Our results suggest that XRCC1 399Gln and XRCC1 280Arg may be independent predictors of radiation-induced toxicity in post-surgical breast cancer patients, and the selection of genotyping method is an important factor in determining risk factors. No evidence for any predictive value of XRCC1 Arg194Trp and XRCC1 -77T>C was found. So, larger and well-designed studies might be required to further evaluate the predictive value of XRCC1 gene variation on radiation-induced side effects in patients undergoing whole breast radiotherapy.

Keywords: XRCC1 - single nucleotide polymorphisms - whole breast radiotherapy - side effect - meta-analysis

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Introduction

Breast cancer is the most common type of cancer among women with an incidence rate of 13% worldwide (Bray et al., 2004; Boyle et al., 2005; Héry et al., 2008). Whole breast radiotherapy, which is widely used after breast-conserving surgery, has been shown to reduce the risk of loco-regional recurrence of breast cancer by approximately 70% and to be as effective as radical mastectomy (Fisher et al., 2002). However, radiation-induced skin reaction is the most common side effect in breast cancer patients and can affect the therapeutic program and worsen the quality of life of patients (Schnur et al., 2011). Acute and late normal tissue effects are known to vary considerably, even between patients treated with identical schedules, ranging from negligible to severe. There is increasing evidence that the heterogeneity of normal tissue reactions in cancer patients treated with radiotherapy may be related to their genetic predisposition, as only 30% of this variation can be attributed to changes in treatment-related parameters (Safwat et al., 2002).

Extensive research efforts are being directed towards the identification of genetic markers such as single nucleotide polymorphisms (SNPs) as predictive factors for the risk of radiation-induced normal tissue toxicity. Because irradiation can cause DNA damage-induced cytotoxicity, inter-individual differences in DNA repair capacity may modify the response of the normal tissue. The base excision repair (BER) pathway plays an important role in the repair of radiation-induced DNA damage (de et al., 2000; Heijmakers er al., 2001; Giotopoulos er al., 2007). The X-ray repair cross-complementing 1 (XRCC1) protein, which functions in the short-patch of the BER pathway, has been implicated in the repair of basic sites through its action as both a scaffold and modulator of the different enzymes involved in BER (Vidal et al., 2001). The most extensively studied variants of the XRCC1 gene are Arg194Trp in exon 6, Arg280His in exon 9, Arg399Gln

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in exon 10 (Lunn et al., 1999; Butkiewicz et al., 2001; Stern et al., 2001), and 77T>C in the 5'-UTR (Shen et al., 1998; Ambrosone et al., 2001; Collett, 2003; Bray et al., 2004; Zhang et al., 2005; Hao et al., 2006).

The association between XRCC1 gene polymorphisms (Arg399Gln, Arg194Trp, -77T>C, and Arg280His) and radiation-induced toxicity has been investigated extensively. However, studies performed to date have shown conflicting evidence of this association. In breast cancer patients treated with radiotherapy after mastectomy or breast-conserving surgery, Andreassen et al. observed a significant correlation between the XRCC1 Arg399Gln polymorphism and the risk of subcutaneous fibrosis (Andreassen et al., 2003) but these findings could not be confirmed in a larger subsequent study (Andreassen et al., 2006). Chang-Claude et al. (2005) reported that the XRCC1 399Gln allele may be protective against the development of acute side-effects after radiotherapy in a study that included a cohort of 446 normal weight Caucasian breast cancer patients who received radiotherapy after breast-conserving surgery. In contrast, Giotopoulos et al. (2007) found an association between the 399Gln allele and an increased risk of developing late side effects. Mongani et al. (2011) reported that the carriers of the XRCC1-Arg194Trp variant allele in combination with the XRCC1-Arg399Gln wild-type allele had a significant risk of acute radiation-induced skin toxicity. However, in patients treated with radiotherapy for gynecologic cancers, a significant protective effect of the XRCC1 Arg194Trp SNP against normal tissue reactions was observed (De et al., 2005). Another study that did not differentiate between early and late adverse reactions to radiotherapy reported an elevated risk in women carrying variant alleles of the Arg194Trp and the Arg399Gln polymorphisms (Moullan et al., 2003). Zhou et al. (2010) reported that the risk of ≥grade 2 acute skin toxicity after radiotherapy could be increased by 2.86-fold in patients carrying the XRCC1-77TC and CC genotypes. Two published studies have addressed the impact of the XRCC1 position 194, 280, and 399 polymorphisms on the risk of late adverse reactions after radiotherapy for prostate cancer. Damaraju et al. (2006) investigated the relationship between 49 DNA repair and steroid metabolism gene polymorphisms and late toxicity in 83 prostate cancer patients. However, these authors could not find a significant association between the investigated SNPs in XRCC1 and grade >2 late toxicity. Furthermore, in an investigation by Burri et al. (2008), a significant association between the XRCC1 194, 280, and 399 polymorphisms and late rectal and urinary morbidity was not detected.

The present meta-analysis was performed to further evaluate the role of XRCC1 gene polymorphisms in predicting high-grade toxicity in patients with breast cancer treated with radiotherapy.

Materials and Methods

Data sources, search strategy, and selection of studies

Efforts were made to review all published studies related to the effects of XRCC1 gene polymorphisms on radiation-associated skin toxicity in breast carcinoma patients. Published articles were searched using different databases such as PubMed, Embase, and CNKI, screening all available information up to the present. Keyword combinations used for screening included "breast" and "cancer" or "carcinoma", "XRCC1" or "X-ray cross-complementing group1" and "polymorphism" or "variation", and "skin reaction" or "side effect" or "toxicity" and "radiotherapy" or "radiation". Studies retrieved by the search were reviewed and their cited references were checked for other potentially relevant publications. Review articles were also scanned to find additional eligible studies.

Selected publications were prescreened and studies were excluded if they met the following criteria: 1. The study did not report clinical outcomes; 2. The clinical outcome reported in the study was not specific to polymorphisms or could not be attributed to a specific polymorphism; 3. The principal investigator declined or was unable to provide relevant information upon request; 4. Insufficient data to allow the estimation of an odds ratio (OR) with a 95% confidence interval (CI). If the same research group published multiple articles on the topic (Falvo et al., 2011; Falvo et al., 2012), we selected the article that used the most samples and the most recent polymorphism data, or provided the most detailed information on each gene polymorphism.

Data extraction and quality assessment

The following information was extracted from each study where available: journal name, first author's surname, publication year, country, total number of patients included in the study, treatment regimens, genotyping methods, standards to evaluate radiation-induced toxicity, and number of endpoint events with differing status of XRCC1 gene variation. Information was carefully extracted from all eligible publications and the quality of each study was assessed independently by four of the authors of the present study (Xiaoxue Xie, Shuyu Ouyang, HK Jin, and Hui Wang) using a predefined scale. Our quality scoring criteria followed the guidelines established by prior studies (Thakkinstian et al., 2005). The QSS (quality score of a given study) was determined using the following 4 factors: genotyping methods, radiotherapy alone or in combination with chemotherapy, radiotherapy regimen, and sample size. With respect to genotyping methods, pyrosequencing, TaqMan, SnaPshot, and PCR-restriction fragment length polymorphism (RFLP) analysis verified by sequencing in all samples were considered to be of higher quality than RFLP verified in 20% or less samples (Xiao et al., 2006; Xiao et al., 2007; Wan et al., 2007; Wang et al., 2008) and fluorescence-based melting curve analysis (FBMCA). Total scores ranged from 0 (worst) to 12 (best). A final QSS score was assigned to each study after consensus was reached between reviewers. A study was considered low (or high) quality if QSS was <9 (or ≥9).

Statistical analysis

A total of 6 genetic models were considered in this meta-analysis, 3 main models (M1, allele comparison, A vs. a; M2, recessive model, AA vs. Aa+aa; or M3, dominant model, AA+Aa vs. aa) and 3 models of multiple pairwise

Table 1. Characteristics of Eligible Studies Considered for Inclusion in the Report

Author (Year)	Subjects	Evaluation criteria	Genotyping methods	Radiotherapy regimen	Combined Chemotherapy	End-points (toxicity)	QSS
Mangoni (2010)	148	NIH	SnaPshot	Mixed*	Partial patients	Acute	9
Chang-Claude (2005)	446	NIH	FBMCA	Conventional	None patients	Acute	10
Zhou (2010)	119	CTCAE,version3.0	PCR-RFLP	Conventional	None patients	Acute	10
Terrazzino (2012)	285	RTOG	PCR-RFLP	Conventional	Partial patients	Acute	10
Raabe (2012)	83	RTOG	PCR-RFLP	Conventional	All patients	Acute	7
Zschenker (2010)	69	LENT/SOMA	PCR-RFLP*	Conventional	Partial patients	Late	9
Falvo (2012)	57	CTCAE,version3.0	Pyrosequencing	APBI	Partial patients	Late	7
Giotopoulos (2007)	82	RTOG and SOMA	PCR-RFLP	Conventional	Partial patients	Late	8
Chang-Claude (2009)	409	RTOG and SOMA	FBMCA	Conventional	None patients	Late	10
Moullan (2003)	254	EORTC	TaqMan	Hypo-fractioned	Partial patients	Both	10
Brem (2006)	247	EORTC	PCR-RFLP	Hypo-fractioned	No referral	Both	8

NIH, Common toxicity criteria of the National Institutes of Health; CTCAE, Common Terminology Criteria for Adverse Events; RTOG, Radiation Therapy Oncology Group; EORTC, European Organization for Research and Treatment of Cancer; LENT/SOMA, Late Effects of Normal Tissue-Subjective Objective Management Analytical; FBMCA, Fluorescence-based melting curve analysis; RFLP, restricted fragment length polymorphisms ; APBI, accelerated partial breast irradiation. PCR-RFLP*, PCR-RFLP+ sequencing(100%); Mixed*, Patients treated by conventional-fractioned radiotherapy and by hypo-fractioned radiotherapy

Table 2. Distribution of XRCC1 Genotypes with Respect to Radiation-induced Toxicity

	Arg194Trp				Arg399Gln				-77T>C				Arg280His			
Author (Year)	TrpTrp	TrpArg	ArgArg	(Trp) Allelic frequency%	GlnGln	GlnArg	ArgArg	(Gln)Allelic frequency%	C/C	T/C	T/T	(C)Allelic frequency%	HisHis	ArgHis	ArgArg	(His)Allelic frequency%
Mangoni (2010)	40954		13/133	—	11/77		4/71	—	—	—	—	—	—	—	—	—
Chang-Claude (2005)	0/2	7/45	70/396	5.53	10/61	36/204	31/181	36.55	—	—	—	—	1/2	5/48	71/395	5.84
Zhou (2010)	6/10	25/42	32/50	30.39	6/10	24/34	39/58	26.47	6/7	18/21	45/74	17.17	2/6	41261	55/80	14.42
Terrazzino (2012)	0/1	41213	79/253	5.79	12/35	42/137	35/113	36.32	10/51	47/136	32/98	41.75	—	—	—	—
Raabe (2012)	—	—	—	—	7/14	20/33	19/36	36.75	—	—	—	—	—	—	—	—
Zschenker (2010)	—	—	—	—	5/15	8/25	4/29	39.86	—	—	—	—	—	—	—	—
Falvo (2012)	—	—	—	—	14/26		14/31	—		—	—	—		—	—	—
Giotopoulos (2007)	—	—	—	—	4/13	9/34	2/35	36.59		—	—	—	—	—	—	—
Chang-Claude (2009)	0/2	10/40	117/359	5.49	14/58	61/183	50/162	37.1	30/75	54/190	43/137	28.3	0/2	9/39	118/362	5.33
Moullan (2003)	1/1	13/34	56/219	7.09	9/32	37/113	24/109	34.84	—	—	—	—	1/1	9/39	60/214	8.07
Brem (2006)	—	—	—	—	—	—	—	—	15/50	27/107	24/90	41.9	—	—	—	—

In front of oblique line is subjects evaluated as radiation-associated toxicity G≥2 according to CTCAE,RTOG,EORTC, LENT/SOMA ,RTOG and SOMAor G≥2c according to NIH;behind oblique line is total subjects

comparisons (M4, AA vs. aa; M5, Aa vs. AA; or M6, AA vs. Aa). Models M1 to M3 were considered primary genetic models of interest (Minelli et al., 2005; Zintzaras et al., 2008). The ORs with 95% CIs were estimated for the incidence of radiation-induced skin toxicity. The odds of incidence were defined as the rate of high-grade toxicity (grade ≥2 according to Common Terminology Criteria for Adverse Events (CTCAE), Radiation Therapy Oncology Group (RTOG), European Organization for Research and Treatment of Cancer (EORTC), LENT/SOMA), or grade ≥2c according to the National Institutes of Health(NIH)) induced by radiotherapy.

For data analyses, Hardy-Weinberg equilibrium (HWE) was assessed using a goodness-of-fit test. Clinical outcome, in particular the OR for risk of radiation-induced toxicity, was estimated using random-effect models with Mantel-Haenszel statistics (Ades et al., 2005). Heterogeneity between studies was assessed visually by scatter plot and estimated by I^2-statistic after the χ^2 test. The same statistical methods were applied in sub-analyses using stratified patient populations. All analyses were conducted using Review Manager version 5.1. P values less than 0.05 were considered statistically significant.

Results

Description of studies

After application of critical search strategies and exclusion criteria, 11 follow-up studies (Moullan et al., 2003; Chang-Claude et al., 2005; Brem et al., 2006; Giotopoulos et al., 2007; Chang-Claude et al., 2009; Zhou et al., 2010; Zschenker et al., 2010; Mangoni et al., 2011; Falvo et al., 2012; Raabe et al., 2012; Terrazzino et al., 2012) comprising 2199 cases were eligible for the present analysis. The baseline characteristics of the included studies are shown in Table 1. Ten of these studies included mainly Caucasian patients and one study included only Chinese patients (Zhou et al., 2010). All of them were published in English-language journals. The sample size of each report ranged from 57 to 446 individuals. The quality score for studies of the association between the Arg399Gln, Arg194Trp, -77T>C and Arg280His polymorphisms and radiation-induced toxicity ranged from 7 to 10, with 70% (7/10), 100% (6/6), 75% (3/4), and 100% (4/4), respectively, of the trials classified as high quality. A total of 6 studies used PCR-RFLP genotyping methods. Genotypes were verified by sequencing of all samples in one study (Zschenker et al., 2010), partially verified in 20% of samples in one study (Zhou et al., 2010), and not verified in the rest of the studies (Brem et al., 2006; Giotopoulos et al., 2007; Zhou et al., 2010; Raabe et al., 2012). All studies used samples of peripheral blood.

Allele frequencies

Table 2 shows the distribution of XRCC1 genotypes

Table 3. Analysis of the Association Between XRCC1 Arg399Gln and Radiation-induced Toxicity

Study groups	M1:Gln vs. Arg					M2: GlnGln vs. GlnArg+ArgArg					M3: GlnGln+GlnArg vs. ArgArg				
	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q[c]	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q[c]	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q[c]
Overall	8	1.24[0.99,1.56].	0.06	42%	0.1	8	0.96[0.70,1.32]	0.82	0%	0.58	10	1.28[1.00,1.64]	0.05	17%	0.29
End-points															
Acute toxicity	4	1.19[0.94,1.52]	0.15	10%	0.35	4	0.96[0.61,1.50]	0.86	0%	0.64	5	1.11[0.82,1.50]	0.49	0%	0.63
Late toxicity	3	1.61[0.76,3.38]	0.21	77%	0.01	3	0.88[0.51,1.49]	0.62	26%	0.26	4	1.12[0.68,1.53]	0.47	0%	0.59
Genotyping [a]															
high quality	2	1.43[1.00,2.05]	0.05	0%	0.32	2	1.21[0.61,2.41]	0.58	0%	0.49	4	1.85[1.20,2.86]	0.005	0%	0.69
low quality	6	1.19[0.90,1.56]	0.22	50%	0.08	6	0.91[0.64,1.30]	0.6	0%	0.46	6	1.10[0.85,1.41]	0.47	2%	0.4
QSS [a]															
≥9	5	1.19[0.96,1.48]	0.12	35%	0.18	6	0.93[0.66,1.30]	0.98	0%	0.58	7	1.21[0.95,1.54]	0.13	7%	0.37
<9	2	1.63 [0.61,4.32]	0.33	72%	0.06	2	1.27[0.43,3.77]	0.67	35%	0.22	3	1.82[0.78,4.25]	0.17	42%	0.18
Chemotherapy															
All not	3	1.13[0.77,1.66]	0.53	60%	0.08	3	0.76[0.48,1.20]	0.24	0%	0.44	3	1.04[0.77,1.41]	0.81	0%	0.99
Partially yes	5	1.35[0.99,1.84]	0.06	38%	0.17	5	1.20[0.78,1.81]	0.41	0%	0.73	7	1.60[1.09,2.33]	0.02	25%	0.23

[a]The detailed criteria is given in Table 1; [b]The detailed references are given in Table 1 and 2; [c]p value of heterogeneity

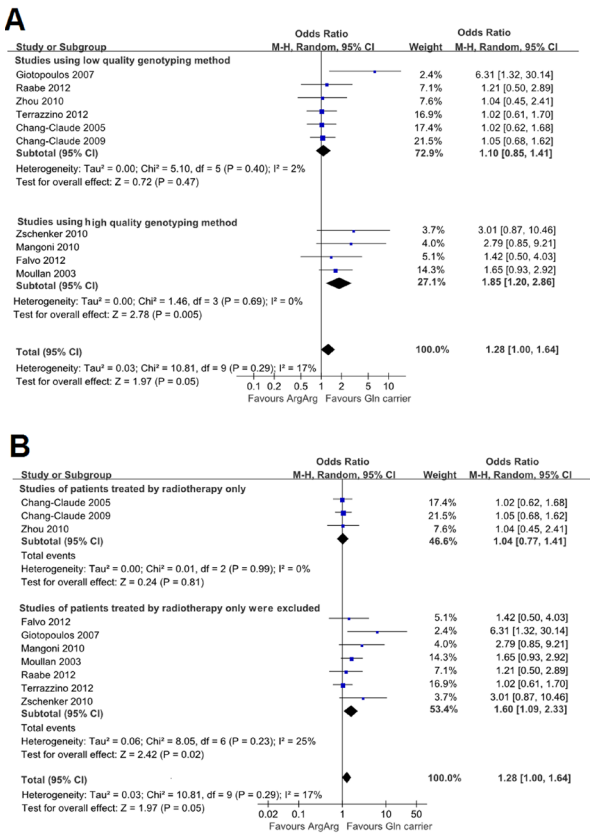

Figure 1. Forest Plots of the Genotype Contrast of XRCC1 Polymorphisms and Radiation-induced Toxicity Stratified by Treatment Regimen (radiotherapy alone or in combination with chemotherapy) (A) and the quality of genotyping methods (B). An OR>1(or OR<1) indicates that 399Gln carriers are more (or less) likely to show grade 2 or above radiation-induced toxicity than ArgArg carriers

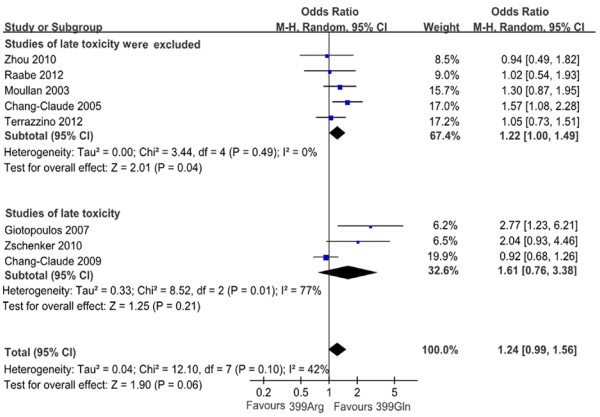

Figure 2. Forest Plots of the Allele Contrast of XRCC1 Polymorphisms and Radiation-induced Toxicity Stratified by Treatment Regimen (radiotherapy alone or in combination with chemotherapy). An OR>1 (or OR<1) indicates that 399Gln carriers are more (or less) likely to show grade 2 or above radiation-induced toxicity than 399Arg carriers

with respect to radiation-induced toxicity and the distribution of the XRCC1 allele frequencies. Using the frequencies of XRCC1 genotypes, all populations were found to be in Hardy-Weinberg Equilibrium (HWE).

The pooled frequency was 35.93% (26.47–39.86) for the 399Gln allele, 7.54% (5.53%–30.39%) for the 194Trp allele, 6.88% (5.33%–14.42%) for the 280His allele, and 32.28% (28.30%–41.90%) for -77T>C. The frequency for 399Gln, 194Trp, 280His, and -77T>C changed to 36.53%, 5.86%, 6.17%, and 37.32%, respectively, after exclusion of the study conducted entirely on Chinese patients (3). For all available studies, heterogeneity was only observed in pooled frequency for the -77C allele (P=0.004) and the 399Gln allele(P=0.10). No heterogeneity was observed for the other two SNPs (194Trp and 280His) with respect to radiation-induced toxicity.

Gene effects

Data concerning the predictive value of the XRCC1 Arg399Gln allele with respect to the risk of radiation-induced toxicity in breast cancer patients were available in 10 trials comprising 1929 individuals (Moullan et al., 2003; Chang-Claude et al., 2005; Giotopoulos et al., 2007; Chang-Claude et al., 2009; Zschenker et al., 2010; Zhou et al., 2010; Mangoni et al., 2011; Terrazzino et al., 2012; Falvo et al., 2012; Raabe et al., 2012). No significant association between the 399Gln allele and radiation-induced toxicity was detected in the overall analysis (Table 3). Nevertheless, subgroup analyses showed that carriers of the variant XRCC1 Arg399Gln were at a higher risk of radiation-induced toxicity in studies using high quality genotyping methods [Gln carriers vs. ArgArg: OR, 1.85; 95% CI, 1.20–2.86] (Figure 1A) and in studies with mixed treatment regimens consisting of radiotherapy alone and

Table 4. Analysis of the Association Between XRCC1 Arg280His and Radiation-induced Toxicity

Study groups	M1:Gln vs. Arg					M2: GlnGln vs. GlnArg+ArgArg					M3: GlnGln+GlnArg vs. ArgArg				
	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q^c	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q^c	No. studies[b]	Random-effect (95% CI)	*P*	I^2	P_Q^c
Overall	4	0.68[0.46,1.00]	0.05	0%	0.66	4	1.08[0.18,6.54]	0.93	47%	0.13	4	0.67[0.44,1.02]	0.06	0%	0.91
End-points															
Acute toxicity	2	0.62[0.35,1.08]	0.09	0%	0.56	2	0.87[0.05,16.60]	0.92	70%	0.07	2	0.63[0.33,1.20]	0.16	0%	0.97
Late toxicity	1	0.56[0.26,1.19]	0.13	—	—	1	0.43[0.02,9.03]	0.59	—	—	1	0.58[0.27,1.26]	0.17	—	—
Genotyping [a]															
high quality	1	0.96[0.47,1.97]	0.91	—	—	1	7.96[0.32,197.83]	0.21	—	—	1	0.69[0.39,1.86]	0.69	—	—
low quality	3	0.59[0.38,0.93]	0.02	0%	0.83	3	0.64[0.10,3.94]	0.63	40%	0.19	3	0.61[0.37,1.00]	0.05	0%	0.99
QSS [a]	—	—	—	—	—		—	—	—	—	—	—	—	—	—
≥9	4	0.68[0.46,1.00]	0.05	0%	0.66	4	1.08[0.18,6.54]	0.93	47%	0.13	4	0.67[0.44,1.02]	0.06	0%	0.91
<9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chemotherapy															
All not	3	0.59[0.38,0.93]	0.02	0%	0.83	3	0.64[0.10,3.94]	0.63	40%	0.19	3	0.61[0.37,1.00]	0.05	0%	0.99
Partially yes	1	0.96[0.47,1.97]	0.91	—	—	1	7.96[0.32,197.83]	0.21	—	—	1	0.69[0.39,1.86]	0.69	—	—

[a]The detailed criteria is given in Table 1; [b]The detailed references are given in Table 1 and 2; [c]p value of heterogeneity

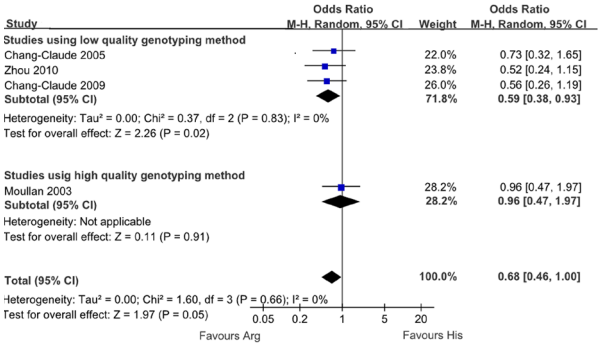

Figure 3. Forest Plots of the Allele Contrast of XRCC1 Polymorphisms and Radiation-induced Toxicity Stratified by the Treatment Regimen (radiotherapy along or in combination with chemotherapy). An OR>1 (or OR<1) indicates that 280His carriers are more (or less) likely to show grade 2 or above radiation-induced toxicity than 280Arg carriers

combined with chemotherapy [Gln carriers vs. ArgArg: OR, 1.60; 95% CI, 1.09–2.23] (Figure 1B). In the stratified analysis, the association between the 399Gln allele and radiation-induced toxicity was significant when studies on late toxicity only were excluded (Figure 2). No significant heterogeneity was detected among the predictive values in these studies. No significant difference was identified with respect to any association between radiation-induced toxicity and the other subgroups.

Data concerning the predictive value of the 280His allele with respect to radiation-induced toxicity were available from 4 studies covering a total of 1088 individuals (Moullan et al., 2003; Chang-Claude et al., 2005; Chang-Claude et al., 2009; Zschenker et al., 2010). Table 4 shows an association between the 280His ("decreasing" allele) and radiation-induced toxicity relative to the 280Arg allele in studies of patients treated by radiotherapy alone, although these studies used low quality genotyping methods (Figure 3). These results suggest that the protective effect of the 280His allele should be further investigated by using high quality testing methods. There was no evidence of heterogeneity with respect to the predictive value.

No significant associations were detected between radiation-induced toxicity and the other two SNPs (Arg194Trp and -77T>C) in the meta-analysis.

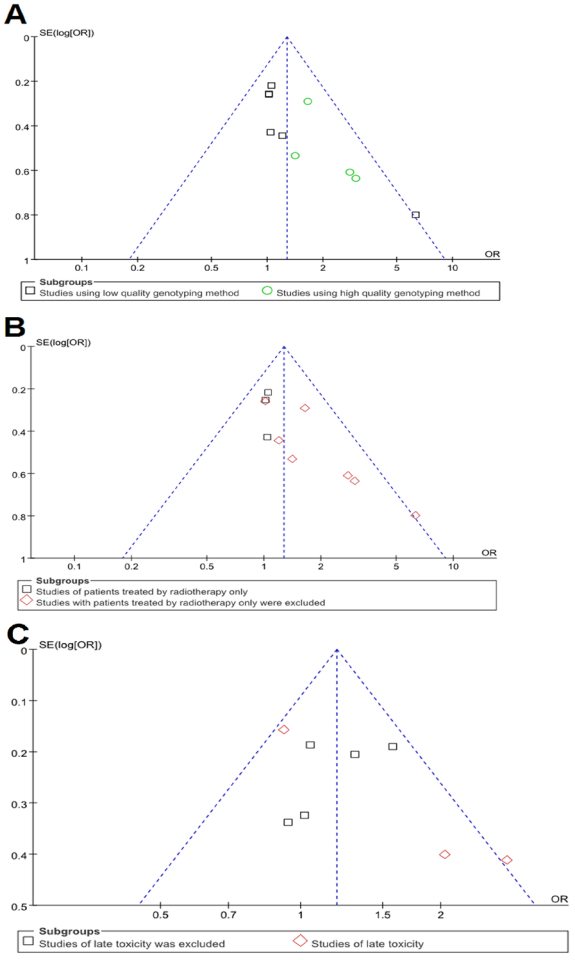

Figure 4. Funnel Plots of the Genotype Contrast of XRCC1 Arg399Gln Polymorphisms and Radiation-induced Toxicity Stratified by Treatment Regimen (radiotherapy alone or in combination with chemotherapy) (A) and quality of genotyping methods (B). Funnel plots of the allele contrast of XRCC1 Arg399Gln polymorphisms and radiation-induced toxicity stratified by treatment regimen (radiotherapy alone or in combination with chemotherapy)(C)

Publication bias

No evidence of publication bias was detected in the funnel plots of the meta-analysis of XRCC1 Arg399Gln (Figure 4).

Discussion

Our analysis shows an association between the XRCC1 399Gln allele and a trend towards an increased risk of high-grade radiation-induced toxicity when a dominant model is used to analyze the data. The dominant effect of the Arg399Gln variant was observed in studies with mixed treatment regimens consisting of radiotherapy alone or combined with chemotherapy. This suggests that the associations among radiation-induced toxicity, XRCC1 Arg399Gln polymorphism, and chemotherapy should be studied further. Our stratified analysis also indicated that the association between XRCC1 399Gln carriers and radiation-induced toxicity was significant only in studies using high quality genotyping methods, which implies that trials should be based on high quality genotyping methods. In addition, the 399Gln allele was positively associated with radiation-induced toxicity when studies on late toxicity only were excluded, implying that the Gln allele may be a better predictive marker for mixed acute and late toxicity than for late toxicity only.

The XRCC1 Arg280His variant allele was found to be negatively associated with radiation-induced toxicity in studies that included patients treated with radiotherapy alone, although these studies were performed using low quality genotyping methods. Our findings suggest that the role of XRCC1 Arg280His in radiation-induced toxicity should be investigated more carefully in future studies by using high quality genotyping methods to ensure a more accurate and robust conclusion.

No significant associations were detected between radiation-induced toxicity and Arg194Trp in the overall analysis and in the subgroup analysis. In a study by Moullan et al. (Moullan et al., 2003), the 194Trp allele of the XRCC1 Arg194Trp polymorphism was found to be significantly associated with the risk of developing an adverse response to radiotherapy when analyzed in combination with the 399Gln allele of the XRCC1 Arg399Gln polymorphism. On the other hand, Mongani et al. (Mangoni et al., 2011) showed that women carrying the combination of the XRCC1-194Trp and the XRCC1-399 Arg alleles were more likely to experience grade 2c or higher toxicity. The original data on such combination studies currently available are not sufficient for inclusion into a meta-analysis, suggesting that more emphasis should be placed on combination or haplotype analysis in future studies.

For all available studies, heterogeneity was detected in the analysis of XRCC1 -77T>C. The presence of heterogeneity indicated variability, which may have been caused by differences between study characteristics such as the endpoints of evaluation, treatment regimen, and the genotyping method used. Hence, stratified analyses are needed to reduce such variability. However, no subpopulations were available for -77T>C even after stratification, which could be due to a lack of sufficient available original studies. Therefore, the conclusions drawn in this meta-analysis for -77T>C should be weighed with caution.

Variation in the quality of studies (or trials) is not uncommon in meta-analyses of genetic associations in genetic epidemiology (Attia et al., 2003; Funke et al., 2008). In the present analysis, we evaluated each study using a QSS as described in Table 1. However, our results showed that the predictive value of XRCC1 gene polymorphisms is influenced only by the quality of the genotyping methods and not by the quality of the studies as determined by the QSS. These results suggest that the use of high-quality genotyping methods is more important than other factors associated with the quality of the study.

Toxicity was assessed using a classification system. The development of acute side effects of grade ≥2c according to the Common Toxicity Criteria of the U.S. NIH or ≥2 according to the CTCAE v3.0 or RTOG was considered to indicate increased sensitivity for acute effects in our study (Chang-Claude et al., 2005; Zhou et al., 2010; Mangoni et al., 2011; Raabe et al., 2012; Terrazzino et al., 2012). The development of late effects of grade ≥2 according to the CTCAE v3.0 or SOMA was considered to indicate increased sensitivity for late effects in our study (Giotopoulos et al., 2007; Chang-Claude et al., 2009; Zschenker et al., 2010; Falvo et al., 2012). Adverse reactions to radiotherapy of grade ≥2 by the EORTC in the 2-year period after the start of radiotherapy were also considered to be indicative of increased sensitivity for side effects in our study (both acute and late) (Moullan et al., 2003; Brem et al., 2006). Two studies addressing acute and late toxicity induced by radiotherapy were included in the present meta-analysis. Moullan et al. (2003) reported an increased risk of radiation-induced toxicity in women carrying both variant alleles of the Arg194Trp and Arg399Gln polymorphisms, and no difference between early and late adverse reactions to radiotherapy was observed. Early and late reactions are not necessarily related and they may be influenced differently by genetic predisposition. Late side-effects from radiotherapy, which are often irreversible, can decrease health-related quality of life and limit radiation dose. However, in the present meta-analysis, the 399Gln allele was only found to be positively associated with radiation-induced toxicity when studies of late toxicity were excluded.

This study has some limitations. In some of the sub-analyses on stratified groups of patients, only one or no trials were available and hence the variability across trials could not be assessed. Because of the nature of meta-analyses, the accuracy of inference and statistical power were usually limited because analyses could only be conducted on secondary data, other than the original data collected directly from individual patients. In addition, the quality of trials could not be controlled directly by researchers conducting the meta-analysis. Further analysis of the combined effect of multiple SNPs and haplotypes is necessary in future studies.

In conclusion: The present study identified XRCC1 gene polymorphisms as predictive factors for high-grade toxicity in patients with breast cancer treated with radiotherapy. For XRCC1 399Gln carriers, the predictive value was found to differ according to the treatment regimen, depending on whether radiotherapy alone or in combination with chemotherapy was used. The XRCC1 390Gln allele was positively correlated with toxicity when studies on late toxicity only were excluded, and the

XRCC1 280His allele was protective against radiation-induced toxicity only in studies that included patients treated with radiotherapy alone. However, no significant associations were detected between radiation-induced toxicity and the other two SNPs (Arg194Trp and -77T>C) in the overall and subgroup analyses. Larger well-designed studies are required to further evaluate the predictive value of XRCC1 gene variation on radiation-induced toxicity in patients undergoing surgery for breast cancer. The quality of the genotyping methods used may be an important factor affecting the predictive value of XRCC1 gene polymorphisms. Functional studies may help validate the effects of these polymorphisms on radiation-associated toxicity.

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