RESEARCH ARTICLE

Association Between XRCC5, 6 and 7 Gene Polymorphisms and the Risk of Breast Cancer: A HuGE Review and Meta-analysis

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Abstract

 <u>Objective</u>: Non-homologous end joining (NHEJ) is a pathway for repairing DNA double-strand breaks. Recent publications indicated that XRCC5, XRCC6 and XRCC7 genes may participate in the pathogenesis of breast cancer. The aim of this Human Genome Epidemiology (HuGE) review and meta-analysis was to investigate associations between XRCC5, XRCC6 and XRCC7 genetic polymorphisms in the NHEJ pathway and breast cancer risk. <u>Methods</u>: Studies focusing on the relationship between genetic polymorphisms in XRCC5, XRCC6 and XRCC7 genes and susceptibility to breast cancer were selected from the Pubmed, Cochrane library, Embase, Web of Science, Springerlink, CNKI and CBM databases. Data were extracted by two independent reviewers. The meta-analysis was performed with Review Manager Version 5.1.6 and STATA Version 12.0 software. The odds ratio (OR) with 95% confidence interval (95%CI) was calculated based on the extracted data. <u>Results</u>: According to the inclusion criteria, we final included seven studies with a total of 2,864 breast cancer cases and 3,060 healthy controls. Meta-analysis results showed that rs3835 (G>A) and rs828907 (G>T) in XRCC5 gene, and rs132793 (G>A) in XRCC6 gene might increase the risk of breast cancer, while rs132788 G>T and rs6002421 (A>G) might be protective factors. However, there was no relationship between XRCC7 genetic polymorphisms and the risk of breast cancer. <u>Conclusion</u>: This meta-analysis suggests that the rs3835 G>A and rs828907 G>T in XRCC5 gene, rs6002421 (A>G), rs132788 (G>T) and rs132793 (G>A) in XRCC6 gene might be risk factors for breast cancer, while the rs132788 (G>T) and rs6002421 (A>G) in XRCC6 gene might be protective.

Keywords: XRCC5 - XRCC6 - XRCC7 - polymorphism - breast cancer - meta-analysis

Introduction

Breast cancer is the most common malignancy to threaten human health throughout the world usually diagnosed in women, sometimes also developed in men (DeSantis et al., 2011; Li et al., 2011). It is the second leading cause of cancer death among women, and the latest estimates suggest that over 1,050,000 new breast cancer cases occur worldwide annually. During the past three decades, the mortality of breast cancer among women has decreased significantly but the incidence has doubled (Coughlin et al., 2009; Agurs-Collins et al., 2010).

DNA repair plays a crucial role in maintaining normal function and genetic material stability of mammalian (Spry et al., 2007; Hakem et al., 2008). After DNA damage caused by endogenous damage such as attack by reactive oxygen species and various exogenous damages such as ultraviolet radiation happened, if is not repaired, sequently it may arise carcinogenesis or apoptosis (Dapic et al., 2005; Hoeijmakers, 2009). DNA double-strand breaks (DNA DSBs) are the most serious damage form, which should be repaired in eukaryotes by two major pathways: homologous recombination (HR) and nonhomologous DNA end joining (NHEJ). (Mao et al., 2008; Shrivastav et al., 2008). The difference between HR and NHEJ is that NHEJ directly rejoins two broken DNA ends and thus does not require homologous sequences for the DSB repair (Yano et al., 2009). NHEJ involves the XRCC4 (X-ray repair complementing defective repair in Chinese hamster cells 4, XRCC4), XRCC5 (X-ray repair complementing defective repair in Chinese hamster cells 5, XRCC5, also known as Ku80), XRCC6 (X-ray repair complementing defective repair in Chinese hamster cells 6, XRCC7, also known as Ku70), XRCC7 (DNA-dependent protein kinase catalytic subunit, DNA-PKcs), XLF (XRCC4-like factor), LIG4 (DNA ligase IV, LIG4) and other genes and their proteins. The process of NHEJ pathway model is that first, the XRCC5/XRCC6 heterodimer recognizes and binds the DSB, which induces inward translocation of Ku and recruits XRCC7 to the ends of the DSB to form DNA-PK (DNA-dependent protein kinase, DNA-PK); second, depending on the type and complexity of the DSB break, the DNA ends are processed by different processing factors such as PNKP (polynucleotide kinase 3'-phosphatase, PNKP), DNA polymerases, or the MRN complex (The MRN complex is heterotrimeric protein complex consisting of Mre11, Rad50 and Nbs1, which recognizes DNA damage and rapidly relocates to DSB

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sites and forms nuclear foci); then, XRCC7 will dissociate from the DSBs. At last, XLF stimulates the XRCC4/LIG4 complex to ligate the DNA ends to repair the DSB (Shrivastav et al., 2008; Yano et al., 2009). DNA-PK is composed of XRCC7 and the XRCC5/XRCC6 subunits, which act as the catalytic and regulatory subunits, respectively (Rivera-Calzada et al., 2007). XRCC5 and XRCC6 heterodimer and XRCC7 are required for NHEJ pathway of DNA repair, and their mutants can influence the association with cancers.

In recent years, relevant studies have commenced the association between XRCC5, XRCC6 and XRCC7 and breast cancer risk. However, the exact roles of these genes and their protein products, such as XRCC5 or XRCC6, in each type of cancer are not well investigated or understood. Goode et al carried out a study on XRCC5 SNPs and one XRCC6 SNP (rs132788) was listed among the 21 SNPs they chose for genotyping and analysis, but with negative association results (Goode et al., 2002). Kuschel et al finding that the same SNP of XRCC6 was not associated with breast cancer risk. Willems et al performed a study in Belgium on SNPs of XRCC6 at rs2267437 to breast cancer, and the results demonstrated a significant odds ratio of 1.85 in sporadic, but not familial breast cancer patients (Kuschel et al., 2002). Given controversial results in those previous studies, we conducted a meta-analysis to investigate the association between XRCC5, XRCC6 and XRCC7 polymorphisms in NHEJ pathway and breast cancer risk.

Materials and Methods

Literature search

We performed an electronic search of the Pubmed, Cochrane library, Embase, Web of science, Springerlink, CNKI and CBM databases extensively to identify relevant studies available up to June 25, 2012. The search terms were used, including ("DNA-PKcs" OR "Ku70" OR "Ku80" OR "XRCC5" OR "XRCC6" OR "RXCC7") AND ("Breast neoplasms" OR "Breast cancer" OR "Breast tumor" OR "Breast carcinoma") AND ("Genetic polymorphism" OR "Single nucleotide polymorphism" OR "SNP" OR "Mutant" OR "Gene variation" OR "Gene mutation"). The references in the eligible studies or textbooks were also reviewed to check through manual searches to find other potentially eligible studies.

Inclusion and exclusion criteria

The included studies had to meet the following criteria: i) Case-control study focused on associations between XRCC5, 6, 7 genetic polymorphisms and breast cancer risk; ii) All patients with the diagnosis of breast cancer confirmed by pathological examination of the surgical specimen; iii) The number and the mutant frequencies of alleles or genotypes case and control groups could be extracted; iv) The publication was in English or Chinese. Studies were excluded when they were: i) Not case-control studies about XRCC5, 6, 7 genes polymorphisms and breast cancer risk; ii) Based on incomplete data; iii) Useless or overlapping data were reported; iv) Meta-analyses, letters, reviews or editorial articles.

Data extraction

Using a standardized form, data from published studies were extracted independently by two reviewers to populate the necessary information. The following information was extracted from each of the articles included: first author, year of publication, country, language, ethnicity, study design, source of cases and controls, number of cases and controls, mean age, sample, pathological type, genotype method, genotype frequency, the rate of mutation and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, an agreement was reached following a discussion with a third reviewer.

Quality assessment of included studies

Two reviewers independently assessed the quality of papers according to modified STROBE quality score systems (von Elm et al., 2007; Zhang et al., 2011). Forty assessment items related with the quality appraisal were used in this meta-analysis, scores ranging from 0 to 40. Scores of 0-20, 20-30 and 30-40 were defined as low, moderate and high quality, respectively. Disagreement was resolved by discussion.

Statistical analysis

The meta-analysis examined the association between XRCC5, 6, 7 genetic polymorphisms and the risk of breast caner for the comparisons of mutation rates in cases and controls. The mutation rates can be classified into total mutation rate (TMR), the ratio of heterozygotes and mutant homozygotes to the total number of genotypes; complete mutation rate (CMR), the ratio of mutant homozygotes to the total number of genotypes; partial mutation rate (PMR), the ratio of heterozygotes to the total number of genotypes. The odds ratio (OR) and 95% confidence interval (95%CI) were calculated using Review Manager Version 5.1.6 (provided by the Cochrane Collaboration, available at: http://ims.cochrane.org/revman/download) and STATA Version 12.0 (Stata Corp, College Station, TX) softwares. Between-study variations and heterogeneities were estimated using Cochran's Q-statistic (Higgins et al., 2002; Zintzaras et al., 2005) (P≤0.05 was considered to be manifestation of statistically significant heterogeneity).

We also quantified the effect of heterogeneity by using I2 test, which ranges from 0 to 100% and represents the proportion of inter-study variability that can be contributed to heterogeneity rather than by chance. When a significant Q-test (P≤0.05) or I^2>50% indicated that heterogeneity among studies existed, the random effects model was conducted for meta-analysis. Otherwise, the fixed effects model was used.

To establish the effect of heterogeneity on meta-analyses' conclusions, subgroup analysis was operated. We tested whether genotype frequencies of controls were in HWE using the χ^2 test. Funnel plots are often used to detect publication bias. However, due to its limitations caused by varied sample sizes and subjective reviews, Egger's linear regression test which measures funnel plot's asymmetry using a natural logarithm scale of OR was used to evaluate the publication bias (Peters et al., 2006). When the P value is less than 0.1, publication bias is considered significant. All the P values were two-sided.

Table 1. Characteristics of the Studies Included in this Meta-analysis

First author	Year	Country	Ethnicity	Number Case	Control	Source of control	Sample	Genotype method	Gene	SNP	Quality scores
Fu et al	2003	China	Asian	254	379	Hospital -based	Blood	MassArray	XRCC6	rs2267437 (C/G)	24
									XRCC6	rs132788 (G/T)	
									XRCC6	rs132793 (G/A)	
									XRCC5	rs3835 (G/A)	
									XRCC5	rs3834 (G/A)	
									XRCC7	rs2231178 (C/T)	
Willems et al	2008	Belgium	Caucasian	172	129	Hospital -based	Blood	PCR-RFLP	XRCC6	rs132788 (G/T)	26
									XRCC5	rs3835 (G/A)	
									XRCC5	rs2440 (G/A)	
									XRCC7	rs2213178 (C/T)	
Han et al	2009	USA	Caucasian	239	477	Population-based	Blood	AS-PCR	XRCC6	rs6002421 (A/G)	25
Wang et al	2009	China	Asian	1272	1272	Population-based	Blood	TapMan	XRCC5	rs828907 (G/T)	27
									XRCC5	rs11685387 (C/T)	
									XRCC5	rs9288518 (A/G)	
Willems et al	2008	Belgium	Caucasian	206	171	Hospital -based	Blood	PCR-RFLP	XRCC6	rs2267437 (C/G)	25
Sobczuk et al	2010	Poland	Caucasian	135	60	Population-based	Blood	TapMan	XRCC6	rs132793 (A/G)	28
He et al	2012	China	Asian	293	301	Hospital -based	Blood	PCR-RFLP	XRCC6	rs2267437 (C/G)	26

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; AS, allele specific

Table 2. The Genotype Distribution of XRCC5, 6 and 7 Gene Polymorphisms

First author	SNP	Case							Control							P	Test
		Total	PM	CM	TM	TMR	CMR	PMR	Total	PM	CM	TM	TMR	CMR	PMR		
Fu et al (2003)	rs2267437 (C/G)	254	55	7	62	0.24	0.03	0.22	379	106	12	118	0.31	0.03	0.28	0.76	HWE
	rs132788 (G/T)	254	85	21	106	0.42	0.08	0.33	379	161	27	188	0.5	0.07	0.42	0.38	HWE
	rs132793 (G/A)	254	23	2	25	0.1	0.01	0.09	379	47	2	49	0.13	0.01	0.12	0.82	HWE
	rs3835 (G/A)	254	42	2	44	0.17	0.01	0.17	379	45	1	46	0.12	0	0.12	0.69	HWE
	rs3834 (G/A)	254	39	2	41	0.16	0.01	0.15	375	44	1	45	0.12	0	0.12	0.71	HWE
	rs2231178 (C/T)	254	54	0	54	0.21	0	0.21	377	73	4	77	0.2	0.01	0.19	0.85	HWE
Willems et al (2008)	rs132788 (G/T)	172	67	13	80	0.47	0.08	0.39	123	48	18	66	0.54	0.15	0.39	0.14	HWE
	rs3835 (G/A)	174	36	5	41	0.24	0.03	0.21	129	22	3	25	0.19	0.02	0.17	0.18	HWE
	rs2440 (G/A)	172	83	23	106	0.62	0.13	0.48	128	64	13	77	0.6	0.1	0.5	0.27	HWE
	rs2213178 (C/T)	168	56	15	71	0.42	0.09	0.33	129	48	10	58	0.45	0.08	0.37	0.64	HWE
Han et al (2009)	rs6002421(A/G)	236	1	0	1	0	0	0	473	14	1	15	0.03	0	0.03	0.02	non-HWE
Wang et al (2009)	rs828907 (G/T)	1272	309	130	439	0.35	0.1	0.24	1272	230	86	316	0.25	0.07	0.18	0.00	non-HWE
	rs11685387 (C/T)	1272	322	795	1117	0.88	0.63	0.25	1272	303	799	1102	0.87	0.63	0.24	0.00	non-HWE
	rs9288518 (A/G)	1272	373	758	1131	0.89	0.6	0.29	1272	402	738	1140	0.9	0.58	0.32	0.00	non-HWE
Willems et al (2009)	rs2267437 (C/G)	206	107	40	147	0.71	0.19	0.52	171	73	27	100	0.58	0.16	0.43	0.26	HWE
Sobczuk et al (2010)	rs132793 (A/G)	135	35	70	105	0.78	0.52	0.26	60	35	15	50	0.83	0.25	0.58	0.18	HWE
He et al (2012)	rs2267437 (C/G)	293	127	25	152	0.52	0.09	0.43	301	113	9	122	0.41	0.03	0.38	0.08	HWE

TM, total mutation; CM, complete mutation; PM, partial mutation; TMR, the rate of total mutation; CMR, the rate of complete mutation; PMR, the rate of partial mutation; SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium
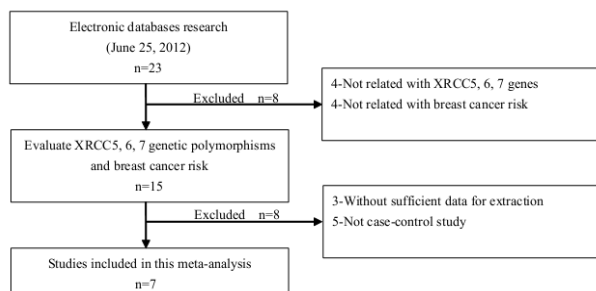

Figure 1. Flow Chart Shows Study Selection Procedure

To ensure the reliability and the accuracy of the results, two reviewers populated the data in the statistical software programs independently and obtained the same results.

Results

Characteristics of included studies

We identified a total of 23 relevant publications after initial screening. According to the inclusion criteria, seven case-control studies (Fu et al., 2003; Willems et al., 2008; Han et al., 2009; Wang et al., 2009; Willems et al., 2009; Sobczuk et al., 2010; He et al., 2012) appeared to have met the inclusion criteria. The flow chart of study selection is shown in Figure 1. A total of 2864 breast cancer cases and 3060 healthy controls from seven studies were included in the pooled analysis. The publication year of involved studies ranged from 2003 to 2012. Overall, there were four studies were conducted in Caucasians, and three studies in Asians. In our meta-analysis, we detected twelve single nucleotide polymorphisms (SNPs) in three genes, separately including six in in XRCC5 gene, rs2440 (G>A), rs3834 (G>A), rs3835 (G>A), rs828907 (G>T), rs9288518 (A>G), rs11685387 (C>T), four in in XRCC6 gene, rs132788 (G>T), rs132793 (A>G), rs2267437 (C>G), rs6002421 (A>G) and two in in XRCC7 gene, rs2213178 (C>T), rs2231178 (C>T). The characteristics and methodological quality of the included studies are summarized in Table 1. The mutation genotypes of XRCC5, 6, 7 genes polymorphisms were presented in Table 2.

Association between XRCC5, 6, 7 gene polymorphisms

Table 3. Meta-analysis of the Association between XRCC5 Gene Polymorphisms and Breast Cancer Risk

Polymorphisms	Cancern/N	Controln/N	OR [95%CI]	P value	Effect model
Rs2440 (G>A)					
TMR	106/172	77/128	1.06 [0.67, 1.70]	0.8	Fixed
CMR	23/172	13/128	1.37 [0.66, 2.81]	0.4	
PMR	83/172	64/128	n	0.77	
Rs3834 (G>A)					
TMR	41/254	45/375	1.41 [0.89, 2.23]	0.14	Fixed
CMR	2/254	1/375	2.97 [0.27, 32.91]	0.38	
PMR	39/254	44/375	1.36 [0.86, 2.17]	0.19	
Rs3835 (G>A)					
TMR	85/428	71/508	1.42 [1.00, 2.01]	0.05	Fixed
CMR	7/428	4/508	1.58 [0.46, 5.44]	0.47	
PMR	78/428	67/508	1.39 [0.97, 1.99]	0.07	
Rs828907 (G>T)					
TMR	439/1272	316/1272	1.59 [1.34, 1.89]	<0.00001	Fixed
CMR	130/1272	/861272	1.57 [1.18, 2.09]	0.002	
PMR	309/1272	230/1272	1.45 [1.20, 1.76]	0.0001	
Rs9288518 (A>G)					
TMR	1131/1272	1140/1272	0.93 [0.72, 1.19]	0.56	Fixed
CMR	758/1272	738/1272	1.07 [0.91, 1.25]	0.42	
PMR	373/1272	402/1272	0.90 [0.76, 1.06]	0.21	
Rs11685387 (C>T)					
TMR	1117/1272	1102/1272	1.11 [0.88, 1.40]	0.37	Fixed
CMR	795/1272	799/1272	0.99 [0.84, 1.16]	0.87	
PMR	322/1272	303/1272	1.08 [0.90, 1.30]	0.38	

TMR, the rate of total mutation; CMR, the rate of complete mutation; PMR, the rate of partial mutation; OR, odds ratio; 95%CI, 95% confidence interval

Table 4. Meta-analysis of the Association between XRCC6 Gene Polymorphisms and Breast Cancer Risk

Polymorphisms	Cancern/N	Controln/N	OR [95%CI]	P value	Effect model
Rs132793 (A>G)					
TMR	130/389	99/438	0.72 [0.47, 1.11]	0.14	Fixed
CMR	72/389	17/438	2.99 [1.59, 5.64]	0.0007	
PMR	58/389	82/438	0.43 [0.16, 1.17]	0.1	
Rs132788 (G>T)					
TMR	186/426	254/502	0.74 [0.56, 0.96]	0.02	Fixed
CMR	34/426	45/502	0.77 [0.32, 1.87]	0.57	
PMR	152/426	209/502	0.77 [0.59, 1.01]	0.06	
Rs2267437 (C>G)					
TMR	361/753	340/851	1.25 [0.72, 2.20]	0.43	Random
CMR	72/753	48/851	1.52 [0.79, 2.94]	0.21	
PMR	289/753	292/851	1.09 [0.72, 1.66]	0.67	
Rs6002421 (A>G)					
TMR	1/236	15/473	0.13 [0.02, 0.99]	0.05	Fixed
CMR	0/236	1/473	0.67 [0.03, 16.41]	0.8	
PMR	1/236	14/473	0.14 [0.02, 1.07]	0.06	

TMR, the rate of total mutation; CMR, the rate of complete mutation; PMR, the rate of partial mutation; OR, odds ratio; 95%CI, 95% confidence interval

and breast cancer risk

A summary of the meta-analysis findings of the association between XRCC5, 6 and 7 genetic polymorphisms and breast cancer risk is separately provided in Table 3, Table 4 and Table 5. Six SNPs in XRCC5 gene were examined, and the rs828907 (G>T) were proved to be associated with breast cancer risk (TMR: OR=1.59, 95%CI: 1.34-1.89, P<0.001; CMR: OR=1.57, 95%CI: 1.18-2.09, P=0.002; PMR: OR=1.45, 95%CI: 1.20-1.76, P=0.001). Besides, the mutants of rs3835 (G>A) also had association with breast cancer risk (TMR: OR=1.42, 95%CI: 1.00-2.01, P=0.05). The rest of four SNPs showed no linkage with the risk of breast cancer (all P>0.05).

Table 5. Meta-analysis of the Association between XRCC7 Gene Polymorphisms and Breast Cancer Risk

Polymorphisms	Cancern/N	Controln/N	OR [95%CI]	P value	Effect model
Rs2213178 (C>T)					
TMR	71/168	58/129	0.90 [0.56, 1.42]	0.64	Fixed
CMR	15/168	10/129	1.17 [0.51, 2.69]	0.72	
PMR	56/168	48/129	0.84 [0.52, 1.36]	0.49	
Rs2231178 (C>T)					
TMR	54/254	77/377	1.05 [0.71, 1.56]	0.8	Fixed
CMR	0/254	4/377	0.16 [0.01, 3.04]	0.22	
PMR	54/254	73/377	1.12 [0.76, 1.67]	0.56	

TMR, the rate of total mutation; CMR, the rate of complete mutation; PMR, the rate of partial mutation; OR, odds ratio; 95%CI, 95% confidence interval
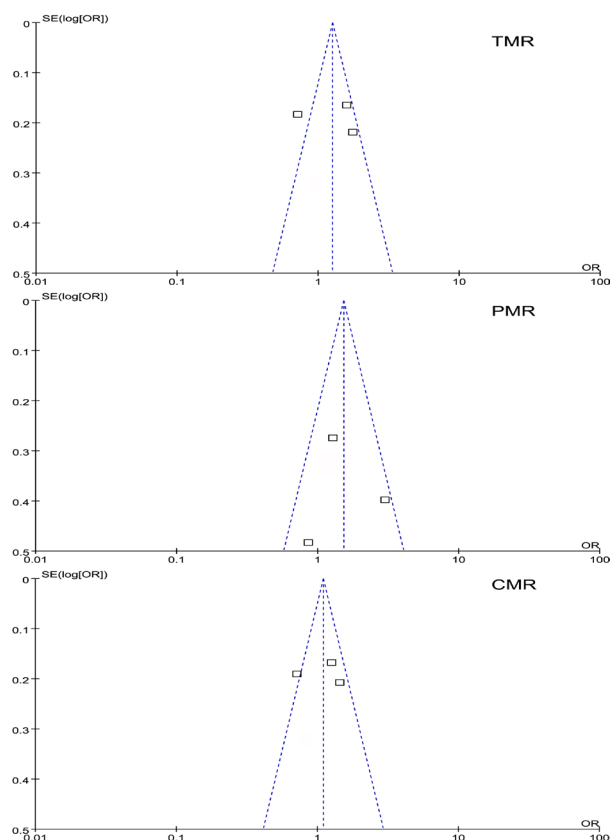

Figure 2. Begger's Funnel Plot of Publication Bias

Interestingly, we found that rs132793 (G>A) in XRCC6 gene might increase the risk of breast cancer (CMR: OR=2.99, 95%CI: 1.59-5.64, P=0.05), while rs132788 G>T and rs6002421A>G might decrease the risk for breast cancer (TMR; OR=0.74, 95%CI: 0.56-0.96, P=0.02; TMR: OR=0.13, 95%CI: 0.02-0.99, P=0.05, respectively). However, there was no evidence that the rs2267437 C>G polymorphism in XRCC6 gene and the rs2213178 (C>T) and rs2231178 (C>T) polymorphisms in XRCC7 gene were associated with the risk of breast cancer (all P>0.05).

In the subgroup analysis by ethnicity, we investigated the associations between mutation genotypes in XRCC5, 6, 7 genes and breast cancer susceptibility in Caucasians and Asians. However, no association was found between XRCC5, 6, 7 genes and breast cancer risk neither in Caucasians nor in Asians (all P>0.05). Additional a subgroup analysis was conducted by HWE, we also found

Table 6. Additional Pooled Analysis and Subgroup Analysis by Ethnicity

Mutation genotypes	Case n/N	Control n/N	OR [95%CI]	P	Heterogeneity P	I²	Effect model
TMR							Random
Asians	3171/5633	3202/6384	1.15 [0.92, 1.45]]	0.22	0.005	81%	
Caucasians	551/1263	391/1213	0.97 [0.56, 1.68]	0.9	0.01	74%	
CMR							Random
Asians	1742/5633	1679/6384	1.30 [0.85, 2.01]	0.23	0.03	71%	
Caucasians	166/1263	87/1213	1.46 [0.78, 2.71]	0.23	0.02	69%	
PMR							Random
Asians	1429/5633	1524/6384	1.05 [0.90, 1.23]	0.51	0.09	59%	
Caucasians	385/1263	304/1213	0.63 [0.30, 1.34]	0.23	<0.01	88%	
TMR							Random
HWE	1034/2844	1021/3308	1.15 [0.87, 1.51]	0.33	0.003	75%	
Non-HWE	2688/4052	2573/4289	0.50 [0.06, 4.08]]	0.52	0.03	78%	
CMR							Random
HWE	225/2844	142/3308	1.55 [0.97, 2.47]	0.07	0.006	72%	
Non-HWE	1683/4052	1624/4289	1.07 [0.97, 1.17]	0.17	0.77	0%	
PMR							Random
HWE	127/2844	879/3308	0.91 [0.66, 1.27]	0.6	<0.01	83%	
Non-HWE	1005/4052	949/4289	0.51 [0.07, 3.61]	0.5	0.05	75%	

TMR, the rate of total mutation; CMR, the rate of complete mutation; PMR, the rate of partial mutation; OR, odds ratio; 95%CI, 95% confidence interval

no association between XRCC5, 6, 7 genes and breast cancer risk in HWE and non-HWE groups (all P>0.05) (Table 6).

Sensitivity analysis and publication bias

Sensitivity analysis was performed by sequential omission of individual studies under various contrasts. However, the significance of pooled OR in all individual analysis and subgroup analysis was not influenced excessively. Publication bias of the literatures was accessed based on rs2267437 (C>G) polymorphisms in XRCC6 gene by Begger's funnel plot and Egger's linear regression test. All graphical funnel plots of included studies appeared to be symmetrical (Figure 2). Egger's test also showed that there was no statistical significance for all evaluations of publication bias.

Discussion

Breast cancer is a kind of malignant tumor that seriously threaten human heath, which is caused by a complex combination of genetic and environmental factors (Parkin et al., 2001). Two major susceptibility genes caused breast cancer were identified to be BRCA1 and BRCA2 (Cao et al., 2009). However only fewer than 2% of all breast cancer cases can attribute to germline mutations in BRCA1 and BRCA2. This means that there could be other breast cancer susceptibility genes that contribute to developing breast cancer (Peto et al., 1999). What's more, the majority of genetic variants that impact susceptibility to sporadic breast cancer are needed to identified (Monsees et al., 2011). DNA DSB is one of the most critical forms of DNA damage and plays a fundamental role in the maintenance of genomic integrity, which is frequently triggered by spontaneous DNA damage or exogenous DNA damage carcinogens such as ionizing radiation or a complex combination of exogenous and exogenous (Pastwa et al., 2003; Grabarz et al., 2012). Failure of

DNA repair mechanisms can lead to sustained damage, potentially resulting in the malfunction of cellular systems and checkpoints, and the ability of a cell to over proliferate or evade apoptosis (Helzlsouer et al., 1995; Parshad et al., 1996). Two pathways can repair DNA DSB, the HR and the NHEJ pathways (Frank-Vaillant et al., 2001). The DNA-dependent protein kinase (DNA-PK), composed of an approximately 470-kDa catalytic subunit (DNA-PKcs) and a DNA end binding component, Ku (Ku70/Ku80), is a key player in the NHEJ pathway of DSB repair and has additional functions in the mammalian cell including telomere maintenance and induction of apoptosis (Burma et al., 2004; Burma et al., 2006). These genetic variations in DNA repair genes have been reported to be associated with breast cancer risk and prognosis (Fu et al., 2003; Bau et al., 2004; Smith et al., 2008). In addiction, He et al provided the evidence that Ku70 polymorphism (rs2267437) was a genetic susceptibility factor for the development of breast cancer in a Chinese Han population. However, the the precise roles of mutations of XRCC5, 6, 7 genes in breast cancer risk are still controversial.

In this meta-analysis, including a total of 2864 breast cancer cases and 3060 healthy controls from seven publications, we mainly examined the association of twelve SNPs in XRCC5, XRCC6, and XRCC7 three genes with breast cancer risk, including rs2440 (G>A), rs3834 (G>A), rs3835 (G>A), rs828907 (G>T), rs9288518 (A>G), rs11685387 (C>T), rs132788 (G>T), rs132793 (A>G), rs2267437 (C>G), rs6002421 (A>G), rs2213178 (C>T) and rs2231178 (C>T). We demonstrated that rs3835 (G>A), rs828907 (G>T) in XRCC5 gene, rs6002421 (A>G), rs132788 (G>T) and rs132793 (G>A) in XRCC6 gene had significant association with breast cancer risk. The rs3835 (G>A), rs828907 (G>T) and rs132793 (G>A) were positively associated with the risk of breast cancer, increasing the risk for breast cancer. However The rs132788 (G>T) and rs6002421 (A>G) were showed negative association with breast cancer, decreasing the

risk of breast cancer. In addition, rs2440 (G>A), rs3834 (G>A), rs9288518 (A>G), rs11685387 (C>T), rs2267437 (C>G), and rs2213178 (C>T), rs2231178 (C>T) did not have an influence on breast. Moreover we also did not found no association between XRCC5, 6, 7 gene and breast cancer risk neither in Caucasians nor in Asians in subgroup analysis by ethnicity. Similarly, in the subgroup analysis by HWE, mutation genotypes of XRCC5, 6, 7 gene in the HWE and non-HWE groups were also showed not any association with breast cancer risk.

However, several limitation of this present study should be noted. For one thing, small sample size is an important limitation, as effects observed in small sample are less likely to be replicated than effects initially observed in large samples. For another thing, as a meta-analysis study, our inclusive patients with breast cancer were enrolled from the hospitals and the controls were selected from the community population or hospital-based populations, inherent selection bias cannot be completely excluded. In addiction, although the funnel plot and Egger's test did not show any publication bias, selection bias could have occurred because only studies published in English or Chinese were included. Moreover, our meta-analysis was based on unadjusted ORs estimates because not all published presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders, such as age, geographic distribution, pathological types, etc. Therefore, large studies are needed to further elucidate the impact of XRCC5, XRCC6 and XRCC7 genetic SNPs on breast cancer susceptibility.

In conclusion, this meta-analysis of seven case-control studies demonstrates that the rs3835 (G>A), rs828907 (G>T) in XRCC5 gene, the rs6002421 (A>G), rs132788 (G>T) and rs132793 (A>G) in XRCC6 gene were significantly associated with breast cancer risk. Mutation genotypes of SNPs might increase or decrease the risk of the molecular mechanism of breast carcinogenesis.

Acknowledgements

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