RESEARCH ARTICLE

Lack of Association Between LIG4 Gene Polymorphisms and the Risk of Breast Cancer: A HuGE Review and Meta-analysis

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Abstract

Objective: Non-homologous end joining (NHEJ) is one of the pathways of repair of DNA double-strand breaks. A number of genes involved in NHEJ have been implicated as breast cancer susceptibility genes such as *LIG4*. However, some studies have generated conflicting results. The aim of this Human Genome Epidemiology (HuGE) review and meta-analysis was to investigate association between LIG4 gene polymorphisms in the NHEJ pathway and breast cancer risk. Methods: Studies focusing on the relationship between LIG4 gene polymorphisms 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 and the meta-analysis was performed with Review Manager Version 5.1.6 and STATA Version 12.0 software, calculating odds ratios (ORs) with 95% confidence intervals (95%CIs). Results: According to the inclusion criteria, we final included seven studies with a total of 10,321 breast cancer cases and 10,160 healthy controls in the meta-analysis. The results showed no association between LIG4 gene polymorphisms (rs1805386 T>C, rs1805389 C>T, rs1805388 C>T and rs2232641 A>G) and breast cancer risk, suggesting that the mutant situation of these SNPs neither increased nor decreased the risk for breast cancer. In the subgroup analysis by Hardy-Weinberg equilibrium (HWE) and ethnicity, we also found no associations between the variants of LIG4 gene and breast cancer risk among HWE, non-HWE, Caucasians, Asians and Africans. Conclusion: This meta-analysis suggests that there is a lack of any association between LIG4 gene polymorphisms and the risk of breast cancer.

Keywords: LIG4 - polymorphism - mutation rate - breast cancer - meta-analysis

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Introduction

Breast cancer is the most common malignancy and the second leading cause of cancer death among women, the incidence accounting for various 7~10% among all the malignant tumors (Li et al., 2011; Lacey et al., 2002). Like other forms of cancer, breast cancer is considered to result from multiple environmental and hereditary risk factors (Wernberg et al., 2009). However, the majority of genetic variants that influence susceptibility to sporadic breast cancer are unknown (Balmain et al., 2003). Common variants may explain a greater proportion of breast cancer morbidity and mortality than rare highly penetrant mutations, such as those in BRCA1 and BRCA2 which account for only 15~20% of familial breast cancer cases (Ponder, 2001).

DNA damage repair is a crucial mechanism to keep mammalian cells genetic material stability (Wang et al., 2012). Unrepaired damage can result in apoptosis or may lead to unregulated cell growth and cancer (Matullo et al., 2006). At least four pathways of DNA repair operate on specific types of damaged DNA, probably the most important is DNA double-strand breaks (DSB) repair (Ferguson and Alt, 2001). DNA DSB are extremely harmful lesions that can lead to genomic instability and cell death (Mao et al., 2008; Shrivastav et al., 2008). There are two principle pathways for DNA DSB repair, namely homologous recombination (HR) and nonhomologous end joining (NHEJ) (Rothkamm et al., 2003). NHEJ has been considered the major pathway of DNA DSB repair in mammalian cells. In recent years, relevant studies have found that DNA DSB repair dysfunction increases the risk of familial and sporadic breast cancer (Hsu et al., 2007). Malfunction of DSB repair mechanisms can result in the fusion of DNA ends that were originally distant from one another in the genome, which generates chromosomal rearrangements such as inversions, translocations and deletions (Monsees et al., 2011). Accumulating evidence indicates that breast cancer pathogenesis is driven by DSB-initiated chromosome instability (Venkitaraman et al., 2002; Yoshida et al., 2004). These evidence makes DSB related genes good candidates for study in relation to breast cancer susceptibility.

LIG4 is a human gene that encodes the protein DNA Ligase IV (Garcia et al., 2011). The protein encoded by this gene is an ATP-dependent DNA ligase that joins DSB

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during the NHEJ repair pathway (Kapusta et al., 2011). LIG4 forms a complex with XRCC4, and further interacts with the DNA-dependent protein kinase (DNA-PK) and XLF, which are also required for NHEJ (Symington and Gautier, 2011). Two case-control studies conducted among Caucasians have showed that genetic variants in LIG4 gene may be associated with breast cancer risk (Kuschel et al, 2002; Rafii et al., 2002). In addiction, Fu et al found that the combined genotypes of DSBR genes (XRCC4, XRCC5, XRCC6, XRCC7 and LIG4) were associated with an elevated risk of breast cancer in Taiwanese women (Fu et al., 2003). Another study suggested that there is an interaction between polymorphisms of DNA repair genes and family history of breast cancer in the etiology of breast cancer (Han et al., 2004). However, the specific associations between LIG4 and breast cancer risk are still controversial. Given controversial results in those previous studies, we conducted a meta-analysis to investigate the association between LIG4 gene 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 15, 2012. The search terms were used, including ("DNA ligase" OR "Lig4 protein" OR "DNA ligase" OR "LIG4" OR "Ligase IV" OR "Ligase 4") 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 LIG4 gene 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 LIG4 gene 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, cancer 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 LIG4 gene 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) ($P \leq 0.05$ was considered to be manifestation of statistically significant heterogeneity). We also quantified the effect of heterogeneity by using I^2 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 ($P \leq 0.05$) 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. 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 12 relevant publications after

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

First author	Year	Country	Ethnicity	Number Case	Number Control	Source of control	Sample	Genotype method	SNP	Quality scores
Kuschel et al	2002	UK	Caucasian	2205	1826	Population-based	Blood	TaqMan	rs1805386 (T>C)	22
Fu et al	2003	China	Asian	254	379	Hospital -based	Blood	MassArray	rs2232641 (A>G) rs1805389 (C>T) rs1805388 (C>T)	24
Han et al	2004	USA	Caucasian	1004	1385	Population-based	Blood	DNA sequencing	rs1805388 (C>T) rs1805386 (T>C)	23
BCAC	2006	UK	Caucasian	2743	2764	NR	Blood	NR	rs1805386 (T>C)	26
Garcia-Closas et al	2006	USA	Caucasian	3368	2880	Population-based	Blood	TaqMan	rs1805388 (C>T) rs1805386 (T>C)	28
Acevedo et al	2009	Colombia	Caucasian	428	636	Population-based	Blood	MassArray	rs1805388 (C>T)	30
Jakubowska et al	2010	Poland	Caucasian	319	290	Population-based	Blood	PCR-RFLP	rs1805386 (T>C)	33

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; BCAC, Breast Cancer Association Consortium; NR, not reported

Table 2. The Genotype Distribution of LIG4 Gene Polymorphisms in Case and Control Groups

First author	SNP	Case							Control							HWE test	
		Total	TM	CM	PM	TMR	CMR	PMR	Total	TM	CM	PM	TMR	CMR	PMR	χ^2	P
Kuschel et al	rs1805386 (T>C)	4419	1325	121	1204	0.30	0.03	0.27	5211	1645	184	1461	0.32	0.04	0.28	5.07	0.02
Fu et al	rs2232641 (A>G)	254	8	0	8	0.03	0.00	0.03	369	9	1	8	0.02	0.00	0.02	13.28	0.00
	rs1805389 (C>T)	254	62	6	56	0.24	0.02	0.22	379	95	8	87	0.25	0.02	0.23	0.19	0.66
	rs1805388 (C>T)	253	116	16	100	0.46	0.06	0.40	376	178	28	150	0.47	0.07	0.40	0.00	0.96
Han et al	rs1805388 (C>T)	978	290	18	272	0.30	0.02	0.28	1276	348	20	328	0.27	0.02	0.26	2.20	0.14
	rs1805386 (T>C)	977	296	22	274	0.30	0.02	0.28	1266	392	32	360	0.31	0.03	0.28	0.50	0.48
BCAC	rs1805386 (T>C)	611	197	19	178	0.32	0.03	0.29	639	207	19	188	0.32	0.03	0.29	1.81	0.18
	rs1805386 (T>C)	266	88	9	79	0.33	0.03	0.30	199	77	7	70	0.39	0.04	0.35	0.63	0.43
	rs1805386 (T>C)	651	211	21	190	0.32	0.03	0.29	954	311	35	276	0.33	0.04	0.29	0.63	0.43
	rs1805386 (T>C)	945	290	36	254	0.31	0.04	0.27	962	275	23	252	0.29	0.02	0.26	0.00	0.98
Garcia-Closas et al	rs1805388 (C>T)	1316	396	57	339	0.30	0.04	0.26	1043	319	42	277	0.31	0.04	0.27	5.42	0.02
	rs1805386 (T>C)	1338	434	55	379	0.32	0.04	0.28	1057	343	34	309	0.32	0.03	0.29	0.01	0.94
Acevedo et al	rs1805388 (C>T)	426	124	11	113	0.29	0.03	0.27	630	180	12	168	0.29	0.02	0.27	0.66	0.42
Jakubowska et al	rs1805386 (T>C)	319	112	112	0	0.35	0.35	0.00	290	94	94	0	0.32	0.32	0.00	290.00	0.00

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; BCAC, Breast Cancer Association Consortium

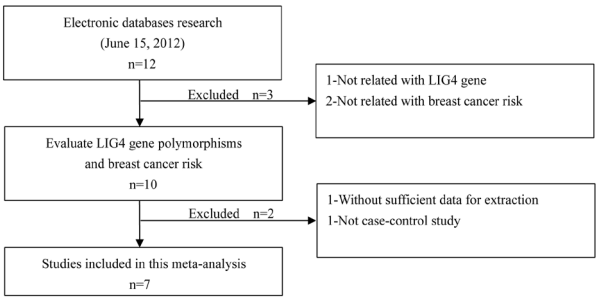

Figure 1. Flow Chart Shows Study Selection Procedure

initial screening. According to the inclusion criteria, seven case-control studies (Kuschel et al., 2002; Fu et al., 2003; Han et al., 2004; Lee et., al 2005; BCAC, 2006; Garcia-Closas et al., 2006; Acevedo et al., 2009; Jakubowska et al., 2010) appeared to have met the inclusion criteria. The flow chart of study selection is shown in Figure 1. A total of 10321 breast cancer cases and 10160 healthy controls from seven studies were included in the pooled analysis. The publication year of involved studies ranged from 2002 to 2010. Overall, there were six studies were conducted in Caucasians, and only one study in Asians. Four single nucleotide polymorphisms (SNPs) were analyzed, including rs1805386 (T>C), rs1805388 (C>T), rs1805389 (C>T) and rs2232641 (A>G). The characteristics and methodological quality of the included studies are summarized in Table 1. The mutation genotypes of LIG4 gene polymorphisms were presented in Table 2.

Association between LIG4 gene polymorphisms and breast cancer risk

A summary of the meta-analysis findings of the association between LIG4 gene polymorphisms and breast cancer risk is provided in Table 3. No heterogeneity was found (all P>0.05), so the fixed effects model was used. Four studies refer to rs1805386 (T>C) polymorphism of LIG4 gene and breast cancer risk, all subjects in these studies were Caucasians. There was no evidence that the rs1805386 (T>C) polymorphism associated with the risk of breast cancer (TMR: OR=0.97, 95%CI: 0.91-1.03, P=0.31; CMR: OR=1.03, 95%CI: 0.81-1.31, P=0.81; PMR: OR=0.97, 95%CI: 0.91-1.03, P=0.37). Similarly, we also found no association among rs1805388 (C>T), rs1805389 (C>T) and rs2232641 (A>G) with the risk of breast cancer (all P>0.05).

In the subgroup analysis by ethnicity, we combined four mutation genotypes in LIG4 gene to investigate associations between the overall mutation rate of LIG4

Table 3. Meta-analysis of the Association between LIG4 Gene Polymorphisms and Breast Cancer Susceptibility

Polymorphisms		Cancer n/N	Control n/N	OR [95%CI]	P	Heterogeneity P	Heterogeneity I^2	Effect model
rs1805386 (T/C)	TMR	2953/9526	3344/10588	0.97 [0.91, 1.03]	0.31	0.52	0%	Fixed
	CMR	395/9526	428/10588	1.03 [0.81, 1.31]	0.81	0.07	57%	
	PMR	2558/9526	2916/10588	0.97 [0.91, 1.03]	0.37	0.90	0%	
rs1805388 (C/T)	TMR	926/2973	1025/3325	1.03 [0.92, 1.15]	0.60	0.68	0%	Fixed
	CMR	102/2973	102/3325	1.07 [0.81, 1.42]	0.63	0.81	0%	
	PMR	824/2973	923/3325	1.02 [0.91, 1.14]	0.73	0.72	0%	
rs1805389 (C/T)	TMR	62/254	95/379	0.97 [0.67, 1.40]	0.85	-	-	Fixed
	CMR	6/254	8/379	1.12 [0.38, 3.27]	0.83	-	-	
	PMR	56/254	87/379	0.95 [0.65, 1.39]	0.79	-	-	
rs2232641 (A/G)	TMR	8/254	9/369	1.30 [0.50, 3.42]	0.59	-	-	Fixed
	CMR	0/254	1/369	0.48 [0.02, 11.90]	0.66	-	-	
	PMR	8/254	8/369	1.47 [0.54, 3.96]	0.45	-	-	

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. Subgroup Analysis by Ethnicity and HWE Test

Subgroups		Case n/N	Control n/N	OR [95%CI]	P	Heterogeneity P	Heterogeneity I^2	Effect model
Caucasians	TMR	3763/12246	4191/13537	0.98 [0.93, 1.04]	0.55	0.63	0%	Fixed
	CMR	481/12246	502/13537	1.00 [0.87, 1.14]	0.95	0.17	35%	
	PMR	3282/12246	3689/13537	0.98 [0.93, 1.04]	0.55	0.87	0%	
Asians	TMR	186/761	282/1124	0.97 [0.78, 1.20]	0.75	-	-	Fixed
	CMR	22/761	37/1124	0.87 [0.51, 1.49]	0.62	-	-	
	PMR	164/761	245/1124	0.99 [0.79, 1.23]	0.90	-	-	
HWE	TMR	2108/6699	2406\7748	1.02 [0.95, 1.09]	0.67	0.98	0%	Fixed
	CMR	213/6699	218/7748	1.11 [0.92, 1.35]	0.28	0.83	0%	
	PMR	1895/6699	2188/7748	1.00 [0.93, 1.08]	0.98	0.95	0%	
Non-HWE	TMR	1841/6308	2067/6913	0.95 [0.88, 1.02]	0.17	0.62	0%	Fixed
	CMR	290/6308	321/6913	0.90 [0.76, 1.07]	0.24	0.22	32%	
	PMR	1551/6308	1746/6913	0.96 [0.89, 1.04]	0.37	0.71	0%	

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

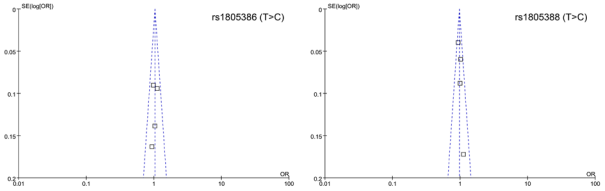

Figure 2. Begger's Funnel Plot of Publication Bias Based on the rs1805386 (T>C) and rs1805388 (C>T) Polymorphisms in LIG4 Gene

gene and breast cancer susceptibility in Caucasians and Asians. However, no association was found between LIG4 gene 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 no association between LIG4 gene and breast cancer risk in HWE and non-HWE groups (all P>0.05) (Table 4).

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 rs1805386 (T>C) and rs1805388 (C>T) polymorphisms in LIG4 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 (P=0.14 for rs1805386; and P=0.64 for rs1805388).

Discussion

It is well known that breast cancer is one of the most common types of cancer, which is caused by a complex combination of genetic and environmental factors (Parkin et al., 2001). BRCA1 and BRCA2 are two major identified susceptibility genes (Cao et al., 2009). Fewer than 2% of all breast cancer cases are due to germline mutations in BRCA1 and BRCA2, and of which less than 20% account for the excess familial risk of breast cancer, implying that there remains other breast cancer susceptibility genes needed to be identified (Peto et al., 1999). DNA DSB are thought to be the most detrimental form of DNA damage, and are frequently triggered by spontaneous DNA damage or exogenous DNA damage carcinogens such as ionizing radiation. They could lead to apoptosis or tumorigenesis by breaking and rearranging chromosome (Pastwa et al., 2003; Grabarz et al., 2012). Two pathways can repair DNA DSB, the HR and the NHEJ pathways (Frank-Vaillant et al., 2001). Therefore, variants in genes involved in DNA DSB repair are considered tobe good candidates for breast cancer susceptibility. LIG4 encoding the protein DNA Ligase IV, is a human ATP-dependent DNA ligase

gene that plays a significant role in joining double-strand breaks during the NHEJ pathway of double-strand break repair (Liang et al., 2008). Han et al have found that Ligase IV C299T (5'UTR) rs1805386 had no overall association with breast cancer risk among 1004 breast cancer cases and 1385 controls. Fu et al had also shown no significant association between LIG4 gene and breast cancer risk (Dapic et al., 2005). However, Kuschel et al indicated that Ligase IV can decrease the risk for breast cancer. This controversy might be explained by several reasons, including differences between pathological types, ethnicity, study designs and sample size, statistical methods, and assay characteristics need to be investigated further.

In this meta-analysis, including a total of 10321 breast cancer cases and 10160 healthy controls from seven publications, we mainly examined the association of four well-characterized polymorphisms with breast cancer risk, including rs1805386 (T>C), rs1805388 (C>T), rs1805389 (C>T) and rs2232641 (A>G) in LIG4 gene. We demonstrated that there was no significant association between rs1805386 (T>C) polymorphism and breast cancer risk. In addition, rs1805388 (C>T), rs1805389 (C>T) and rs2232641 (A>G) in LIG4 gene also did not appear to have an influence on cancer risk. Ethnicity may influence cancer risk by different genetic backgrounds and environmental exposures through gene-gene and gene-environment interactions. From subgroup analysis by ethnicity, we also found no association between LIG4 gene and breast cancer risk neither in Caucasians nor in Asians. Similarly, in the subgroup analysis by HWE, mutation genotypes of LIG4 gene in the HWE and non-HWE groups were also showed any association with breast cancer risk. Perhaps, the LIG4 gene might not be involved in the molecular mechanism of breast carcinogenesis.

In interpreting our results of the current meta-analysis, some limitations need to be addressed. Firstly, 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. Secondly, the numbers of published studies were still not sufficiently large for the analysis of some mutation genotypes of LIG4 gene. Thirdly, 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. In addiction, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that may have influenced our results.

In conclusion, this meta-analysis of seven case-control studies demonstrates that there was lack of association between LIG4 gene polymorphisms and the risk of breast cancer. Mutation genotypes of LIG4 gene might not be involved in the molecular mechanism of breast carcinogenesis.

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