

Association between the RAD51 135 G>C Polymorphism and Risk of Cancer: A Meta-Analysis of 19,068 Cases and 22,630 Controls

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Abstract

Background: RAD51 135G>C can modify promoter activity and the penetrance of BRCA1/2 mutations, which plays vital roles in the etiology of various cancer. To date, previous published data on the association between RAD51 135G>C polymorphism and cancer risk remained controversial. Recent meta-analysis only analyzed RAD51 135G>C polymorphism with breast cancer risk, but the results were also inconsistent.

Methods: A meta-analysis based on 39 case-control studies was performed to investigate the association between cancer susceptibility and RAD51 135G>C. Odds ratios (OR) with 95% confidence intervals (CIs) were used to assess the association in different inheritance models. Heterogeneity among studies was tested and sensitivity analysis was applied.

Results: Overall, no significant association was found between RAD51 135G>C polymorphism and cancer susceptibility in any genetic model. In further stratified analysis, significantly elevated breast cancer risk was observed in BRCA2 mutation carriers (recessive model: OR = 4.88, 95% CI = 1.10–21.67; additive model: OR = 4.92, 95% CI = 1.11–21.83).

Conclusions: This meta-analysis suggests that RAD51 variant 135C homozygote is associated with elevated breast cancer risk among BRCA2 mutation carriers. Moreover, our work also points out the importance of new studies for RAD51 135G>C association in acute myeloid leukemia, especially in Caucasians, where at least some of the covariates responsible for heterogeneity could be controlled, to obtain a more conclusive understanding about the function of the RAD51 135G>C polymorphism in cancer development.

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Introduction

Recently, there is growing evidence that radicals such as reactive oxidative stress produced during metabolic process play an important role in the DNA damage which could also be caused by UV, ionizing radiation, as well as environmental chemical agents and then initiate human cancer [1]. Moreover, mutagens in living environment can produce DNA adducts, DNA damage, and DNA strand breaks [2]. If these mutagens to DNA structures are left un-repaired, genetic changes can accumulate, which may result in cell-cycle dysregulation, autonomous growth and development of invasive mechanisms, leading to carcinoma [3]. In order to maintain the integrity of the genome, mammalian cells have developed several DNA-repair mechanisms that each deal with a specific type of DNA damage. DNA-repair genes are, like

detoxification enzymes, responsible for preventing cancer by protecting the integrity of the genome and are therefore considered as cancer susceptibility genes [4,5]. The association between defective DNA-repair caused by highly penetrant mutations in DNA repair genes on the one hand, and chromosomal instability and cancer predisposition on the other, is well documented for rare familial cancer syndromes like pigmentosum (XP) and ataxia telangiectasia (A-T) [5]. In contrast to the occurrence of these rare and highly penetrant mutations, the human genome contains a large number of low-penetrant single-nucleotide polymorphisms (SNPs), which make up 90% of the naturally occurring sequence variations [6,7]. An attack from reactive oxygen species (ROS) can result in cleavage of both DNA strands, causing DNA double-strand breaks (DSBs). Double-strand breaks (DSB) damage, causing cell death or loss of genetic

material, is the most injurious lesion and responsible for cancer development.

The RAD51 gene, a homologue of *recA* in *Escherichia coli*, has been mapped to chromosome 15q15.1 in humans [8]. It spans >39 kb, contains 10 exons and encodes a 339 amino acid protein (genomic accession no: NM_133487). The RAD51 gene makes a protein also called RAD51, which is essential for the repair of damaged DNA. The protein made by the BRCA2 gene binds to and regulates the RAD51 protein to fix breaks in DNA [9]. These breaks can be caused by natural or medical radiation. They also occur when chromosomes exchange genetic material (when pieces of chromosomes trade places) in preparation for cell division. The BRCA2 protein transports the RAD51 protein to sites of DNA damage in the cell nucleus. RAD51 then binds to the damaged DNA and encases it in a protein sheath, which is an essential first step in the repair process. In addition to its association with BRCA2, the RAD51 protein also interacts with the protein made by the BRCA1 gene. By repairing DNA, these three proteins play a role in maintaining the stability of the human genome. Changes in RAD51 biosynthesis are usually preceded by changes in its gene transcription and mRNA level. Gene variability could contribute to the level of the RAD51 biosynthesis. A single nucleotide polymorphism in the 5'-untranslated region (5'-UTR) of RAD51 (a G to C substitution at position 135, the G/C polymorphism) can influence cancer risk among BRCA1/BRCA2 mutation carriers [10,11]. In view of the potential significant role of RAD51 for tumor development, it is important to know, whether this polymorphism can account for the development and/or progression of cancer.

To date, a number of molecular epidemiological studies have been done to evaluate the association between RAD51 135G>C polymorphism and different types of cancer risk in diverse populations [12–64]. However, the results were inconsistent or even contradictory. Some recent meta-analysis only analyzed RAD51 135G>C polymorphism with breast cancer risk [65–69], but the results were also inconsistent. Gao et al. [65] found that the CC genotype was associated with a significantly increased risk of breast cancer when compared with the GG, CG, and CG/GG genotypes. Subgroup analyses showed that individuals carrying the CC genotype were associated with an elevated tumor risk in European populations and in sporadic breast cancer. Wang et al. [66] observed an overall significant increased breast cancer risk (for the recessive model CC vs. GG/CG: OR = 1.35, 95% CI = 1.05–1.74, *P* (heterogeneity) = 0.06). Yu et al. [67] found that there was no evidence for a significant association between 135G>C and breast cancer risk in non-BRCA1/2 mutation. The study of Sun et al. [68] had 17 studies, with significantly decreased breast cancer risk being observed in the additive model (OR = 0.995, 95% CI = 0.991–0.998) and recessive model (OR = 0.994, 95% CI = 0.991–0.998). Zhou et al. [69] suggested that RAD51 variant 135C homozygote was associated with elevated breast cancer risk among BRCA2 mutation carriers. Since then, additional several studies with a large sample size about RAD51 135G>C polymorphism with cancer risk have not been reported. Therefore, we performed a comprehensive meta-analysis by including the most recent and relevant articles to identify statistical evidence of the association between RAD51 135G>C polymorphism and risk of all cancers that have been investigated.

Materials and Methods

Identification and eligibility of relevant studies

A comprehensive literature search was performed using the PubMed database for relevant articles published (the last search update was July 5, 2012) with the following key words “RAD51,” “polymorphism,” and “Cancer” or “Carcinoma.” The search was limited to human studies. In addition, studies were identified by a manual search of the reference lists of reviews and retrieved studies. We included all the case–control studies and cohort studies that investigated the association between RAD51 135G>C polymorphism and cancer risk with genotyping data. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. When the same sample was used in several publications, only the most complete study was included following careful examination.

Inclusion criteria

All human-associated studies were included if they met the following criteria: (1) only the case–control studies or cohort studies were considered; (2) evaluated the RAD51 135G>C polymorphism and the risk of cancer; (3) the genotype distribution of the polymorphism in cases and controls were described in details and the results were expressed as odds ratio (OR) and corresponding 95% confidence interval (95% CI). Major reasons for exclusion of studies were as follows: (1) not for cancer research; (2) only case population; (3) duplicate of previous publication; and (4) the distribution of genotypes among controls are not in Hardy–Weinberg equilibrium (*P* < 0.01).

Data extraction

Information was carefully extracted from all eligible studies independently by two investigators according to the inclusion criteria listed above. The following data were collected from each study: first author's name, year of publication, country of origin, ethnicity, source of controls (population-based controls and hospital-based controls), genotyping method, sample size, and numbers of cases and controls in the RAD51 135G>C genotypes whenever possible. Ethnicity was categorized as “Caucasian”, “Asian”, and “African”. When one study did not state which ethnic groups was included or if it was impossible to separate participants according to phenotype, the sample was termed as “mixed population.” Meanwhile, studies investigating more than one kind of cancer were counted as individual data set only in subgroup analyses by cancer type. We did not define any minimum number of patients to include in this meta-analysis. Articles that reported different ethnic groups and different countries or locations, we considered them different study samples for each category cited above.

Statistical analysis

Crude odds ratios (ORs) together with their corresponding 95% CIs were used to assess the strength of association between the RAD51 135 G>C polymorphism and the risk of cancer. Following published recommendations for quality assessment in meta-analyses of genetic associations, we examined: choice of genetic models (we adopted three genetic models, avoiding assuming only one “wrong” genetic model). The pooled ORs were performed for dominant model (GC+CC *versus* GG), recessive model (GG+GC *versus* CC), additive model (GG *versus* CC), respectively. Between-study heterogeneity was assessed by calculating *Q*-statistic (Heterogeneity was considered statistically significant if *P* < 0.10) [70] and quantified using the *I*² value, a value that describes the percentage of variation across studies that

are due to heterogeneity rather than chance, where $I^2 = 0\%$ indicates no observed heterogeneity, with 25% regarded as low, 50% as moderate, and 75% as high [71]. If results were not heterogeneous, the pooled ORs were calculated by the fixed-effect model (we used the Q -statistic, which represents the magnitude of heterogeneity between-studies) [72]. Otherwise, a random-effect model was used (when the heterogeneity between-studies were significant) [73]. In addition to the comparison among all subjects, we also performed stratification analyses by cancer type (if one cancer type contained less than three individual studies, it was combined into the “other cancers” group), ethnicity, BRCA1/2 mutation status, and source of controls. Lung, bladder, esophageal, head and neck, and pancreatic cancers were defined as smoking-related cancers because tobacco smoking is an established risk factor for these cancers [74,75–77]. In addition, given the roles of estrogens in the etiology of breast, cervical and ovarian cancers, these cancers were defined as estrogen-related [78,79]. We examined whether the RAD51 135G>C polymorphism was associated with the risk of these cancers as a group as well. Moreover, sensitivity analysis was performed, including studies whose allele frequencies in controls exhibited significant deviation from the Hardy–Weinberg equilibrium (HWE), given that the deviation may denote bias. In addition, we also performed by excluding a single study each time. Last, we also ranked studies according to sample size, and then repeated this meta-analysis. HWE was calculated by using the goodness-of-fit test, and deviation was considered when $P < 0.01$. Begg’s funnel plots [80] and Egger’s linear regression test [81] were used to assess publication bias. All of the calculations were performed using STATA version 10.0 (STATA Corporation, College Station, TX).

Results

Eligible studies and meta-analysis databases

Figure 1 graphically illustrates the trial flow chart. A total of 128 articles regarding RAD51 135 G>C polymorphism with respect to cancer were identified. After screening the titles and abstracts, 75 articles were excluded because they were review articles, case reports, other polymorphisms of RAD51, or irrelevant to the current study. In addition, genotype distributions in the controls of all the eligible studies were in agreement with HWE except for four studies [45,50,58,64]. Last, of these studies, 13 publications [12,13,15,22,27,28,30,33,34,41,53,55,59] were excluded because of their populations overlapped with another six included studies [20,29,39,42,50,62]. The study of Webb et al. [17] including different case–control groups were considered as four separate studies each. Hence, as summarized in Table 1, 36 publications including 39 studies were selected among the meta-analysis, including 19,068 cases and 22,630 controls. Among the 39 studies, five studies were included in the dominant model only because they provided the genotypes of GC+CC *versus* GG as a whole. Of these, there were 20 hospital-based studies and 10 population-based studies. There were 14 breast cancer studies, 7 acute myeloid leukemia studies, 6 ovarian cancer studies, and 12 studies with the “other cancers”. Twenty-four of 39 studies were conducted in Caucasians and six studies were conducted in Asians. The remained nine studies were populations with mixed ethnicity. In addition, there were 21 estrogen-related cancers studies and 3 smoking-related cancers studies. All of the cases were pathologically confirmed.

Quantitative synthesis

There was a wide variation in the C-allele frequency of the RAD51 135G>C polymorphism among the controls across

different ethnicities. For Asian populations, the C-allele frequency was 14.06% (95% CI = 11.46%–18.18%), which was significantly higher than that in Caucasians (8.34%, 95% CI = 7.33%–18.04%, $P < 0.001$). The evaluations of the association of RAD51 135G>C polymorphism with cancer risk are shown in Table 2. Overall, no significant association was found between RAD51 135G>C polymorphism and cancer susceptibility in any genetic model (dominant model: OR = 1.06, 95% CI = 0.96–1.08, P value of heterogeneity test [P_h] < 0.001, $I^2 = 61.4\%$; recessive model: OR = 1.35, 95% CI = 0.89–2.03, $P_h < 0.001$, $I^2 = 80.8\%$; additive model: OR = 1.46, 95% CI = 0.94–2.27, $P_h < 0.001$, $I^2 = 72.8\%$). However, there was significant heterogeneity between studies. Hence, we then performed subgroup analysis by cancer type, smoking-related cancer, and estrogen-related cancer, there was still no significant association detected in all genetic models. We further examined the association of the RAD51 135G>C polymorphism and cancer risk according to cancer type and ethnicity (Table 3) because there was significant heterogeneity between studies. There was still no significant association detected in any ethnicity. Next, the effect of RAD51 135G>C polymorphism was evaluated in subgroup analysis according to BRCA1/2 mutation status and breast cancer (Table 4. A significant association was found only among BRCA2 mutation carriers (recessive model: OR = 4.88, 95% CI = 1.10–21.67; additive model: OR = 4.92, 95% CI = 1.11–21.83).

Test of heterogeneity and sensitivity

There was significant heterogeneity among these studies for dominant model comparison (GC+CC *versus* GG: $P_{het} < 0.001$), recessive model comparison (GG+GC *versus* CC: $P_{het} < 0.001$), and additive model comparison (GG *versus* CC: $P_{het} < 0.001$). Then, we assessed the source of heterogeneity for dominant model comparison (GC+CC *versus* GG) by ethnicity, cancer type, and source of controls. We found that cancer type ($P = 0.717$), ethnicity ($P = 0.724$), and the source of controls ($P = 0.832$) did not contribute to substantial heterogeneity among the meta-analysis. Although the sample size for cases and controls in all eligible studies ranged from 38 to 8,512, the corresponding pooled ORs were not qualitatively altered with or without the study of small sample. Examining genotype frequencies in the controls, significant deviation from HWE was detected in the four studies [45,50,58,64]. After the inclusion of the four studies [45,50,58,64] significantly departing from HWE, the results of RAD51 135G>C remained practically unchanged in the overall analysis (data not shown).

Publication bias

Both Begg’s funnel plot and Egger’s test were performed to assess the publication bias of literatures. Fig.2 lists Begg’s funnel plot of allele comparison for publication bias of RAD51 135G>C (dominant model and additive model). The Egger’s test results ($P = 0.111$ for dominant model, $P = 0.120$ for recessive model, and $P = 0.525$ for additive model) and Begg’s funnel plot suggested no evidence of publication bias, indicating that our results were statistically robust.

Discussion

DNA repair systems have been considered to maintain genomic integrity by countering threats posed by DNA lesions. Deficiency in the DNA repair pathways might make these lesions unrepaired or repaired incorrectly, eventually leading to genome instability or mutations which may contribute directly to cancer. Thus, genetic differences, such as single nucleotide polymorphism (SNP) may

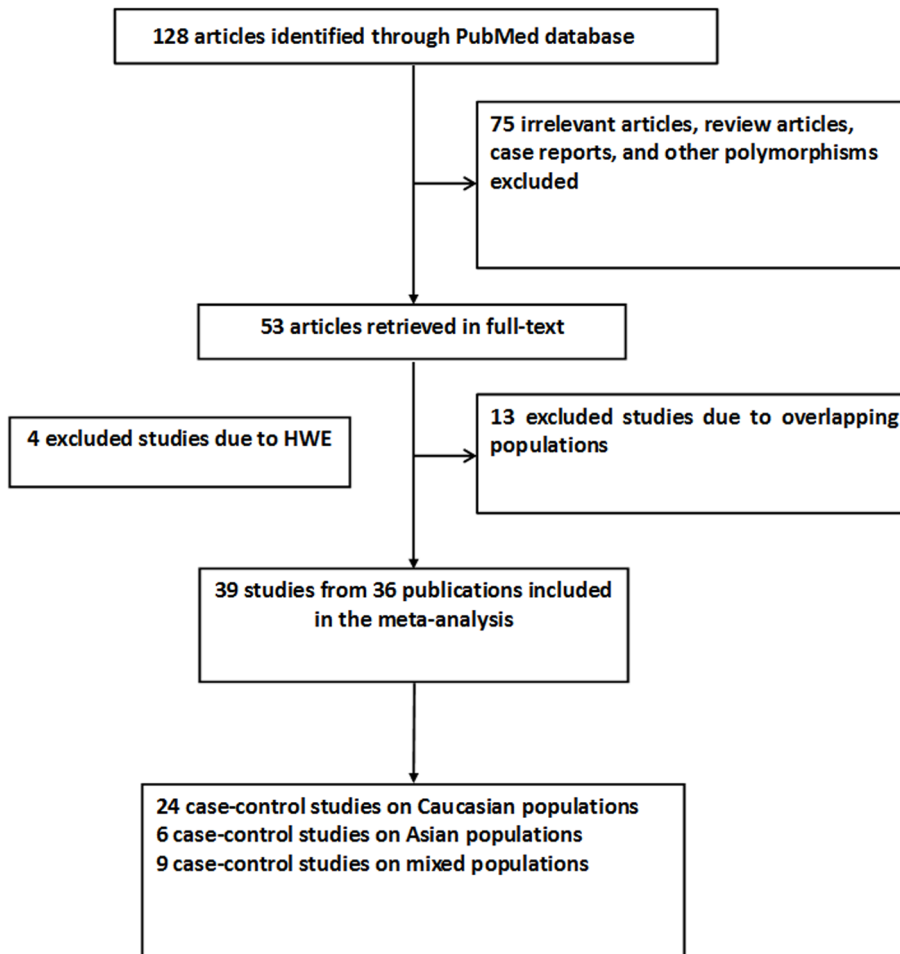


Figure 1. Study flow chart explaining the selection of the 39 eligible case-control studies included in the meta-analysis.
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contribute to carcinogenesis [82,83]. Previous studies have already found certain kinds of polymorphisms in DNA repair proteins are associated with cancer, such as XRCC3 (Thr241Met), OGG1 (Ser326Cys) and XPD (Lys751Gln) with breast cancer, XRCC2 (R188H G>A), and XRCC3 (T241M C>T) with ovarian cancer, XPC C/A (i11) with sporadic colorectal cancer and so on. Therefore, great interests have been aroused in the exploration of the association of SNP of DNA repair proteins and cancer risk to provide better prediction of cancer.

Homologous recombination repair (HRR), an important part of DNA repair system, is involved in the repair of double strand breaks (DSBs) [84]. Genetic polymorphisms in HRR genes, which can lead to protein haploinsufficiency have also been associated with cancer risk [85]. Double-strand break (DSB) damage, causing cell death or loss of genetic material, is the most injurious lesion and responsible for cancer development. However, it can be repaired by several DSB repair genes such as BRCA1/2 in which mutations have been proven to contribute to high risk of cancer in women [86]. RAD51 is located at chromosome position 15q15.1 [87], a region that exhibits loss of heterozygosity in a large range of cancers, including those of the lung, the colorectum, and the breast [88]. RAD51 plays a crucial role in the HRR pathway. The RAD51 135G>C polymorphism at position 135 in the 5' UTR region may be related with RAD51 protein over-expression and DNA repair increase [89–91]. RAD51, a homolog of *Escherichia*

coli RecA, is another important DSB repair gene and can interact with BRCA1 and BRCA2 proteins, functioning through homologous recombination and nonhomologous end joining [92,93]. A number of epidemiological studies have evaluated the association between RAD51 135G>C polymorphism and cancer risk, but the results remained inconclusive. In order to resolve this conflict, this meta-analysis of 39 eligible studies including 19,068 cases and 22,630 controls was performed to derive a more precise estimation of the association between RAD51 135G>C polymorphism and risk of different types of cancer.

Overall, no significant association was found between RAD51 135G>C polymorphism and cancer susceptibility in any genetic model. In the stratified analysis by cancer type, we did not also find significant association among AML, breast cancer, and ovarian cancer. Krupa et al. [55], Jakubowska et al. [46], Chang et al. [24], Romanowicz-Makowska et al. [22], Sliwinski et al. [20], Blasiak et al. [12], Webb et al. [17], Brooks et al. [49], Kuschel et al. [27], Lee et al. [19], and Hu et al. [54] reported that the RAD51 135G>C polymorphism was not associated with the risk of breast cancer. Webb et al. [17], Dhillon [60] 2011, and Auranen et al. [18] reported that the RAD51 135G>C polymorphism was not associated with the risk of ovarian cancer. Seedhouse et al. [14], Bhatla et al. [43], and Zhang et al. [56] reported that the RAD51 135G>C polymorphism was not associated with the risk of AML. The results of our meta-analysis

Table 1. Main characteristics of all studies included in the meta-analysis.

First author/year	Country	Ethnicity	Cancer Type	Case-control	SC	GM	Genotype distribution (case/control)			HWE
							GG	GC	CC	
Levy-Lahad [25] 2001	Israel	Caucasian	Ovarian	42–90	HB	PCR	38/85	4/5		NA
Wang [26] 2001	Multiple	Mixed	Ovarian	44–263	PB	PCR-RFLP	42/238	2/25		NA
Seedhouse [14] 2004	UK	Caucasian	AML	206–186	NR	PCR-RFLP	171/166	32/18	3/2	0.171
Wang [16] 2004	USA	Caucasian	Glioma	309–342	HB	PCR-RFLP	265/301	40/41	4/0	0.840
Webb [17] 2005	Australia	Mixed	Breast	149–128	PB	PCR-RFLP	121/101	24/27	4/0	0.757
Webb [17] 2005	Australia	Caucasian	Breast	1,295–650	PB	PCR-RFLP	1100/575	188/77	7/8	0.021
Webb [17] 2005	Australia	Mixed	Ovarian	95–173	PB	PCR-RFLP	74/141	20/32	1/0	0.765
Webb [17] 2005	Australia	Caucasian	Ovarian	448–953	PB	PCR-RFLP	383/830	65/113	3/10	0.028
Auranen [18] 2005	Multiple	Caucasian	Ovarian	1,629–2,805	PB	TaqMan	1419/2440	201/355	9/10	0.746
Lee [19] 2005	Korea	Asian	Breast	782–587	HB	PCR	611/450	143/123	28/14	0.287
Sliwinski [20] 2005	Poland	Caucasian	Breast	150–150	NR	PCR-RFLP	108/106	38/41	4/3	0.912
Dufloth [21] 2005	Brazil	SA	Breast	169–119	HB	PCR-RFLP	144/103	24/13	1/3	0.026
Tarasov [23] 2006	Russia	Caucasian	Breast	151–191	NR	PCR-RFLP	111/148	36/41	4/2	0.903
Chang [24] 2006	China	Asian	Breast	189–421	HB	PCR	116/284	73/137		NA
Poplawski [31] 2006	Poland	Caucasian	Gastric	18–20	HB	PCR-RFLP	8/14	10/5	0/1	0.935
Rollinson [32] 2007	UK	Caucasian	AML	466–936	NR	TaqMan	431/817	34/115	1/4	1.000
Costa [35] 2007	Portugal	Caucasian	Breast	365–435	HB	PCR-RFLP	216/381	45/53	4/1	0.845
Lu [36] 2007	USA	Caucasian	HNSCC	716–719	HB	PCR-RFLP	624/622	91/96	1/1	0.393
Jakubowska [37] 2007	Poland	Caucasian	Ovarian	127–127	PB	PCR-RFLP	104/89	23/38		NA
Figueroa [38] 2007	Spanish	Caucasian	Bladder	1,085–1,032	HB	TaqMan	932/909	147/116	6/7	0.322
Voso [40] 2007	Italy	Caucasian	AML	160–161	NR	PCR-RFLP	125/142	33/18	2/1	0.968
Antoniou [42] 2007	Multiple	Mixed	Breast	4,443–4,069	NR	RT-PCR	3838/3485	567/565	38/17	0.747
Pharoah [29] 2007	Multiple	Caucasian	Breast	2,160–2,266	PB	TaqMan	1911/1995	236/257	13/14	0.199
Bhatla [43] 2008	USA	Mixed	AML	452–646	PB	PCR	374/555	73/85	5/6	0.418
Brooks [49] 2008	USA	Mixed	Breast	611–611	N	PCR-RFLP	516/513	88/88	7/10	0.031
Werbrouck [51] 2008	Belgium	Caucasian	HNSCC	152–157	HB	PCR-RFLP	136/134	15/23	1/0	0.848
Hu [54] 2008	China	Asian	Breast	71–85	NR	PCR-RFLP	51/59	18/23	2/3	0.930
Jakubowska [46] 2009	Poland	Caucasian	Breast	1,007–1,069	PB	PCR-RFLP	785/822	207/232	15/15	0.959
Zhang [56] 2009	China	Asian	AML	166–458	NR	PCR-RFLP	117/315	47/123	2/20	0.214
Wiśniewska-Jarosińska [58] 2009	Poland	Caucasian	Colorectal	100–236	HB	PCR-RFLP	61/169	36/44	3/23	<0.001
Palanca [48] 2010	Spain	Caucasian	BC and OC	182–208	HB	PCR	155/175	27/33		NA
Sliwinski [64] 2010	Poland	Caucasian	HNSCC	288–353	HB	PCR-RFLP	138/258	145/64	5/32	<0.001
Jara [62] 2010	Chile	SA	Breast	267–500	HB	PCR	232/441	33/58	2/1	0.835
Krupa [63] 2011	Poland	Caucasian	Endometrial	30–30	HB	PCR-RFLP	6/19	8/9	16/2	0.808
Krupa [61] 2011	Poland	Caucasian	Colorectal	100–100	HB	PCR-RFLP	61/36	36/35	3/29	0.012
Dhillon [60] 2011	Australia	Caucasian	Prostate	116–132	HB	PCR-RFLP	97/119	18/13	1/0	0.929
Liu [57] 2011	China	Asian	AML	105–704	HB	PCR-RFLP	72/511	25/175	8/18	0.809
Romanowicz-Makowska [50] 2011	Poland	Caucasian	Breast	700–708	NR	PCR-RFLP	130/178	74/396	496/134	0.005
Hamdy [47] 2011	Egypt	African	AML	50–30	HB	PCR-RFLP	39/26	9/3	2/1	0.184
Pasaje [44] 2011	Korea	Asian	Liver	285–727	HB	PCR	237/569	42/150	6/8	0.864
Smolarz [39] 2011	Poland	Caucasian	Endometrial	240–240	HB	PCR-RFLP	25/65	30/138	185/37	0.037
Gil [52] 2012	Poland	Caucasian	Colorectal	133–100	HB	PCR-RFLP	100/73	29/27	4/0	0.675
Sobti [45] 2012	India	Asian	Bladder	270–252	HB	PCR-RFLP	159/134	82/81	29/37	<0.001

HNSCC head and neck squamous cell carcinoma, NR not reported, AML acute myeloid leukemia, SA South American, N nested case-control study, HWE Hardy-Weinberg equilibrium, HB hospital-based study, PB population-based study, SC source of control, GM Genotype method.
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supported the negative association between RAD51 135G>C polymorphisms and AML, breast cancer, and ovarian cancer. In the stratified analysis by Smoking-related cancers, estrogen-related

cancers, ethnicity, and BRCA1/BRCA2 mutation status, significant association was only observed between RAD51 135G>C and breast cancer risk for BRCA2 mutation carriers (Table 4; recessive

Table 2. Stratified analysis of RAD51 135G>C polymorphism on cancer risk.¹

Variables	No. comparisons (SZ case/control)	Dominant model		Recessive model		Additive model	
		OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²
Overall	39 (19,068/22,630)	1.06 (0.96–1.08)	<0.001/61.4%	1.35 (0.89–2.03)	<0.001/80.7%	1.46 (0.94–2.27)	<0.001/72.8%
Cancer type							
AML	7 (1,605/3,121)	1.17 (0.84–1.65)	0.003/70.2%	1.12 (0.67–1.88)	0.123/40.2%	1.14 (0.68–1.92)	0.125/39.9%
Breast cancer	14 (11,709/11,291)	1.00 (0.93–1.07)	0.521/0.0%	1.27 (0.98–1.67)	0.198/24.3%	1.26 (0.97–1.65)	0.215/22.6%
Ovarian cancer	6 (2,388/4,411)	1.00 (0.86–1.15)	0.140/39.9%	1.23 (0.62–2.47)	0.348/5.3%	1.25 (0.62–2.49)	0.359/2.4%
Other cancer	12 (3,366/3,807)	²	<0.001/79.8%	²	<0.001/90.0%	²	<0.001/87.3%
Smoking-related	3 (1,953/1,908)	1.06 (0.88–1.27)	0.203/37.3%	0.97 (0.37–2.50)	0.738/0.0%	0.98 (0.38–2.54)	0.765/0.0%
estrogen-related	21 (14,279/15,910)	0.99 (0.93–1.06)	0.429/2.3%	1.27 (0.99–1.63)	0.265/16.5%	1.26 (0.98–1.62)	0.287/14.5%

¹All summary ORs were calculated using fixed-effects models. In the case of significant heterogeneity (indicated by *), ORs were calculated using random-effects models. ²The results were excluded due to high heterogeneity. The bold values indicate that the results are statistically significant. doi:10.1371/journal.pone.0075153.t002

Table 3. Summary ORs (95% CI) and value of value of the heterogeneity of RAD51 135G>C polymorphism for studies according to ethnicity and cancer type.¹

Ethnicity	Cancer type	No. comparisons (SZ case/control)	Dominant model		Recessive model		Additive model	
			OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²
Caucasian	AML	3 (832/1283)	²	<0.001/88.2%	1.08 (0.34–3.35)	0.672/0.0%	1.11 (0.36–3.44)	0.606/0.0%
	Breast cancer	6 (5028/4771)	1.04 (0.93–1.16)	0.195/32.1%	1.06 (0.70–1.60)	0.246/25.1%	1.06 (0.70–1.60)	0.243/25.5%
	Ovarian cancer	4 (2249/3975)	1.04 (0.88–1.21)	0.308/3.8%	0.77 (0.50–1.18)	0.133/46.4%	1.13 (0.55–2.34)	0.281/14.1%
Asian	Breast cancer	3 (1042/1093)	0.92 (0.72–1.16)	0.931/0.0%	1.33 (0.98–1.81)	0.785/0.0%	1.37 (0.74–2.52)	0.514/0.0%
Mixed	Breast cancer	5 (5639/5427)	0.96 (0.86–1.06)	0.924/0.0%	1.48 (0.96–2.28)	0.120/45.3%	1.46 (0.95–2.26)	0.133/43.4%

¹All summary ORs were calculated using fixed-effects models. In the case of significant heterogeneity (indicated by *), ORs were calculated using random-effects models. ²The results were excluded due to high heterogeneity. The bold values indicate that the results are statistically significant. doi:10.1371/journal.pone.0075153.t003

model: OR = 4.88, 95% CI = 1.10–21.67; additive model: OR = 4.92, 95% CI = 1.11–21.83). As described above, the RAD51 gene product acts together with BRCA1 and BRCA2 proteins in homologous recombination and DSB repair. It is reasonable to assume that RAD51 and BRCA1/2 mutations may have interactive effects on breast cancer risk. Some previous studies presented an association of RAD51 variant allele 135C with an elevated breast cancer risk only in BRCA2 mutation carrier, but not in BRCA1 mutation carriers or non-carriers or unselected populations [15,25,26,42]. In contrast, Jakubowska et

al. [13,22] observed a significantly reduced risk of breast cancer among Polish female carriers of RAD51 135C allele and BRCA1 founder mutations. Subgroup analysis on BRCA1/2 mutation status in this meta-analysis, however, confirmed the former result.

In the present meta-analysis, highly heterogeneity was observed in acute myeloid leukemia, especially in Caucasians. The reason may be acute myeloid leukemia including the hospital-based studies. The hospital-based studies have some biases because such controls may contain certain benign diseases which are prone to develop malignancy and may not be very representative of the

Table 4. Meta-analysis of RAD51 135G>C polymorphism and breast cancer association according to BRCA1/BRCA2 mutation.

BRCA1/2 mutation status	Sample size (case/control)	Dominant model		Recessive model		Additive model	
		OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²	OR (95% CI)	<i>P_h</i> / <i>I</i> ²
BRCA1 mutation	1 (2876/2902)	0.89 (0.77–1.03)	–	1.49 (0.80–2.76)	–	1.46 (0.79–2.71)	–
BRCA2 mutation	1 (1574/1174)	1.12 (0.89–1.41)	–	4.88 (1.10–21.67)	–	4.92 (1.11–21.83)	–
Non BRCA1/BRCA2 mutation	3 (1853/1443)	1.11 (0.90–1.36)	0.996/0.0%	0.94 (0.40–2.19)	0.218/34.1%	0.95 (0.41–2.23)	0.220/33.4%
Mixed	12 (5711/6160)	0.99 (0.89–1.09)	0.564/0.0%	1.22 (0.96–1.56)	0.570/0.0%	1.15 (0.82–1.60)	0.492/0.0%

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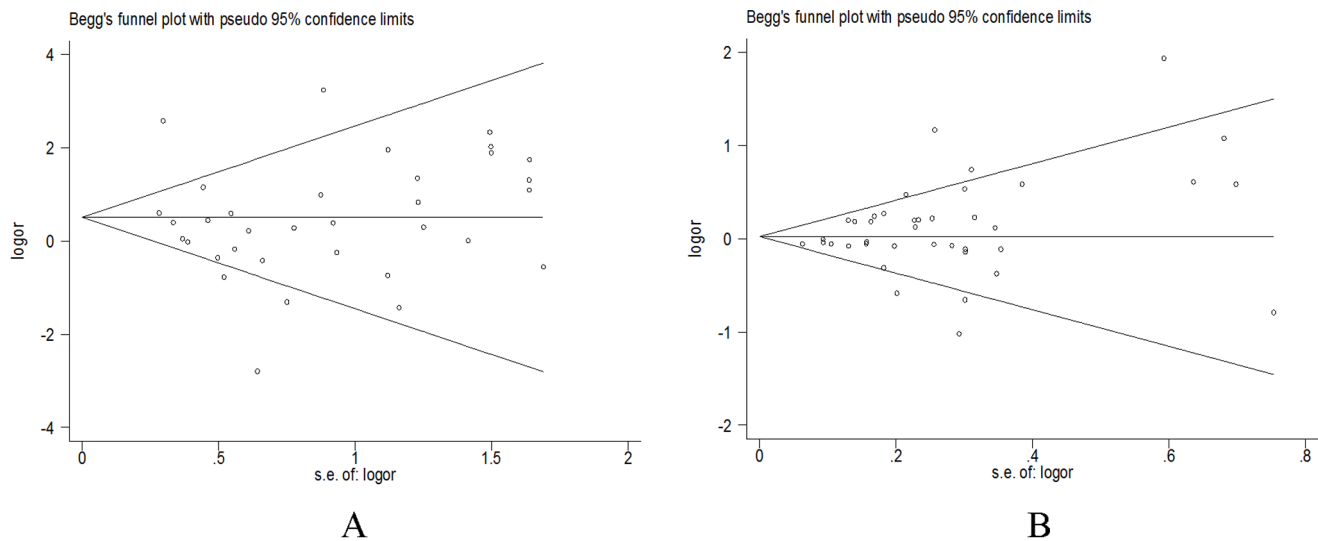


Figure 2. Begg's funnel plot for publication bias test in Additive model (A) and dominant model (B). Each point represents a separate study for the indicated association. Log (OR), natural logarithm of OR. doi:10.1371/journal.pone.0075153.g002

general population. Thus, the use of a proper and representative cancer-free control subjects is very important in reducing biases in such genotype association studies. Highly heterogeneity was also observed in mix cancers, the reason may be the same polymorphisms play different roles among different cancers, because cancer is a complicated multi-genetic disease, and different genetic backgrounds may contribute to the discrepancy. Possible sources of heterogeneity, such as controls source, cancer type and ethnicity did not demonstrate the evidence of any significant variation by meta-regression. It is possible that other limitations of recruited studies may partially contribute to the observed heterogeneity. And this indicates that it may be not appropriate to use an overall estimation of the relationship between RAD51 135 G>C polymorphism and cancer risk.

Although we have put considerable efforts and resources into testing possible association between RAD51 135G>C polymorphism and cancer risk, there are still some limitations inherited from the published studies. First, our results were based on single-factor estimates without adjustment for other risk factors including alcohol usage, environmental factors and other lifestyle. At lower levels of alcohol consumption, the difference in cancer risk between the various gene carriers was less striking. And higher levels of alcohol consumption result in production of more acetaldehyde which then can exert its carcinogenic effect [94]. Second, the subgroup analysis may have had insufficient statistical power to check an association. Third, the controls were not uniformly defined. Some studies used a healthy population as the reference group, whereas others selected hospital patients without organic cancer as the reference group. Therefore, non-differential misclassification bias is possible because these studies may have included the control groups who have different risks of developing cancer of various organs. Our meta-analysis also has several

strengths. First, a systematic review of the association of RAD51 135G>C polymorphism with cancer risk is statistically more powerful than any single study. Second, the quality of eligible studies included in current meta-analysis was satisfactory and met our inclusion criterion.

In conclusion, this meta-analysis suggests that RAD51 variant 135C homozygote is associated with elevated breast cancer risk among BRCA2 mutation carriers. Moreover, our work also points out the importance of new studies for RAD51 135G>C association in acute myeloid leukemia, especially in Caucasians, where at least some of the covariates responsible for heterogeneity could be controlled, to obtain a more conclusive understanding about the function of the RAD51 135G>C polymorphism in cancer development. However, it is necessary to conduct large sample studies using standardized unbiased genotyping methods, homogeneous cancer patients and well-matched controls. Moreover, further studies estimating the effect of gene-gene and gene-environment interactions may eventually lead to our better, comprehensive understanding of the association between RAD51 135G>C polymorphism and cancer risk.

Supporting Information

Checklist S1 Prisma checklist. (DOC)

Author Contributions

Conceived and designed the experiments: BYW. Performed the experiments: WW JLL. Analyzed the data: XFH APL. Contributed reagents/materials/analysis tools: YLC NX SMS. Wrote the paper: WW JLL. Data acquisition: XFH APL YLC NX SMS. Manuscript editing and final version approval: BYW.

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