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.

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 GG/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 = 0.991–0.999) 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 GC), respectively. Between-study heterogeneity was assessed by calculating Q-statistic (Heterogeneity was considered statistically significant if P<0.10) [70] and quantified using the I² 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 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 versus CC. Fourteen 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%–18.19%), which was significantly higher than that in Caucasians (8.34%, 95% CI = 7.33%–10.04%, $P<0.001$). 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: $P_h<0.001$), recessive model comparison (GG+GC versus CC: $P_h<0.001$), and additive model comparison (GG versus CC: $P_h<0.001$). 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 contributed 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
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 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

Figure 1. Study flow chart explaining the selection of the 39 eligible case–control studies included in the meta-analysis.
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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
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 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)                      | \(P_h/I^2\)    | OR (95% CI)     | \(P_h/I^2\)   |
| 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% |
| 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% |
| 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% |
| Ovarian cancer          | 6 (2,388/4,411)                  | 1.00 (0.86–1.15) | 0.140/39.9%     | 1.23 (0.62–2.47) | 0.348/53.3% |
| Other cancer            | 12 (3,366/3,807)                 | 2               | <0.001/79.8%    | 2             |
| 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% |
| estrogen-related        | 21 (14,279/15,910)               | 0.99 (0.93–1.06) | 0.429/23.3%     | 1.27 (0.99–1.63) | 0.265/16.5% |

1 All summary ORs were calculated using fixed-effects models. In the case of significant heterogeneity (indicated by *), ORs were calculated using random-effects models.

| Ethnicity   | Cancer type | No. comparisons (SZ case/control) | Dominant model | Recessive model | Additive model |
|-------------|-------------|----------------------------------|----------------|-----------------|---------------|
|             |             | OR (95% CI)                      | \(P_h/I^2\)    | OR (95% CI)     | \(P_h/I^2\)   |
| Caucasian   | AML         | 3 (832/1283)                     | 2              | <0.001/88.2%    | 1.08 (0.34–3.35) | 0.672/0.0% |
|             | 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% |
|             | 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% |
| 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% |
| 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 All summary ORs were calculated using fixed-effects models. In the case of significant heterogeneity (indicated by *), ORs were calculated using random-effects models.

| BRCA1/2 mutation status | Sample size (case/control) | Dominant model | Recessive model | Additive model |
|-------------------------|---------------------------|----------------|-----------------|---------------|
|                         | OR (95% CI)                  | \(P_h/I^2\)    | OR (95% CI)     | \(P_h/I^2\)   |
| BRCA1 mutation          | 1 (2876/2902)              | 0.89 (0.77–1.03) | –               | 1.49 (0.80–2.76) | –             |
| BRCA2 mutation          | 1 (1574/1174)              | 1.12 (0.89–1.41) | –               | 4.88 (1.10–21.67) | –             |
| 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% |
| 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 All summary ORs were calculated using fixed-effects models. In the case of significant heterogeneity (indicated by *), ORs were calculated using random-effects models.

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

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

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