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135G/C polymorphism in the RAD51 gene and acute myeloid leukemia risk: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the 135G/C polymorphism in the RAD51 gene and risk of acute myeloid leukemia (AML), but the results have been inconsistent. The aim of this study is to precisely examine the association between the 135G/C polymorphism in the RAD51 gene and AML risk through a meta-analysis. PubMed, Google Scholar, and Web of Science databases were systematically searched to identify relevant studies from their inception to June 2015. Pooled odds ratios (OR) with 95% confidence intervals (95%CI) were calculated using fixed- or random-effect models. A total of 6 case-control studies containing 1432 patients and 2750 controls were used in this meta-analysis, and our results showed no association between the 135G/C polymorphism in the RAD51 gene and AML risk (CC *vs* GG: OR = 1.67, 95%CI = 0.93-3.02; GC *vs* GG: OR = 1.24, 95%CI = 0.80-1.92; the dominant model: OR = 1.26, 95%CI = 0.83-1.91; the recessive model: OR = 1.63, 95%CI = 0.90-2.95). No publication bias was found in this study. In summary, the present meta-analysis suggests that the 135G/C polymorphism in the RAD51 gene

may not be associated with AML risk. However, further studies with larger cohorts are needed to confirm this conclusion.

Key words: RAD51; 135G/C polymorphism; Acute myeloid leukemia; Meta-analysis

INTRODUCTION

Acute myeloid leukemia (AML) is a type of cancer in which hematopoietic progenitor cells lose the ability to differentiate normally and respond to normal regulators of proliferation and apoptosis (Ferrara and Schiffer, 2013). The etiology of AML was found to be multifactorial and there are some environmental factors that may potentially play a role in susceptibility, including radiation, smoking, obesity, and exposure to chemical carcinogens (Ilhan et al., 2006). However, AML only develops in a small proportion of individuals exposed to these environmental risk factors, indicating that genetic factors also play an important role in its development. Previous meta-analyses indicated that the Thr241Met polymorphism in the X-ray repair complementing defective repair in Chinese hamster cells 3 (XRCC3) gene might be associated with risk of AML (Qin et al., 2014).

DNA repair pathways are responsible for maintaining genomic stability and play a key role in protecting against genetic mutations (Dixon and Kopras, 2004). Deficiencies in the DNA repair system may affect genome integrity, leading to the development of cancers, including AML (Seedhouse et al., 2004). DNA double-strand breaks are the most injurious lesions because they cause cell death or loss of genetic material. Two major pathways have evolved in repairing DNA double-strand breaks: homologous recombination (HR) and non-homologous end-joining.

As one of the key proteins involved in HR, RAD51 is essential to meiotic and mitotic recombination and plays a crucial role in HR repair (Richardson, 2005). The human RAD51 gene is located on chromosome 15q15.1 and is thought to participate in a common double-strand break repair pathway. A functional single nucleotide polymorphism termed 135G/C (NCBI accession number D14134, rs1801320) has been identified in the 5'-untranslated region of the RAD51 gene. This polymorphism was shown to affect gene transcriptional activity (Hasselbach et al., 2005).

In the past decade, a number of molecular epidemiological studies have been donew to evaluate the association between the 135G/C polymorphism in the RAD51 gene and AML risk, but the results remain controversial. A single study may have lower statistical power to detect the overall effects due to small sample size, but a quantitative synthesis of all included studies will provide valuable evidence on the association between the 135G/C polymorphism in the RAD51 gene and AML risk. Therefore, we performed a comprehensive meta-analysis by including relevant articles to identify statistical evidence of the association between the 135G/C polymorphism in the RAD51 gene and risk of AML.

MATERIAL AND METHODS

Search strategies

All relevant studies published before June 1, 2015 were identified through an extended computer-based search of PubMed, Google Scholar, and Web of Science. The search terms were as follows: “135G/C polymorphism”, “acute myeloid leukemia/AML”, “RAD51”, “single nucleotide polymorphism”, and “genetic polymorphism”. The search was restricted to human studies without

language limitations. If the same case series was included in multiple studies published with the same list of authors, only the most informative study with the largest number of subjects was used.

Study selection

Studies were included if they met the following criteria: i) case-control study that addressed AML patients and healthy controls; ii) study that evaluated the association between the 135G/C polymorphism and AML risk; and iii) genotype frequencies of healthy controls were in Hardy-Weinberg equilibrium (HWE). The following were causes for study exclusion: i) not a case-control study that evaluated the association between the 135G/C polymorphism and AML risk; ii) case reports, letters, reviews, and editorial articles; iii) studies that were based on incomplete raw data and no usable data reported; iv) duplicate data were contained in the studies; and v) healthy controls were not in HWE.

Data extraction

Two investigators independently extracted data using a standardized data collection form. Study characteristics extracted from each article were as follows: first author, year of publication, geographical area, number of cases and controls, genotype frequencies in cases and controls, and evidence of HWE in controls. For conflicting evaluations, an agreement was reached following a discussion.

Statistical analysis

We assessed HWE in the controls for each study using a chi-square test and $P < 0.05$ was considered as statistically significant. The pooled odds ratio (OR) with corresponding 95% confidence intervals (95%CI) were calculated to assess the strength of the association between the 135G/C polymorphism in the RAD51 gene and AML risk under a homozygote comparison (CC *vs* GG), a heterozygote comparison (GC *vs* GG), a dominant model (CC + GC *vs* GG), and a recessive model (CC *vs* GC + GG) between groups. The variation caused by heterogeneity was estimated by calculating the inconsistency index (I^2), with I^2 values of 25, 25-75 and 75% representing low, moderate or high degrees of inconsistency, respectively (Higgins and Thompson, 2002). When $I^2 > 50\%$ indicated heterogeneity across studies, the random-effect model was used for meta-analysis, otherwise the fixed-effect model was used. When heterogeneity was observed, the Galbraith plot was used to detect the possible sources of heterogeneity. Sensitivity analysis was performed by altering the statistical models to ensure the stability of the results. Additionally, the Begg test by visual inspection of the funnel plot was carried out to address the potential publication bias and $P < 0.05$ was considered as an indicator of significant publication bias. Meta-analysis was performed using the STATA package version 12.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Characteristics of included studies

There were 89 papers found that were relevant to the search words used in this study. Through screening the title and reading the abstract and the entire article, 6 eligible articles were

selected for this meta-analysis (Seedhouse et al., 2004; Rollinson et al., 2007; Voso et al., 2007; Bhatla et al., 2008; Hamdy et al., 2011; Liu et al., 2011). The flow chart for the study selection is summarized in Figure 1, including a total of 1432 cases and 2750 controls. There were 4 studies (Seedhouse et al., 2004; Rollinson et al., 2007; Voso et al., 2007; Hamdy et al., 2011) of Caucasian descent, 1 study (Liu et al., 2011) of Asian descent, and one study (Bhatla et al., 2008) of mixed descent. The HWE test was performed on genotype distribution of the controls and all were in agreement with HWE. The main characteristics of eligible studies are summarized in Table 1.

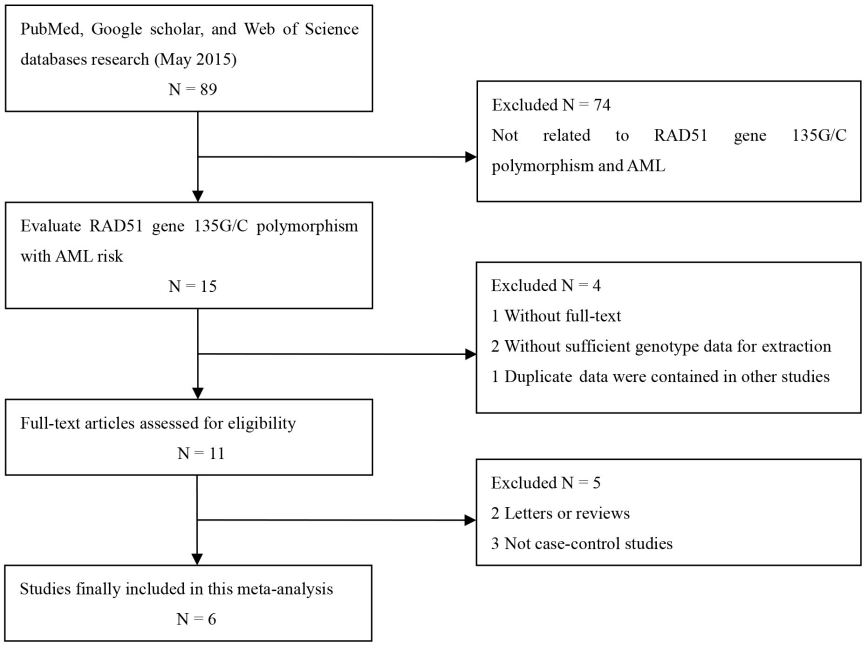

Figure 1. Detailed process of identifying eligible studies.

Table 1. Characteristics of the included studies for meta-analysis.

Study included	Geographical area	Race	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test
				GG	GC	CC	GG	GC	CC	
Seedhouse et al. (2004)	UK	Caucasian	186/257	210	44	3	166	18	2	0.08
Voso et al. (2007)	Italy	Caucasian	160/161	125	33	2	142	18	1	0.61
Rollinson et al. (2007)	UK	Caucasian	479/952	431	34	1	817	115	4	0.98
Bhatla et al. (2008)	USA	Mixed	452/646	374	73	5	555	85	6	0.18
Hamdy et al. (2011)	Egypt	Caucasian	50/30	39	9	2	26	3	1	0.06
Liu et al. (2011)	China	Asians	105/704	72	25	8	511	175	18	0.52

HWE = Hardy-Weinberg equilibrium.

Meta-analysis

The combined results of the 135G/C polymorphism in the RAD51 gene with risk of AML are displayed in Figures 2-5 and Table 2. Meta-analysis results showed that there was no association between the 135G/C polymorphism in the RAD51 gene and AML risk (CC *vs* GG: OR = 1.67, 95%CI = 0.93-3.02; GC *vs* GG: OR = 1.24, 95%CI = 0.80-1.92; the dominant model: OR = 1.26,

95%CI = 0.83-1.91; the recessive model: OR = 1.63, 95%CI = 0.90-2.95). We used a Galbraith plot to analyze the heterogeneity and no reports were excluded (Figure 6). Sensitivity analysis was performed by altering the statistical models and the result was unchanged, indicating that the results of meta-analysis were statistically significant.

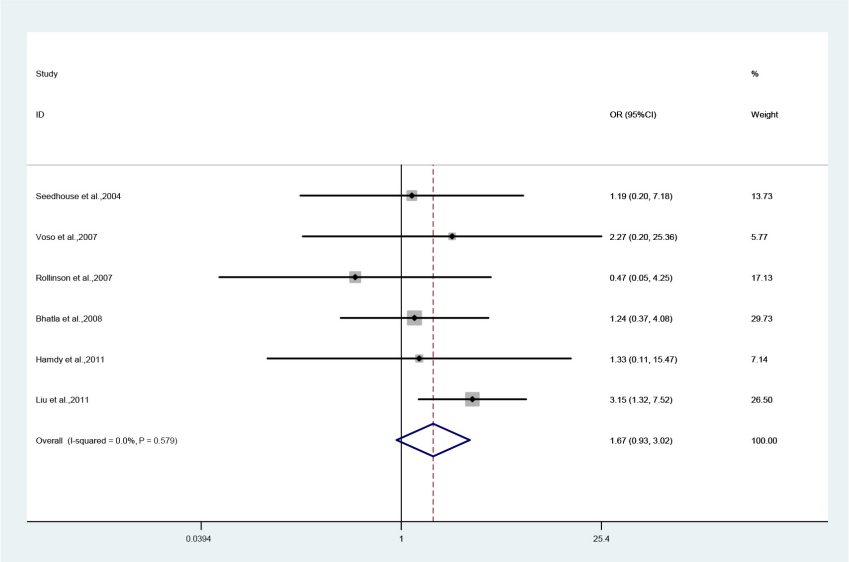

Figure 2. Meta-analysis of the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk (CC *vs* GG).

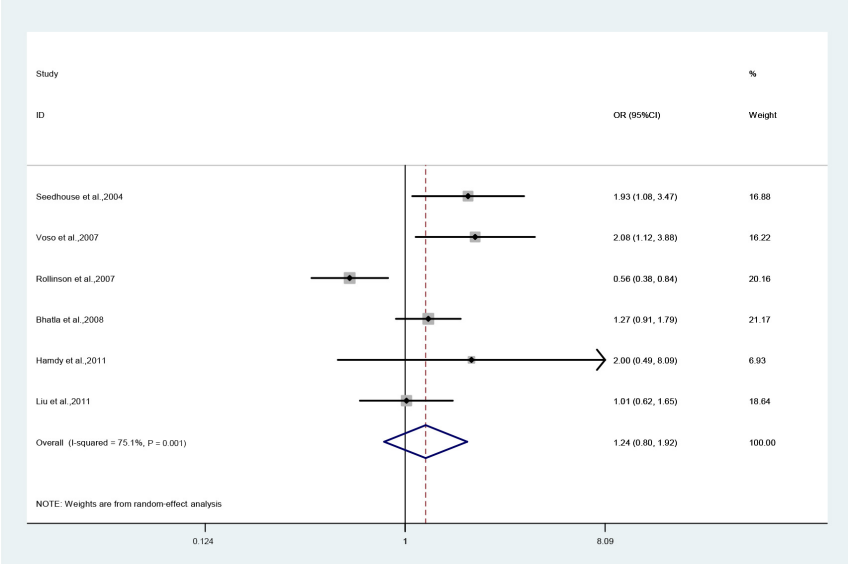

Figure 3. Meta-analysis of the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk (GC *vs* GG).

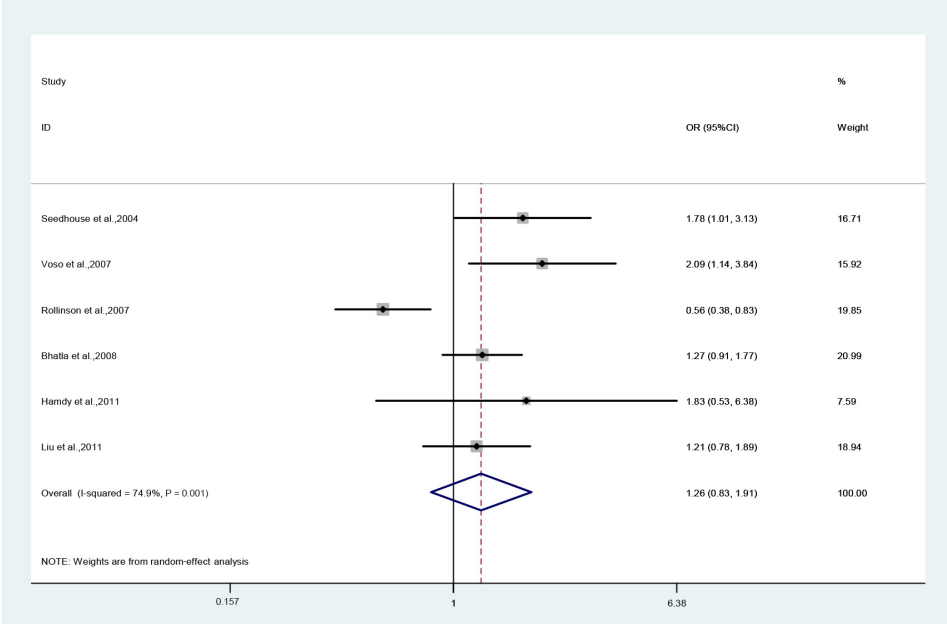

Figure 4. Meta-analysis of the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk (dominant model).

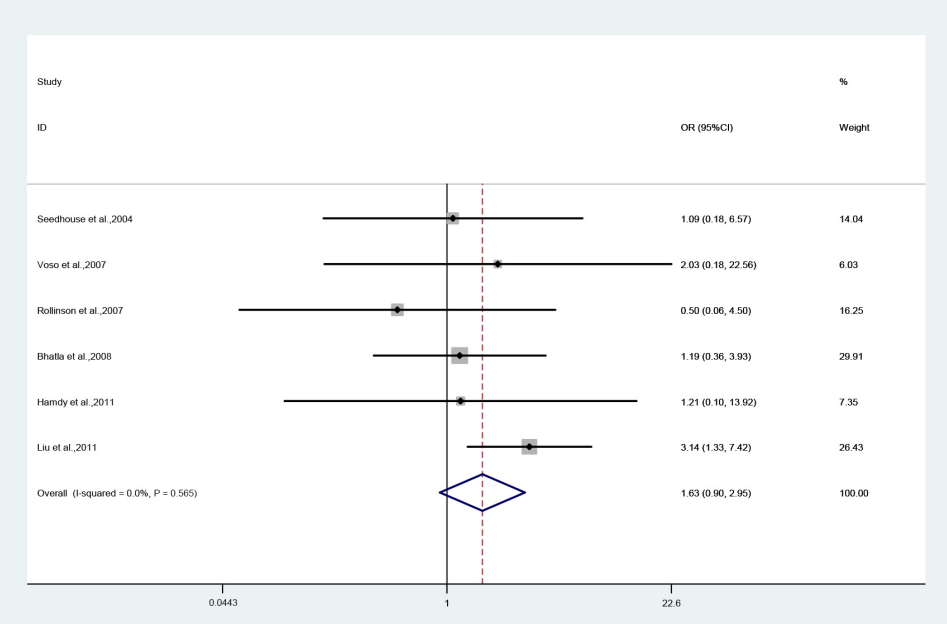

Figure 5. Meta-analysis of the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk (recessive model).

Table 2. Summary ORs and 95%CIs of the 135G/C polymorphism in the RAD51 gene and AML risk.

Genetic model	Sample size	Type of model	Test of heterogeneity	Test of association	Test of publication bias
	Case Control		I^2 P	OR (95%CI)	z P
CC *vs* GG	1432 2750	Fixed	0.0% 0.58	1.67 (0.93-3.02)	0.00 1.00
GC *vs* GG		Random	75.1% 0.00	1.24 (0.80-1.92)	0.00 1.00
Dominant model		Random	74.9% 0.00	1.26 (0.83-1.91)	0.00 1.00
Recessive model		Fixed	93.6% 0.00	1.63 (0.90-2.95)	0.00 1.00

OR = odds ratio; CI = confidence interval.

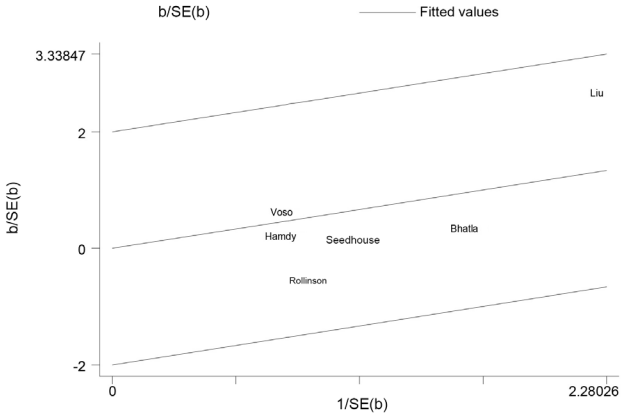

Figure 6. Galbraith plot of the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk.

Publication bias

Begg's funnel plot was used to assess the publication bias of the selected literature. There was no evidence of publication bias in our study (all P > 0.05; Figure 7).

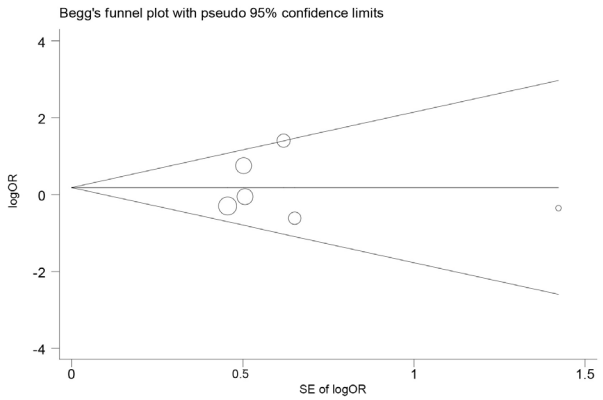

Figure 7. Begg's funnel plot for publication bias test.

DISCUSSION

AML is a heterogeneous clonal disorder in which hematopoietic progenitor cells lose their ability to differentiate normally and respond to normal regulators of proliferation and apoptosis (Ferrara and Schiffer, 2013). DNA repair systems have a key role in maintaining genome integrity and stability. It is well known that defects in repair pathways are associated with genetic instability, which in turn may promote the development of cancer (Wood et al., 2001). Thus, genetic abnormalities, including single nucleotide polymorphisms, may contribute to carcinogenesis (Mendoza et al., 2013). In the past decade, a number of epidemiological studies have evaluated the association between the 135G/C polymorphism in the RAD51 gene and AML risk, but the results remain inconclusive. Therefore, we conducted a meta-analysis of previously published studies to assess the relationship between the 135G/C polymorphism in the RAD51 gene and AML risk.

Our meta-analysis quantitatively assessed the association between the 135G/C polymorphism in the RAD51 gene and AML risk. Finally, 6 case-control studies were included and assessed, involving a total of 1432 cases and 2750 healthy controls. There was only one study in an Asian population; therefore, further studies in Asians should be performed. There was no evidence of publication bias in this meta-analysis. The meta-analysis results showed no association between the 135G/C polymorphism in the RAD51 gene and susceptibility to AML. However, our study was based on a single-factor estimate, which lacks combination of multiple-polymorphism analysis. The potential function of the 135G/C polymorphism in the RAD51 gene might be via gene-gene interactions. A previous meta-analysis demonstrated that the Thr241Met polymorphism in the XRCC3 gene is associated with AML susceptibility (Qin et al., 2014), and the Thr241Met polymorphism in the XRCC3 gene in combination with the 135G/C polymorphism in the RAD51 gene synergistically increased AML risk (Seedhouse et al., 2004). In addition, a recent study found a significantly increased risk of AML associated with the combination of the 135G/C polymorphism in the RAD51 gene and the A290G polymorphism in the CYP3A4 gene (Voso et al., 2007). Further studies examining the 135G/C polymorphism should be taken into consideration to investigate the possible relationships between these single nucleotide polymorphisms.

Several limitations should be acknowledged in this meta-analysis. First, we only selected the published articles to acquire data for analysis and the effect of unpublished articles is unknown. Thus, it is necessary to conduct a systematic review to avoid this potential effect in analysis. Second, the results may be affected by additional confounding factors, such as gender and age, but this subgroup analysis could not be investigated because of incomplete data. Additionally, lacking the original data limited our further evaluation of potential gene-gene and gene-environment interactions.

In conclusion, our results suggest that the 135G/C polymorphism in the RAD51 gene may be not associated with susceptibility to AML. Moreover, further studies examining the effect of gene-gene and gene-environment interactions may eventually provide a more comprehensive understanding of any potential association.

Conflicts of interest

The authors declare no conflict of interest.

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