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# Correlations between MDM2 gene SNP309 Polymorphism and Susceptibility to Leukemia

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCEG **Wen-Bin Ou**

Out-Patient Department, Air Force General Hospital, Beijing, China

**Corresponding Author:** Wen-Bin Ou, e-mail: zhan\_xiaohu@yeah.net  
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**Background:** The objective of this study was to perform a systematic review of correlations between the single-nucleotide polymorphism at nucleotide 309 (single-nucleotide polymorphism, SNP309) in the murine double-minute 2 (MDM2) gene promoter and susceptibility to leukemia.




**Material/Methods:** We performed a computer search of relevant case-control studies published from January 1990 to Jan 2014 in databases such as Ovid, EBSCO, PubMed, CNKI, CBMDISC, VIP, and WanFang Data. The literature was screened based on inclusion and exclusion criteria. The data were retrieved, and the quality of the methodology used in the studies was evaluated. A meta-analysis was performed by calculating the combined odds ratios (OR) and 95% confidence intervals (CI) using RevMan 5.0 and Stata 10.0 software. Sensitivity was analyzed and publication bias was assessed.

**Results:** A total of ten case-control studies from nine research papers were selected in this study, which included 1889 cases and 5707 controls. Meta-analysis showed that people who carried the G allele had increased susceptibility to leukemia compared to people who carried the T allele [OR=1.24, 95% CI (1.06, 1.45), P=0.007]. In a recessive model, the GG homozygotic population had a higher risk of leukemia than the heterozygotic GT+TT population [OR=1.47, 95% CI (1.11, 1.96), P=0.008]. We did not find significant difference in a dominant model [GG+GT vs. TT: OR=1.22, 95% CI (0.98, 1.52), P=0.07]. Publication bias was not significant.

**Conclusions:** SNP309 polymorphism in the MDM2 gene is associated with susceptibility to leukemia. The G allele may be a risk factor for leukemia.

**MeSH Keywords:** **Core Binding Factor Alpha 2 Subunit • Meta-Analysis as Topic • Polymorphism, Single Nucleotide**

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## Background

Leukemia is a group of malignant clonal diseases with a high degree of heterogeneity. The pathological basis of leukemia is changes in pathways regulating cell proliferation, differentiation, and apoptosis due to gene mutation [1]. However, the cause of leukemia is not yet fully understood. During the development of leukemia, the tumor suppressor gene p53 plays an important role in regulating cellular functions. The TP53 tumor suppressor pathway helps to maintain genomic integrity, thereby preventing tumor formation [2]. The murine double-minute gene 2 (MDM2) plays a key role in this pathway. MDM2 directly binds to the p53 protein and inhibits p53 activity [3]. In addition, MDM2 regulates the TP53 pathway through the ubiquitination and degradation of p53. In contrast, p53 upregulates MDM2 expression, thus forming a negative feedback loop [4,5]. In the MDM2 gene promoter region, the single-nucleotide polymorphism at nucleotide 309 (SNP309, 309T>G) can enhance the binding affinity of transcription factor Sp1, thereby increasing MDM2 mRNA and protein expression levels and decreasing the tumor suppression function of p53 [6]. Currently, there are several case-control studies on the relationship between SNP309T>G and susceptibility to leukemia. However, these studies have certain limitations, including variations in research quality, small sample size, and different regional and ethnic backgrounds of the subjects. Thus, the conclusions drawn from these studies are not very firm and have limited credibility. Our study aimed to perform a meta-analysis of the relationships among MDM2 SNP309 polymorphism and susceptibility to leukemia by using data from case-control studies in China and other countries. The results from this study will provide more reliable evidence for basic research and clinical treatment.

## Material and Methods

### Inclusion and exclusion criteria

#### *Inclusion criteria*

1) The study design is case-control; 2) the association between MDM2 SNP309 polymorphism and susceptibility to leukemia was assessed; 3) the disease group consisted of clinically and pathologically diagnosed leukemia patients, while the control group consisted of healthy individuals; 4) among studies published by the same authors, the studies with the highest quality and largest sample size were selected; 5) studies were selected that reported proper statistical methods and highly reliable data, definitive results, various genotype data, OR, and 95% CI (or where such values could be calculated from the original data).

#### *Exclusion criteria*

Studies that were not on human subjects were excluded from the study.

#### **Search strategy**

A Medical Subject Headings (MeSH) method was used to retrieve studies from databases. “SNP309” or “MDM2 Polymorphism(s)” or “MDM2 variants” or “MDM2 genotype” and “leukemia” were used as keywords. Studies published between January 1990 and January 2014 were searched.

#### **Quality assessment and data retrieval**

The research quality of the selected case-control studies was assessed using the Oxford Critical Appraisal Skill program (Oxford-CASP, 2004). The criteria include the following: 1) whether the diagnostic criteria were clearly indicated; 2) the methods of randomization and matching; 3) whether the controls were comparable with the cases; 4) whether the gene detection method was appropriate; 5) whether the sample size was adequate; and 6) whether the data were adequate. Two investigators assessed the quality of the studies and retrieved data from the literature based on the same quality standards and then performed a cross-check. Any disagreement was resolved by discussion or by a third investigator [7].

#### **Statistical methods**

Meta-analysis was performed using the RevMan 5.0 and Stata 10.0 software. Cochran's Q was used for the analysis of heterogeneity between the results of each study (test level=0.10). When there was no heterogeneity between studies, a fixed-effects model was used for the meta-analysis. When there was heterogeneity, a random-effects model was used for the meta-analysis. The OR and 95% CI of each allele and genotype frequency were calculated for each study. The Hardy-Weinberg equilibrium of the control group was calculated.  $P < 0.05$  was considered statistically significant. Sensitivity analysis was conducted using the individual exclusion method. The overall effects were re-assessed and compared with the overall effects prior to exclusion. Begg's test and Egger's test were applied to determine whether there was publication bias in the studies.

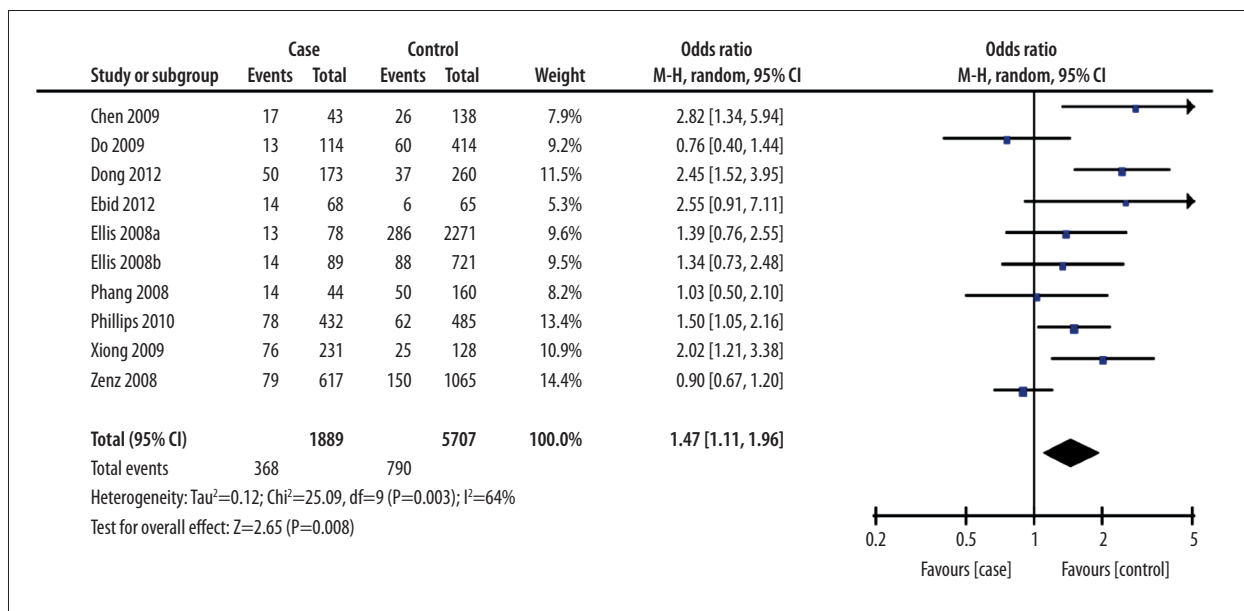
## Results

### **Literature search and quality evaluation**

Eighty-nine papers were initially retrieved. After screening based on the criteria, ten case-control studies from nine

**Table 1.** The characteristics of included studies.

Included studies	Country	Ethnicities	Methods for genotyping	Number (case/control)	Genotypes (case)			Genotypes (control)		
					GG	GT	TT	GG	GT	TT
Ellis 2008a	United States	Caucasian	TaqMan	78/2271	13	34	31	286	1027	958
Ellis 2008b	Britain	Caucasian	TaqMan	89/721	14	40	35	88	303	330
Zenz 2008	Germany	Caucasian	DHPLC	617/1065	79	299	239	150	470	445
Phang 2008	Singapore	Asian	TaqMan	44/160	14	13	17	50	80	30
Chen 2009	China Taiwan	Asian	SSCP-CE	43/138	17	20	6	26	83	29
Do 2009	China	Asian	AS-PCR	231/128	76	123	32	25	68	35
Xiong 2009	Canada	Caucasian	TaqMan	114/414	13	55	46	60	167	187
Phillips 2010	United States	Caucasian	TaqMan	432/485	78	178	176	62	229	194
Dong 2012	China	Asian	PCR-RFLP	173/260	50	84	39	37	141	82
Ebid 2012	Egypt	Caucasian	PCR-RFLP	77/77	14	33	21	6	29	30

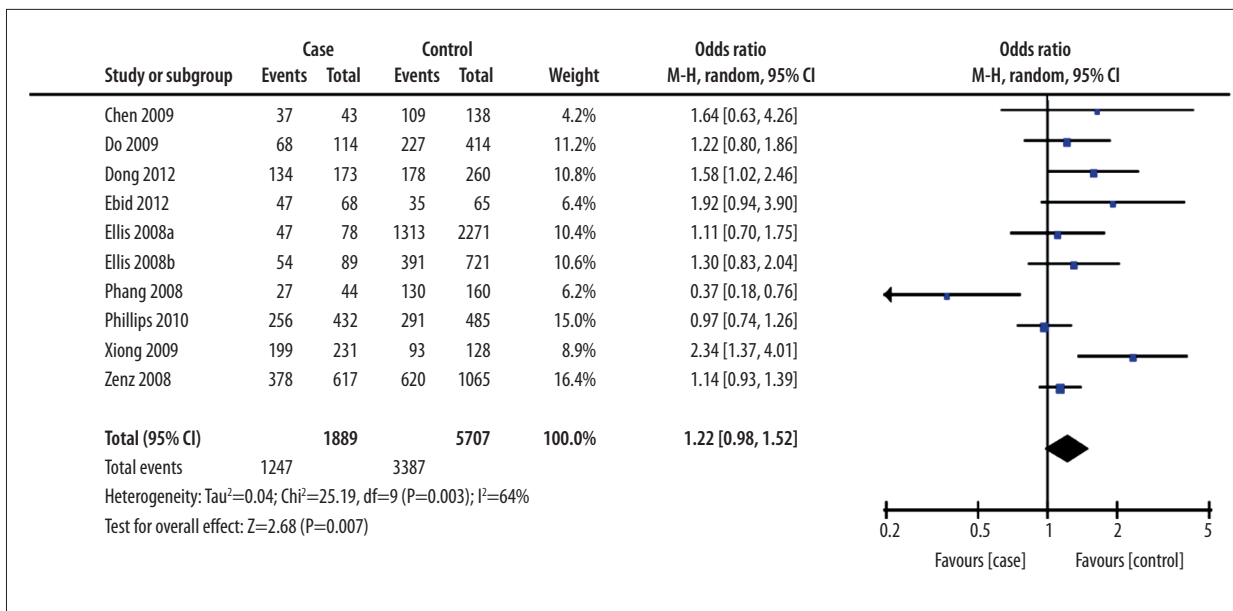


**Figure 1.** Forest plot of leukemia susceptibility and SNP309 of MDM2 gene (a recessive model: GG vs. GT+TT), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95%CI.

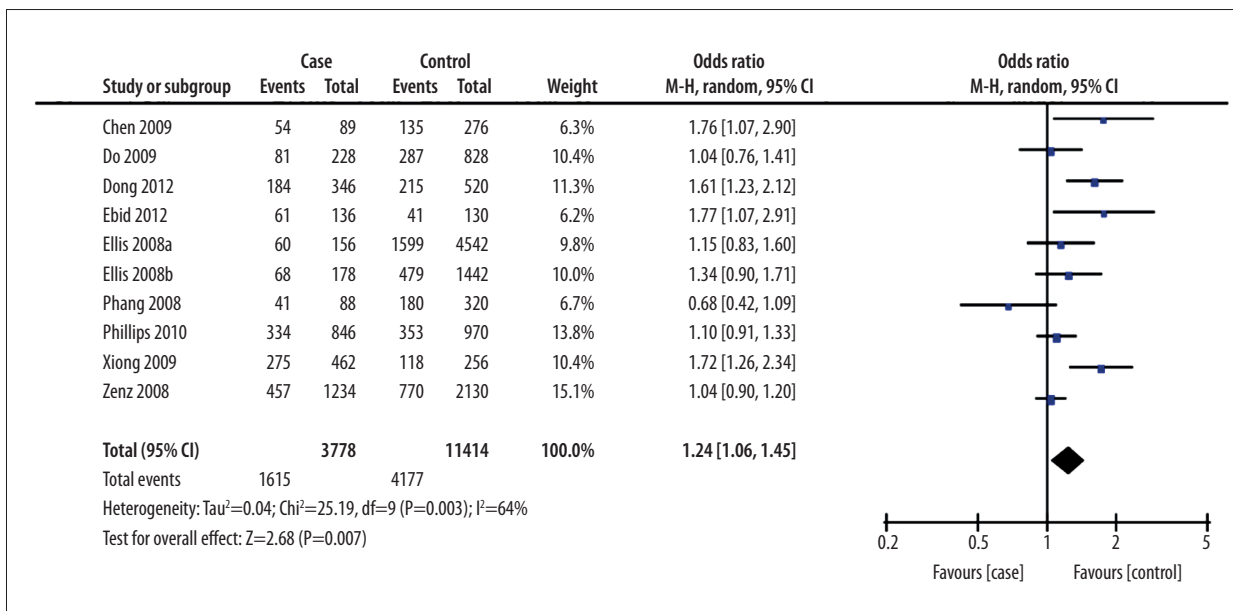
papers were included [8–16]. This study included 1889 cases and 5707 controls. The basic characteristics of the cases included are shown in Table 1. The quality assessment results showed that the studies included had clear diagnostic criteria; comparable patient and control groups; appropriate genetic testing methods; and clearly documented data and results. Six studies recruited Caucasian subjects, and another four studies recruited Asian subjects. All ten studies contained allelic data. All studies were consistent with the Hardy-Weinberg equilibrium.

**Meta-analysis**

Heterogeneity analysis showed significant heterogeneity among the studies (I<sup>2</sup>=64%, P=0.0006). Therefore, a random effects model for pooled analysis was applied. The GG genotype was defined as the exposure factor, and the TT genotype was defined as the non-exposure factor. The meta-analysis of a recessive model showed a significant difference between the GG genotype and the GT+TT genotype in terms of leukemia risk [OR=1.47, 95% CI (1.11, 1.96), P=0.008] (Figure 1).The



**Figure 2.** Forest plot of leukemia susceptibility and SNP309 of MDM2 gene (a dominant model: TT vs. GG+GT), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95%CI.

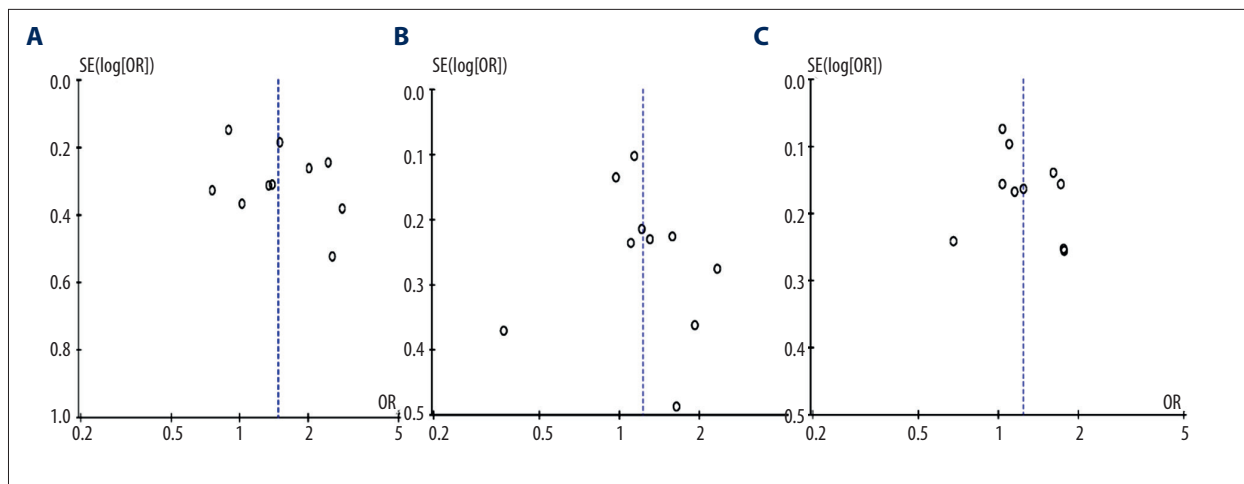


**Figure 3.** Forest plot of leukemia susceptibility and SNP309 of MDM2 gene (an allele model: G vs. T), the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95%CI.

meta-analysis of a dominant model showed no significant difference between the GG+GT genotype and the TT genotype in terms of leukemia risk [OR=1.22, 95% CI (0.98, 1.52),  $P=0.07$ ]. (Figure 2). The meta-analysis shows that people with the G allele had increased susceptibility to leukemia compared to people with the T allele [OR=1.24, 95% CI (1.06, 1.45),  $P=0.007$ ] (Figure 3).

**Sensitivity analysis**

The individual exclusion method was used for the sensitivity analysis in both the GG and TT genotypes. The smallest combined OR after exclusion was 1.32 [95% CI (0.94, 1.96)], and the largest combined OR after exclusion was 1.62 [95% CI (1.14, 2.29)]. Comparisons of the meta-analysis results before and



**Figure 4.** Begg's funnel plot for publication bias tests. Each point represents a separate study for the indicated association. Log or represents natural logarithm of OR. Vertical line represents the mean effects size.

after this exclusion revealed no significant differences, indicating that the analysis exhibited relatively low sensitivity and that the analysis results are therefore relatively robust and credible.

### Publication bias

Funnel plot and Egger's test were performed to assess the publication bias of the literature. Egger's test further confirmed the absence of publication bias in this meta-analysis ( $P > 0.05$ ) (Figure 4).

In this study, we performed a systematic review of the associations between MDM2 gene polymorphisms and susceptibility to leukemia. Our results indicate that at the MDM2 gene SNP309 polymorphism *loci*, the OR of the relative susceptibility to leukemia of the G allele compared to the T allele was 1.24 [95% CI (1.06, 1.45),  $P = 0.007$ ] and was statistically significant.

People who carried the homozygous GG allele had 1.47 times the risk of leukemia compared to people who carried the TT or GT genotype. These results indicate that the G allele significantly increases susceptibility to leukemia. The biological mechanisms of this susceptibility may be as follows. It is known that MDM2 has two promoters, namely, the P1 and P2 promoters [17–20]. The P1 promoter is a constitutive promoter that does not affect the expression levels of MDM2. The P2 promoter is a p53-dependent intronic promoter containing two adjacent p53 binding elements. P2 may affect the expression levels of MDM2. The MDM2 gene contains several polymorphic *loci*, among which SNP309T>G is located on the P2 promoter. Compared to the wild-type T allele, the G allele can significantly increase the affinity of MDM2 to transcriptional activator SPL. The transcriptional activity of MDM2 is increased, resulting in increased mRNA transcription and protein expression of MDM2. MDM2 directly inhibits the p53 tumor

suppressor pathway [21], thereby increasing the susceptibility to leukemia.

The heterogeneity analysis of the included studies showed the presence of heterogeneity among the studies. In the present study we included studies involving both Caucasian and Asian populations; the genetic background may be a major cause of heterogeneity among studies. In addition, the different genotyping methods were used in the included studies, which may result in heterogeneity between each study.

Publication bias was not found using a funnel plot of the qualitative Begg's test. Therefore, our study carries considerable credibility. However, our study may still have the following limitations. First, our study was restricted to published studies, and the languages were limited to Chinese and English. Therefore, potential publication and language biases could interfere with the meta-analysis. Second, most current research on the associations between MDM2 SNP309 polymorphisms and cancer susceptibility focus on solid tumors [22,23]. Studies on hematologic oncology are limited. Thus, only a limited number of studies were included in our study, resulting in a small number of patients and controls. The generalizability of our conclusions is potentially affected. Additionally, because our study only included genotype data on Asian and Caucasian populations, but not on other ethnic groups such as Africans, the results are not comprehensive.

### Conclusions

In summary, MDM2 SNP309 polymorphism is associated with susceptibility to leukemia. The G allele may be a risk factor for leukemia. More multicenter case-control studies with large sample sizes and high homogeneity are expected in the

future. These studies will more accurately evaluate the correlations between MDM2 polymorphisms and susceptibility to

leukemia. More reliable evidence will be provided for both basic research and clinical treatment.

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