

## Original Article

# Impact of *MDM2* 309T>G polymorphism on sarcomagenesis

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**Abstract:** Background: A series of epidemiological studies have attempted to evaluate the impact of 309T>G polymorphism in *MDM2* gene frequently identified as a susceptibility loci for various cancers on malignant sarcomas, however the reported conclusions remain inconsistent and elusive. We pooled all usable data sets in order to systematically assess the association between 309T>G polymorphism and sarcoma risk. Methods: To identify as many informative studies with complete data as possible, we searched a number of databases (PubMed, EBSCO, BIOSIS, the Cochrane Library, ISI Web of Science, Wiley Online Library and Embase). Inclusion criteria were defined to select the eligible studies. The fixed effects meta-analysis was properly used to calculate the pooled ORs and 95% CIs. Major findings: We eventually identified six studies evaluating the association of sarcoma risk with 309T>G polymorphism. People with 309-GG were found to have 43% greater risk of sarcoma relative to people with 309-TT (OR, 1.43; 95% CI, 1.01~2.03; Pheterogeneity, 0.45). In the G vs. T genetic model, the risk reduced to 19% (OR, 1.19; 95% CI, 1.01~1.40; Pheterogeneity, 0.50). Statistical data showed no significant heterogeneity or publication bias in the meta-analysis. Conclusion: These data demonstrate that 309T>G polymorphism located within the *MDM2* gene may act as modifier factor for sarcomas. A weakness of this analysis is that the findings cannot be explainable when the subtypes are separated and additional larger investigations are needed to identify the role of 309T>G polymorphism in each form of sarcoma.

**Keywords:** Sarcoma, *MDM2*, polymorphism, risk

### Introduction

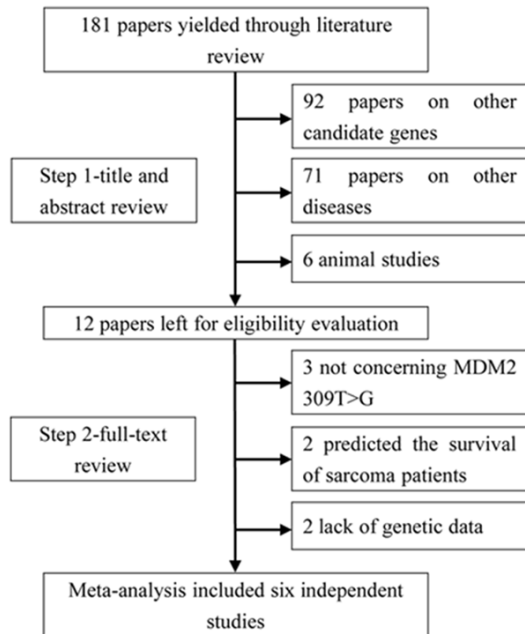
Sarcoma is a relatively rare form of cancer with a low prevalence around the world. In the United States, there are estimated 15,000 newly diagnosed cases each year, accounting for about 1% of total new cancer diagnoses [1, 2]. Sarcoma has many subdivisions (Askin's tumor, chondrosarcoma, Ewing's sarcoma, sarcoma botryoides, malignant hemangioendothelioma, malignant schwannoma, osteosarcoma, and soft tissue sarcomas) that affect people of different ages, with chondrosarcoma, gastrointestinal stromal tumor and leiomyosarcoma favoring adults, and osteosarcoma and Ewing's more prevalent in children and young adults. Collective data have suggested a reference of single nucleotide polymorphisms at *CYP19A1*, *RASSF1A*, *EWS*, and *CTLA-4* loci to the incidence of sarcomas [3-6]. This led to the hypothesis that inherited genetic factors may act as modifiers for these invasive diseases.

p53 is a well-characterized tumor suppressor known as the 'the guardian of the genome' [7], due to its fundamental role in maintaining chromosome stability by preventing genome mutation. Many signaling pathways involved in apoptosis, senescence, DNA reconstruction, and cell cycle arrest, are mediated by p53 and appropriately activated in the presence of carcinogenic agents and DNA damage, thereby inhibiting normal cells from malignant transformation [8, 9]. Mouse double minute 2 homolog (*MDM2*) is a protein encoded by the *MDM2* gene [10], a key mediator negatively regulating p53 in several conditions, such as DNA repair capability, cell death induction, and tumor suppression. *MDM2* protein has dual role as a suppressor of p53 activation at the transcriptional level and as an E3 ubiquitin ligase to recognize the N-terminal transactivation domain of p53 which, in turn activates the transcription of *MDM2*, and thereby increases the *MDM2* pro-

**Table 1.** Baseline information of the studies included in this meta-analysis

Study	Publication year	No. of cases/controls	Type	Genotyping assay	Ethnicity	Country of origin	MAF in controls (%)
Tornesello et al. (a) [13]	2011	30/88	Kaposi's sarcoma	PCR-RFLP	African	Italy	11.3
Alhopuro et al. [19]	2005	68/185	Uterine leiomyosarcoma	PCR-RFLP	Caucasian	Finland	43.2
Thurou et al. [20]	2013	24/91	Ewing sarcoma	PCR-RFLP	Caucasian	Brazil	28
Tornesello et al. (b) [13]	2011	56/122	Kaposi's sarcoma	PCR-RFLP	Caucasian	Italy	35.2
Ito et al. [21]	2011	155/37	Mixed	Taqman	Caucasian	Australia	29.7
Toffoli et al. [22]	2009	201/250	Osteosarcoma	Pyrosequencing	Caucasian	Italy	34.2

MAF: minor allele frequency.



**Figure 1.** Study flow chart for study exclusion/inclusion with specification of reasons.

tein levels (<http://en.wikipedia.org/wiki/Mdm2>).

There is a common single-nucleotide polymorphism with a T>G substitution located in the first intron of *MDM2* (309T>G, rs2279744). It enhances the affinity for Sp1 binding and subsequently upregulates the gene expression [11] and attenuates the p53 pathway. This functionally important polymorphism has been linked to earlier onset of both sporadic and inherited cancers in human [12]. *MDM2* 309T>G polymorphism is relatively seldom investigated in the field of sarcomas, especially the common forms. In addition, the published studies were conducted in a limited number of subjects of distinct ancestries, making the genetic contribution unclear and elusive. We thus summarized all available data and performed a meta-

analysis to maximize the statistical power and provide stronger evidence of the association between 309T>G polymorphism and sarcoma.

**Materials and methods**

*Identification and eligibility of relevant studies*

We exhaustively searched the PubMed, EBS-CO, BIOSIS, the Cochrane Library, ISI Web of Science, Wiley Online Library and Embase to retrieve all studies reporting on the association of 309T>G polymorphism with any type of sarcoma. Search terms were ‘mouse double minute 2 homolog’ or ‘*MDM2*’, ‘polymorphism’ or ‘polymorphisms’ or ‘variants’ or ‘genotypes’, and ‘sarcoma’. After having retrieved all potentially relevant studies, we screened the references of the publications involving both *MDM2* polymorphisms and sarcoma to identify additional papers. Selection of the eligible studies was based on the following conditions: a) written in English or in Chinese, b) a case-control or cohort study, c) investigating the genetic contribution of *MDM2* 309T>G polymorphism to sarcoma, 4) adequate genotyping information to evaluate the overall risk of sarcoma (pooled OR (odds ratio) and 95% CI (confidence interval)). Exclusion criteria included: (1) without control population; (2) without eligible genotype frequencies; (3) duplicated publications. When the same patient population was included in several studies published by the same author, we selected the most informative study with available genotype frequency.

*Data extraction*

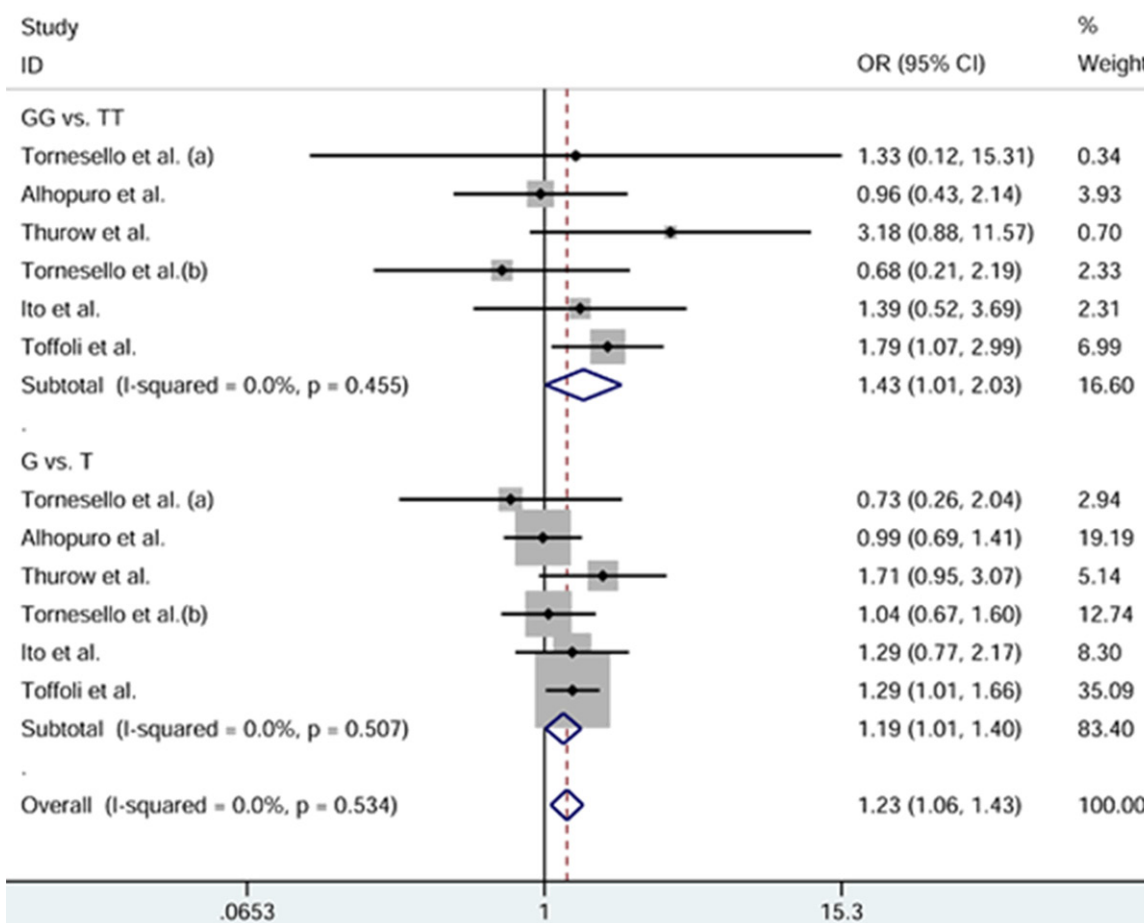
Baseline characteristics including first author's name, journal and year of publication, study country, ethnicity of the investigated populations, number of cases and controls, type of sarcoma, minor allele frequency in controls, source of controls, assay used in genotype deter-

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**Table 2.** Association of *MDM2* 309T>G polymorphism with sarcoma

Genetic models	Models used for OR calculations	$P_{\text{heterogeneous}}/I^2$ (%) <sup>*</sup>	Test of association			Publication bias	
			OR	95% CI	$P_{\text{OR}}$	$P_{\text{Begg}}$	$P_{\text{Egger}}$
GG vs. TT	Fixed effects	0.45/0	1.43	(1.01, 2.03)	0.04	0.70	0.67
GG + TG vs. TT <sup>†</sup>	Fixed effects	0.78/0	1.16	(0.95, 1.41)	0.14	1.00	0.80
GG vs. TG + TT <sup>‡</sup>	Fixed effects	0.25/24.0	1.31	(0.94, 1.81)	0.11	1.00	0.39
G vs. T	Fixed effects	0.50	1.19	(1.01, 1.40)	0.03	0.70	0.97
TG vs. TT	Fixed effects	0.69	1.17	(0.93, 1.46)	0.18	1.00	0.98

<sup>\*</sup>Heterogeneity across studies. <sup>†</sup>Dominant model. <sup>‡</sup>Recessive model.



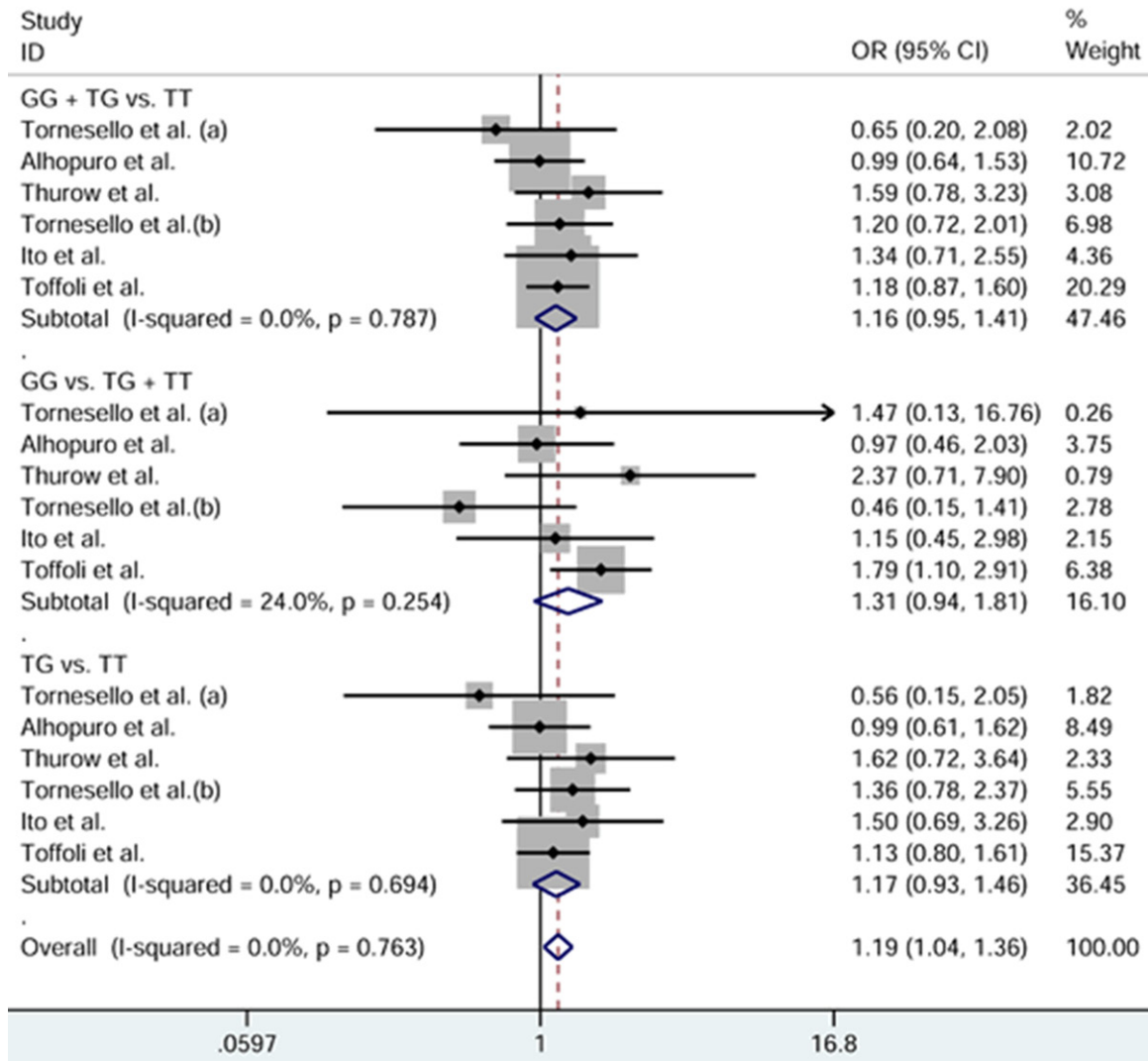
**Figure 2.** ORs of overall sarcoma risk associated with *MDM2* 309T>G polymorphism under GG vs. TT model and the G vs. T model. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI.

mination, and count of TT, TG, GG genotypes were separately collected by two investigators. In cases of discrepancies, an expert in this field was invited to find a final solution. As Tornesello et al. provided detailed information on two independent populations with different ethnicities [13], we appropriately categorized the populations into the ethnic groups and their data were collected in separation.

### Statistical methods

The association between 309T>G polymorphism located in *MDM2* and sarcoma risk was assessed using OR and 95% CI. The pooled ORs were evaluated under the assumption of GG vs. TT, GG + TG vs. TT, GG vs. TG + TT, G vs. T, and TG vs. TT. The significance was determined using the Z test. Between-study hetero-

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**Figure 3.** ORs of overall sarcoma risk associated with *MDM2* 309T>G polymorphism under the dominant model, the recessive model and the TG vs. TT model. For each study, the estimates of OR and its 95% CI were plotted with a box and a horizontal line. The symbol filled diamond indicates pooled OR and its 95% CI.

geneity was measured by the Q statistic and the  $I^2$  statistic, a value expressed between 0% and 100% (0-25%: low heterogeneity, 25%-50%: moderate heterogeneity, 50%-100%: considerable heterogeneity) [14]. In a condition that  $P$  values  $>0.05$  and  $I^2$  statistic  $<50\%$ , the combined values were calculated with a fixed effects meta-analysis (the Mantel-Haenszel method), while a random effects meta-analysis (the DerSimonian and Laird method) was used when the  $P$  values  $\leq 0.05$  and the  $I^2$  statistic  $\geq 50\%$  [15, 16].

The consistency with Hardy-Weinberg equilibrium (HWE) was checked in controls by  $\chi^2$  analysis. Potential publication bias was determined

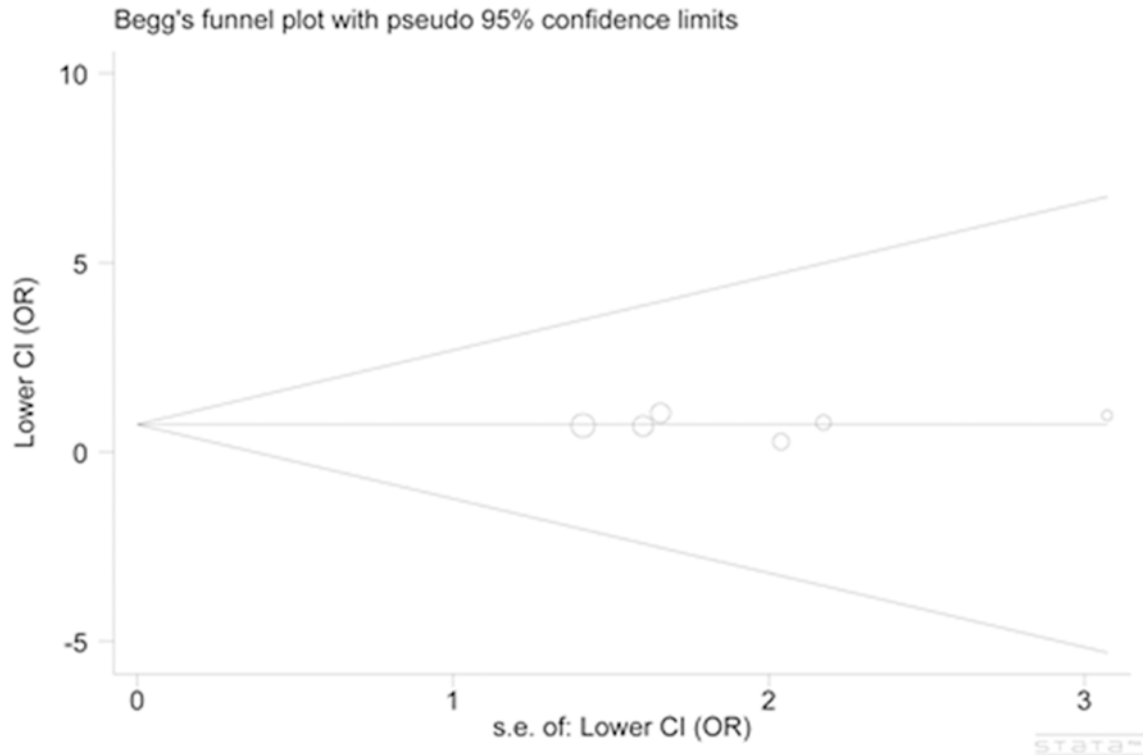
by constructing funnel plots and the symmetry was examined by Egger's test [17]. We also conducted the leave-one-out sensitivity analysis to examine robustness of the combined effect estimates [18]. All tests were two-tailed.

Statistical data were analyzed by use of Stata software (version 12.0). The significance level was fixed at a  $P$  value less than 0.05 for all analyses unless otherwise emphasized.

### Results

#### *Eligible studies and studies' characteristics*

The literature review yielded 181 papers. We reviewed all titles and abstract to exclude the



**Figure 4.** Funnel plot analysis to detect publication bias.

papers apparently irrelevant to or marginally associated with the current subject (n=169). The full-texts of the remaining 12 papers were retrieved and screened. 7 papers were excluded due to the following reasons: concerning *MDM2* polymorphisms other than 309T>G, using 309T>G to predict the survival of sarcoma patients, and lack of the genetic data that could help to calculate pooled ORs and 95% CIs. As a result, five papers fulfilled all pre-defined inclusion criteria and included in the meta-analysis [13, 19-22] (**Table 1**). **Figure 1** presents a flow chart of study exclusion/inclusion with specification of reasons. Of the included papers published between 2005 to 2013, one focused on Kaposi's sarcoma, one on Uterine leiomyosarcoma, one on Ewing sarcoma, one on osteosarcoma and one investigated the association of 309T>G polymorphism with various types of sarcoma. PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotype determination in all publications except for Ito et al. and Toffoli et al., in which Taqman and pyrosequencing were used, respectively [21, 22]. Departure from HWE was observed in one study [21] (**Table 2**).

#### Quantitative synthesis

The meta-analysis results are displayed in detail in **Table 2**.

When all available data were pooled into one data set, we found a significant association between *MDM2* 309T>G polymorphism and sarcoma risk. The association was more pronounced in the GG vs. TT model, showing that people with 309-GG genotype were 43% more likely than people with 309-TT genotype to develop sarcoma (OR, 1.43; 95% CI, 1.01~2.03; P heterogeneity, 0.45, **Figure 2**). Using the G vs. T genetic model, we found 19% higher risk among people carrying the 309-G allele (OR, 1.19; 95% CI, 1.01~1.40; P heterogeneity, 0.50, **Figure 2**). The risk estimates revealed under the dominant model, the recessive model and the TG vs. TT model were not statistically significant (**Figure 3**).

#### Sensitivity analysis

To check robustness of the combined estimates, we performed the leave-one-out sensitivity analysis. The pooled ORs remained unaltered during the sequential removals of the



single studies. This indicated that our findings are robust (data not shown).

### Heterogeneity test

There was no indication of significant heterogeneity in the meta-analysis evaluating sarcoma risk in association with *MDM2* 309T>G polymorphism ( $P>0.05$  and  $I^2<50\%$ , **Table 2**). Therefore, the fixed effects meta-analysis was appropriately selected for pooled OR and 95% CI calculations.

### Publication bias

The funnel plots for all tested genetic models seemed symmetrical. **Figure 4** displays the funnel plot constructed under the G vs. T model. To confirm the visual symmetry, we performed the Egger's test, with the statistical evidence suggesting no publication bias in this study ( $P>0.05$ ). The details are presented in **Table 2**.

### Discussion

The purpose of this study was to test the hypothesis that 309T>G polymorphism in the *MDM2* gene is associated with sarcomagenesis. We identified six studies with available data and carried out a meta-analysis, demonstrating that the people carrying either two 309-G alleles or the single G allele were at higher risk of sarcoma compared with those who harbored two T alleles or merely one T allele. To the best of our knowledge, this is the largest study to evaluate the effects *MDM2* 309T>G polymorphism confers on sarcoma, and it is the maximized sample size along with absence of publication bias and between-study heterogeneity that makes these risk estimates more reliable and powerful.

The derived significant estimates are sustained by most published studies examining the association of 309T>G polymorphism with sarcoma risk. Tornesello et al. suggested that G allele of the *MDM2* promoter polymorphism that increases transcription of this major negative regulator is related to 2.38-fold (95% CI, 1.0-5.5) increased risk of classic Kaposi's sarcoma among Caucasian population, but not African subjects [13]. An obviously higher risk was identified by Toffoli et al., who found 4.26-fold (95% CI, 1.61~11.25) elevated risk of developing high-grade osteosarcoma in females

harboring the GG genotype [22]. A most recent study, conducted by Thurow et al., demonstrated evidence of almost 3 times (OR, 2.97; 95% CI, 1.03~8.58) higher risk of Ewing Sarcoma among people carrying both TG and GG genotypes [20]. These results are in line with our findings which supports the hypothesis of a causal correlation between 309T>G polymorphism and sarcoma.

However, Alhopuro et al. showed different observations. They found that 309T>G polymorphism has no significant contribution to uterine leiomyosarcoma formation [19]. This is biologically possible, as substantiated by Post et al. observing upregulated *MDM2* protein and mRNA levels attributable to the presence of 309-GG genotype in all investigated tissues with the possible exception of brain and uterus [23]. This may suggest that the promoter polymorphism mediates *MDM2* activities in a tissue-specific manner. Therefore, it is substantially important to identify its pathogenic role by independently investigating the subtypes of sarcoma in a substantially large number to provide strong evidence, thus facilitating sarcoma prevention, protection and therapy.

Sarcoma is a class of invasive cancers. Hence previous reports on human carcinogenesis may have some implications. A large-scale meta-analysis containing 26,160 cancer cases and 33,046 controls identified substantially declined risk of prostate cancer and significantly elevated risk of bladder, colorectal, lung, and gastric cancers in relation to 309T>G polymorphism [24]. Many groups have recently confirmed such a positive association [25-27]. Based on the earlier association studies, meta-analyses and the present work, it seems that 309T>G polymorphism acts as modifier factor for some human diseases, but not all.

Several limitations are suggested to be taken into consideration in explaining our results. First, since existing studies have shown that the *MDM2* promoter polymorphism confers genetic susceptibility in a tissue-specific fashion, the risk of sarcomas should be assessed in isolation in case of sufficient data. Second, sarcomas may develop with considerable geographical variation in their respective prevalence. Thus it is likely that the ethnic populations are not equally susceptible to these diseases. However, we are not able to confirm the differ-

ence based on the current data we have summarized. Third, the polymorphisms usually do not work alone in predisposing individual susceptibility. A more precise estimation could be derived when gene-to-gene and gene-environment interactions are considered.

In conclusion, 309T>G polymorphism in the *MDM2* promoter appears to be an important risk factor in the etiology of sarcoma. Its role in sarcomagenesis requires to be confirmed in additional studies among different populations.

#### Disclosure of conflict of interest

None.

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