MDM2 Promoter Polymorphism SNP309 Contributes to Tumor Susceptibility: Evidence from 21 Case-Control Studies

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Abstract

Since the identification of a well-characterized functional polymorphism named SNP309 in *MDM2*, abundant studies were published in the last 2 years to evaluate the association between SNP309 and tumor risk in diverse populations. However, the results remain conflicting rather than conclusive. Because a single study may have been underpowered to detect the effect of low-penetrance genes, a quantitative synthesis to accumulate data from different studies may provide better evidence on the association of genetic variant with tumor susceptibility. We conducted a meta-analysis on 14,770 cases with different tumor types and 14,524 controls from 25 published case-control studies to estimate the effect of SNP309 on tumor risk, as well as to quantify the potential between-study heterogeneity. We found that variant homozygote 309GG was associated with a significantly increased risk of all types of tumors [homozygote comparison: odds ratio (OR), 1.17, 95% confidential interval (95% CI), 1.04-1.33, P = 0.0002 for heterogeneity test; recessive model comparison: OR, 1.15, 95% CI, 1.03-1.28, P = 0.0005 for heterogeneity test]. Tumor type and ethnicity contributed to the substantial heterogeneity (69.5% for homozygote comparison and 77.2% for recessive model comparison). The analyses suggest that *MDM2* SNP309 serves as a low-penetrance susceptibility tumor marker. Further large studies incorporate quantitative detection of different *p53*-responsible environmental stresses, *p53* mutation status, and also functional genetic variants in p53-MDM2–related genes are warranted. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2717–23)

Introduction

The *p53* tumor suppressor gene is most frequently inactivated in human malignancies. It can play an important role in tumor etiology because the dysfunction of p53 leads to the accumulation of genetic errors through ineffective orchestration of multiple biological processes, including cell cycle arrest, DNA repair, cell senescence, and apoptosis (1, 2). MDM2 directly binds to p53 and acts as a crucial negative modulator for maintaining function of p53 through regulating its location, stability, and activity (3). A subset of tumors overexpresses MDM2, which is associated with accelerated cancer progression (4) and poor prognosis (5). Overexpression of MDM2 could be mutually exclusive to p53 mutation, suggesting that overexpression of MDM2 can substitute for inactivating the mutation of p53 (6-8). Although the p53-independent tumorigenicity of MDM2 is not fully understood, MDM2 binds to a number of proteins with various functions (9) and has implications in both cancer prevention and therapy.

Human MDM2 protein shares ~78% homology with mice, and the genomic structures and coding sequences of MDM2 mRNA between mice and human beings are similar (Genbank accession no. NM_002392 for human or U40145 for mice; Fig. 1). Two promoters were reported for *mdm2* in mice (10). One is an internal *mdm2* promoter (P2), located near the 3′-end of intron 1, and can be activated by p53 through its tandem p53-binding motifs. Meanwhile, the upstream *mdm2* promoter (P1) is only mildly affected by p53. In humans, a functional p53-responsive intronic promoter was also found within the first intron (ref. 11; Fig. 1). Recently, a T-to-G substitution at the 309th nucleotide (SNP309) was identified in this region, resulting in higher levels of MDM2 mRNA/protein for the mutant G-allele through specifically interacting with a transcriptional activator Sp1 (12). The stressed *MDM2* GG homozygote cell lines were associated with an attenuated p53 pathway and reduced levels of wild-type p53 (12). Moreover, in the GG fibroblast cell line derived from tumor-prone individuals with Li-Fraumeni syndrome (one *p53* allele mutated), significant higher levels of MDM2 were also found, and the p53 pathway was further weakened (12). In both hereditary Li-Fraumeni syndrome patients and adult sporadic soft tissue sarcoma patients, the presence of the SNP309

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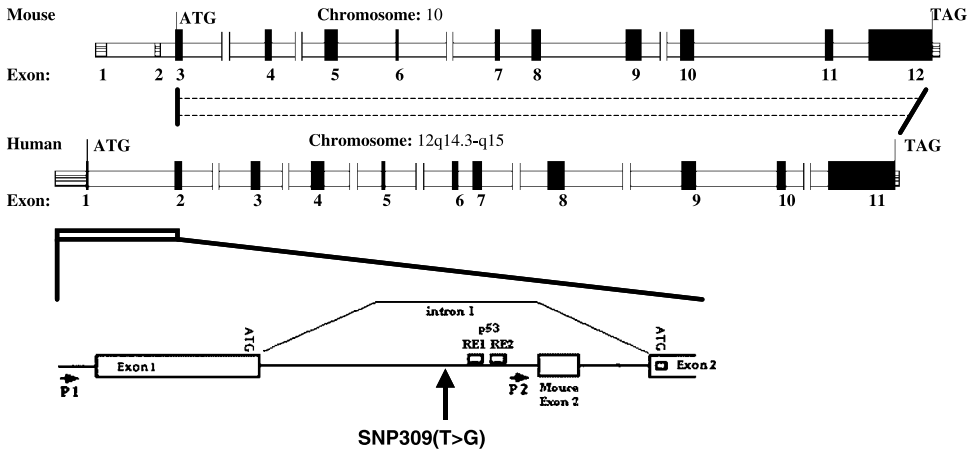

Figure 1. Gene structures of mouse and human *MDM2* and location of SNP309.

G-allele accelerated tumor formation as a rate-limiting event, and in tumor-prone Li-Fraumeni syndrome individuals, SNP309 can cause the occurrence of multiple primary tumors in a lifetime (12).

Thereafter, emerging studies have been done in the last 2 years to evaluate the association between *MDM2* SNP309 and tumor risk in diverse populations (13-37). The tumor types in the case populations included lung, breast, colorectal, bladder, ovarian, head and neck, hepatocellular, gastric, and so on. In consideration of the extensive role of MDM2 in the carcinogenic process, we carried out a systematic review and meta-analysis on

Table 1. Characteristics of literatures included in the meta-analysis

Reference	Tumor type	Country of origin	Ethnicity	Matching criteria	Sample size (case/control)	MAF in controls	Detection of *p53* mutation status
Alhopuro et al., 2005 (13)	Uterine leiomyosarcoma	Finland	European	—	68/185	0.43	No
	Colorectal cancer	Finland	European	—	969/185	0.43	No
	Squamous cell carcinoma of the head and neck	Finland	European	—	157/185	0.43	No
Allazzouzi et al., 2006 (14)	Colorectal cancer	Spain	European	—	152/184	0.30	Yes
Boersma et al., 2006 (15)	Breast cancer	America	European	Age	125/136	0.33	Yes
Campbell et al., 2006 (16)	Breast cancer	England	European	Area	351/258	0.38	No
	Ovarian cancer	England	European	Area	302/258	0.38	No
Li et al., 2006 (17)	Lung cancer	America	European	Age, sex, and smoking status	1,026/1,145	0.39	No
Lind et al., 2006 (18)	Lung cancer	Norway	European	—	341/412	0.36	Yes
Menin et al., 2006 (19)	Colorectal cancer	Italy	European	Area	153/92	0.35	Yes
Millikan et al., 2006 (20)	Breast cancer	America	European	Age	1,270/1,133	0.37	Yes
Onat et al., 2006 (21)	Bladder cancer	Turkey	European	Age	75/103	0.44	No
Petenkaya et al., 2006 (22)	Breast cancer	Turkey	European	Age	223/149	0.52	No
Pine et al., 2006 (23)	Lung cancer	America	European	Age and sex	371/421	0.35	No
Wasielewski et al., 2006 (24)	Breast cancer	Netherlands	European	Area	343/126	0.43	No
Wilkening et al., 2006 (25)	Breast cancer	Germany	European	—	549/1,065	0.36	No
Wilkening et al, 2007 (26)	Basal cell carcinoma of the skin	Germany	European	Gender and area	509/513	0.38	No
Dharel et al., 2006 (27)	Hepatocellular carcinoma	Japan	Asian	—	187/48	0.54	No
Hong et al., 2005 (28)	Esophageal squamous cell carcinoma	China	Asian	Age and sex	758/1,420	0.46	No
Hu et al., 2006 (29)	Lung cancer	China	Asian	Age, sex, and area	717/1,083	0.50	No
Ma et al., 2006 (30)	Breast cancer	China	Asian	Age	366/605	0.51	No
Ohmiya et al., 2006 (31)	Gastric carcinoma	Japan	Asian	Sex	410/438	0.50	Yes
Park et al., 2006 (32)	Lung cancer	Korea	Asian	Age and sex	582/582	0.53	No
Zhang et al., 2006 (33)	Lung cancer	China	Asian	Age and sex	1,106/1,420	0.46	No
Hirata et al., 2007 (34)	Renal cell carcinoma	Japan	Asian	Age and sex	200/200	0.45	No
Zhou et al., 2007 (35)	Nasopharyngeal carcinoma	China	Asian	—	803/763	0.52	No
Boersma et al., 2006 (15)	Breast cancer	America	African	Age	165/178	0.08	Yes
Millikan et al., 2006 (20)	Breast cancer	America	African	Age	767/680	0.11	Yes
Pine et al., 2006 (23)	Lung cancer	America	African	Age and sex	133/255	0.11	No
Walsh et al., 2007 (36)	Endometrial cancer	America	Mixed	—	73/79	0.35	No
Cox et al., 2007 (37)	Breast cancer	America	Mixed	Age and menopausal status	1,519/2,271	0.35	No

all eligible case-control studies to estimate the overall tumor risk of *MDM2* SNP309 polymorphism and to quantify the potential between-study heterogeneity.

Materials and Methods

Identification and Eligibility of Relevant Studies. We included all the case-control studies published to date on the association between *MDM2* SNP309 and tumor risk. Eligible studies were identified by searching the electronic literature MEDLINE for relevant reports (last search update August 31, 2007, using the search terms ''*MDM2* polymorphism(s) and tumor'') by two independent investigators (Z.H. and G.J.). Additional studies were identified by a hand search of references of original studies or review articles on this topic. If studies had partly overlapped subjects, only the one with a larger sample size was selected (16, 38). For the studies with shared controls (28, 33), they were separated into two studies only in subgroup analysis by tumor types. Hence, the data for this analysis were available from 25 case-control studies, including 14,770 cases with different types of tumor and 14,524 controls.

Data Extraction. Two investigators independently extracted the data and reached consensus on all items. The following information was sought from each publications: the first author's name, year of publication, tumor type, country of origin, ethnicity, matching criteria, number of cases and controls, minor allele frequency (MAF) in controls, *p53* mutation status, genotype frequency for cases and controls, characteristics for cases, source of DNA, genotyping methods, and quality control (Table 1 and Supplementary Table). Different ethnicity descents were categorized as European, Asian, and African. When studies included subjects of more than one ethnicity (15, 20, 23), genotype data were extracted separately according to ethnicities for subgroup analyses. Two studies without exact ethnic information for different genotypes were excluded in the subgroup analyses (36, 37).

Statistical Analysis. The risks of tumors associated with the *MDM2* SNP309 polymorphism was estimated for each study. The fixed-effects model and the random-effects model, based on the Mantel-Haenszel method and the DerSimonian and Laird method, respectively, were used to pool the data from different studies (39). These two models provide similar results when heterogeneity between studies is absent; otherwise, the random-effects model is more appropriate. We first estimated the risks of the variant genotype GG and GT, compared with the wild-type TT homozygote, and then evaluated the risks of (GG + GT) versus TT and GG versus (GT + TT), assuming dominant and recessive effects of the variant G allele, respectively. Subgroup analyses, according to tumor type (if one tumor type contains less than three individual studies, it was combined into the ''other tumors'' group), ethnicity, *p53* mutation, and sample size (subjects more than 300 in both cases and controls) were also done. Statistical heterogeneity between studies was assessed with the χ^2-based Q test, and the heterogeneity was considered significant when $P < 0.1$ (40). Sources of heterogeneity were determined by using random-effects meta-regression models with restricted maximum likelihood estimation. The interstudy variance (τ^2) was used to quantify the degree of heterogeneity between studies, and the percentage of τ^2 was used to describe the extent of explained heterogeneity of the characteristics (41). Publication bias was evaluated with the funnel plot and the linear regression asymmetry test by Egger et al. (42). A significance level of 0.1 was used as an indication for the presence of potential publication bias. All analyses were done using the SAS software (v.9.1.3) and Review Manage (v.4.2).

Results

Characteristics of Studies. Twenty-five publications on *MDM2* SNP309 genotypes and tumor risk were identified. The selected study characteristics were summarized in Table 1. All studies were case-control studies, including eight breast cancer studies, six lung cancer

Table 2. Summary ORs of the *MDM2* T309G polymorphism and tumor risk

	Comparisons	Cases/controls	GG versus TT, OR (95% CI)	P*	Cases/controls	Dominant model (GG/TG versus TT), OR (95% CI)	P*	Recessive model (GG versus TT/TG), OR (95% CI)	P*
Total	24	8,110/7,954	1.17 (1.04-1.33)†	0.0002	14,770/14,524	1.07 (1.00-1.16)†	0.01	1.15 (1.03-1.28)†	0.0005
Tumor types									
Breast cancer	8	3,284/3,853	1.00 (0.89-1.12)	0.55	5,678/6,601	1.04 (0.96-1.12)	0.88	0.98 (0.88-1.09)	0.52
Lung cancer	6	2,247/2,756	1.26 (0.97-1.62)†	0.001	4,276/5,318	1.11 (0.91-1.34)†	0.0005	1.20 (1.01-1.44)†	0.02
Colorectal cancer	3	670/260	0.90 (0.63-1.29)	0.74	1,274/461	1.00 (0.79-1.27)	0.13	0.93 (0.68-1.29)	0.59
Other cancers	10	1,909/2,028	1.35 (1.09-1.68)†	0.03	3,542/4,007	1.09 (0.94-1.28)†	0.06	1.35 (1.14-1.59)†	0.06
Smoking-related cancer	8	2,778/2,889	1.26 (0.99-1.61)†	0.0004	5,266/5,606	1.08 (0.91-1.27)†	0.002	1.24 (1.03-1.49)†	0.004
Ethnicity									
European	14	3,746/3,178	1.05 (0.90-1.23)†	0.06	6,984/5,922	0.98 (0.91-1.06)	0.14	1.06 (0.92-1.23)†	0.05
Asian	8	2,625/2,570	1.37 (1.23-1.53)	0.11	5,129/5,139	1.21 (1.10-1.32)	0.36	1.25 (1.09-1.45)†	0.03
African	3	848/921	0.75 (0.40-1.39)	0.85	1,065/1,113	1.15 (0.94-1.42)	0.10	0.73 (0.39-1.35)	0.81
p53 mutation status									
Positive	4	262/575	1.33 (0.72-2.45)†	0.03	472/1,126	1.19 (0.94-1.50)	0.62	1.29 (0.71-2.34)†	0.01
Negative	4	195/575	1.05 (0.72-1.55)	0.84	363/1,126	1.08 (0.84-1.40)	0.56	1.09 (0.77-1.54)	0.39

*Test for heterogeneity.
† Random-effects model was used when P value for heterogeneity test <0.1; otherwise, fixed-effects model was used.

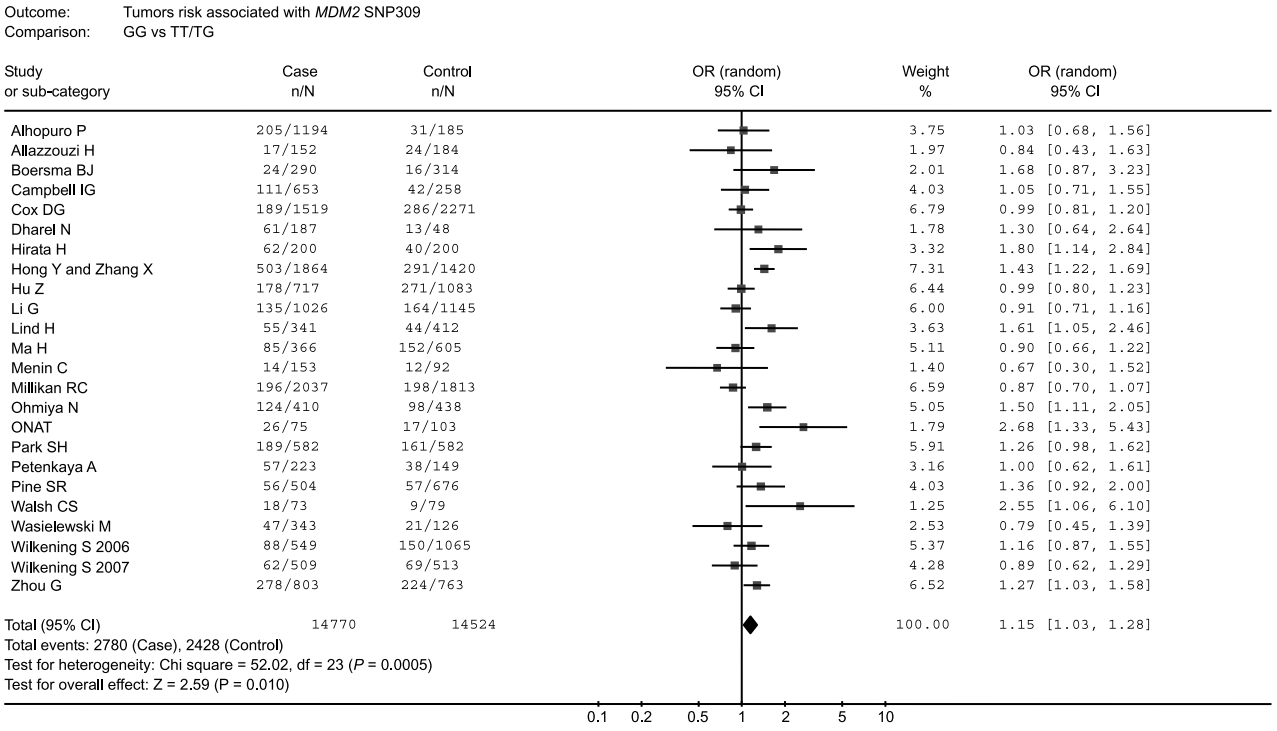

Figure 2. ORs (log scale) of tumors associated with *MDM2* SNP309 for the GG genotype compared with the TT/TG genotypes.

studies, three colorectal cancer studies, and the others were categorized into the ''other tumor'' group. There were eleven studies of European descent, nine studies of Asian descent (two studies shared controls, refs. 28, 33), three studies of both European and African descent, and two studies of mixed ethnicity descent. Only six studies detected *p53* mutation status in tumor tissues from cases (14, 15, 18-20, 31), but two of them did not present *MDM2* SNP309 genotype distributions according to the *p53* mutation status (15, 20). Cases in most of the studies were histologically diagnosed, and three studies obtained DNA from tumor tissue of breast cancer (15), colorectal cancer (19), and uterine leiomyosarcoma (13). Diverse genotyping methods were used, including PCR-RFLP, TaqMan, direct sequencing, amplification refractory mutation system-PCR, primer-introduced restriction analysis-PCR, and PCR-single-strand conformational polymorphism; however, only 72% (18/25) of the studies mentioned quality control of the genotyping, such as blindness to the case-control status, random repeat, or validation using a different genotyping method. The distribution of genotypes in the controls was consistent with Hardy-Weinberg equilibrium in all studies except for three (14, 20, 31).

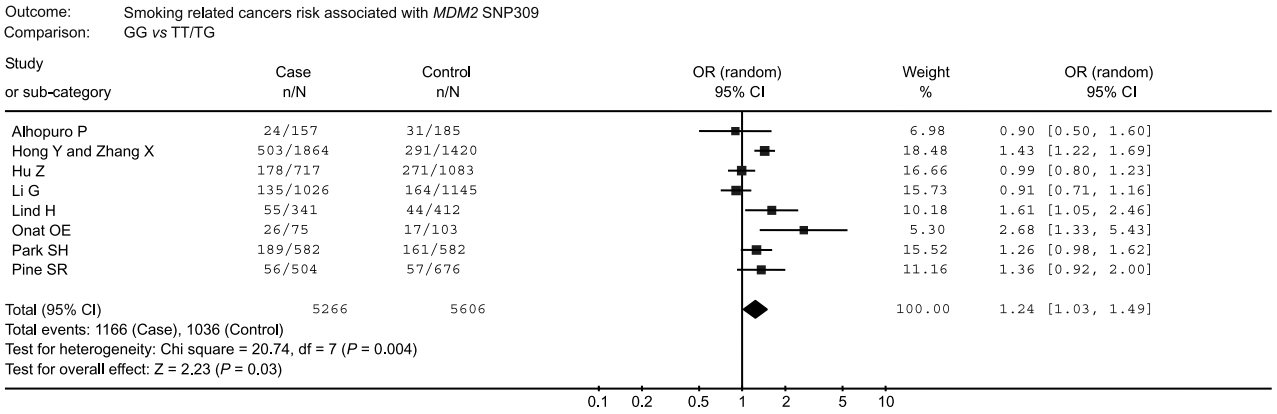

Figure 3. ORs (log scale) of smoking-related cancers associated with *MDM2* SNP309 for the GG genotype compared with the TT/TG genotypes.

Quantitative Synthesis. There was a wide variation in the *MDM2* 309G allele frequency across different ethnicities, ranging from 0.08 in an African population (15) to 0.54 in an Asian population (27). The mean frequency of 309G allele was 0.38 for European, 0.49 for Asian, and 0.11 for African.

When all the eligible studies were pooled into the meta-analysis, the variant genotypes were associated with increased tumor risk in different genetic models. As shown in Table 2, the variant homozygote (309GG) was associated with a significantly increased risk of all types of tumors when compared with wild-type homozygote [309TT; odds ratio (OR), 1.17; 95% confidential interval (95% CI), 1.04-1.33; $P = 0.0002$ for heterogeneity test]. However, the variant heterozygote (309TG) seemed to be only a minor modifier on tumor risk (OR, 1.04; 95% CI, 0.98-1.09; $P = 0.11$ for heterogeneity test). Significant main effects were also shown both in dominant and recessive models (dominant model: OR, 1.07; 95% CI, 1.00-1.16; $P = 0.01$ for heterogeneity test; recessive model: OR, 1.15; 95% CI, 1.03-1.28; $P = 0.0005$ for heterogeneity test; Table 2).

We then evaluated the effects of *MDM2* SNP309 according to specific tumor types, different ethnicities, and different *p53* mutation status. We found that individuals with the 309GG genotype were associated with elevated risks of lung cancer and the ''other tumor'' but not of breast or colorectal cancers when compared with subjects with combined TT/TG genotypes (recessive model; Table 2, Fig. 2). When we combined lung cancer, squamous cell carcinoma of the head and neck (including esophageal cancer), and bladder cancer as smoking-related cancers, significant increased risk was also observed for GG genotype, compared with TT/TG genotypes (OR, 1.24; 95% CI, 1.03-1.49; $P = 0.004$ for heterogeneity test; Table 2, Fig. 3).

In the subgroup analysis on ethnicity, significantly elevated risks were associated with SNP309 variant genotypes in the Asian population in all models tested (GG versus TT: OR, 1.37; 95% CI, 1.23-1.53; $P = 0.11$ for heterogeneity test; dominant model: OR, 1.21; 95% CI, 1.10-1.32; $P = 0.36$ for heterogeneity test; recessive model: OR, 1.25; 95% CI, 1.09-1.45; $P = 0.03$ for heterogeneity test). However, no significant associations were found for European and African populations (Table 2).

Only four studies had detailed genotype information according to *p53* mutation status in the cases. We then dichotomized cases to *p53* mutation-positive and *p53* mutation-negative subgroups to compare them with controls, but we did not find significant associations in any models tested (Table 2).

Test of Heterogeneity. Heterogeneity between studies was observed in overall comparisons and also subgroup analyses. We evaluated the source of heterogeneity for the GG genotype (GG versus TT and GG versus TT/TG) by tumor type, ethnicity, *p53* mutation status, and sample size. We found that tumor type (GG versus TT: $\chi^2 = 17.48$, $df = 3$, $P = 0.0006$; GG versus TT/TG: $\chi^2 = 22.12$, 22.12, $df = 3$, $P < 0.0001$) and ethnicity (GG versus TT: $\chi^2 = 10.54$, $df = 2$, $P = 0.005$; GG versus TT/TG: $\chi^2 = 7.89$, $df = 2$, $P = 0.02$) do contribute to substantial altered heterogeneity, but not the *p53* mutation status and sample size. Furthermore, meta-regression analyses revealed that tumor type can explain 30.5% (GG versus TT, $P = 0.0078$) or 60.4% (GG versus TT/TG, $P = 0.0006$) of the τ^2, whereas ethnicity can explain 68.1% (GG versus TT, $P < 0.0001$) or 55.4% (GG versus TT/TG, $P = 0.0003$) of the τ^2, respectively. Interestingly, 69.5% ($P = 0.0005$) and 77.2% ($P = 0.0002$) of the between-studies heterogeneity could be explained by tumor type and ethnicity for the homozygote comparison and recessive model comparison, respectively. In contrast, sample size could not explain any of the between-studies heterogeneity in different comparisons.

Publication Bias. Funnel plot and Egger's test were done to access the publication bias of literatures. As shown in Fig. 4, the shapes of the funnel plots seemed asymmetrical in both homozygote comparison and recessive model comparison, suggesting the presence of publication bias. Then, an Egger's test was used to provide statistical evidence for funnel plot asymmetry, which is more pronounced when the larger of the intercept deviated from zero in linear regression analysis. We obtained the intercept value of 1.47 and 1.28 for homozygote and recessive model comparisons ($t = 2.97$ and $P = 0.007$ for GG versus TT and $t = 2.36$ and $P = 0.027$ for GG versus TT/TG), respectively.

Discussion

On the basis of 25 case-control studies focused on *MDM2* SNP309 and tumor risk, our meta-analysis provided evidence that variant homozygote GG of *MDM2* SNP309

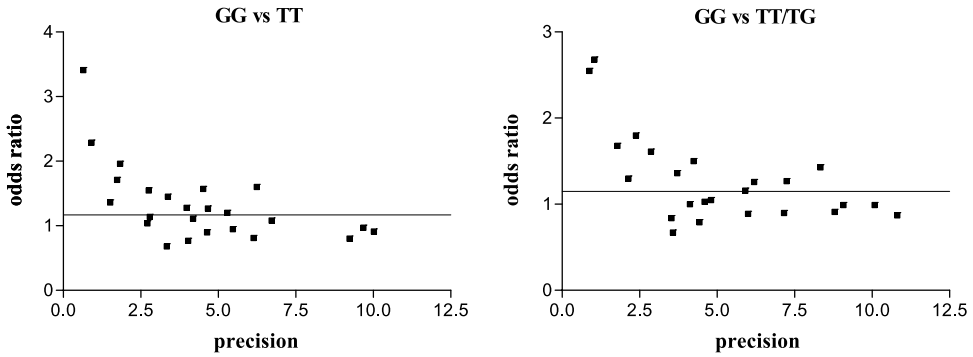

Figure 4. Funnel plot analysis to detect publication bias. Each point represents a separate study for the indicated association. For each study, the OR is plotted on a logarithmic scale against the precision (the reciprocal of the SE).

was associated with a modest but significantly increased risk of tumors, especially of lung cancer or smoking-related cancers.

MDM2 is one of the central nodes in the p53 pathway. The proper regulation of MDM2 levels has been shown to be vital for p53 tumor suppression, and even a modest change in levels could affect the p53 pathway and, subsequently, cancer development in mouse models (43). The study by Bond et al. (12) revealed that SNP309 GG cell lines expressed higher levels of MDM2 (on average 8-fold mRNA and 4-fold protein levels) than TT cell lines, whereas intermediate protein levels (on average 1.9-fold) were observed in four heterozygous (TG) cell lines. Furthermore, Hong et al. showed that SNP309 GG carriers had significantly higher *MDM2* mRNA expression in esophageal tissue than TT carriers, but the TG heterozygote did not confer an increased *MDM2* transcription (28). More recently, Hirata et al. showed that the renal cell carcinoma tissues from GG carriers were more frequently positively stained for MDM2 than those with TT genotype (50% versus 13%), whereas 26% positive staining was detected in TG genotype (34). Consistent with these observations, our meta-analysis showed that, in the population level, individuals carrying the GG genotype were associated with a higher tumor risk than subjects with the TT genotype, but the increased risk of the TG genotype was less significant.

Tobacco smoking is one of the most common carcinogenic exposures eliciting many kinds of cellular stresses. Under cellular stresses, *p53* could be activated, but *MDM2* SNP309 can serve as an important mediator upon this response. For example, 309TT cells have a 5- to 14-fold increased p53 protein level upon the stress signal, whereas 309GG cells only have a 2- to 3-fold increase (43). Furthermore, Bond et al. (12) also showed that MDM2 targets p53 for degradation only in stressed GG cells, and in nonstressed cells, heightened levels of MDM2 do not further reduce the levels of wide-type p53. Thus, it is biologically plausible that different tumors with different carcinogenic mechanism and environmental exposures had disparate responses to the basis of SNP309 genotypes. In our meta-analysis, we found that the effects of SNP309 were more evident in lung cancer and smoking-related cancers but not in breast cancer and colorectal cancer. Additive interactions between SNP309 and smoking dose were observed in both esophageal squamous cell carcinoma and lung cancer (28, 33). Furthermore, the estrogen receptor (ER) status may be an interpretation for the null association between *MDM2* SNP309 and breast cancer because estrogen-signaling pathway plays an important role in *MDM2* regulation and breast cancer carcinogenic process (43). Several reports showed that higher MDM2 levels were expressed in ER-positive tumors or cell lines than ER-negative ones (8, 44–47). The effect of the estrogen-signaling pathway on MDM2 transcription was mediated by *MDM2* SNP309 that estrogen preferentially stimulated transcription of *MDM2* from the SNP309 G allele and higher MDM2 levels in SNP309 homozygous cells (48), which may result in earlier and more breast cancer cases with SNP309G alleles in ER-positive tumors (20, 49). In addition, gender should be taken into consideration in further studies, which may account for the lack of observed effects of SNP309 on colorectal cancer, because SNP309 were more frequent in women than in men affected with colorectal cancer (13), and a more significant effect of SNP309 on lung cancer was also observed in females (18).

p53 is the most frequently mutated gene in human tumors (50). In view of the robust effect of *p53* mutation in carcinogenesis, the impact of SNP309 on the Li-Fraumeni syndrome has been characterized in several studies, showing that SNP309 G-allele accelerated tumor formation and caused the occurrence of multiple primary tumors in a lifetime for *P53* mutation carriers (12, 51-53). Therefore, it is necessary to incorporate the mutation status of *p53* when the effects of *MDM2* SNP309 on tumors are explored. Thus far, we had only four studies to pool the genotypes in cases according to *p53* mutations (14, 15, 19, 31). However, no significant discrepancy was found in the two *p53* mutation subgroups (Table 2), probably because of the insufficient statistical power. In a lung cancer study (18) and a gastric cancer study (31), significant higher risks were associated with SNP309 GG genotype (recessive model) among the p53 mutation-positive subgroup. Furthermore, the potentially functional SNP (codon 72) in *p53* had been implicated to interact with SNP309 in carcinogenesis of esophageal squamous cell carcinoma and lung cancer (28, 33). Further functional and molecular epidemiologic studies were suggested to explore the joint/interaction effects between functional polymorphisms in p53-MDM2–related genes and *p53* mutation status in cancer susceptibility.

A clear association between SNP309 and tumor risk was indicated in Asians but not in Europeans or in Africans (Table 2). Although the exact mechanism for this ethnic difference was not clear, several concerns may account for it. First, four of the nine (two shared controls) Asian studies investigated smoking-related cancer (weighted 60.9% and 59.7% in comparisons of GG versus TT and GG versus TT/TG), whereas only four out of 14 studies focused on smoking-related cancer in the European population (weighted 32.9% and 31.2% in comparisons of GG versus TT and GG versus TT/TG). Second, different genetic background and environmental exposures, as projected by the marked difference of SNP309 MAF among the three populations, may play a role because the highly different MAF might be a reflection of natural selection pressures (stresses) or a balance by other related functional genetic variants. Of course, given the multiplicity of possible comparisons and the unavoidable flexibility of choosing and defining the correlates, associations may have been detected by chance alone. For example, selection bias, matching criteria and adjustment in the statistical analyses, misclassifications on disease status and genotyping, and publication bias all may be involved.

In conclusion, the result of this meta-analysis is consistent with the functional evaluation on *MDM2* SNP309 (12), supporting the hypothesis that SNP309 serves as a low-penetrance susceptibility tumor marker.

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