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MDM2 SNP309 polymorphism is associated with colorectal cancer risk

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The human murine double minute 2 (MDM2) is known as an oncoprotein through inhibiting P53 transcriptional activity and mediating P53 ubiquitination. Therefore, the amplification of MDM2 may attenuate the P53 pathway and promote tumorigenesis. The SNP309 T>G polymorphism (rs2279744), which is located in the intronic promoter of *MDM2* gene, was reported to contribute to the increased level of MDM2 protein. In this hospital-based case-control study, which consisted of 573 cases and 588 controls, we evaluated the association between *MDM2* SNP309 and the risk of colorectal cancer (CRC) in a Chinese population by using the TaqMan method to genotype the polymorphism. We found that the *MDM2* SNP309 polymorphism was significantly associated with CRC risk. In addition, in our meta-analysis, we found a significant association between *MDM2* SNP309 and CRC risk among Asians, which was consistent with our results. In conclusion, we demonstrated that the *MDM2* SNP309 polymorphism increased the susceptibility of CRC in Asian populations.

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of death from cancer worldwide, which accounts for an estimated 1,330,000 new cases and 608,000 cancer deaths in 2008[1]. The incidence rates are high in Australia/New Zealand and Western Europe, low in Africa and South-Central Asia, and intermediate in Latin America[1]. In the USA, CRC was the third leading cancer type for estimated new cancer cases and deaths in 2013[2]. In China, epidemiological data showed that there was an annual increase of 3.33% in CRC incidence and 3.05% in CRC mortality during 2003~2007[3]. The mechanisms underlying the development of CRC are complex. Both environmental and genetic factors play an important role in the occurrence and progression of CRC[4]. Genetic epidemiology and twin studies demonstrate that upwards of 35% of the CRC cases may be due to inherited factors, which indicates the importance of inherited genetic susceptibility in carcinogenesis[5].

P53, the tumor suppressor protein, plays a crucial role in multi-cellular functions, including gene transcription, DNA synthesis and repair, growth arrest, cell senescence, and apoptosis[6]. *p53* mutations that disrupt the balance between cell apoptosis and repair are found in at least half of all human cancers, which highlight a critical role of P53 in tumor suppression[7]. The human homolog of the mouse double minute 2 (MDM2) functions as an important negative regulator of P53 through an autoregulatory feedback loop. The elevated nuclear P53 level will activate *MDM2* gene transcription and increase the protein expression of MDM2. MDM2 will inhibit the transcriptional activity of P53 through its direct binding to P53 and also serve as an E3 ubiquitin ligase, promoting the degradation of P53[8–11]. Thus, MDM2 overexpression may disturb this feedback loop and cause the deficiency of P53, which will result in inefficient growth arrest and/or apoptosis. Amplification of MDM2 is observed in many human tumor tissues, including CRC[12–14]. Consequently, up-regulated expression of MDM2 and attenuation of P53 pathway has been observed[15].

MDM2 SNP309 (rs 2279744), which is located in the promoter of *MDM2* gene, was identified as a functional single nucleotide polymorphism (SNP). This SNP is a novel T to G substitution located at the 309th nucleotide in the first intron, showing a greater binding affinity for the transcription factor Sp1[15]. Therefore, it was hypothesized that the genetic variant might have an impact on the expression of MDM2 and affect the individual's susceptibility to developing tumors. Many studies have evaluated this association in different tumors, but their

results are conflicting[16–18]. Some studies have reported a direct connection between *MDM2* SNP309 and CRC risk[19–21]; however others have shown the opposite[22,23].

Recently, Zhang et al. has shown no direct association between *MDM2* SNP309 and CRC, but a combined effect of *TP53* Arg72Pro and *MDM2* SNP309 showed an increased CRC risk in a Chinese population[24]. Considering this conclusion is only based on the central Chinese demographics, more studies are needed to confirm this finding. Therefore, in this study, we genotyped the *MDM2* SNP309 and evaluated its association with CRC risk in a population from the southeast of China.

Results

Study characteristics. The characteristics of our study are shown in Table 1. No significant differences were found between cases and controls for age [cases *vs.* controls (mean ± SD), 60.3 ± 12.5 *vs.* 59.3 ± 9.8 years; $P = 0.136$], sex ($P = 0.824$), smoking status ($P = 0.191$), and alcohol use ($P = 0.082$). And these variables were adjusted for in the multivariate logistic regression analysis. As expected, however, CRC patients had a higher rate of family history of cancer than that of the controls ($P < 0.001$). Of the 573 CRC cases, the frequencies of the Dukes A, B, C and D stage were 9.1%, 40.6%, 35.1%, and 15.2%, respectively. For tumor grade, 6.5% of patients were with poor-differentiated tumors; 74.9% and 18.6% were found with moderate and well-differentiated tumors, respectively.

Association between *MDM2* SNP309 and CRC risk. The genotype distributions of *MDM2* SNP309 in the control group were in accordance with the HWE ($P = 0.805$). The genotype frequencies of *MDM2* SNP309 were 19.4% (TT), 51.5% (TG), and 29.1% (GG) in cases, which were statistically different from that in the control group (25.5% TT, 49.5% TG, and 29.1% GG) ($P = 0.031$). After adjusting for age, sex, smoking status, and drinking status, multivariate logistic regression analysis revealed that the individuals carrying the TG or GG genotype had an increased CRC risk (OR = 1.36, 95% CI = 1.01–1.82 for TG *vs.* TT; OR = 1.53, 95% CI = 1.10–2.13 for GG *vs.* TT), compared with the TT genotype. We also found that the *MDM2* SNP309 TG/GG genotypes were associated with higher CRC susceptibility (OR = 1.41, 95% CI = 1.07–1.87) (Table 2). In the stratified analyses based on the dominant model, we found individuals carrying *MDM2* SNP309 (TG/GG) were associated with increased risk among older subjects (OR = 1.76, 95% CI = 1.17–2.64), males (OR = 1.52, 95% CI = 1.06–2.19), smokers (OR = 1.90, 95% CI = 1.11–3.27), and non-drinkers (OR = 1.42, 95% CI = 1.03–1.96) (Table 3). Furthermore, we also assessed the association between the *MDM2* SNP309 polymorphism and clinicopathological characteristics of CRC. As shown in Table 4, the individuals carrying the TG/GG genotypes were found to have an increased risk in rectal cancer (OR = 1.50, 95% CI = 1.06–2.14), well-differentiated CRC (OR = 2.07, 95% CI = 1.16–3.69), and early stage cancer (Dukes A and B) (OR = 1.55, 95% CI = 1.08–2.21). In addition, the median age of tumor onset according to the genotype of *MDM2* SNP309 was evaluated. No significant differences were found in the median ages among men [62.0 for TT, 63.0 for TG and 62.0 for GG ($P = 0.895$)]. Moreover, neither younger women (≤57 years) [46.0 for TT, 47.0 for TG, and 48.0 for GG ($P = 0.246$)], nor older women (> 57 years) [66.5 for TT, 68.0 for TG, and 68.5 for GG ($P = 0.371$)] showed statistical differences in the median ages of tumor onset.

Table 1 | Distribution of selected variables in colorectal cancer cases and caner-free controls

	Cases (n = 573)		Controls (n = 588)		
Variables	N	%	N	%	*P*[a]
Age (years) mean ± SD	60.3 ± 12.5		59.3 ± 9.8		0.136
Sex					
Male	354	61.8	367	62.4	0.824
Female	219	38.2	221	37.6	
Smoking status					
No	377	65.8	408	69.4	0.191
Yes	196	34.2	180	30.6	
Drinking status					
No	414	72.3	451	76.7	0.082
Yes	159	27.7	137	23.3	
Family history of cancer					
No	443	77.3	546	92.9	< 0.001
Yes	130	22.7	42	7.1	
Tumor site					
Colon	279	48.7			
Rectum	294	51.3			
Duke's stage					
A	52	9.1			
B	233	40.6			
C	201	35.1			
D	87	15.2			
Tumor grade					
Low	37	6.5			
Intermediate	429	74.9			
High	107	18.6			

[a]Two-sided Student's *t*-test or χ^2 test.

Meta-analysis of *MDM2* SNP309 and CRC risk. We performed a meta-analysis to evaluate the association between *MDM2* SNP309 and CRC risk. A total of 11 studies were selected, which included 4 studies of Asian population and 7 studies in Europeans (Table 5). Then we pooled the previous published studies and our present study together, and this meta-analysis consisted of 3744 cases and 3185 controls.

The *MDM2* SNP309 (TG/GG) carriers among Asians were associated with higher CRC risks (OR = 1.20, 95% CI = 1.03–1.38) (Fig. 1C). And significantly increased risks of CRC were also observed in Asians with TG (OR = 1.20, 95% CI = 1.03–1.40) (Fig. 1A) or GG (OR = 1.21, 95% CI = 1.01–1.45) (Fig. 1B), when compared with SNP309 TT. However, these results were not found in Europeans (Table 6). In the total population, no statistical association between the *MDM2* SNP309 polymorphism and CRC risk were found in all genetic models under random-effects model (P value for heterogeneity < 0.1). Thus we used a Galbraith plot to investigate the source of heterogeneity and found one article with an European population[21], which could potentially be the cause of high heterogeneity (Fig. 2). After excluding that specific study, we analyzed the data again. With low heterogeneity, statistical associations with risk of CRC were found in the dominant model (Fig. 1), but the associations were still not observed in Europeans. In addition, publication bias was assessed by the Begg's and Egger's tests, and no evidence of publication bias in all genetic models was found (t = 0.15, $P = 0.880$ for TG *vs.* TT; t = −0.19, $P = 0.851$ for GG *vs.* TT; t = 0.08, $P = 0.937$ for dominant model; t = −0.44, $P = 0.672$ for recessive model).

Discussion

As reported, MDM2 can directly bind to P53 and down-regulate its function as a tumor suppressor. The oncogenic properties of MDM2 are thought to be P53-dependent. However, some studies have shown that MDM2 may form complexes with other tumor suppressor proteins independent of P53 *in vitro* and in P53-deficient cells[34,35]. These findings demonstrate the oncogenic potential of MDM2 in P53-independent pathways. In addition, although *MDM2* SNP309 is located on a P53-response intronic promoter, the P53-independent overexpression of MDM2 was still observed[36]. Moreover, MDM2 amplification might also be regulated in post-transcriptional ways[37,38]. All aforementioned findings indicate that

Table 2 | Distribution of genotypes of *MDM2* SNP 309 among colorectal cancer cases and cancer-free controls

	Cases (n=573)		Controls (n=588)				
Genotypes	n	%	n	%	P^a	Crude OR (95%CI)	Adjusted OR (95%CI)[b]
Co-dominant model							
TT	111	19.4	150	25.5		1.00 (reference)	1.00 (reference)
TG	295	51.5	291	49.5	**0.036**	**1.37 (1.02–1.84)**	**1.36 (1.01–1.82)**
GG	167	29.1	147	25.0	**0.011**	**1.54 (1.10–2.14)**	**1.53 (1.10–2.13)**
G allele	0.549		0.497		**0.013**		
P_{trend}						**0.013**	
Additive model					**0.031**	**1.23 (1.05–1.45)**	**1.23 (1.04–1.45)**
Dominant model							
TT	111	19.4	150	25.5		1.00 (reference)	1.00 (reference)
TG/GG	462	80.6	438	74.5	**0.012**	**1.43 (1.08–1.88)**	**1.41 (1.07–1.87)**

[a]*P* for χ^2test.
[b]Adjusted for age, sex, smoking status, and alcohol use in logistic regression models.
OR, odds ratio; CI, confidence interval.

complex mechanisms underlie the regulation of *MDM2* gene during tumorigenesis. Considering that *MDM2* SNP309 may regulate the MDM2 expression, it is meaningful for us to evaluate its association with cancer risk.

In CRC, whether *MDM2* SNP309 has a direct effect on carcinogenesis is still controversial. Some studies show that the TG genotype is associated with higher CRC risk than the TT genotype[19–21], whereas, others show no association[22,23]. Recently, Zhang *et al.* reported a combined effect of *TP53* Arg72Pro and *MDM2* SNP309 in a dose-response fashion, increasing CRC risk in the population from the central region of China, but no association between *MDM2* SNP309 alone and CRC risk was found[24]. In our study population, the *MDM2* SNP309 carriers, either with TG or GG, were found to have an increased CRC risk compared with those carrying TT genotype. To resolve this conflict and further validate our results, we pooled the published data and our current data together, and then did a meta-analysis. In this meta-analysis, we found that *MDM2* SNP309 contributed to an increased CRC susceptibility in Asians, which was consistent with our present study. And the similar association in the dominant model was also observed in the combined populations across studies if we excluded one article which was the main cause of the high heterogeneity. Interestingly, a meta-analysis published by Cao *et al.* also found a significantly increased CRC risk among the individuals with TG genotype, especially among Asians, when compared with TT genotype[39]. However, we found no association between *MDM2* SNP309 and CRC risk among Europeans. Considering the frequencies of the *MDM2* SNP309 G allele among the cases and controls were different by ethnicity (MAF: 0.47 in Asians and 0.39 in Europeans), it has indicated a possible ethnic difference in genetic backgrounds. Moreover, a second *MDM2* promoter polymorphism named SNP285 (a G to C transversion located only 24 bp upstream of SNP309), which is present only among Caucasions, was reported to reduce Sp1 binding and antagonize the affinity of Sp1 with an enhanced effect by SNP309[40]. Thus, *MDM2* SNP285 may be another explanation for the differential effect of *MDM2* SNP309 between ethnicities.

The effect of *MDM2* SNP309 on CRC risk was found more pronounced among the older people, which may reflect the accumulative effects of risk factors, such as prolonged red meat consumption[41]. Increased CRC risk associated with *MDM2* SNP309 was found only in men but not in women, which was consistent with a previous study[20], and we also did not have a biological explanation. Some authors reported that the interaction between *TP53* Arg72Pro and *MDM2* SNP309 was associated with elevated CRC risk in smokers

Table 3 | Stratification analyses between *MDM2* SNP309 genotypes and CRC risk

		Genotypes (cases/controls)					
	Cases/controls	TT		TG/GG		TG/GG *vs.* TT	
Variables	N	N	%	N	%	Adjusted OR (95% CI)[a]	P^a
Age (years)							
≤60	277/348	57/82	20.6/23.6	220/266	79.4/76.4	1.15 (0.78–1.70)	0.468
> 60	296/240	54/68	18.2/28.3	242/172	81.8/71.7	**1.76 (1.17–2.64)**	**0.007**
Sex							
Male	354/367	64/93	18.1/25.3	290/274	81.9/74.7	**1.52 (1.06–2.19)**	**0.023**
Female	219/221	47/57	21.5/25.8	172/164	78.5/74.2	1.28 (0.82–2.01)	0.274
Smoking status							
No	377/408	85/109	22.6/26.7	292/299	77.5/73.3	1.28 (0.92–1.79)	0.141
Yes	196/180	26/41	13.3/22.8	170/139	86.7/77.2	**1.90 (1.11–3.27)**	**0.020**
Drinking status							
No	414/451	82/118	19.8/26.2	332/333	80.2/73.8	**1.42 (1.03–1.96)**	**0.035**
Yes	159/137	29/32	18.2/23.4	130/105	81.8/76.6	1.44 (0.81–2.55)	0.218
Family history of cancer							
No	443/546	93/141	21.0/25.8	350/405	79.0/74.2	1.33 (0.98–1.80)	0.063
Yes	130/42	18/9	13.9/21.4	112/33	86.1/78.6	1.31 (0.51–3.37)	0.578

[a]OR (odds ratio), CI (confidence interval), and *P* values were calculated in dominant model with adjustment for age, sex, smoking status, and alcohol use.

Table 4 | Associations between the *MDM2* SNP309 polymorphism and clinicopathologic parameters of CRC

	TT		TG/GG		TG/GG *vs.* TT	
Variables	N	%	N	%	Adjusted OR (95% CI)[a]	*P*[a]
Controls (n=588)	150	25.5	438	74.5	1.00 (reference)	
Cases (n=573)						
Duke's stage						
A/B	52	18.3	233	81.7	**1.55 (1.08–2.21)**	**0.016**
C/D	59	20.5	229	79.5	1.31 (0.93–1.84)	0.128
Tumor grade						
Poor/Moderate	96	20.6	370	79.4	1.32 (0.98–1.77)	0.064
Well	15	14.0	92	86.0	**2.07 (1.16–3.69)**	**0.014**
Tumor site						
Colon	57	18.3	222	81.7	1.33 (0.94–1.88)	0.105
Rectum	54	20.5	240	79.5	**1.50 (1.06–2.14)**	**0.023**

[a]OR (odds ratio), CI (confidence interval), and *P* values were calculated in dominant model with adjustment for age, sex, smoking status, and alcohol use.

but not in non-smokers[24]. In the stratified analysis, we found *MDM2* SNP309 had a direct connection with CRC risk in smokers and also not in non-smokers. Long-term smoking has been reported as a risk factor for CRC[42]. *MDM2* SNP309 might influence the activity of P53, and then increase the possibility that some colon cells damaged by tobacco carcinogens might escape the apoptosis triggered by P53. Therefore, smokers carrying *MDM2* SNP309 are expected to have a higher risk of CRC but further validation is still needed. Alcohol consumption is also associated with CRC risk[43], and has already been reported to be related with *p*53 mutations in breast cancer[44]. Therefore, drinkers with *MDM2* SNP309 should be associated with higher CRC risk. However, in our study, this association was not found. The relative small sample size after stratifying for drinking status may be the reason. After stratifying the tumor stage and grade, we observed that the *MDM2* SNP309 was associated with an increased risk in CRC patients with Duke's A/B stage or well-differentiated tumor grade, which indicated the involvement of SNP309 in the early stages of CRC. The family history of cancer in our study is not matched, and it might be important for the better understanding of the genetic variants. However, in our analysis, the effect of family history on the association between *MDM2* SNP309 and CRC risk was not observed.

A significant earlier age of onset was observed to be associated with *MDM2* SNP309 in several tumors[15]. In CRC, several studies showed this association especially in women, but not in men[22,45]. The *MDM2* promoter, where SNP309 is located, is regulated by hormonal signaling pathways. Therefore, it is hypothesized that the increased affinity of female-specific hormones such as estrogen, caused by the gene variant, might accelerate tumor formation[46]. And higher frequencies of the SNP309 G allele in CRC were found in women at a younger or premenopausal age than in women at a older or menopausal age, and in men[46], which supported the hypothesis in some extent. Because we did not have the data of menopausal age, we only compared the onset age of CRC in younger and older women based on the median age (60 years) separately. However, no statistical difference was observed between CRC onset age of the SNP309 carriers and individuals with TT genotypes in younger or older women. Several studies have shown conclusions consistent with ours[20]. But there is still one more thing we should consider. Menin *et al.* reported that *MDM2* SNP309 may affect the age of cancer onset only in the tumors with wild-type P53[27]. The lack of the information of the *p53* mutation status in the tumors might influence our results. Thus, further studies about *p*53 mutations are required to resolve this conflict.

In conclusion, we demonstrated that *MDM2* SNP309 was associated with increased CRC risk in a Chinese population, which was concordant with our meta-analysis. Additionally, in the stratified analyses, we found that increased risk was more pronounced in males, older people, smokers, non-drinkers, people diagnosed with rectal cancer, and patients with Duke's A/B stage or well-differentiated tumor grade. Moreover, the earlier age of cancer onset in patients carrying *MDM2* SNP309 was not found in our study. Considering the correlation between MDM2 and P53, the status of P53 is necessary for further studies. Further validation of large population-based studies in different ethnicities is still needed.

Methods

Ethics statement. The study was approved by the institutional review board of Nanjing Medical University. Informed written consent was obtained from all subjects. The experimental protocol was carried out in accordance with the approved guidelines.

Study subjects. The characteristics of the CRC patients and cancer-free controls in this study have been previously described in detail[25]. Briefly, this study consisted of 573 patients with CRC and 588 cancer-free controls. All the patients with histologically-confirmed CRC were consecutively recruited from September 2010 at the First Affiliated Hospital of Nanjing Medical University, Nanjing, China, without age or sex restrictions. The cancer-free control patients, who were genetically unrelated to the CRC patients, were matched by age (±5 years) and sex to the CRC patients. A trained personnel interviewed each participant after obtaining the signed informed consent and a structured questionnaire on demographic information and environmental exposures. Individuals who smoked daily for at least one year were defined as smokers. People who consumed one or more alcoholic drinks per week for more than one year were defined as drinkers. After the interview, a 5 ml venous blood sample was obtained from each patient for genomic DNA extraction.

Table 5 | Characteristics of the studies selected in the meta-analysis

Author	Years	Country	Ethnicity	Genotying methods	Source of controls	Sample size (cases/controls)	Cases (TT/TG/GG)	Controls (TT/TG/GG)
Alhopuro	2005	Finland	European	PCR-RFLP	Population	969/185	334/465/170	56/98/31
Sotamaa	2005	Finland	European	PCR-RFLP	Population	121/209	38/66/17	78/94/37
	2005	United States	European	PCR-RFLP	Population	30/118	15/11/4	45/52/21
Menin	2006	Italy	European	PCR-RFLP	Population	153/92	69/70/14	40/40/12
Talseth	2006	Australia and Poland	European	TaqMan PCR	Hospital	116/98	45/57/14	40/51/7
Alazzouzi	2007	Spain	European	PCR-SSCP	Hospital	152/184	66/69/17	97/63/24
Chen	2009	China	Asian	PCR-CE	Population	123/138	27/66/30	29/83/26
Sugano	2010	Japan	Asian	LH-MSAs	Population	211/59	61/95/55	12/27/20
Joshi	2011	Japan	Asian	PCR-RFLP	Population	685/778	129/373/183	177/384/217
Chaar	2012	Tunisia	European	PCR-RFLP	Population	167/167	11/86/70	64/56/47
Zhang	2012	China	Asian	MALDI-TOF MS	Hospital	444/569	131/223/90	180/281/108

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-stranded conformation polymorphism; CE, capillary electrophoresis; LH-MSAs, Loop-hybrid mobility shift assay; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry.

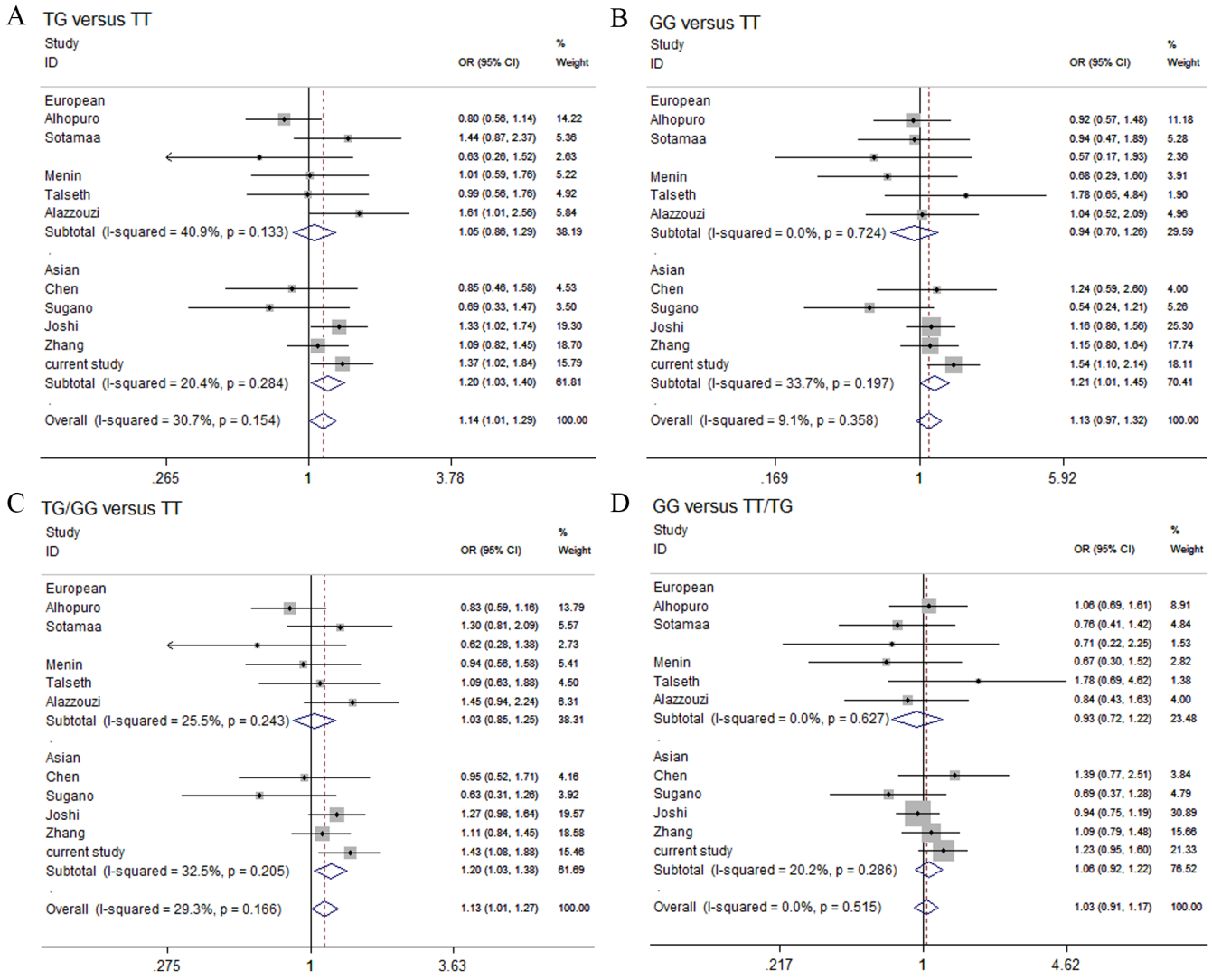

Figure 1 | Forest plot on the association between *MDM2* SNP309 and the risk of colorectal cancer. (A) TG versus TT, (B) GG versus TT, (C) TG/GG versus TT, (D) GG versus TT/TG.

DNA extraction and genotyping. Genomic DNA was obtained from white-blood-cell fractions by using the Qiagen Blood Kit (Qiagen) following the manufacturer's protocol. We used the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) for the TaqMan SNP Genotyping assay. Two people achieved this genotype analysis independently in a blind fashion. We also randomly selected 10% of our samples for repeated genotyping to assess the reproducibility, and the concordant rate was 100%.

Statistical analysis. Hardy-Weinberg equilibrium (HWE) of alleles was evaluated by using a goodness-of-fit chi-square test. The differences in demographic

Table 6 | Meta-analysis of *MDM2* SNP309 on colorectal cancer risk

		GG *vs.* TT		TG *vs.* TT		GG/TG *vs.* TT		GG *vs.* TT/TG	
Variables	n[a]	OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]	OR (95%CI)	P[b]
Total	12	1.21 (0.89–1.66)[c]	<0.001	1.23 (0.94–1.60)[c]	<0.001	1.21 (0.94–1.56)[c]	<0.001	1.07 (0.92–1.25)	0.183
Total[d]	11	1.13 (0.97–1.32)	0.358	**1.14 (1.01–1.29)**	0.154	**1.13 (1.01–1.27)**	0.166	1.03 (0.91–1.17)	0.515
Ethnicity									
Asian	5	**1.21 (1.01–1.45)**	0.197	**1.20 (1.03–1.40)**	0.284	**1.20 (1.03–1.38)**	0.205	1.06 (0.92–1.22)	0.286
European	7	1.30 (0.66–2.54)[c]	<0.001	1.38 (0.82–2.33)[c]	<0.001	1.34 (0.81–2.23)[c]	<0.001	1.11 (0.88–1.39)	0.130
European[d]	6	0.94 (0.70–1.28)	0.724	1.05 (0.88–1.29)	0.133	1.03 (0.85–1.25)	0.243	0.93 (0.72–1.22)	0.627
Source of controls									
Population-based	8	1.14 (0.68–1.91)[c]	<0.001	1.22 (0.79–1.90)[c]	<0.001	1.19 (0.83–1.70)[c]	<0.001	1.02 (0.87–1.19)	0.109
Population-based[d]	7	0.98 (0.80–1.21)	0.518	1.06 (0.90–1.25)	0.134	1.03 (0.88–1.20)	0.184	0.93 (0.79–1.11)	0.641
Hospital-based	4	**1.33 (1.06–1.66)**	0.534	**1.24 (1.04–1.49)**	0.389	**1.26 (1.07–1.49)**	0.503	1.16 (0.96–1.40)	0.552

[a]Number of comparisons.
[b]*P* value of *Q*-test for heterogeneity test.
[c]When *P* value for heterogeneity test < 0.10, random-effects model was used; otherwise, fix-effects model was used.
[d]Analysis without the study contributing to the high heterogeneity.
OR, odds ratio; CI, confidence interval.

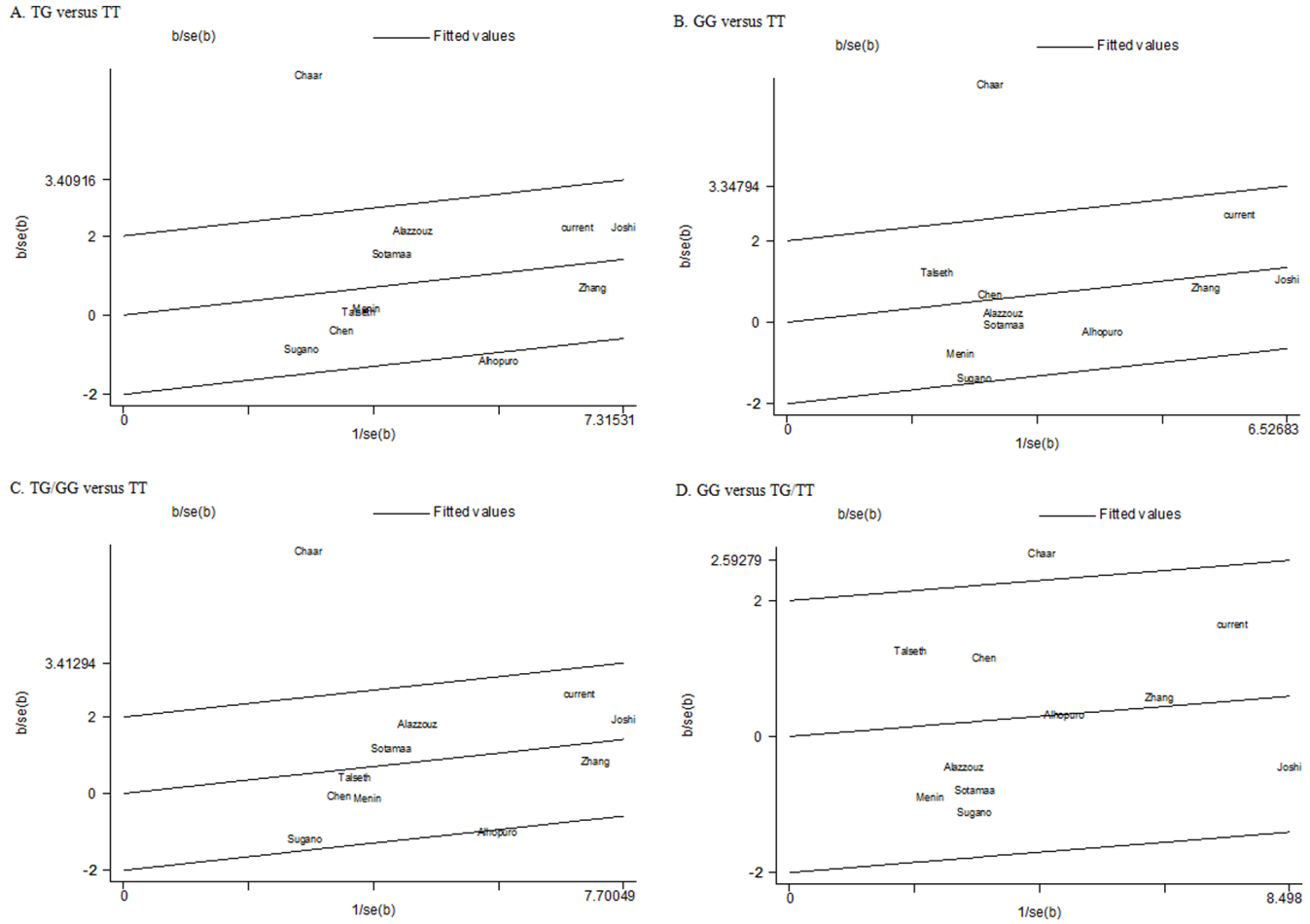

Figure 2 | Galbraith plot for investigating the source of heterogeneity. The studies outside the parallel lines were considered contributing to the heterogeneity. (A) TG versus TT, (B) GG versus TT, (C) TG/GG versus TT, (D) GG versus TT/TG.

characteristics, selected variables and frequencies of the genotypes were tested using a Student's *t*-test (for continuous variables) or Pearson's chi-square test (for categorical variables). The Kruskal-Wallis Test was used to compare the age of tumor onset according to the genotype of *MDM2* SNP309. The association between *MDM2* SNP309 and CRC risk was assessed by odds ratios (ORs) and 95% confidence intervals (CI) using unconditional logistic regression analysis with the adjustment for possible confounders. All data analyses were two-sided and performed with Statistical Analysis System software (version 9.1.3; SAS Institute Inc, Cary, NC, USA).

Meta-analysis. To further evaluate the association between the *MDM2* SNP309 and CRC risk, we performed a meta-analysis based on the previous published studies and our current study. The databases of PubMed, Embase and Web of Science updated on April 1, 2013, were searched for articles based on the human associated case-control studies in English, using the terms: "MDM2", "polymorphism(s) or genetic variation(s)", "colorectal" and "cancer or carcinoma or tumor" as well as their combinations. Finally, we collected 11 studies consisting of a total of 3171 cases and 2597 controls[19–24,26–29]. Because the studie published by Chaar[21] was found to be the potential cause of high heterogeneity, we excluded this study and analyzed the rest again. ORs and 95% CIs were used to determine the strength of association between *MDM2* SNP309 and CRC risk, and we used *Z*-tests to estimate the statistical significance of the pooled OR. A fixed-effects model was used, unless the heterogeneity of the study results tested by the Cochran's *Q*-test was considered significant ($P < 0.1$). Then, the random-effects model was used[30,31]. Weighting was applied to results calculated by the fix-effects or random-effects model, which represented the contribution of each study to the pooled analysis. We used a Galbraith plot to find the source of heterogeneity. Begg's test and Egger's test were used to assess the publication bias[32,33]. All analyses were calculated with Stata software (version 10.1; StataCorp LP, College Station, TX), using two-sided *P* values.

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Author contributions

Z.Z., Z.X. and M.W. conceived and designed the experiments. W.W., M.D. and D.G. performed the experiments. W.W., M.D. and L.Z. analyzed the data. M.W., H.C. and N.T. contributed reagents/materials/analysis tools. W.W. wrote the paper.

Additional information

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