Original Article

Interaction between common variants of *MDM2* and *PPP1R13L* and *CD3EAP* and *TP53* SNPs in relation to lung cancer risk among Chinese

Jiaoyang Yin[1], Wei Hou[2], Ulla Vogel[3], Yegang Ma[4], Chunhong Wang[1], Huiwen Wang[1], Zhenxiang Sun[1]

[1]Key Laboratory of Environment and Population Health of Liaoning Education Ministry (Shenyang Medical College), Shenyang, China; [2]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Pathology, Peking University Cancer Hospital & Institute, Beijing, China; [3]National Research Centre for the Working Environment, Copenhagen, Denmark; [4]Department of Thoracic Surgery, Liaoning Cancer Hospital, Shenyang, China

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Correspondence to: Jiaoyang Yin. Shenyang Medical College, Shenyang 110034, China. Email: yinjye@163.com.

Background: Lung cancer is a complex disease that diagnosed the most common cancer and led cause of cancer death. *MDM2* (MDM2 proto-oncogene) encodes a nuclear-localized E3 ubiquitin ligase. The encoded protein can promote tumor formation by targeting tumor suppressor proteins, such as TP53, for proteasomal degradation. Epidemiology studies have investigated the association of *MDM2* single nucleotide polymorphisms (SNP) and interaction between genetic and environmental factors with lung cancer.

Methods: This Chinese case-control study comprised 627 cases and 633 controls explored the role of *MDM2* five htSNPs (rs1690924, rs1846402, rs2291857, rs3730581 and rs3730635, haplotype-tagging SNP) tagging 95% of the common haplotypes across the gene and the interactions of *MDM2*, *PPP1R13L*, *CD3EAP* and *TP53* in the same pathological pathway on lung cancer risk, together with smoking-duration.

Results: None of the htSNPs in *MDM2* were associated with lung cancer risk in co-dominant, dominant, recessive, and log-additive models (adjusted for smoking-duration). Haplotype analysis showed that global haplotype association was statistically significant (P=0.0036, adjusted for smoking-duration) and haplotype5 (rs1690924^{A}-rs1846402^{G}-rs2291857^{C}-rs3730581^{G}-rs3730635^{A}) was associated with reduced risk of lung cancer [OR (95%) =0.52 (0.33–0.82), P=0.0053, adjusted for smoking-duration]. MDR interaction analysis showed that two the best significant models and strong synergy between *MDM2* and *TP53*.

Conclusions: *MDM2* five-htSNPs haplotype exhibited association with lung cancer susceptibility, interaction of *MDM2* and *TP53* htSNPs and smoking-duration contributed to lung cancer risk and strong synergy between *MDM2* and *TP53* htSNPs influenced lung cancer predisposition. Our results suggest that *MDM2*, *TP53* and smoking-duration interact in relation to lung carcinogenesis.

Keywords: *MDM2* and *PPP1R13L* and *CD3EAP* and *TP53*; genetic variants; smoking duration; interaction; lung cancer

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Introduction

Cancer incidence and mortality are rapidly growing worldwide. Lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths) (1). Lung cancer is complex disease affected by many genetic factors and environmental exposures. Nicotine and carbon monoxide caused by cigarette smoking have been considered as causative environmental factors for the development of lung cancer. Another possible mechanism may involve interactions between smoking and various susceptibility genes in relation to lung cancer (2).

MDM2 (MDM2 proto-oncogene) (Gene ID: 4193) is located on chromosome 12q15. The gene consists of 13 exons and encodes a nuclear-localized E3 ubiquitin ligase. The encoded protein promotes tumor formation by targeting tumor suppressor proteins, such as TP53, for proteasomal degradation. This gene is itself transcriptionally-regulated by TP53. Over-expression or amplification of *MDM2* is detected in many human malignancies, including lung cancer (https://www.ncbi.nlm.nih.gov/gene/4193) (3). The effects of single nucleotide polymorphisms (SNP) at *MDM2* have been investigated in relation to lung cancer with inconsistent results (4-15).

The two genes *PPP1R13L* [protein phosphatase 1, regulatory (inhibitor) subunit 13 like)] (Gene ID: 10848) and *CD3EAP* (CD3e molecule, epsilon-associated protein) (Gene ID: 10849) located on chromosome 19q13.3 relate to DNA repair and cell survival and cell proliferation, respectively. The gene *TP53* (tumor protein p53) located on chromosome 17p13.1 encodes the tumor suppressor p53, which in response to diverse types of cellular stress regulates expression of target genes. We previously reported that *PPP1R13L* rs1970764, *CD3EAP* rs967591 and rs735482, and *TP53* htNP2 were associated with lung cancer or interacted in relation to lung cancer risk among both Caucasian Danes and Chinese [(16-20), Yin *et al.* submitted and revised].

MDM2, *TP53*, *PPP1R13L*, and *CD3EAP* all belong to the pathway of gene expression. *MDM2*, *TP53* and *PPP1R13L* belong to the pathways of gene expression and p53 pathway. Both *MDM2* and *TP53* share the pathways of gene expression, p53 pathway and TP53 Network (https://www.ncbi.nlm.nih.gov/gene/4193).

Previous epidemiology studies concerning *MDM2* SNPs and lung cancer risk were mainly focused on single SNP and interactions (4-15). No systematical investigations have been reported on the associations between *MDM2* htSNPs (Haplotype-tagging SNP) and lung cancer risk. In this Chinese case-control study we explored the role of htSNPs tagging 95% common haplotypes across the *MDM2* gene and assess gene-gene and gene-gene-environment interactions in the same pathological pathway related to lung cancer risk, including the interaction between *MDM2* htSNPs, *TP53* htSNPs, and *PPP1R13L* and *CD3EAP* risk SNPs. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-19-4784).

Methods

Ethics permission

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by Chinese Administration Office of Human Genetic Resources {no. [2001]015}. Subjects were informed about the study and written or oral informed consent was achieved from all study participants.

Study population

A total of 1,260 subjects were enrolled in this hospital-based case-control study, comprising 627 cases and 633 controls. The patients with lung cancer were diagnosed using standard clinical and histological criteria. Qualified cases were previously untreated (no chemotherapy or radiotherapy for cancer prior to recruitment). Cancer-free controls were selected from the orthopedics wards in the same area. All subjects were unrelated ethnic Han Chinese. Demographic and covariate data were acquired from medical records and questionnaires through personal interview with a professional physician. Stratification criteria were determined as follows age (10 years an interval), gender, family history and smoking duration (20 years an interval).

Determined MDM2 htSNPs

Using the TagSNPs software online and approaches of the algorithm-Tagger-pairwiseTagging, htSNPs of *MDM2* gene from the International HapMap Project were determined in the relevant region of chromosome 12 (http://www.hapmap.org, HapMap Data Rel 27 PhaseII+III, Feb09, on NCBI B36 assembly, dbSNP b26). Qualified criteria were: r2-cut off of 0.8 and MAF (minor

Table 1 Characteristics for *MDM2* htSNPs selected and SNPs in *PPP1R13L* and *CD3EAP* and htSNPs in *TP53*[a]

dbSNP ID	Position	Location	Base change	Allele frequency in HapMap HCB[b]	MAF[c] in controls for current study
Chr12q15					
MDM2					
rs1690924	68811541	Intron	A/G	A: 0.814/G: 0.186	G: 0.24
rs1846402	68814798	Intron	G/T	G: 0.826/T: 0.174	T: 0.16
rs2291857	68824258	Intron	C/A	C: 0.700/A: 0.300	A: 0.32
rs3730581	68825712	Intron	A/G	A: 0.581/G: 0.419	G: 0.49
rs3730635	68835343	Intron	A/G	A: 0.946/G: 0.054	G: 0.02
Chr19q13.3					
PPP1R13L[a]					
rs1970764	45387615	Intron	A/G	No	G: 0.48
CD3EAP[a]					
rs967591	45406676	5'UTR	G/A	G: 0.525/A: 0.475[d]	A: 0.42
rs735482	45408744	Exon3	A/C	A: 0.558/C: 0.442	C: 0.45
		Codon 261 (K [Lys] [AAA] ⇒ T [Thr] [ACA]) (missense)			
Chr17p13.1					
TP53					
rs12951053	7674089	Intron	A/C	A: 0.667/C: 0.333	C: 0.34[e]
rs1042522	7676154	Exon4	G/C	G: 0.511/C: 0.489	C: 0.45[e]
		Codon 72 (R [Arg] [CGC]) ⇒ P [Pro] [CCC] (missense)			
rs8079544	7676734	Intron	C/T	C: 0.878/T: 0.122	T: 0.08[e]
rs12602273	7679695	Intron	C/G	C: 0.678/G: 0.322	G: 0.28[e]
rs8064946	7685993	Intron	G/C	G: 0.622/C: 0.378	C: 0.32[e]

[a], information from NCBI SNP database (GRCh38.p7) and HapMap database; [b], Han Chinese in Beijing; [c], minor allele frequency; [d], CHB+ JPT (Han Chinese in Beijing + Japanese from 1000 GENOMES); [e], from previous result, here this is employed for interaction analysis.

allele frequency)-cut off of 0.05 in HCB (Han Chinese in Beijing) samples. Five htSNPs (rs1690924, rs1846402, rs2291857, rs3730581 and rs3730635) were selected, tagging 95% of the common haplotype diversity across the *MDM2* gene. *Table 1* shows the information of *MDM2* five htSNPs, three risk SNPs of *PPP1R13L* and *CD3EAP* and *TP53* five htSNPs. Three risk SNPs of *PPP1R13L* and *CD3EAP* were previously reported (20,21) while we increased the number of included samples in present study. The genotype data of *PPP1R13L* and *CD3EAP* three risk SNPs and *TP53* five htSNPs were employed for interaction analyses of gene-gene and gene-gene-environment in this study.

DNA isolation and genotyping

A volume of 5 mL of peripheral blood was taken from each volunteer. Genomic DNA of peripheral blood samples was extracted with the Puregene DNA Isolation Kit or FlexiGene DNA kit 250 (Gentra Systems, Minneapolis, MN, USA or Qiagen, Germany) following the manufacturer's instructions. Genotyping of rs1690924

Table 2 The sequences (5'-3') of primers and probes for *MDM2* 5 htSNPs examined

rs number	Primers and probes
rs1690924	Forward primer: TGTAATGGAAAGCCATCAGTAT
	Reverse primer: TCTCCTGTCCCAAGATCTTGC
	Common probe:-P-GCTATAAAAGATAATAGCATTTGTA-FAM-
	Discriminating probe G: TTTAAAGACATGTATTAATGAGAAAACG
	Discriminating probe A: AAAGACATGTATTAATGAGAAAACA
rs1846402	Forward primer: TAAGTGGGAGAGACAGAGAAC
	Reverse primer: CCAGGTTAAGAACTTCTGCAC
	Common probe:-P-GCTCAATCTGTCACTGAAAATCATGTTT-FAM-
	Discriminating probe T: TTTTTTTTCACACTGAAATTCTGCCTAAGGTT
	Discriminating probe G: TTTTTCACACTGAAATTCTGCCTAAGGTG
rs2291857	Forward primer: CTACTCAGTAGATATGCTATCAG
	Reverse primer: CAACATTTAGTATGAGGATGC
	Common probe:-P-ACCTTTCAATAAACATTAAACATGT-HEX-
	Discriminating probe A: CTAACACAAACCCTTATGCAATTTA
	Discriminating probe C: TTTCTAACACAAACCCTTATGCAATTTC
rs3730581	Forward primer: AGAAAATAGTTGACAGAGAGAA
	Reverse primer: GCATGTACGAGATTCTGGTCT
	Common probe:-P-TAGTAGACGAGAAGGCTGTTGCCTGTTT-HEX-
	Discriminating probe G: TTTTTTTAATAGTTGAGAACAGTTAGTAGACG
	Discriminating probe A: TTTTAATAGTTGAGAACAGTTAGTAGACA
rs3730635	Forward primer: AAGGTGGAAGAGCCTTTTCAG
	Reverse primer: CGAAAGTACCTACAGTGTGAC
	Common probe:-P-GTTAGAGGGGGAAAGTGTGGAAGTT-FAM-
	Discriminating probe G: TTTGGATTTTGAAACTGAAATTATTCTG
	Discriminating probe A: GGATTTTGAAACTGAAATTATTCTA

(A > G), rs1846402 (G > T), rs2291857 (C > A), rs3730581 (A > G), and rs3730635 (A > G) of the *MDM2* gene was performed using ligase detection reaction coupled with polymerase chain reaction (LDR-PCR) as previously published (22) in Shanghai Generay Biotechnology Co. Ltd. (China). Genotypes of *PPP1R13L* rs1970764 (A > G) and *CD3EAP* rs967591(G > A) and rs735482 (A > C) have been previously reported (20,21). This study only genotyped the loci for the increased samples. The sequences (5'-3') of primers and probes of *MDM2* five htSNPs are listed in *Table 2*. Each group of LDR probes consisted of 1 common probe and 2 discriminating probes for the 2 alleles. The steps for genotyping were in short: performed PCR reactions, completed LDR reactions and sequenced LDR products. The genotyping call-rate was 96% on average for the *MDM2* five htSNPs. As a quality control, some samples were genotyped in duplicate. Repeated genotyping yielded 100% identity.

Statistical analysis

Characteristics of cases and controls, allele frequencies, genotype frequencies, Hardy-Weinberg equilibrium, co-dominant model; dominant model; recessive model and

Table 3 Distribution of selected characteristics in the case-control study population

Characteristics	Cases, n (%)	Controls, n (%)	P value
Overall	627	633	
Age (years)			
Mean (±SD)	58 (±10.4)	58 (±10.5)	0.9[a]
≤40	29 (4.6)	29 (4.6)	
41–50	109 (17.4)	125 (19.7)	
51–60	222 (35.4)	214 (33.8)	0.748[b]
>60	267 (42.6)	265 (41.9)	
Gender			
Female	185 (29.5)	184 (29.1)	
Male	442 (70.5)	449 (70.9)	0.86[b]
Family history[c]			
No	536 (85.5)	628 (99.2)	
Yes	91 (14.5)	5 (0.8)	<0.0001[b,d]
Smoking duration			
Never	241 (38.4)	333 (52.6)	
≤20 (years)	104 (16.6)	98 (15.5)	<0.0001[b,d]
>20 (years)	282 (45.0)	202 (31.9)	

[a], for *t*-test; [b], for χ^2 test (two-sided); [c], family history of cancer; [d], statistical significance.

log-additive model for case-control association of each single-locus, haplotype associations, and pair-wise linkage disequilibrium (LD), unconditional logistic regression for measurement of odd ratio, 95% confidence interval (OR, 95% CI) after adjustment for smoking-duration were explored employing SPSS© v16.0 (SPSS Inc., Chicago, IL, USA) or SNPStats program (23) or Haploview software 4.2 (24). Haplotypes with frequency <0.01 among both cases and controls were excluded from the analysis. The interaction analyses of gene-gene and gene-gene-smoking duration in relation to lung cancer risk were implemented employing platform of multifactor dimensionality reduction (MDR). This software (3.0.3. dev. Jar) (25) is an updated version where permutation testing has been added into the main MDR program. The MDR method is nonparametric and free model. MDR has rational power to recognize interactions between two or more loci in relatively small samples. MDR has excellent power for identifying high-order gene-gene interactions. MDR is directly usable to case-control and discordant-sib-pair studies (25). If P value was less than 0.05, the difference was considered to be statistically significant. Power test was determined employing online statistical software: Unmatched Case/Control Studies (https://www.stat.ubc.ca/~rollin/stats/ssize/caco.html).

Results

Study population

The *MDM2* five htSNPs were genotyped in a Chinese hospital-based case-control study of 627 lung cancer patients and 633 control subjects. There were no statistically significant differences for the distribution of age and gender between cases and controls. However, more cases had a family history of cancer and cases had longer smoking history than controls (>20 years) (both P<0.0001) (*Table 3*).

Allele frequencies of MDM2 five htSNPs

The minor-allele frequencies (MAF) among the controls (G =0.24, T =0.16, A =0.32, G =0.49 and G =0.02 for rs1690924, rs1846402, rs2291857, rs3730581 and rs3730635, respectively), were similar to the MAF of HapMap-HCB reported by NCBI SNP database (https://www.ncbi.nlm.nih.gov/snp) (P=0.154, 0.552, 0.523, 0.078, respectively) except for rs3730635 (P=0.017) (*Table 1*). The genotype distribution in control population was in Hardy-Weinberg equilibrium for rs1690924 (P=0.29), rs1846402 (P=0.54), rs2291857(P=0.93), rs3730581 (P=0.23) and rs3730635 (P=1).

Association between MDM2 five htSNPs and lung cancer risk

No significant associations were found between genotype distributions and lung cancer risk for *MDM2* five htSNPs in co-dominant, dominant, recessive, and log-additive models after adjustment for smoking status (*Table 4*). Next, LD analysis was implemented. Strong LD was found between the five htSNPs (D' values from 0.768 to 0.9984 for pair-wise LD) except for rs1690924 and rs2291857 (D'=0.3291) and rs2291857 and rs3730635 (D'=0.4139) in middle LD (*Table 5*, *Figure 1A*). Five-locus haplotype analysis was performed (*Table 6*). Among 19 possible haplotypes, 9 commonly occurring haplotypes were identified (frequency: about or above 1%), capturing 97.28% (cumulative frequency) of all possible haplotypes. The haplotype analysis revealed that a statistically significant

Table 4 Associations of single htSNP in *MDM2* and *PPP1R13L* and *CD3EAP* with lung cancer risk[a,b]

Gene/rs Ca/Co	Co–dominant (AB *vs*. AA)/(BB *vs*. AA)/P	Dominant (AB + BB *vs*. AA)/P	Recessive (BB *vs*. AA + AB)/P	Log–additive – –/P
MDM2				
rs1690924(A>G)				
571/568	1.24 (0.96–1.59)/1.24 (0.77–2.00)/0.22	1.24 (0.97–1.57)/0.08	1.14 (0.71–1.83)/0.58	1.17 (0.97–1.42)/0.11
rs1846402(G>T)				
608/603	1.20 (0.93–1.56)/0.74 (0.35–1.59)/0.25	1.16 (0.90–1.48)/0.25	0.70 (0.33–1.50)/0.36	1.09 (0.87–1.36)/0.46
rs2291857(C>A)				
611/613	1.17(0.92–1.49)/1.02 (0.69–1.51)/0.42	1.14 (0.91–1.43)/0.26	0.94 (0.65–1.37)/0.76	1.06 (0.90–1.27)/0.47
rs3730581(A>G)				
613/621	1.18 (0.90–1.55)/0.99 (0.72–1.36)/0.35	1.11 (0.86–1.44)/0.42	0.89 (0.68–1.16)/0.39	1.00 (0.85–1.17)/0.99
rs3730635(A>G)				
612/625	1.24 (0.74–2.10)/NA (0.00–NA)/0.17	1.32 (0.79–2.21)/0.29	NA (0.00–NA)/0.092	1.37 (0.84–2.26)/0.21
PPP1R13L				
rs1970764(A>G)				
594/590	1.06 (0.79–1.40)/1.39(0.99–1.94)/0.11	1.15 (0.88–1.50)/0.32	1.34 (1.02–1.76)/0.037[c]	1.18 (0.99–1.39)/0.057
CD3EAP				
rs967591(G>A)				
594/590	1.31 (1.01–1.70)[c]/1.28 (0.92–1.78)/0.11	1.30 (1.01–1.67)/0.038[c]	1.09 (0.81–1.45)/0.58	1.15 (0.98–1.35)/0.092
rs735482(A>C)				
603/595	1.13 (0.87–1.48)/1.14 (0.82–1.57)/0.62	1.13 (0.88–1.46)/0.32	1.05 (0.79–1.39)/0.73	1.07 (0.91–1.26)/0.4

[a], Dominant model: AB (Heterozygote) + BB (Homozygous variant-type) versus AA (Homozygous wild-type), Recessive model: BB versus AA + AB, Co-dominant model: AB versus AA and BB versus AA, Log-additive model: analysis of trend where AA is '0', AB is '1' and BB is '2'; [b], OR (95% CI), adjusted for smoking duration; [c], statistical significance.

Table 5 D' statistics of linkage disequilibrium analysis for *MDM2* htSNPs[a]

rs number	rs1690924	rs1846402	rs2291857	rs3730581	rs3730635
rs1690924	–	0.9984	0.3291	0.8816	0.7944
rs1846402	<2e-16	–	0.768	0.9125	0.9903
rs2291857	<2e-16	<2e-16	–	0.9121	0.4139
rs3730581	<2e-16	<2e-16	<2e-16	–	0.9452
rs3730635	0.000339	0.000501	1.92e-06	5.55e-15	–

[a], above is the D' value, below is the P value.

global haplotype association after adjustment for smoking duration (P=0.0036) and that haplotype5 (rs1690924^{A}-rs1846402^{G}-rs2291857^{C}-rs3730581^{G}-rs3730635^{A}) was associated with reduced risk of lung cancer after adjustment for smoking duration (0.0279 for cases and 0.0552 for controls) [OR (95% CI) =0.52 (0.33–0.82), P=0.0053]. Of minor importance, the combined group of 10 rare haplotypes was associated with increased risk of lung cancer

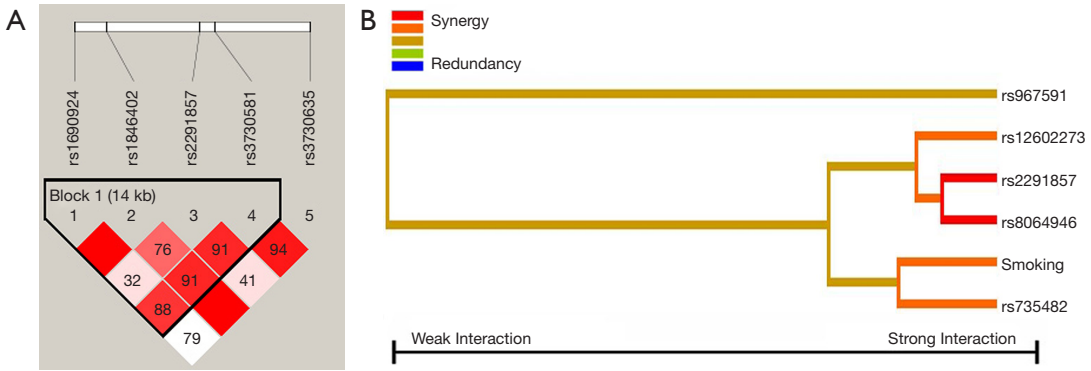

Figure 1 (A) D' LD map and LD plot of *MDM2* five htSNPs generated by Haploview 4.2. One block was detected. The criteria of block partition were based on solid spine LD. The digit in the boxes represents D' value (e.g., 99 means 0.99; 1 means 0.01; empty boxes means 1.0). Deep red boxes designate strong evidence of LD. Light red boxes designate uninformative. White boxes designate strong evidence of recombination. (B) Interaction dendrogram resulting from MDR analysis of 14 attributors in *MDM2*, *PPP1R13L*, *CD3EAP*, *TP53* and smoking-duration [entropy-based IG (the value of information gain) for the SNP pairs]. Red bar and orange bar indicate the high-level synergies on the phenotype, while the brown indicate a medium-level interaction, green and blue connections indicate redundancy or lack of synergistic interactions between the markers.

Table 6 Haplotype association of *MDM2* htSNPs with lung cancer risk[a]

Haplotype[b]	Construction[c]	Case frequency	Control frequency	OR (95% CI)	P value
1	AGCAA	0.476	0.4844	1.00 (Reference)	–
2	ATAGA	0.1303	0.1403	1.00 (0.77–1.28)	0.98
3	GGAGA	0.1456	0.1219	1.17 (0.91–1.51)	0.23
4	GGCGA	0.1021	0.1073	0.97 (0.73–1.29)	0.82
5	AGCGA	0.0279	0.0552	0.52 (0.33–0.82)[d]	0.0053[d]
6	AGAGA	0.0284	0.0311	1.07 (0.64–1.79)	0.79
7	ATCGA	0.0242	0.0142	1.64 (0.81–3.31)	0.17
8	AGAGG	0.0196	0.0121	1.41 (0.72–2.76)	0.32
9	AGAAA	0.009	0.0164	0.54 (0.23–1.29)	0.17
Rare	–	NA	NA	2.23 (1.21–4.09)[d]	0.0099[d]

[a], global haplotype association P value: 0.0036, adjusted for smoking duration; [b], five-locus order: rs1690924-rs1846402-rs2291857-rs3730581-rs3730635; [c], underlined indicates minor allele; [d], statistical significance.

after adjustment for smoking duration [OR (95% CI) =2.23 (1.21–4.09), P=0.0099]. No evidence was found for individual htSNPs of *MDM2* in smoking-subgroup analyses (data not shown).

Association of PPP1R13L and CD3EAP SNPs and lung cancer

PPP1R13L rs1970764(A > G) in recessive model [OR (95% CI) =1.34 (1.02–1.76), P=0.037] and *CD3EAP* rs967591(G > A) in dominant model [OR (95% CI) =1.30 (1.01–1.67), P=0.038] were associated with increased risk of lung cancer after controlling for smoking duration in the current expanded study (*Table 4*).

Interactions of gene-gene-smoking

Table 7 summarizes the best significant candidate models of

Table 7 The best candidate models for interactions of gene-gene-smoking duration from MDR analysis[a,b]

Model	Attributes	Bal. Acc.[c] overall	Bal. Acc. CV[d] training	Bal. Acc. CV testing	CV consistency	P value[e]
MDM2 + *PPP1R13L* + *CD3EAP* + smoking-duration						
One-locus	Smoking	0.5708	0.5708	0.5708	10/10	0.0020–0.0030
Two-locus	Smoking					
	rs735482	0.5831	0.5833	0.5657	8/10	0.0040–0.0050
MDM2 + *PPP1R13L* + *CD3EAP* + *TP53* + smoking-duration						
One-locus	Smoking	0.5708	0.5708	0.5708	10/10	0.0080–0.0090
Two-locus	Smoking					
	rs735482	0.5831	0.5833	0.5657	8/10	0.0130–0.0140
MDM2 + *TP53* + smoking-duration						
One-locus	Smoking	0.5708	0.5708	0.5708	10/10	0.0030–0.0040
Two-locus	Smoking					
	rs1846402	0.579	0.5807	0.557	7/10	0.0170–0.0180
Three-locus	Smoking					
	rs2291857					
	rs8064946	0.6073	0.609	0.57	8/10	0.0030–0.0040
Fourth-locus	Smoking					
	rs1690924					
	rs2291857					
	rs8064946	0.6446	0.6486	0.5535	8/10	0.0250–0.0260

[a], analyzed by MDR 3.0.3. dev. Jar, data of *TP53* from previous study (Yin *et al.* submitted and revised); [b], only list statistical significant models; [C], balanced accuracy; [d], cross-validation; [e], P value based on 1,000 permutation test, statistical significant P value.

gene-gene-smoking duration interactions for combinations of htSNPs in *MDM2*, *PPP1R13L*, *CD3EAP* and smoking-duration, for combinations of htSNPs in *MDM2*, *PPP1R13L*, *CD3EAP*, *TP53*, and smoking-duration and for combinations of htSNPs in *MDM2*, *TP53*, and smoking-duration from MDR analysis. In the analysis for *MDM2*, *TP53*, and smoking-duration, one three-locus model and one four-locus model had relative higher values of balanced accuracy overall (0.6073 or 0.6446) and cross-validation consistency (8/10) that were significant at the 0.0030-0.0040 or 0.0250-0.0260 level. The three-locus model and four-locus model consisted of *MDM2*, *TP53* and smoking duration. One two-locus model including *MDM2* rs1846402 was significant. In the analysis for *MDM2*, *PPP1R13L*, *CD3EAP*, and smoking-duration or *MDM2*, *PPP1R13L*, *CD3EAP*, *TP53*, and smoking-duration, one two-locus (*CD3EAP* rs735482 and smoking duration) had values of balanced accuracy overall of 0.5831 and cross-validation consistency of 8/10 that was significant at the 0.0040-0.0050 or 0.0130-0.0140 level. No significant interaction model was found when smoking-duration was excluded.

Figure 1B shows the dendrogram of 14 attributes in the interaction analysis of *MDM2*, *PPP1R13L*, *CD3EAP*, *TP53*, and smoking-duration built using the MDR software. The entropy-based dendrogram indicated that a high degree of synergy interaction exists between *MDM2* rs2291857 and *TP53* rs8064946. There was a lesser degree of synergy interaction between *CD3EAP* rs735482 and smoking duration.

Discussion

We evaluated five htSNPs of *MDM2* gene in relation to lung cancer risk in current study. Only one of these SNPs, rs1690924, has previously been assessed in a study of the

chemotherapy outcome in lung cancer patients (26-29). To the best of our knowledge, none of the remaining four htSNPs have been assessed previously in relation to lung cancer risk. The majority of the *MDM2* SNP studies have focused on *MDM2* rs2279744 (SNP T309G) in relation to lung cancer risk with inconsistent results (4-15).

An Asian-Korean study reported that *MDM2* rs2279744 was associated with increased risk of lung adenocarcinoma [GG versus TT, adjusted OR (95% CI) =1.91 (1.16–3.14), P=0.01] and the risk of lung adenocarcinoma increased as the number of rs2279744 G alleles increased [P (trend) =0.01] (4). An Asian-Chinese study reported that *MDM2* rs2279744 was associated with an increased lung cancer risk [GG versus TT, OR (95% CI) =1.83 (1.45–2.32)] and [TG versus TT, OR (95% CI) =1.33 (1.09–1.63)] and that the interactions between *MDM2* and *TP53* polymorphisms increased lung cancer risk [for the presence of both *MDM2* rs2279744 GG and *TP53* rs1042522 CC, OR (95% CI) =4.56 (2.76–7.54)] and interactions of the polymorphisms (respectively and jointly) and smoking [smokers with both the *MDM2* rs2279744 GG and *TP53* rs1042522 CC, OR (95% CI) =10.41 (5.26–20.58)] (5). A Caucasian-Norwegian study reported that the *MDM2* rs2279744 GG genotype was associated with risk of non-small cell lung cancer (NSCLC) [OR (95% CI) =1.62 (1.06–2.50)] and the GG genotype was associated with higher age at diagnosis in individuals with *TP53* mutations (P=0.037) (6). A Caucasian-Norwegian study found a slightly reduced risk for lung cancer among individuals harboring the *MDM2* rs2279744 G allele [TG/GG versus TT, OR (95% CI) =0.86 (0.67–0.98)] (7). An Asian-Singaporean study reported that *MDM2* rs2279744 TT genotype was associated with increased risk of lung cancer [TT versus GG, OR (95% CI) =2.1 (1.01–4.36)] and carriers of this genotype with the *TP53* rs1042522 C allele had a 2.5-fold increased risk [OR (95% CI) =2.5 (1.2–5.0)] among non-smoking Chinese women (8). An Asian-Chinese study reported increased risk for carriers of the *MDM2* rs2279744 GG genotype in relation to lung adenocarcinoma risk [GG versus TT, adjusted OR (95% CI) =1.68 (1.27–2.21)]. The combination of *TP53* rs1042522 CC and *MDM2* rs2279744 GG genotypes [adjusted OR (95% CI) =2.66 (1.54–4.60)] interacted in relation to lung adenocarcinoma risk (9). An Asian-Chinese study reported that the *P73* rs2273953 and rs1801173 AT/AT [AT/AT versus GC/GC, OR (95% CI) =0.46 (0.22–0.97)] and *MDM2* rs2279744 TT [TT versus GG, OR (95% CI) =0.48 (0.26–0.86)] genotypes were associated with a decreased risk of developing NSCLC, and interaction between the P73 and MDM2 polymorphisms such that carriers of both the *P73* AT/AT and *MDM2* TT genotypes were at reduced risk of developing NSCLC [OR (95% CI) =0.13 (0.03–0.59)] (10).

A meta-analysis including 7 studies encompassing in total 4,276 cases and 5,318 controls revealed that *MDM2* rs2279744 (SNP T309G) was associated with increased risk of lung cancer for homozygous G-allele carriers [GG versus TT, OR (95% CI) =1.27 (1.12–1.44)] (11). Recently, a meta-analysis including 11 articles with a total 6,470 NSCLC patients and 8,027 controls concluded that the *MDM2* rs2279744 (SNP T309G) polymorphism may contribute to NSCLC susceptibility, especially for Asians and women (12).

A Caucasian-Canada study found no overall association between the *MDM2* rs2279744 genotypes and NSCLC risk [T/G versus TT, adjusted OR (95% CI) =0.82 (0.6–1.1)] and [G/G versus TT, adjusted OR (95% CI) =1.32 (0.9–2.0)] and but reported interaction (P=0.01) between smoking and *MDM2* rs2279744 genotypes (13). A study on a population consisting of Caucasians in the United States and African-Americans reported that *MDM2* rs2279744 (SNP T309G) and *MDM2* rs769412 (SNP A354G) were not associated with lung cancer risk (14). An Asian-Japanese study reported no association between *MDM2* rs2279744 (SNP T309G) and lung cancer risk (15).

An Asian-Japanese study reported reduced overall survival of carriers of the *MDM2* rs2279744 TT genotype as compared to carriers of the TG or GG genotypes (P=0.02) for patients with stage I lung adenocarcinoma (26). An Asian-Chinese study reported that carriers of *MDM2* rs1690924 AG genotype were more sensitive to gastrointestinal toxicity than carriers of the wild-type homozygote GG [OR (95% CI) =2.32 (1.30–4.14), P=0.004)], suggesting *MDM2* rs1690924 could be used to predict the toxicities of platinum-based chemotherapy in patients with advanced NSCLC (27). Recently, a Caucasian-Spanish study reported that *MDM2* rs1690924 GG genotype presented higher risk of death [HR (95% CI) =1.99 (1.05–3.80), P=0.0345], suggesting one may significantly act as predictive factors of survival among NSCLC patients treated with platinum-based chemotherapy (28). A Caucasian-Spanish study did not find the influence of *MDM2* rs1690924 on platinum-based chemotherapy toxicity for NSCLC patients (29).

To the best of our knowledge, the current study is the first systematically assess the association and interaction of *MDM2* common variants in relation to lung cancer risk. In this study, the role of five htSNPs (rs1690924, rs1846402,

rs2291857, rs3730581 and rs3730635) across *MDM2* gene tagging 95% common haplotype diversity was evaluated. The five htSNPs are all located in intron regions. The five htSNPs selected did not include *MDM2* rs2279744 (SNP T309G) SNP which is the most extensively studied polymorphism in previous epidemiological studies because the htSNP choice wads random. The htSNPs were selected solely based on their linkage with other SNPs in *MDM2*.

In the present study, we documented positive association of global five-locus haplotype and negative association of haplotype5 (rs1690924^{A}-rs1846402^{G}-rs2291857^{C}-rs3730581^{G}-rs3730635^{A}) containing one variant-allele of rs3730581 with risk of lung cancer after adjusting smoking-duration. This finding is close to findings reported by others that carriers at least one variant-allele of *MDM2* SNP [rs2279744 (SNP T309G)] were at reduced risk of lung cancer in a study of Caucasian-Norwegians (7).

We also documented that smoking-duration was the most important risk factor for lung cancer (one-locus model) and found interactions between *MDM2* rs2291857 and *TP53* rs8064946 (three-locus model), or *MDM2* rs1690924, rs2291857 and *TP53* rs8064946 (four-locus model), and or *MDM2* rs1846402 (two-locus model) with smoking-duration in relation to lung cancer risk. Furthermore, we documented strong synergy interaction between *MDM2* rs2291857 and *TP53* rs8064946 in relation to smoking-induced lung cancer. These findings are in agreement with previous Chinese studies showing interactions between *MDM2* and *TP53* polymorphisms and smoking in relation to lung cancer risk (5,9). Smoking-duration has been considered a more important risk factor for lung cancer developing than other smoking-related factors such as cumulative smoking (17). Tobacco carcinogens may induce various types of DNA damage and increase genomic instability during long-term smoking. The *TP53* tumor suppressor gene, cellular gatekeeper and guardian of genome, coordinates protective cellular responses to oncogenic stressors, such as DNA damage (30). TP53 activity is tightly controlled by MDM2. MDM2 is a primary negative regulatory factor for TP53 (3). The TP53-MDM2 negative feedback loop constitutes the core module of a network of regulatory interactions activated under cellular stress. In normal cells, the level of TP53 proteins is kept low through negative regulation by MDM2 (31). Through its N-terminal domain, MDM2 binds to TP53 and forms the MDM2–TP53 complex. The binding process obscures the *TP53* transcription activation region and reduces *TP53* transcription activity. MDM2 exerts the inhibitory effect not only through blocking its transcriptional activity, but also through directly eliminating it from the cell for down-regulating TP53 (3). Overall, the observed interactions support the notion that smoking-duration interact with genetically determined variation in *MDM2* activity to modulate the individual's predisposition towards smoking-induced lung cancer.

In this expanded study group including 83 new cases and 78 new controls, we were also able to reproduce our previous findings that *PPP1R13L* rs1970764 and *CD3EAP* rs967591 were associated with lung cancer risk and a previously reported interaction between *CD3EAP* rs735482 and smoking-duration in relation to lung cancer risk (19-21).

The present study included 1,260 participants and power analyses showed that we had 89%, 92%, 94%, 86% and 34% chance for rs1690924, rs1846402, rs2291857, rs3730581 and rs3730635, respectively, to detect OR =1.5 at the 0.05 significant level using two-sided tests under the dominant model. The low statistical power for rs3730635 is due to the low MAF of 0.02 in present controls population, which is significantly lower than reported HCB frequency in NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp). Thus, further larger population-based studies are warranted to confirm present findings.

Conclusions

In conclusion, *MDM2* five-htSNPs haplotype exhibited association with lung cancer susceptibility, interaction of *MDM2* and *TP53* htSNPs and smoking-duration contributed to lung cancer risk and strong synergy between *MDM2* and *TP53* htSNPs influenced lung cancer predisposition. Our results suggest that *MDM2*, *TP53* and smoking-duration interact in relation to lung carcinogenesis.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Chinese Administration Office of Human Genetic Resources {no. [2001]015} and informed consent was taken from all the participants.

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