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

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**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 (rs1690924A-rs1846402G-rs2291857C-rs3730581G-rs3730635A) 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 the two best significant models and strong synergy between *MDM2* and *TP53*.

**Conclusions:** *MDM2* five-hSNPs 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

Submitted Dec 31, 2019. Accepted for publication Jun 30, 2020.
doi: 10.21037/atm-19-4784

View this article at: http://dx.doi.org/10.21037/atm-19-4784
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 a 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-oncoprotein) (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** hSNP2 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** hSNPs (Haplotype-tagging SNP) and lung cancer risk. In this Chinese case-control study we explored the role of hSNPs 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** hSNPs, **TP53** hSNPs, 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 hSNPs

Using the TagSNPs software online and approaches of the algorithm-Tagger-pairwiseTagging, hSNPs 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
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 \textit{MDM2} gene. \textbf{Table 1} shows the information of \textit{MDM2} five htSNPs, three risk SNPs of \textit{PPP1R13L} and \textit{CD3EAP} and \textit{TP53} five htSNPs. Three risk SNPs of \textit{PPP1R13L} and \textit{CD3EAP} were previously reported (20,21) while we increased the number of included samples in present study. The genotype data of \textit{PPP1R13L} and \textit{CD3EAP} three risk SNPs and \textit{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

\begin{table}[ht]
\centering
\begin{tabular}{llllll}
\hline
\textbf{dbSNP ID} & \textbf{Position} & \textbf{Location} & \textbf{Base change} & \textbf{Allele frequency in HapMap HCB} & \textbf{MAF in controls for current study} \\
\hline
\textit{MDM2} & & & & & \\
\textbf{Chr12q15} & & & & & \\
rs1690924 & 68811541 & Intron & A/G & A: 0.814/G: 0.186 & G: 0.24 \\
r1846402 & 68814798 & Intron & G/T & G: 0.826/T: 0.174 & T: 0.16 \\
r2291857 & 68824258 & Intron & C/A & C: 0.700/A: 0.300 & A: 0.32 \\
r3730581 & 68825712 & Intron & A/G & A: 0.581/G: 0.419 & G: 0.49 \\
r3730635 & 68835343 & Intron & A/G & A: 0.946/G: 0.054 & G: 0.02 \\
\textit{PPP1R13L} & & & & & \\
r1970764 & 45387615 & Intron & A/G & No & G: 0.48 \\
\textit{CD3EAP} & & & & & \\
r967591 & 45406676 & 5'UTR & G/A & G: 0.525/A: 0.475 & A: 0.42 \\
r735482 & 45408744 & Exon3 & A/C & A: 0.558/C: 0.442 & C: 0.45 \\
& & & Codon 261 (K [Lys] [AAA] \Rightarrow T [Thr] [ACA]) (missense) & & \\
\textit{TP53} & & & & & \\
r12951053 & 7674089 & Intron & A/C & A: 0.667/C: 0.333 & C: 0.34 * \\
r1042522 & 7676154 & Exon4 & G/C & G: 0.511/C: 0.489 & C: 0.45 * \\
& & & Codon 72 (R [Arg] [CGC]) \Rightarrow P [Pro] [CCC] (missense) & & \\
r8079544 & 767634 & Intron & C/T & C: 0.878/T: 0.122 & T: 0.08 * \\
r12602273 & 7679695 & Intron & C/G & C: 0.678/G: 0.322 & G: 0.28 * \\
r8064946 & 7685993 & Intron & G/C & G: 0.622/C: 0.378 & C: 0.32 * \\
\hline
\end{tabular}
\caption{Characteristics for \textit{MDM2} htSNPs selected and SNPs in \textit{PPP1R13L} and \textit{CD3EAP} and htSNPs in \textit{TP53} 
\textbf{a}, information from NCBI SNP database (GRCh38.p7) and HapMap database; \textbf{b}, Han Chinese in Beijing; \textbf{c}, minor allele frequency; \textbf{d}, CHB+JPT (Han Chinese in Beijing + Japanese from 1000 GENOMES); \textbf{e}, from previous result, here this is employed for interaction analysis.}
\end{table}
The sequences (5'-3') of primers and probes for MDM2 5 htSNPs examined

| rs number   | Primers and probes                                                                 |
|-------------|-----------------------------------------------------------------------------------|
| rs1690924   | Forward primer: TGTAATGGAAAGCCATCAGTAT  
Reverse primer: TCTCCTGTCACAAGATCTTGC  
Common probe: P-GCTATAAAAGATAATAGCATTTGTA-FAM  
Discriminating probe G: TTTAAAGACATGTATTAATGAGAAAACG  
Discriminating probe A: AAAGACATGTATTAATGAGAAAACA |
| rs1846402   | Forward primer: TAAGTGGGAGAGACAGAGAC  
Reverse primer: CCAGGTAAAGACACTCTGCAC  
Common probe: P-GCTCAATCTGTCACTGAAAATCATGTTT-FAM  
Discriminating probe T: TTTTTTTCACTGAAGAACATCGTCT  
Discriminating probe G: TTTTTCACACTGAAATCTGGTT |
| rs2291857   | Forward primer: CTACTCATAGATATGCTAC  
Reverse primer: CAACATTTAGTATGAGATGC  
Common probe: P-ACCTTTCAATACATACATGAT-HEX  
Discriminating probe A: CTAACACAAACCCTCTATGCAATTTA  
Discriminating probe C: TTTTCAACAAACCCTTTGATGCAATTC |
| rs3730581   | Forward primer: AGAAAAATAGTTGACAGAGAA  
Reverse primer: GCATGTACGAGATCATCTGCT  
Common probe: P-TAGTAGACGAGATCTGTTT-FAM  
Discriminating probe G: TTTTTTTAATAGTTGAGAACAGTTAGACG  
Discriminating probe A: TTTTATAAGTTGAGACAGTTAGACAG |
| rs3730635   | Forward primer: AAGGTGGAAGAGCCTTTTACC  
Reverse primer: CGAAAGTACCTACAGTGAC  
Common probe: P-GTTAGAGGGAAAGTGTGGAAGTT-FAM  
Discriminating probe G: TTTGGATTTTGAAACTGAAATTATTCTG  
Discriminating probe A: GGATTTTGAAACTGAAATTATTCTA |

(A > G), rs1846402 (G > T), rs2291857 (C > G), 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 PP1R13L 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, codominant model; dominant model; recessive model and
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

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**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)      | 0.9a    |
| ≤40                     | 29 (4.6)     | 29 (4.6)        |         |
| 41–50                   | 109 (17.4)   | 125 (19.7)      |         |
| 51–60                   | 222 (35.4)   | 214 (33.8)      | 0.748b  |
| >60                     | 267 (42.6)   | 265 (41.9)      |         |
| Gender                  |              |                 |         |
| Female                  | 185 (29.5)   | 184 (29.1)      |         |
| Male                    | 442 (70.5)   | 449 (70.9)      | 0.86b   |
| Family historyc         |              |                 |         |
| No                      | 536 (85.5)   | 628 (99.2)      |         |
| Yes                     | 91 (14.5)    | 5 (0.8)         | <0.0001h,d |
| Smoking duration        |              |                 |         |
| Never                   | 241 (38.4)   | 333 (52.6)      |         |
| ≤20 (years)             | 104 (16.6)   | 98 (15.5)       | <0.0001h,d |
| >20 (years)             | 282 (45.0)   | 202 (31.9)      |         |

*a*, for t-test; *b*, for χ² test (two-sided); *c*, family history of cancer; *d*, statistical significance.
Table 4  Associations of single htSNP in MDM2 and PPP1R13L and CD3EAP with lung cancer risk\(^a\,b\)

| Gene/rs         | 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)  | 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)  | 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)  | 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)  | 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)  | 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)  | 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)   | 1.31 (1.01–1.70)\(^j\)/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)   | 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 (Homzygous 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\(^4\)-rs1846402\(^4\)-rs2291857\(^4\)-rs3730581\(^4\)-rs3730635\(^4\)) 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.
Table 6  Haplotype association of MDM2 htSNPs with lung cancer risk

| Haplotype | Construction | 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         | GCCGA        | 0.1021         | 0.1073            | 0.97 (0.73–1.29)     | 0.82    |
| 5         | AGCA         | 0.0279         | 0.0552            | 0.52 (0.33–0.82)     | 0.0053  |
| 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)     | 0.0099  |

* 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.

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.

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
gene-gene-smoking duration interactions for combinations of hSNPs in MDM2, PPP1R13L, CD3EAP, and smoking-duration, for combinations of hSNPs in MDM2, PPP1R13L, CD3EAP, TP53, and smoking-duration and for combinations of hSNPs 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, 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 hSNPs 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 GG/GG, 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 was 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 (rs1690924A-rs1846402G-rs2291857C-rs3730581C-rs3730635G) 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.

Acknowledgments

Funding: This study was supported by the National Natural Science Foundation of China (Grant No. 30571016 and No. 81072384).
Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-19-4784

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-19-4784). The authors have no conflicts of interest to declare.

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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Cite this article as: Yin J, Hou W, Vogel U, Ma Y, Wang C, Wang H, Sun Z. Interaction between common variants of MDM2 and PPP1R13L and CD3EAP and TP53 SNPs in relation to lung cancer risk among Chinese. Ann Transl Med 2020;8(15):934. doi: 10.21037/atm-19-4784