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Original Article

TP53 common variants and interaction with PPP1R13L and CD3EAP SNPs and lung cancer risk and smoking behavior in a Chinese population

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ABSTRACT

Background: *TP53* encodes a tumor suppressor protein containing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. The effect of *TP53* inactivation is well-known, and genetically determined smaller variations in *TP53* activity are related to cancer. Lung cancer causes the highest rates of morbidity and mortality in the world. Epidemiology studies have assessed the association of *TP53* single nucleotide polymorphisms with lung cancer.

Methods: We systematically examined the association of five htSNPs (haplotype-tagging single nucleotide polymorphism) (rs12951053, rs1042522, rs8079544, rs12602273 and rs8064946) across the entire *TP53* locus and interaction between genes *TP53* and *PPP1R13L* and *CD3EAP* and smoking-duration related to lung cancer risk in this Chinese study including 544 cases and 550 controls.

Results: No significant associations were observed in analysis of alleles and genotypes with co-dominant, dominant, recessive, and log-additive models after adjustment for smoking status. Haplotype analysis showed that haplotype9 ($rs12951053^{A}$-$rs1042522^{C}$-$rs8079544^{C}$-$rs12602273^{G}$-$rs8064946^{C}$) [OR (95% CI) = 0.13 (0.03–0.59), $p = 0.0079$] was associated with decreased risk of lung cancer after adjusted for smoking-duration. The analysis of smoking-duration within *TP53* haplotypes showed that there were more carriers of haplotype1 (AGCCG), 2 (CCCGC) and 4 (CCCCG) in smoking-subgroup of >20 (years) (all $p < 0.05$). MDR testing analysis identified two significant models (both $p < 0.0010$) of gene-gene-environment interaction in relation to lung cancer risk in whole study group.

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Conclusion: The present results provide novel evidence that the haplotype of *TP53* htSNPs and interaction between genetic variation in *TP53* and *CD3EAP* and smoking-duration may associate with lung cancer risk, and provide additional evidence of association between *TP53* htSNP haplotypes and long-term smoking-related behavior.

At a glance commentary

Scientific background on the subject

The *TP53* is the most commonly mutated gene in human cancers. Epidemiology studies have assessed the association of *TP53* SNPs and lung cancer with inconsistent results. This hospital-based case-control study systematically assessed the association of *TP53* htSNPs with lung cancer risk as well as gene-gene and gene-gene-smoking interactions.

What this study adds to the field

The present results suggest novel evidence that the haplotype of *TP53* htSNPs and interaction between genetic variation in *TP53* and *CD3EAP* and smoking-duration may associate with lung cancer risk, and suggest association between *TP53* htSNP haplotypes and long-term smoking-related behavior.

Lung cancer is malignant tumors that cause the highest rates of morbidity and mortality in the world [1]. Lung cancer is a complex polygenic disease. Smoking is the most important risk factor for lung cancer. Most patients with lung cancer have developed genetic mutations due to environmental exposure to carcinogens including smoking. Hereditary, genetic, and environmental factors interact in its genesis [2].

The gene tumor protein p53 (*TP53*, Aliases: *BCC7*, *LFS1*, *P53*, *TRP53*) (Gene ID: 7157) is located on chromosome 17p13.1 and includes 12 exons. *TP53* encodes the tumor suppressor p53 containing transcriptional activation, DNA binding, and oligomerization domains. p53 responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. *TP53* is the most commonly mutated gene in human cancers. Approximately half of all human malignancies exhibit *TP53* mutations {https://www.ncbi.nlm.nih.gov/gene/7157, [3]}. While the effect of *TP53* inactivation is well-known, genetically determined smaller variations in *TP53* activity are also related to risk of cancer. Epidemiology studies have assessed the association of *TP53* SNPs (single nucleotide polymorphism) with lung cancer [4–12]. However, the published study results are inconsistent [7,13,14].

Two genes governing biological function on Chr19q13.3, *PPP1R13L* [protein phosphatase 1, regulatory (inhibitor) subunit 13 like] (Gene ID: 10848), one of the most evolutionarily conserved inhibitors of *TP53*, is related to DNA repair and cell survival and *CD3EAP* (CD3e molecule, epsilon-associated protein) (Gene ID: 10849) may be related to cell proliferation. SNPs of *PPP1R13L* rs1970764 and *CD3EAP* rs967591 and rs735482 have been associated with lung cancer risk among both Caucasian Danes and Chinese in our previous studies [15–19].

TP53 and *PPP1R13L* and *CD3EAP* all belong to pathway of gene expression. *TP53* and *PPP1R13L* share the same 7 pathways such as gene expression, generic transcription pathway, integrated pancreatic cancer pathway, regulation of TP53 activity, regulation of TP53 activity through association with co-factors, transcriptional regulation by TP53 and p53 pathway [https://www.ncbi.nlm.nih.gov/gene/7157, /10848, and /10849, assessed July 2019].

Furthermore, genetic factor of the *TP53* htSNPs (haplotype-tagging single nucleotide polymorphism) and interactions of gene-gene and gene-environment related to lung cancer in the same biological pathways will provide important information about carcinogenesis and etiology of the disease. In the present Chinese case-control study of lung cancer, we assessed the association of *TP53* htSNPs with lung cancer risk as well as gene-gene and gene-gene-smoking interactions. In addition, we explored potential association between *TP53* htSNP haplotypes and smoking-related behaviors.

Materials and methods

Ethical consideration

The Human Genetic Resource Administration of China, Ministry of Science and Technology of the People's Republic of China (Beijing, P. R. China) approved this study. Academic Committee of Shenyang Medical College (Shenyang, P. R. China) approved the review of human medical ethics for this study. The study was in accordance with the principles of the Declaration of Helsinki. Written or oral informed consent was obtained from all study participants.

Study population

In total, 1094 subjects (544 lung cancer cases and 550 cancer-free controls) were recruited to participate in this retrospective hospital-based case-control study as previously described [17,20]. Briefly, this study population was recruited during the period January 2002 to Match 2009. Case specimens were collected from Liaoning Cancer Hospital, P. R. China. Standard clinical and histological criteria were used for lung cancer diagnosis. Qualified cases were previously untreated (no

Table 1 Data for *TP53* htSNPs selected and SNPs in *PPP1R13L* and *CD3EAP*[a].

dbSNP ID	Position	Location	Base change	Allele frequency in HapMap HCB[c]	MAF[b] in controls for current study
Chr17p13.1					
TP53					
rs12951053	7674089	intron	A/C	A0.667/C0.333	C: 0.34
rs1042522	7676154	exon4	G/C	G0.511/C0.489	C: 0.45
Codon 72 (R [Arg] [CGC]) ⇒ P [Pro] [CCC] (missense)					
rs8079544	7676734	intron	C/T	C0.878/T0.122	T: 0.08
rs12602273	7679695	intron	C/G	C0.678/G0.322	G: 0.28
rs8064946	7685993	intron	G/C	G0.622/C0.378	C: 0.32
Chr19q13.3					
PPP1R13L[a]					
rs1970764	45387615	intron	A/G	No	G: 0.46
CD3EAP[a]					
rs967591	45406676	5′ UTR	G/A	G0.525/A0.475[d]	A: 0.39
rs735482	45408744	exon3	A/C	A0.556/C0.444	C: 0.45
Codon 261 (K [Lys] [AAA] ⇒ T [Thr] [ACA]) (missense)					

[a] Information from NCBI SNP database (GRCh38.p7) and HapMap database.
[b] Minor allele frequency.
[c] Han Chinese in Beijing.
[d] CHB+JPT (Han Chinese in Beijing+ Japanese from 1000 GENOMES).

chemotherapy or radiotherapy for cancer prior to recruitment). Cancer-free controls were selected from the orthopedics wards of Second Affiliated Hospital, Shenyang Medical College, P. R. China. Randomly selected controls were matched to the cases (1:1) by age (±3 years), gender (same) and ethnicity (same). All participants were unrelated ethnic Han Chinese. Stratification criteria were determined as follows: age (10 years an interval), smoking duration (20 years an interval) and histology (3 subgroups). All covariate data were obtained from questionnaires (or medical record) by interview (or extract) of professional doctors.

htSNP choice in TP53

We chose htSNPs of *TP53* gene from the International HapMap Project (http://www.hapmap.org, HapMap Data Rel 27 PhaseII+III, Feb09, on NCBI B36 assembly, dbSNP b26) using the TagSNPs software online and approaches of the algorithm-Tagger-pairwiseTagging on chr17:7512445..7531642, qualified criteria: r^2-cut off of 0.8 and MAF (minor allele frequency)-cut off of 0.05 in CHB (Han Chinese in Beijing) samples. Five htSNPs (rs12951053, rs1042522, rs8079544, rs12602273 and rs8064946) were selected across the *TP53* gene, representing 95% of the common haplotype diversity. [Table 1] shows the information of *TP53* five htSNPs and risk SNPs on Chr19q13.3 sub-region (*PPP1R13L* rs1970764 and *CD3EAP* rs967591 and rs735482). The genotype data of three risk SNPs on Chr19q13.3 were employed for interaction analyses of gene-gene and gene-gene-environment in current study. The genotype data of three risk SNPs of Chr19q13.3 were previously reported [17,20]. *CD3EAP* rs736482 was re-genotyped for individuals who genotyping failed in the previous study [17].

DNA isolation and genotyping

Genomic DNA of peripheral blood samples was extracted using the Puregene DNA Isolation Kit or FlexiGene DNA kit 250 (Gentra Systems, Minneapolis, MN, USA or Qiagen, Germany). The status of *TP53* rs12951053, rs1042522, rs8079544, rs12602273, and rs8064946 and *CD3EAP* rs735482 was determined in the study participants using the genotyping assay of ligase detection reaction coupled with polymerase chain reaction (LDR-PCR) as previously published [20,21] in Shanghai Generay Biotechnology Co. Ltd. (P. R. China). The sequences (5′–3′) of primers and probes of *TP53* htSNPs and *CD3EAP* rs735482 are showed in Supplementary Table S1. Each group of LDR probes contained 1 common probe and 2 discriminating probes for the 2 alleles. In brief: performed PCR reactions, completed LDR reactions and sequenced LDR products. The call rate of the genotyping was 93% on average for the five *TP53* htSNPs. Repeated genotyping of a subset of the samples yielded 100% identity.

Statistical analysis

We conducted tests of general characteristics, allele frequencies, genotype frequencies, Hardy-Weinberg equilibrium, haplotype associations, and LD (pair-wise linkage disequilibrium) employing SPSS© v11.5 (SPSS Inc, Chicago, IL, USA), SNPStats program [22] and SHEsis software online [23]. We performed co-dominant model, dominant model, recessive model and log-additive model for case-control association of each single-locus employing SNPStats program [22]. We applied unconditional logistic regression for measurement of OR, 95% CI (odd ratio, 95% confidence interval) after adjustment for smoking duration. We excluded haplotypes with frequency < 0.01 among both cases and controls from the analysis. We completed the analyses of SNP-SNP and SNP-SNP-smoking duration interactions in relation to lung cancer risk employing MDR (multifactor dimensionality reduction) version 3.0.3. dev. Jar [24]. This software (3.0.3. dev. Jar) is an evolvement version which has added permutation testing into the main MDR program. The MDR method is nonparametric and free model. MDR is directly useable to

Table 2 Distribution of selected characteristics in the case-control study population.

Characteristics	Cases		Controls		*p* value
	n	%	n	%	
Over all	544		550		
Age (years)					
Mean (±SD)	58 (±11)		58 (±11)		0.806[a]
≤40	29	5.3	28	5.1	
41–50	99	18.2	114	20.7	
51–60	193	35.5	189	34.4	0.77[b]
>60	223	41.0	219	39.8	
Gender					
Female	158	29.0	161	29.3	
Male	386	71.0	389	70.7	0.93[b]
Family history[c]					
No	463	85.1	545	99.1	
Yes	81	14.9	5	0.9	**<0.0001**[b]
Smoking duration					
Never	196	36.0	294	53.5	
≤20 (years)	96	17.6	91	16.5	
>20 (years)	252	46.3	165	30.0	**<0.0001**[b]
Histology					
Squamous cell carcinoma	232	42.6			
Adenocarcinoma	223	41.0			
Other	89	16.4			

[a] For t-test.
[b] For χ^2 test (two-sided), boldface indicates statistical significance.
[c] Family history of cancer.

case-control and discordant-sib-pair studies. MDR has rational power to recognize interactions among two or more loci in relatively small samples [24]. If the *p* value is less than 0.05, we considered the difference as statistically significant.

Results

This study population comprised 544 lung cancer cases and 550 cancer-free controls. The general characteristics of the studied population are summarized in [Table 2]. There were no statistically significant differences for the distribution of age and gender between case group and control group. However, there were more cases than controls with family history of cancer and cases had longer smoking history (>20 years) than controls (both $p < 0.0001$).

In previous studies, *CD3EAP* rs735482 has been associated with lung cancer risk [18,19,17]. We therefore included this SNP in this expanded study population. [Table 1] shows the following minor allele frequencies among controls in this population: rs12951053 C: 0.34, rs1042522 C: 0.45, rs8079544 T: 0.08, rs12602273 G: 0.28, and rs8064946 C: 0.32. These data are similar to the frequencies published in the HapMap-CHB of NCBI SNP database. All studied six SNPs were in Hardy-Weinberg equilibrium among controls (data not shown).

There were no significant associations between genotype distributions and lung cancer risk for any of the studied polymorphisms in co-dominant, dominant, recessive, and log-additive models after adjustment for smoking status [Table 3]. D′ values of pair-wise LD varied from 0.721 to 0.928 for *TP53* five htSNPs among controls, indicating strong linkage between the htSNPs (Supplementary Table S2). We therefore performed haplotype analysis. The haplotype distribution of the five *TP53* htSNPs was associated with lung cancer risk (Global haplotype association *p*-value = 0.0011) and haplotype9 (rs12951053^{A}-rs1042522^{C}-rs8079544^{C}-rs12602273^{G}-rs8064946^{C}) [OR (95% CI) = 0.13 (0.03–0.59), $p = 0.0079$] was associated with decreased risk of lung cancer after adjusted for smoking duration [Table 4]. The analysis of smoking duration within *TP53* haplotypes for 1037 subjects showed that there were more carriers of haplotype1 (AGCCG), 2 (CCCGC) and 4 (CCCCG) in the subgroup of smokers >20 (years) [OR (95% CI) = 1.90 (1.17–3.09), 2.22 (1.47–3.37), 2.65 (1.08–6.51), respectively; all $p < 0.05$] [Table 5]. Combinatorial rare haplotypes consisting of different structures and very low frequencies showed statistical significances in both haplotype analyses [Tables 4 and 5]. MDR testing analysis of *TP53*, *PPP1R13L*, *CD3EAP* and smoking

Table 3 Associations of single htSNP in *TP53* and *CD3EAP* rs735482 with lung cancer risk[a,b].

Gene/rs	Co-dominant	Dominant	Recessive	Log-additive
Ca/Co	(AB vs AA)/(BB vs AA)/*p*	(AB+BB vs AA)/*p*	(BB vs AA+AB)/*p*	- -/*p*
TP53				
rs12951053 (A>C)				
509/516	0.97 (0.74–1.26)/0.91 (0.59–1.41)/0.91	0.96 (0.74–1.23)/0.73	0.93 (0.61–1.40)/0.72	0.96 (0.79–1.16)/0.67
rs1042522 (G>C)				
489/489	1.03 (0.77–1.38)/1.00 (0.69–1.44)/0.97	1.02 (0.77–1.35)/0.89	0.98 (0.71–1.34)/0.90	1.00 (0.84–1.20)/0.99
rs8079544 (C>T)				
509/516	1.03 (0.73–1.45)/2.57 (0.23–28.86)/0.72	1.05 (0.74–1.47)/0.80	2.56 (0.23–28.73)/0.43	1.06 (0.76–1.48)/0.72
rs12602273(C>G)				
509/516	0.94 (0.72–1.23)/0.69 (0.43–1.10)/0.30	0.89 (0.69–1.15)/0.37	0.70 (0.44–1.12)/0.13	0.88 (0.72–1.06)/0.18
rs8064946 (G>C)				
509/516	0.92 (0.71–1.19)/0.68 (0.44–1.06)/0.23	0.87 (0.68–1.12)/0.27	0.71 (0.46–1.09)/0.11	0.86 (0.71–1.04)/0.12
CD3EAP				
rs735482 (A>C)				
522/511	1.15 (0.86–1.54)/1.25 (0.88–1.78)/0.43	1.18 (0.90–1.55)/0.23	1.15 (0.85–1.55)/0.37	1.12 (0.94–1.33)/0.20

[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.

Table 4 Association of *TP53* htSNP haplotypes with lung cancer risk[a].

Number	Haplotype[b]	Case frequency	Control frequency	OR (95% CI)	*p* value
1	AGCCG	0.5071	0.4753	1.0	–
2	CCCGC	0.2237	0.2210	0.96 (0.77–1.21)	0.75
3	ACTCG	0.0754	0.0632	1.11 (0.77–1.61)	0.58
4	CCCCG	0.0572	0.0560	0.96 (0.63–1.47)	0.86
5	ACCCG	0.0432	0.0469	0.93 (0.59–1.44)	0.73
6	CCCCC	0.0404	0.0333	1.11 (0.66–1.87)	0.68
7	AGCGC	0.0160	0.0163	1.05 (0.47–2.34)	0.90
8	CGCCG	0.0128	0.0103	1.17 (0.42–3.22)	0.76
9	ACCGC	0.0003	0.0197	**0.13 (0.03–0.59)**[c]	**0.0079**[c]
10	Rare	0.0240	0.0580	**0.36 (0.21–0.64)**[c]	**0.0005**[c]

[a] Adjusted by smoking duration, Global haplotype association *p*-value = 0.0011.
[b] SNP order: rs12951053-rs1042522-rs8079544-rs12602273-rs8064946.
[c] Boldface means association with decreased risk of lung cancer.

Table 5 Smoking duration within *TP53* htSNP haplotypes among 1037 subjects.

Number	Haplotype[a]	Frequency	OR (95% CI)	OR (95% CI)	OR (95% CI)
			Never	≤20 (years)	>20 (years)
1	AGCCG	0.4915	1.0	1.26 (0.68–2.34)	**1.90 (1.17–3.09)**[b]
2	CCCGC	0.2228	1.0	1.61 (0.90–2.87)	**2.22 (1.47–3.37)**[b]
3	ACTCG	0.0694	1.0	2.18 (0.83–5.72)	2.16 (0.99–4.72)
4	CCCCG	0.0562	1.0	1.72 (0.56–5.24)	**2.65 (1.08–6.51)**[b]
5	ACCCG	0.0405	1.0	1.01 (0.27–3.83)	1.60 (0.64–4.00)
6	CCCCC	0.0367	1.0	1.64 (0.41–6.58)	1.30 (0.44–3.85)
7	AGCGC	0.0159	1.0	2.17 (0.30–15.76)	7.02 (0.91–53.99)
8	CGCCG	0.0119	1.0	0.77 (0.05–13.12)	0.84 (0.10–7.07)
9	ACCGC	0.0112	1.0	–	–
10	Rare	0.0394	1.0	1.44 (0.22–9.53)	**4.34 (1.21–15.48)**[b]

[a] SNP order: rs12951053-rs1042522-rs8079544-rs12602273-rs8064946.
[b] Boldface indicates statistical significance (*p* value < 0.05).

duration identified the best candidate models of gene-gene-environment interaction for lung cancer occurrence [Table 6]. In whole group, smoking history ($p < 0.0010$ on 1000 permutation test) was the main factor in the interaction analysis of 9 attributes, and the first was a two-way model (CV = 9/10, $p < 0.0010$ on 1000 permutation test) and the second was a three-way model (CV = 6/10, $p = 0.0060–0.0070$ on 1000 permutation test) in relation to lung cancer risk [Table 6]. When stratifying by histology subgroups, significant models only related to lung squamous cell carcinoma ([Table 6]: CV = 10/10, $p < 0.001$ for one-way; CV = 9/10, $p < 0.001$ for two-way and CV = 7/10, $p < 0.001$ for three way, all *p* on 1000 permutation test). No significant interaction was found for MDR analysis when smoking history was excluded in whole group or histology subgroup (data not shown).

Discussion

Studies addressing TP53 SNPs in lung cancer

The previous association studies on *TP53* SNPs and lung cancer risk mainly assessed associations of SNP, haplotype/diplotype and gene-gene and gene-gene-environment interactions [4–14] [Table 7].

Variant-homozygote of *TP53* rs1042522 was at significantly increased risk of lung squamous cell carcinoma [CC versus GG: OR (95% CI) = 2.2 (1.3–3.9), $p = 0.005$] in Asian Japanese [4]. *TP53* rs1042522 was associated with significantly increased lung cancer risk in the total population [recessive model: CC versus Any G, adjusted OR (95% CI) = 1.57 (1.11–2.21)] and minor-allele carriers (TC or CC) of *TP53* rs2078486 were significantly increased lung cancer risk among smokers [adjusted OR (95% CI) = 1.70 (1.08–2.67)] in Asian Chinese [5]. The *TP53* rs1042522 C-allele were significantly associated with increased lung cancer risk [GC or CC versus GG: OR (95% CI) = 2.51 (1.38–4.82) and OR (95% CI) = 4.62 (2.31–9.52), respectively] in Asian Bengalese [6]. A study including Caucasians and African Americans reported that among African Americans, carriers of the haplotype $rs1042522^{C}$-$rs9895829^{T}$-$rs2909430^{A}$-$rs1625895^{G}$-$rs12951053^{G}$ had increased risk for lung cancer [OR (95% CI) = 2.32 (1.18–4.57)] and a worsened lung cancer prognosis [HR (hazards ratio) (95% CI) = 2.38 (1.38–4.10)] compared with carriers of the haplotype 1042522^{G}-$rs9895829^{T}$-$rs2909430^{A}$-$rs1625895^{G}$-$rs12951053^{T}$ [7].

Variant C-allele of *TP53* rs1042522 was significantly associated with increased risk of lung squamous cell carcinoma [CC+GC versus GG: OR (95%) = 1.65 (1.10–2.47), $p = 0.016$], the risk was markedly increased in heavy smokers with lung squamous cell carcinoma [CC versus GG: OR (95%) = 2.80 (1.19–6.58), $p = 0.019$] and combined effect of *TP53*

Table 6 The best candidate models for smoking duration-gene-gene interactions from MDR analysis[a].

Model	Attribute included	Bal. ACC. Overall	Bal. ACC. CV Training	Bal. ACC. CV Testing	CV consistency	*p* value[b]
Whole group						
One-way	Smoking	0.5871	0.5872	0.5807	10/10	< **0.001**[c]
Two-way	Smoking					
	rs735482	0.6011	0.6012	0.5930	9/10	<**0.001**[c]
Three-way	Smoking					
	rs967591					
	rs8064946	0.6174	0.6204	0.5678	6/10	**0.006** –**0.007**[c]
Four-way	Smoking					
	rs1970764					
	rs735482					
	rs1042522	0.6509	0.6572	0.5294	8/10	0.347–0.348
Histology subgroup						
Squamous cell carcinoma						
One-way	Smoking	0.6466	0.6471	0.6366	10/10	<**0.001**[c]
Two-way	Smoking					
	rs967591	0.6626	0.6627	0.6555	9/10	<**0.001**[c]
Three-way	Smoking					
	rs967591					
	rs1042522	0.6877	0.6907	0.6323	7/10	<**0.001**[c]
Four-way	Smoking					
	rs1970764					
	rs1042522					
	rs8064946	0.7159	0.7257	0.5778	4/10	**0.018** –**0.019**[c]
Adenocarcinoma						
One-way	rs967591	0.5476	0.5483	0.5283	10/10	0.475–0.476
Two-way	rs967591					
	rs12951053	0.5751	0.5763	0.5237	6/10	0.536–0.537
Three-way	rs1970764					
	rs735482					
	rs1042522	0.61	0.6131	0.5246	5/10	0.529–0.53
Four-way	rs1970764					
	rs735482					
	rs12951053					
	rs1042522	0.6627	0.6686	0.5194	5/10	0.6–0.601
Other						
One-way	Smoking	0.5931	0.5931	0.5931	10/10	0.073–0.074
Two-way	Smoking					
	rs967591	0.6449	0.6472	0.6009	9/10	0.05–0.051
Three-way	Smoking					
	rs967591					
	rs1042522	0.692	0.696	0.6	9/10	0.053–0.054
Four-way	Smoking					
	rs1970764					
	rs735482					
	rs1042522	0.7552	0.763	0.5349	10/10	0.566–0.567

[a] Analyzed by MDR 3.0.3. dev. Jar, data for *PPP1R13L* and *CD3EAP* from previous reports [17].
[b] *p* value based on 1000 permutation test.
[c] Boldface means statistical significance.

rs1042522 C-allele and *P21/CDKN1A* (cyclin dependent kinase inhibitor 1 A) rs1801270 CC-genotype was most pronounced in heavy smokers with lung squamous cell carcinoma [*TP53* rs1042522$^{CC+\ CG}$/*P21* rs1801270^{CC} versus *TP53* rs1042522^{GG}/*P21* rs1801270^{AA+AC}: OR (95%) = 3.84 (1.46–10.1), $p = 0.007$] in Caucasians Germans [8]. The *TP53* rs1042522 was significantly associated with increased risk of lung adenocarcinoma [CC versus GG: adjusted OR (95% CI) = 1.55, (1.17–2.06)] and gene-gene interaction was found for the combination of *TP53* rs1042522^{CC} and *MDM2* (MDM2 proto-oncogene) rs2279744^{GG} genotypes [adjusted OR (95% CI) = 2.66 (1.54–4.60)] related to risk of lung adenocarcinoma in Asian-Chinese female non-smokers [9]. *TP53* rs1042522 was associated with risk of NSCLC (non-small-cell lung cancer), both independently [dominant model: OR (95% CI) = 1.809 (1.159–2.825), $p < 0.05$; recessive model: OR (95% CI) = 1.933 (1.096–3.409), $p < 0.05$] and in combination with miR-502-binding site SNP (rs16917496) in the 3′ UTR of *SET8* (set domain-containing

Table 7 Results of *TP53* single nucleotide polymorphisms and risk of lung cancer from epidemiological studies[a].

Lung cancer[b]	Reference	SNP	Location/Population[C]	Cases/Controls	Comparison[d]	OR (95% CI)	*P* value[e]
LC	Sakiyama et al. [4]	rs1042522	Japan/Hospital-based case-control	1002/685	CC vs. GG/SQC	2.2 (1.3–3.9)	0.005
LC	Li et al. [5]	rs1042522	China/Hospital-based case-control	399/466	CC vs. Any G	1.57 (1.11–2.21)	–
		rs2078486			TC + CC vs. TT/Smoker	1.70 (1.08–2.67)	–
LC	Mostaid et al. [6]	rs1042522	Bangladesh/Population-based case-control	106/116	GC or CC vs. GG	2.51 (1.38–4.82)/4.62 (2.31–9.52)	–
LC	Mechanic et al. [7]	rs1042522	USA/Hospital-based Case-control/AFA	120/204	Haplotype with C vs. G	2.32 (1.18–4.57)	–
			rs1042522^{C}- rs9895829^{T}- rs2909430^{A}- rs1625895^{G}- rs12951053^{G} vs. $^{G-T-A-G-T}$				
LC	Popanda et al. [8]	rs1042522	Genmany/Hospital-based case-control	405/404	SQC		
					CC+GC vs. GG	1.65 (1.10–2.47)	0.016
					CC versus GG/HS	2.80 (1.19–6.58)	0.019
			TP53 rs1042522$^{CC+\ CG}$/*P21* rs1801270^{CC} versus *TP53* rs1042522^{GG}/*P21* rs1801270^{AA+AC}				
						3.84 (1.46–10.1)	0.007
ADC	Ren et al. [9]	rs1042522	China/Hospital-based case-control/FNS	764/983	CC vs. GG	1.55 (1.17–2.06)	0.002
					Combination genotypes with CC	2.66 (1.54–4.60)	<0.001
			TP53 rs1042522^{CC}+*MDM2* rs2279744^{GG} vs. *TP53* rs1042522^{GG}+*MDM2* rs2279744^{TT}				
NSCLC	Yang et al. [10]	rs1042522	China/Hospital-based case-control	164/199	Dominant model	1.809 (1.159–2.825)	<0.05
					Recessive model	1.933 (1.096–3.409)	<0.05
					Combination genotypes with GG	3.032 (1.580–5.816)	–
					SET8 rs16917496^{TT}-*TP53* rs1042522^{GG} vs. $^{CC+CT-CC+CG}$		
LC	Myneni et al. [11]	rs1042522	China//Population-based case-control	399/466	Diplotype with CC vs. GG+GC	3.68 (1.43–9.45)	–
					*ATM*rs227060^{TT}–*ATM* rs228589^{AA}-*TP53* rs1042522^{CC} vs. $^{CC+CT-TT+TA-GG+GC}$		
LC	Chua et al. [12]	rs1042522	Singapore/Hospital-based case-control	126/162	Combination genotypes with C	2.5 (1.2–5.0)	–
					MDM2 rs2279744^{TT} vs. *TP53* rs1042522$^{GC/CC}$ +*MDM2* rs2279744 $^{GG/TG}$		
LC	Mechanic et al. [7]	rs1042522	USA/Hospital-based case-control/CA	323/343	AB or BB or AB+BB vs. AA:	1.23 (0.86–1.76)/	–
		rs9895829				0.87 (0.41–1.84)/1.18 (0.84–1.66),	
		rs2909430				1.48 (0.78–2.82)/not determined/ 1.48 (0.78–2.82), 1.17 (0.77–1.78)/	
		rs1625895				1.08 (0.31–3.76)/1.16 (0.77–1.74), 1.12 (0.74–1.68)/0.93 (0.25–3.41)/	
		rs12951053				1.10 (0.74–1.64), 0.91 (0.56–1.49)/ 1.97 (0.19–20.6)/0.94 (0.58–1.52)	
LC	Guan et al. [13]	rs78378222	USA/Hospital-based case-control/NHW 1014/1076		AC vs. AA	0.84 (0.51–1.37)	0.379

(*continued on next page*)

Table 7 – (continued)

Lung cancer[b]	Reference	SNP	Location/Population[C]	Cases/Controls	Comparison[d]	OR (95% CI)	P value[e]
LC	Zhang et al. [14]	rs1042522	China/Hospital-based case-control	640/650	CG or GG or CG+GG vs. CC	1.02 (0.79–1.31) 0.882/0.99 (0.72–1.37) 0.963/1.1 (0.80–1.29) 0.924	–
LC	Yin et al. [current]	rs1042522	China/Hospital-based case-control	544/550	Haplotype with C vs. G	0.13 (0.03–0.59)	0.0079
		rs12951053 rs8079544 rs12602273 rs8064946			rs12951053^{A}-rs1042522^{C}-rs8079544^{C}-rs12602273^{G}-rs8064946^{C} vs. $^{A-G-C-G}$ Interaction of gene-gene-smoking duration Whole group: Three-way: *TP53* rs8064946, *CD3EAP* rs967591, Smoking		0.006–0.007
					SQC group: Three-way: *TP53* rs1042522, *CD3EAP* rs967591, Smoking		<0.001

[a] Seeing Discussion for details.
[b] LC: Lung cancer; ADC: Adenocarcinoma; NSCLC: Non-small-cell lung cancer.
[c] AFA: African-American, FNS: Female non-smokers, CA: Caucasians Americans; NHW: Non-Hispanic Whites.
[d] vs.: versus; SQC: Squamous cell carcinoma; HS: Heavy smokers; AB: Heterozygote; BB: Homozygous variant-type; AA: Homozygous wild-type.
[e] -: Not reported.

protein 8) [*SET8* rs16917496^{TT}-*TP53* rs1042522^{GG} versus *SET8* rs16917496^{CC+CT}-*TP53* rs1042522^{CC+CG}: OR (95% CI) = 3.032 (1.58–5.816)] in Asian Chinese [10].

Carriers of *TP53* rs1042522^{CC} who were also carriers of diplotype *ATM* (ATM serine/threonine kinase) rs227060^{TT}-*ATM* rs228589^{AA}-*TP53* rs1042522^{CC} were at much higher risk of lung cancer [adjusted OR (95% CI) = 3.68 (1.43–9.45)] than carriers of variant genotypes of any one of the above three SNPs in Asian Chinese [11]. The TT-genotype of *MDM2* rs2279744 was associated with risk of lung cancer [TT versus GG: OR (95% CI) = 2.1 (1.01–4.36)], and carriers of this genotype in combination with the *TP53* rs1042522 C-allele were at increased lung cancer risk [OR (95% CI) = 2.5 (1.2–5.0)] in Asian Singaporean [12].

Null results have also been reported for *TP53* SNP and lung cancer. No associations of *TP53* single polymorphisms (rs1042522, rs9895829, rs2909430, rs1625895 and rs12951053) with lung cancer were observed in Caucasians Americans [7]. No association was found between the rare novel *TP53* rs78378222 variant and lung cancer risk in non-Hispanic white American [adjusted OR (95% CI) = 0.84 (0.51–1.37), $p = 0.379$] [13]. *TP53* rs1042522 was not associated with lung cancer risk in Asian Chinese [14].

MDM2 SNP rs2279744 [25], cyclin amplifications [*CCNE1* (cyclin E1) and *CCND1* (cyclin D1)] [26] and the haplotypes consisting of *CHRNA5/CHRNA3* (cholinergic receptor nicotinic alpha 5 subunit/cholinergic receptor nicotinic alpha 3 subunit) [27] were associated to *TP53* mutations in Caucasian lung cancer populations.

Main findings, implications and strengths of current study

In the present study, we report no association with lung cancer risk for the individual *TP53* htSNPs (including *TP53* rs1042522) [Table 3]. This is in agreement with a previously report regarding *TP53* rs1042522 in Asian-Chinese Han population [14]. *TP53* five htSNPs were in stronger pair-wise LD for our study population (Supplementary Table S2). Haplotype analysis could increase the estimated effect. Haplotype encompassing rs1042522 and other 4 htSNPs of *TP53* showed association evidence. Haplotype9 (rs12951053^{A}-rs1042522^{C}-rs8079544^{C}-rs12602273^{G}-rs8064946^{C}) with 2% frequency in the controls was associated with lowered risk of lung cancer [Table 4]. This significant observation is not consistent with previously significant associated findings in an African-Americans population [7]. The difference is that the haplotype encompassing rs1042522^{C} was protective in current Chinese population, while the haplotype encompassing rs1042522^{C} was risky in African Americans. The polymorphisms included in the haplotypes studied differed between the studies and only rs1042522 and rs12951053 were included in both haplotypes in the two studies. There were statistically significant differences of the two alleles frequencies in control groups among current Chinese and African Americans for rs1042522 (C = 0.45 and C = 0.55, this was in inversion for minor allele and major allele, $\chi^2 = 5.733$, $p = 0.017$) and rs12951053 (C = 0.34 and C = 0.1, $\chi^2 = 38.512$, $p < 0.001$). Thus the observed discrepancy may result from differences of SNPs or allele frequencies composing hapoltype or differences of LD status and haplotype frequency in

the specific chromosome region between different ethnic populations.

In addition, the analysis of smoking duration within *TP53* haplotypes among 1037 subjects exhibited carriers with haplotype1 (AGCCG), haplotype2 (CCCGC) and haplotype4 (CCCCG) were over-represented in smoking subgroup of >20 (years). This showed that the three haplotypes played coincident roles with respect to smoking duration. It suggested that three haplotypes (AGCCG, CCCGC and CCCCG) consisting of *TP53* htSNPs (htSNPs order: rs12951053-rs1042522-rs8079544-rs12602273-rs8064946) may be a potentially genetic predisposing factor for behavior of long-term smoking.

We have previously reported that *CD3EAP* rs735482 were associated with increased risk of lung cancer [18]. *CD3EAP* rs967591 has been shown to be functional. In Asian Koreans: *CD3EAP* rs967591 A-allele resulted in increased *CD3EAP* promoter activity [A versus G: $p = 0.002$], but did not influence *PPP1R13L* promoter activity. *CD3EAP* rs967591 was also associated with *CD3EAP* mRNA expression levels in lung tissue ($p = 0.01$). *CD3EAP* rs967591 AA-genotype was associated with shorter overall survival [adjusted HR (95% CI) = 1.69 (1.29–2.20), $p = 0.0001$ for early-stage NSCLC [28].

Endogenous PPP1R13L is as a negative regulator of *TP53* function. TP53 accumulation and activity after DNA damage is compromised by *PPP1R13L* expression [29]. Two-stage approach among Caucasian or Hispanic smokers (lung cancer-free) identified that *TP53* rs1641511 was associated with reduction of *TP53* expression of promoter methylation (dominant model: GG +AG versus AA: $p = 0.01$ or 0.02) [30]. Smoking is the strongest known risk factor for lung cancer. We chose to use smoking-duration as a measure of smoking history because duration is more strongly associated with lung cancer than other smoking variables, such as smoking-intensity (dosage) and current smoking-status [15]. In the MDR analysis of whole population [Table 6], we observed significant interaction between smoking duration and *TP53* rs8064946 and *CD3EAP* rs967591 on lung cancer risk. We again observed significant interaction between smoking duration and *CD3EAP* rs735482 on lung cancer risk [17]. We found no interaction between *PPP1R13L* rs1970764 and smoking duration and other SNPs studied on lung cancer risk. Smoking duration was an independent predictor of lung cancer risk. Overall testing accuracy was 58.71% using smoking duration as predictor. When smoking duration was combined with *CD3EAP* rs735482 (two-way) or *TP53* rs8064946 and *CD3EAP* rs967591 (three-way), the overall testing accuracy increased to 60.11% or 61.74% [Table 6]. This indicates that *CD3EAP* polymorphism or combination of *TP53* and *CD3EAP* polymorphisms could modify smoking-induced lung cancer risk. In MDR analysis of histological subgroups, we observed smoking duration as an independent risk factor and interaction of smoking duration and *CD3EAP* rs967591 or smoking duration, *TP53* rs1042522 and *CD3EAP* rs967591 were only associated with squamous cell carcinoma but neither adenocarcinoma nor other. The observed interaction between histological type and smoking duration is in line with the literature reporting that lung squamous cell carcinoma is related to smoking or interaction of smoking-genes and that lung adenocarcinoma appears to be affecting never smokers [4,8,31].

We assessed the possible functionality of the studied polymorphisms using the web tool: SNPinfo [32]. This analysis indicated that *TP53* rs12951053 (Regulatory Potential Score = 0.058167), rs1042522 (nsSNP: Yes, Polyphen: benign, Regulatory Potential Score = 0.31032, Conservation Score = 0.002), rs8079544 (Regulatory Potential Score = 0.204487) and rs8064946 (Transcription Factor Binding Sites: Yes, Regulatory Potential Score = 0.118648) may all be biologically functional, whereas rs12602273 was not. Rs1042522 was the most important functional htSNP, and lead to a non-conservative Arg to Pro amino acid substitution.

Limitations

With current genotypes we had 88%, 79%, 70%, 90% and 89% and 82% chance of detecting OR = 1.5 at 0.05 significant level and two sided test under dominant model for *TP53* rs12951053, rs1042522, rs8079544, rs12602273 and rs8064946 and *CD3EAP* rs735482, respectively. Further studies with larger sample sizes are warranted. The matching concerning age, gender and ethnicity between cases and controls was insufficient to exclude potential confounding factors such as smoking in this study.

Conclusion

In conclusion, the present results provide novel evidence that the haplotype of *TP53* htSNPs and interaction between genetic variation in *TP53* and *CD3EAP* and smoking-duration may associate with lung cancer risk, and provide additional evidence of association between *TP53* htSNP haplotypes and long-term smoking-related behavior.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2021.01.006.

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