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Research Article

Associations between 25 Lung Cancer Risk–Related SNPs and Polycyclic Aromatic Hydrocarbon–Induced Genetic Damage in Coke Oven Workers

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Abstract

Background: Genome-wide association studies (GWAS) have identified multiple single-nucleotide polymorphisms (SNP) associated with lung cancer. However, whether these SNPs are associated with genetic damage, a crucial event in cancer initiation and evolution, is still unknown. We aimed to establish associations between these SNPs and genetic damage caused by the ubiquitous carcinogens, polycyclic aromatic hydrocarbons (PAH).

Methods: We cross-sectionally investigated the associations between SNPs from published GWAS for lung cancer in Asians and PAH-induced genetic damage in 1,557 coke oven workers in China. Urinary PAH metabolites, plasma benzo[a]pyrene-r-7,t-8,c-10-tetrahydrotetrol-albumin (BPDE-Alb) adducts, urinary 8-hydroxydeoxyguanosine (8-OHdG), and micronuclei (MN) frequency were determined by gas chromatography-mass spectrometry, sandwich ELISA, high-performance liquid chromatography, and cytokinesis-block micronucleus assay, respectively.

Results: 13q12.12-rs753955C was suggestively associated with elevated 8-OHdG levels ($P = 0.003$). Higher 8-OHdG levels were observed in individuals with rare allele homozygotes (CC) than in TT homozygotes (β, 0.297; 95% confidence interval, 0.124–0.471; $P = 0.001$). 9p21-rs1333040C, 10p14-rs1663689G, and 15q25.1-rs3813572G were significantly associated with lower MN frequency (P values were 0.002, 0.001, and 0.005, respectively). 10p14-rs1663689G polymorphism downregulated the relationship of the total concentration of PAH metabolites to 8-OHdG levels ($P_{interaction} = 0.002$). *TERT*-rs2736100G and *VTI1A*-rs7086803A aggravated the relationship of BPDE-Alb adducts to MN frequency, whereas *BPTF*-rs7216064G attenuated that correlation (all $P_{interaction} < 0.001$).

Conclusions: Lung cancer risk–associated SNPs and their correlations with PAH exposure were associated with 8-OHdG levels and MN frequency.

Impact: Lung cancer risk–associated SNPs might influence one's susceptibility to genetic damage caused by PAHs. *Cancer Epidemiol Biomarkers Prev; 23(6); 986–96. ©2014 AACR.*

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, including China (1, 2), and is a complex disease caused both by genetic and environmental factors and by their interactions (3, 4). Genome-wide association studies (GWAS) have identified multiple single-nucleotide polymorphisms (SNP) that are associated with lung cancer risks, supporting the suggestion that inherited genetic factors play a significant role in lung cancer development. Environmental pollutants such as polycyclic aromatic hydrocarbons (PAH) also contribute to lung cancer risks (5–7). In China, the population attributable fraction for lung cancer caused by inhalation exposure to PAHs was >44% in isolated locations near small-scale coke oven operations, dramatically exceeding that in the overall population (1.6%; ref. 8). However, despite the fact that more than 80% of lung cancers are attributed to tobacco, only a fraction of smokers (~10%) will ultimately develop lung cancer in their lifetimes (9), indicating that inherited genetic factors modify the effects of carcinogenic compounds in the tobacco smoke.

Genetic damage, including DNA and chromosomal damage, is a key initial event in the carcinogenic potential

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of PAHs (10, 11) and a crucial event in the initiation and evolution of cancer (12). Elevated genetic damage levels were observed both in individuals exposed to PAHs (13, 14) and in lung cancer patients (15) compared with controls. Of note, 8-hydroxydeoxyguanosine (8-OHdG) was a biomarker reflecting the levels of oxidative DNA damage and micronuclei (MN) frequency was widely used in the measurement of chromosomal damage (16, 17). Both were also reported to be associated with an increased lung cancer risk (18, 19). However, published evidence showed that individuals who lived in similar environments had different levels of 8-OHdG (20) and MN frequency (21), as well as different risks for lung cancer, suggesting that genetic factors were important in the chemical carcinogenesis of PAHs. However, shared genetic variants for both DNA and chromosomal damage and lung cancer risks still need to be investigated further, and it remains unknown whether lung cancer–related genetic variants contribute to disease susceptibility via the initiation of damage to DNA and chromosomes. Moreover, it is also yet to be determined whether or not these genetic variants can modify the carcinogenic effects of PAHs. GWAS has identified multiple SNPs associated with lung cancer risks, yet whether those SNPs are associated with levels of PAH-induced DNA and chromosomal damage is still unknown.

Thus, in the present study, we hypothesize that lung cancer risk–related SNPs identified from GWAS themselves may be associated with DNA and chromosomal damage and may also aggravate the magnitude of PAH-induced genetic damage. As coke oven workers had high levels of genetic damage and were at high risk for lung cancer due to their long-term occupational exposure to PAHs, we tested this hypothesis by cross-sectionally investigating the associations of SNPs identified from GWAS for lung cancer in Asians with the levels of 8-OHdG and MN frequency in coke oven workers. We then explored the relationships between these SNPs and PAH exposure, which were assessed by PAH metabolites and plasma benzo[a]pyrene-r-7,t-8,t-9,c-10-tetrahydotetrol-albumin (BPDE-Alb) adducts.

Materials and Methods

Study participants

A total of 1,715 workers were recruited from a coke oven plant in Wuhan (Hubei, China) in the autumn of 2010. All of them were classified into three groups according to their work places: Participants who worked at the top, side, and bottom of coke ovens were referred to as the high-exposure group. Individuals who worked in adjunct workplaces and in the logistics departments and offices were defined as the low-exposure and control groups, respectively. All subjects provided written informed consent and the Ethics and Human Subject Committee of Tongji Medical College approved this research project. Each participant was interviewed one-on-one to collect information on demographic characteristics, health status, body weight and height, smoking status, alcohol consumption, occupational location, and lengths of employment by means of a standardized occupational questionnaire. For each subject, a 6-mL venous blood sample was drawn and distributed into two coded vacuum tubes (1 mL for EDTA-K2 anticoagulant and 5 mL for heparinized anticoagulant) and 20 mL of morning urine samples were collected in sterile conical tubes. The main inclusion criteria for this study were that participants should have worked not less than 1 year, had no physician-diagnosed cancer, and that both blood and urine were available. Finally, 1,557 subjects (1,333 males and 224 females) who met these criteria were enrolled for further analysis.

SNP selection and genotyping

We included common SNPs (MAF > 0.05) identified from published GWAS for lung cancer risk in Asians (3, 22–29), with the exception of one research project published after our experiment was finished (30). Considering the ethnic differences, the SNPs restricted to non-Asians were ignored. Another four SNPs on 15q25 (rs2036534, rs667282, rs12910984, and rs6495309) identified in a Chinese population were also recruited (31). As rs401681, rs11080466, and rs6495309 were in high linkage disequilibrium (LD; $r^2 \geq 0.8$) with rs402710, rs11663246, and rs12910984, respectively, the latter ones were discarded. Finally, a total of 28 SNPs were included in this study. The iPLEX system (Sequenom) was used for genotyping. Due to the failure of primer design, we replaced rs17728461, rs2036534, rs2395185, and rs10433328 (called original SNPs) with rs16988393, rs3813572, rs9272346, and rs4677662, respectively, according to the following criteria: (i) the SNPs were in moderate LD (logarithm of odds of >3 and D′ of >0.99) with original SNPs; (ii) the SNPs were located in the regions from 1,000 kb upstream to 1,000 kb downstream of the original SNPs; (iii) when multiple SNPs met the above two criteria, SNPs residing in functional regions, including exons, untranslated regions, and promoters regions, were prioritized. If none of these SNPs were located in functional regions, the strongest LD SNP was prioritized.

Determination of urinary PAH metabolites

We determined 12 urinary PAH metabolites, including 1-, 2-hydroxynaphthalene, 2-, 9-hydroxyfluorene, 1-hydroxypyrene, 1-, 2-, 3-, 4-, 9-hydroxyphenanthrene, 6-hydroxychrysene, and 3-hydroxybenzo[a]pyrene by gas chromatography-mass spectrometry, which was reported in detail previously (32) with minor modifications as described in our previous study (33). We failed to detect 6-hydroxychrysene and 3-hydroxybenzo[a]pyrene because their concentrations were less than the limits of detection (LOD). The LOD of the remained 10 PAH metabolites ranged from 0.1 to 1.4 μg/L. For measurements below LOD, we used half of LOD as default values. We calibrated the concentrations of urinary PAH metabolites by levels of urinary creatinine and expressed them as μg/mmol creatinine.

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Determination of plasma BPDE-Alb adducts

The concentrations of BPDE-Alb adducts in heparin-anticoagulated plasma were detected by sandwich ELISA described in detail previously (33). Briefly, the 96-well plates were coated with rabbit anti-mouse immunoglobulin G-Fc antibody and then blocked with nonfat dry milk. After that, monoclonal antibody 8E11 and biotin-conjugated rabbit anti-albumin antibody were used as primary and secondary antibodies, respectively, according to standard ELISA procedures. Each standard or sample was assayed in duplicate. We calibrated concentrations of BPDE-Alb adducts by plasma albumin and expressed them as ng/mg albumin. The LOD of this method was approximately 1 ng BPDE-Alb adducts/mg albumin. Half of LOD were used as default values for measurements below LOD.

Determination of genetic damage

The oxidative DNA-damage levels were assessed by urinary 8-OHdG using high-performance liquid chromatography reported previously (34). The detailed experimental procedure was described in our previous study (33). The LOD of 8-OHdG was 7 nmol/L. For measurements below LOD, we used half of LOD as default values. The urinary concentrations of 8-OHdG were calibrated by levels of urinary creatinine and expressed as nmol/mmol creatinine.

The chromosomal damage was measured by cytokinesis-block micronucleus assay according to the standardized protocol reported previously (35). A total of 1,000 binucleated cells in each of two duplicative slides were examined microscopically for MN according to the scoring criteria outlined by the HUman MicroNucleus Project (17). The MN frequency of each subject was reported as the mean number of MN cells per 1,000 binucleated cells.

Bioinformatics

Because all the polymorphisms significantly associated with the genetic biomarkers were located in noncoding regions, their possible effects on transcription factor–binding sites were analyzed *in silico*, using the SNPInspector tool of the Genomatix software suite GEMS Launcher (Genomatix; ref. 36), which was designed to evaluate SNPs situated in noncoding regions.

Statistical analysis

One-way ANOVA, Kruskal–Wallis, and χ^2 tests were used to access the differences among the control, low- and high-exposure groups with regard to continuous variables in normal distribution [age, years worked, and body mass index (BMI)], continuous variables in skewed distribution [packing year, plasma BPDE-Alb adducts, PAH metabolites and total concentrations of PAH metabolites (ΣOH-PAHs)], and categorical variables (gender, smoking, and drinking), respectively. A goodness-of-fit χ^2 analysis was used to test the Hardy–Weinberg equilibrium. The LD was evaluated using Haploview 4.1. Association analysis was performed by general linear models or poisson log-linear models, in which genotypes with three levels can be modeled as nominal variables without assuming additive models. False discovery rate (FDR) adjustment was used for multiple comparisons and FDR < 0.05 was defined as the significance level.

The relationships of SNPs and internal exposure levels (ΣOH-PAHs and BPDE-Alb adducts) with genetic damage were tested by introducing the SNP × internal exposure into the models. All statistical analyses were performed using SPSS 12.0 software.

Results

General characteristics of the subjects

General characteristics of the study population with levels of urinary PAH metabolites, plasma BPDE-Alb adducts, and genetic damage were summarized in Table 1. All subjects were classified into three groups according to their work places (for detailed information, see Materials and Methods). Higher proportions of males, drinkers and smokers, and pack-years were found in the exposure groups, compared with those in the control group (all $P < 0.05$, Table 1). In addition, exposure groups had higher levels of BPDE-Alb adducts and most PAH metabolites except 4-hydroxyphenanthrene. No differences were observed in the distribution of age, years worked, and BMI among these three groups. Multivariate linear regression analysis revealed that 8-OHdG and MN frequency were significantly associated with increased environmental PAH levels, adjusting for age, gender, BMI, smoking, and drinking ($P_{trend} < 0.001$).

In addition, we classified the population into two subgroups according to the median age (41 years) and further investigated the relationships of age, gender, and smoking status with the levels of PAH metabolites, BPDE-Alb adducts, and genetic damage. As Supplementary Table S1 showed, lower levels of 2-hydroxyfluorene, 3-hydroxyphenanthrene, and higher MN frequency were observed in the older age group (all $P < 0.004$); levels of 1-hydroxypyrene and genetic damage were significantly higher in females than in males (all $P < 0.004$). Smokers had significantly higher levels of 1- and 2-hydroxynaphthalene than ex- and nonsmokers ($P < 0.001$). Slight but not statistically significant lower levels of other PAH metabolites, BPDE-Alb adducts, and genetic damage were observed in smokers, compared with nonsmokers. The potential interpretations are as follows: (i) tobacco smoke contains much higher concentrations of naphthalene than that of other PAHs such as phenanthrene and benzo[a]pyrene (37), which significantly increases the levels of urinary 1- and 2-hydroxynaphthalene in smokers; (ii) cytochrome P450 enzymes might be downregulated by smoking (38). To some extent, the generation of BPDE-Alb adducts was, thus, reduced; (iii) the genetic damage of coke oven workers in this study might be determined by high occupational exposure to PAHs rather than tobacco smoke.

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Table 1. General characteristics, levels of urinary PAH metabolites, plasma BPDE-Alb adducts, and genetic damage of the workers in control and exposed groups

Variables	Control group[e] ($n = 497$)	Low-exposure group[e] ($n = 628$)	High-exposure group[e] ($n = 432$)	P
General characteristics				
Age (y; mean ± SD)	42.48 ± 8.04	41.76 ± 8.95	42.22 ± 7.78	0.342[a]
Gender (% female)	22.7	13.2	6.5	<0.001[b]
Years worked (y; mean ± SD)	21.32 ± 9.19	20.26 ± 10.71	20.88 ± 9.22	0.197[a]
Smoking (smoking/quit/never), %	50/3.4/46.6	55.5/4.9/39.6	66.2/3.7/30.1	0.001[b]
Pack-years (mean ± SD)	10.83 ± 14.33	10.87 ± 15.01	13.40 ± 15.35	0.010[a]
Drinking (drinking/quit/never), %	29.4/3.2/67.4	33.0/1.8/65.2	38.3/1.4/60.3	0.020[b]
BMI (kg/m^2, mean ± SD)	23.72 ± 3.14	23.81 ± 3.64	23.37 ± 4.39	0.160[a]
PAH metabolites [μg/mmol creatinine, median (5%–95%)]				
1-hydroxynaphthalene	1.25 (0.34–5.12)	1.61 (0.48–7.43)	2.27 (0.66–8.42)	<0.001[c]
2-hydroxynaphthalene	1.26 (0.23–4.18)	1.49 (0.40–5.39)	2.29 (0.61–7.24)	<0.001[c]
2-hydroxyfluorene	0.71 (0.27–2.06)	0.92 (0.33–3.92)	1.12 (0.31–4.28)	<0.001[c]
9-hydroxyfluorene	0.48 (0.01–4.71)	0.58 (0.01–4.50)	0.63 (0.01–3.71)	0.045[c]
1-hydroxyphenanthrene	0.75 (0.07–4.23)	0.83 (0.10–3.34)	1.15 (0.24–4.67)	<0.001[c]
2-hydroxyphenanthrene	0.25 (0.04–1.22)	0.31 (0.05–1.23)	0.38 (0.09–1.64)	<0.001[c]
3-hydroxyphenanthrene	0.29 (0.03–1.15)	0.36 (0.04–1.56)	0.47 (0.04–2.11)	<0.001[c]
4-hydroxyphenanthrene	0.37 (0.003–2.13)	0.32 (0.004–2.21)	0.30 (0.003–1.98)	0.332[c]
9-hydroxyphenanthrene	0.62 (0.14–3.71)	0.74 (0.17–4.20)	0.86 (0.21–3.88)	<0.001[c]
1-hydroxypyrene	2.54 (0.65–13.14)	3.23 (0.91–13.11)	4.22 (1.22–17.86)	<0.001[c]
ΣOH-PAHs	10.00 (3.62–36.08)	11.65 (4.74–40.03)	14.78 (5.58–50.35)	<0.001[c]
BPDE-Alb adducts [ng/mg albumin, median (5%–95%)]	3.93 (3.09–5.76)	4.30 (1.75–7.39)	4.73 (1.75–9.95)	<0.001[c]
Genetic damage biomarkers (mean ± SD)				P_{trend}
8-OHdG (nmol/mmol creatinine)	4.47 ± 1.26	4.62 ± 1.13	4.83 ± 1.05	<0.001[d]
MN frequency (%)	3.69 ± 2.70	3.78 ± 3.02	4.02 ± 2.57	<0.001[d]

[a]One-way ANOVA for differences among the different groups.
[b]χ^2 tests for differences in the distribution frequencies among different groups.
[c]The Kruskal–Wallis test for differences among different groups for nonnormal variables.
[d]Multivariate linear regression and the poisson log-linear model for the trend of 8-OHdG and MN frequency, respectively, with adjustment for age, gender, BMI, smoking, and drinking.
[e]Control group, workers worked in logistics departments and offices; low-exposure group, workers worked in adjunct workplaces; high-exposure group, workers worked at the top, side, and bottom of coke ovens.

Relationships of lung cancer–related SNPs to genetic damage

Genotyping was successful for 27/28 SNPs. SNP rs9272346 and rs4677657 were not in the Hardy–Weinberg equilibrium. Thus, the remaining 25 SNPs with a genotyping rate of >95% were included in the following statistical analysis. Detailed information of these SNPs was summarized in Table 2.

The associations between lung cancer risk–related SNPs and genetic damage were shown in Tables 3 and 4. 13q12.12-rs753955 was suggestively associated with elevated 8-OHdG levels after FDR adjustment [$P = 0.003$ for the genotype term in the adjusted model; β, 0.297; 95% confidence interval (CI), 0.124–0.471; $P = 0.001$ for the comparison of CC vs. TT; Table 3]. Lower LSM ± SD of 8-OHdG levels were observed in subjects with TC or TT genotypes, compared with those carrying the rare CC genotype (TC, 4.87 ± 0.09; TT, 4.81 ± 0.09; CC, 5.11 ± 0.12; Supplementary Table S2). Bioinformatic analyses indicated that rs753955 T>C substitution led to the loss of one transcription factor–binding site (Avian C-type LTR TATA box).

Three SNPs were significantly associated with decreased MN frequency (Table 4). Subjects carrying 9p21-rs1333040 TC genotype had lower MN frequency than those with wild TT genotype (β, −0.09; 95% CI, −0.146 to −0.035; $P = 0.001$ for TC vs. TT; Table 4]. When comparing TC+CC with TT; β, −0.066; 95% CI, −0.118 to −0.014; and $P = 0.013$. *In silico* analysis indicated that rs1333040 T>C substitution resulted in a gain of two transcription factor–binding sites (Drosophila motif 10 element and odd-skipped related 1) and a loss of another three sites (Doublesex and mab-3–related transcription factor 1, TEA domain-containing factors, and X-box–binding protein RFX1). 10p14-rs1663689 was significantly associated with decreased MN frequency ($P = 0.001$ for the genotype term in the adjusted model; β,

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Table 2. Characteristics of the SNPs associated with lung cancer risk in Asians

SNPs	Region	Reported gene	Location	A/a	N (AA/Aa/aa)	MAF	Call rate	P_{HWE}
rs10937405	3q28	*TP63*	Intron	C/T	768/622/127	0.289	0.974	0.925
rs11080466	18p11.22	*PIEZO2*	Intron	A/G	923/504/65	0.212	0.958	0.716
rs1333040	9p21	*CDKN2B-AS1*	Intron	T/C	738/631/148	0.306	0.974	0.407
rs1663689	10p14	*GATA3*	Intergenic	A/G	544/710/260	0.406	0.972	0.370
rs2131877	3q29	*XXYLT1*	Intron	T/C	458/754/306	0.450	0.975	0.984
rs247008	5q31	*IL3-CSF2-P4HA2*	Intergenic	C/T	397/750/370	0.491	0.974	0.804
rs2736100	5p15.33	*TERT*	Intron	T/G	515/757/244	0.411	0.974	0.178
rs2853677	5p15.33	*TERT*	Intron	T/C	569/741/207	0.381	0.974	0.105
rs2895680	5q32	*PPP2R2B-STK32A-DPYSL3*	Intron	T/C	743/621/153	0.306	0.974	0.285
rs36600	22q12.2	*MTMR3*	Intron	C/T	1246/256/16	0.095	0.975	0.492
rs3813572	15q25.1	*PSMA4*	5′ near gene	A/G	998/462/49	0.186	0.969	0.668
rs3817963	6p21.3	*BTNL2*	Intron	A/G	926/518/71	0.218	0.973	0.710
rs401681	5p15.33	*CLPTM1L*	Intron	C/T	668/681/168	0.335	0.974	0.787
rs4488809	3q28	*TP63*	Intron	C/T	395/747/372	0.492	0.972	0.610
rs465498	5p15.33	*TERT-CLPTM1L*	Intron	T/C	1044/424/51	0.173	0.976	0.313
rs4677657	3q29	*XXYLT1*	Intron	C/T	1101/206/210	0.206	0.974	<0.001
rs4677662	3q29	*XXYLT1*	Intron	T/C	757/633/126	0.292	0.974	0.886
rs4809957	20q13	*CYP24A1*	3′UTR	G/A	560/704/253	0.399	0.974	0.158
rs6495309	15q25.1	*CHRNA3*	5′ near gene	C/T	476/745/291	0.439	0.971	0.878
rs667282	15q25.1	*CHRNA5*	Intron	T/C	477/730/309	0.445	0.974	0.350
rs7086803	10q25.2	*VTI1A*	Intron	G/A	762/641/113	0.286	0.974	0.178
rs7216064	17q24.3	*BPTF*	Intron	A/G	633/682/200	0.358	0.974	0.368
rs753955	13q12.12	*TNFRSF19-MIPEP*	Intergenic	T/C	755/623/140	0.297	0.975	0.404
rs9272346	6p21.32	*HLA-DQA1*	5′ near gene	G/A	893/112/333	0.709	0.859	<0.001
rs9387478	6q22.2	*ROS1-DCBLD1*	Intergenic	C/A	390/734/358	0.489	0.952	0.728
rs9439519	1p36	*AJAP1-NPHP4*	Intergenic	T/C	792/599/128	0.281	0.976	0.941
rs16988393	22q12.2	*MTMR3-HORMAD2-LIF*	Intergene	T/C	1223/279/16	0.102	0.975	0.925
rs952481	3q29	*XXYLT1*	Intron	A/G	[a]	[a]	[a]	[a]

Abbreviation: A/a, major/minor allele; UTR, untranslated region.
[a]Data were missing due to genotyping failure.

−0.145; 95% CI, −0.224 to −0.065; $P < 0.001$ for GG vs. AA; Table 4). Persons with a rare GG genotype showed lower MN frequency than those with wild homozygous genotype AA (LSM ± SE: GG, 3.71 ± 0.27; AA, 4.23 ± 0.23; Supplementary Table S2). SNP-rs1663689 A>G substitution results in the loss of three transcription factor–binding sites: jumonji AT rich interactive domain 2, homeodomain transcription factor HOXC13, and serum response factor. People carrying at least one 15q25.1-rs3813572 G-allele showed lower observed MN frequency than those with AA genotype ($P = 0.005$ for the genotype term in the adjusted model; β, −0.094; 95% CI, −0.151 to −0.038; $P = 0.001$ for GG+AG vs. AA; Table 4). Bioinformatic analyses indicated that the A>G substitution resulted in the loss of five transcription factor–binding sites, including retinoic acid receptor, peroxisome proliferator–activated receptor gamma, SPI-1 proto-oncogene, myeloid zinc finger protein, and PTF1-binding sites and the gain of another three sites (two in C2H2 zinc finger transcription factors 7 and one in Myc-associated zinc finger protein). No significant associations of other SNPs with 8-OHdG levels or MN frequency were observed.

Relationships of SNPs and PAHs to genetic damage

To investigate the relationships of the lung cancer risk–associated SNPs and PAH exposure to genetic damage, we first analyzed the relationships between internal PAH exposure and levels of genetic damage. The correlation coefficient of ΣOH–PAHs for 8-OHdG was larger than that for MN frequency ($r = 0.494$ vs. $r = 0.097$; Supplementary Table S3). In addition, the MN frequency was more related with BPDE-Alb adducts than with ΣOH–PAHs ($r = 0.297$ vs. $r = 0.154$; Supplementary Table S3). Thus, we introduced SNP × ΣOH–PAHs and SNP × BPDE-Alb adducts into the statistical models to investigate the gene–environment relationship to 8-OHdG and MN frequency, respectively.

A significant relationship was observed between 10p14-rs1663689 and ΣOH–PAHs to 8-OHdG levels ($P_{interaction} = 0.002$; Fig. 1 A; Supplementary Table S4). The ΣOH–PAHs had much less correlation with urinary 8-OHdG in

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Table 3. Multivariate analyses for lung cancer risk–related SNPs on urinary 8-OHdG levels of coke oven workers

SNPs [major (A)/minor allele (a)]	Aa vs. AA		aa vs. AA		Aa+aa vs. AA			
	β (95% CI)[a]	*P*[a]	β (95% CI)[a]	*P*[a]	β (95% CI)[a]	*P*[a]	*P*[b]	FDR
rs10937405 (C/T)	−0.102 (−0.206 to 0.002)	0.053	−0.105 (−0.288 to 0.079)	0.264	−0.068 (−0.179 to 0.043)	0.231	0.125	0.511
rs11080466 (A/G)	−0.046 (−0.153 to 0.061)	0.397	−0.234 (−0.471 to 0.003)	0.053	−0.048 (−0.163 to 0.067)	0.415	0.132	0.511
rs1333040 (T/C)	0.039 (−0.065 to 0.143)	0.461	0.152 (−0.019 to 0.322)	0.081	0.023 (−0.088 to 0.134)	0.685	0.211	0.511
rs2131877 (T/C)	−0.032 (−0.147 to 0.082)	0.581	−0.124 (−0.267 to 0.020)	0.091	−0.045 (−0.167 to 0.077)	0.466	0.225	0.511
rs247008 (C/T)	−0.135 (−0.254 to −0.016)	0.026	−0.112 (−0.251 to 0.028)	0.117	−0.146 (−0.274 to −0.019)	0.025	0.078	0.511
rs2736100 (T/G)	−0.134 (−0.244 to −0.025)	0.016	−0.080 (−0.231 to 0.072)	0.303	−0.100 (−0.217 to 0.018)	0.096	0.056	0.511
rs2853677 (T/C)	−0.091 (−0.198 to 0.016)	0.097	0.003 (−0.154 to 0.160)	0.967	−0.057 (−0.172 to 0.058)	0.331	0.191	0.511
rs36600 (C/T)	−0.126 (−0.256 to 0.003)	0.056	0.088 (−0.401 to 0.577)	0.725	−0.117 (−0.261 to 0.027)	0.110	0.146	0.511
rs3813572 (A/G)	−0.075 (−0.184 to 0.034)	0.180	−0.047 (−0.335 to 0.240)	0.746	−0.141 (−0.259 to −0.022)	0.020	0.401	0.689
rs465498 (T/C)	0.001 (−0.111 to 0.113)	0.981	−0.093 (−0.374 to 0.188)	0.515	−0.081 (−0.201 to 0.040)	0.188	0.805	0.875
rs4677662 (T/C)	−0.005 (−0.109 to 0.099)	0.923	−0.132 (−0.314 to 0.051)	0.158	−0.039 (−0.151 to 0.072)	0.487	0.355	0.689
rs6495309 (C/T)	0.060 (−0.053 to 0.173)	0.300	0.126 (−0.018 to 0.270)	0.085	0.085 (−0.035 to 0.206)	0.164	0.221	0.511
rs667282 (T/C)	0.069 (−0.044 to 0.182)	0.231	0.131 (−0.009 to 0.272)	0.066	0.109 (−0.010 to 0.229)	0.073	0.174	0.511
rs753955 (T/C)	0.029 (−0.076 to 0.133)	0.591	0.297 (0.124–0.471)	0.001	0.074 (−0.037 to 0.186)	0.192	0.003	0.075

[a]β (95% CI) and *P* values were derived from the comparisons with the reference genotype (AA) using a general linear model for ln-transformed 8-OHdG, adjusting for covariants (gender, years worked, smoking status, alcohol use, BMI, ΣOH–PAHs, and BPDE-albumin adducts).

[b]A *P* value is for the genotype term in the model, adjusting for all covariants mentioned above.

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Table 4. Multivariate analyses for lung cancer risk–related SNPs on MN frequency of coke oven workers

SNP [major (A)/minor allele (a)]	Aa vs. AA		aa vs. AA		Aa+aa vs. AA			
	β (95% CI)[a]	*P*[a]	β (95% CI)[a]	*P*[a]	β (95% CI)[a]	*P*[a]	*P*[b]	FDR
rs10937405 (C/T)	−0.009 (−0.063 to 0.046)	0.756	−0.102 (−0.205 to 0.001)	0.050	−0.022 (−0.075 to 0.030)	0.403	0.144	0.360
rs11080466 (A/G)	0.037 (−0.019 to 0.094)	0.190	−0.020 (−0.151 to 0.111)	0.764	0.029 (−0.025 to 0.083)	0.293	0.373	0.547
rs1333040 (T/C)	−0.090 (−0.146 to −0.035)	0.001	0.014 (−0.076 to 0.103)	0.765	−0.066 (−0.118 to −0.014)	0.013	0.002	0.025
rs1663689 (A/G)	−0.036 (−0.094 to 0.021)	0.213	−0.145 (−0.224 to −0.065)	<0.001	−0.067 (−0.121 to −0.013)	0.016	0.001	0.025
rs2131877 (T/C)	0.044 (−0.017 to 0.105)	0.159	0.080 (0.005–0.156)	0.036	0.060 (0.003–0.118)	0.040	0.103	0.291
rs247008 (C/T)	0.063 (−0.001 to 0.127)	0.052	0.016 (−0.059 to 0.091)	0.674	0.045 (−0.015 to 0.106)	0.145	0.105	0.291
rs2895680 (T/C)	−0.048 (−0.104 to 0.007)	0.089	0.007 (−0.083 to 0.097)	0.884	−0.033 (−0.085 to 0.020)	0.221	0.191	0.398
rs36600 (C/T)	0.040 (−0.029 to 0.109)	0.256	0.102 (−0.145 to 0.350)	0.416	0.045 (−0.022 to 0.112)	0.191	0.394	0.547
rs3813572 (A/G)	−0.083 (−0.142 to −0.025)	0.005	−0.146 (−0.299 to 0.007)	0.062	−0.094 (−0.151 to −0.038)	0.001	0.005	0.042
rs4488809 (C/T)	0.024 (−0.039 to 0.087)	0.456	−0.064 (−0.139 to 0.010)	0.091	−0.002 (−0.062 to 0.057)	0.937	0.029	0.181
rs4677662 (T/C)	0.041 (−0.014 to 0.096)	0.140	0.074 (−0.022 to 0.170)	0.131	0.047 (−0.005 to 0.099)	0.077	0.170	0.386
rs4809957 (G/A)	−0.064 (−0.122 to −0.007)	0.029	−0.053 (−0.129 to 0.024)	0.177	−0.070 (−0.124 to −0.016)	0.011	0.081	0.289
rs753955 (T/C)	0.020 (−0.035 to 0.075)	0.475	−0.106 (−0.203 to −0.009)	0.032	−0.002 (−0.055 to 0.050)	0.932	0.042	0.210
rs16988393 (T/C)	−0.007 (−0.075 to 0.061)	0.838	−0.387 (−0.710 to −0.063)	0.019	−0.019 (−0.086 to 0.048)	0.580	0.064	0.267

[a]β (95% CI) and *P* values were derived from the comparisons with the reference genotype (AA) using a poisson log-linear model for MN frequency, adjusting for covariants (gender, years worked, smoking status, alcohol use, BMI, ΣOH–PAHs, and BPDE-albumin adducts).

[b]A *P* value is for the genotype term in the model, adjusting for all covariants mentioned above.

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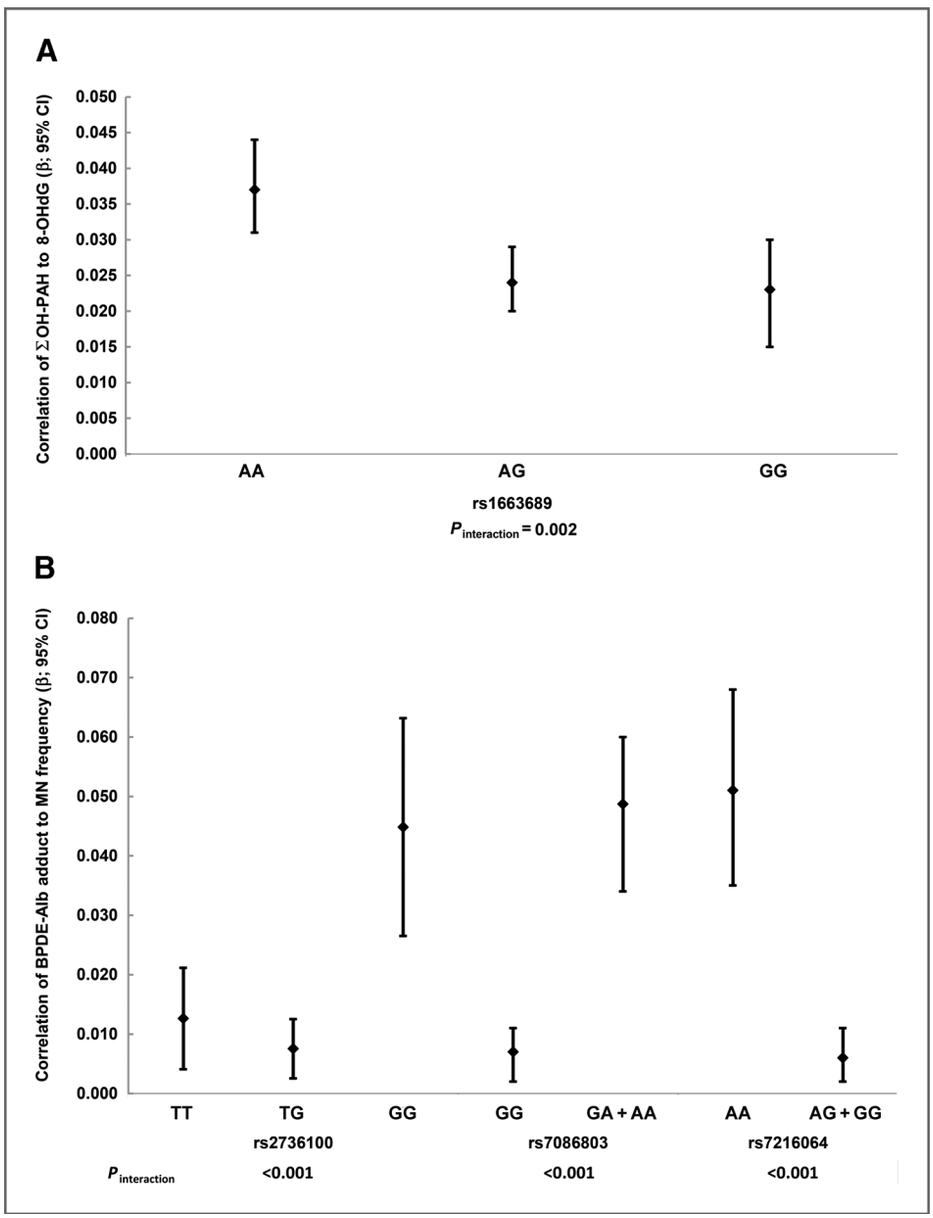

Figure 1. Correlations of PAHs exposure and lung cancer risk–associated SNPs to genetic damage of coke oven workers. A, correlations of ΣOH–PAHs and SNPs to the levels of 8-OHdG, the levels of 8-OHdG were ln-transformed; B, correlations of BPDE-Alb adducts and SNPs with MN frequency.

individuals carrying at least one mutant allele G of rs1663689 than those with wild homozygous genotype AA (GG: β, 0.023; 95% CI, 0.015–0.030; AG: β, 0.024; 95% CI, 0.020–0.029; AA: β, 0.037; 95% CI, 0.031–0.044; Fig. 1A; Supplementary Table S4). The relationships between three SNPs (rs2736100 at *TERT*, rs7086803 at *VTI1A*, and rs7216064 at *BPTF*) and BPDE-Alb adducts to the MN frequency were observed (all $P_{interaction} < 0.001$; Fig. 1B; Supplementary Table S4). Larger increased correlations of BPDE-Alb adducts to MN frequency were observed in individuals with rs2736100 GG (β, 0.045; 95% CI, 0.026–0.063; $P < 0.001$), rs7086803 GA+AA (β, 0.047; 95% CI, 0.034–0.060; $P < 0.001$), and rs7216064 AA (β, 0.051; 95% CI, 0.035–0.068; $P < 0.001$) than those with rs2736100 TT/TG (TT: β, 0.013; 95% CI, 0.004–0.021; $P = 0.004$; TG: β, 0.008; 95% CI, 0.003–0.013; $P = 0.003$), rs7086803 GG (β, 0.007; 95% CI, 0.002–0.011; $P = 0.005$), and rs7216064 AG+GG genotype (β, 0.006; 95% CI, 0.002–0.011; $P = 0.006$), respectively.

Because there were correlations between age and years worked, and pack-years and smoking ($r > 0.5$; Supplementary Table S3), we did not include age and pack-years in the models mentioned above to avoid overadjustment. However, we conducted a sensitivity analysis and compared model estimates, including pack-years and age as covariations instead of years worked and pack-years. Results were generally consistent with those adjusted for years worked and smoking (data not shown).

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Discussion

In this study, we provided evidence that lung cancer risk–related SNPs influenced genetic damage in coke oven workers who were exposed to PAHs. 13q12.12-rs753955 was suggestively associated with elevated urinary 8-OHdG levels and three SNPs (9p21-rs1333040, 10p14-rs1663689, and 15q25.1-rs3813572) were associated with decreased MN frequency. Significant relationships between 10p14-rs1663689 and ΣOH-PAHs to 8-OHdG, and three SNPs (rs2736100 at *TERT*, rs7086803 at *VTI1A*, and rs7216064 at *BPTF*) and plasma BPDE-Alb adducts to MN frequency were observed, suggesting that lung cancer risk–associated SNPs modulate the associations between PAH exposure and genetic damage in coke oven workers. Bioinformatic analyses indicated that several lung cancer risk–related SNPs associated with genetic damage altered putative transcription factor–binding sites, suggesting that they might influence gene expression.

13q12.12-rs753955 is located between *MIPEP* and *TNFRSF19*. Risk-allele homozygotes (CC) at rs753955 were associated with increased 8-OHdG levels, consistent with its increased effect on lung cancer risks (26). The product of *MIPEP* is primarily involved in the maturation of oxidative phosphorylation (OXPHOS)–related proteins. The protein encoded by *TNFRSF19* is a member of the TNF receptor superfamily, which was involved in activating the JNK (c-*jun*–NH_2–kinase) signaling pathway (39). Although OXPHOS was involved in the formation of reactive oxygen species (40) and the JNK signaling pathway was a key modulator in the process of DNA oxidative damage (41), little is known about the potential effect of rs753955 on *MIPEP* and *TNFRSF19* expression. Further studies are needed to explore the functional impact of this polymorphism.

9p21-rs1333040 located about 74 kb upstream of *CDKN2B* and within intron 12 of *CDKN1B* antisense *RNA 1* or *ANRIL*. A 1.41-fold change in *ANRIL* mRNA expression was observed in minor allele homozygous (CC) for rs1333040 to major allele homozygous in peripheral blood (42). *ANRIL* was transcriptionally upregulated following DNA damage and elevated ANRIL suppressed the expression of cyclin-dependent kinase inhibitors (INK4a, INK4b, and ARF) at the late stage of DNA-damage response, allowing the cells to return to normal at the completion of the DNA repair (43). In this study, we observed decreased MN frequency in individuals with CC+TC and TC genotype, compared with those with wild TT genotype. *In silico* analysis showed that SNP-rs1333040 T>C substitution resulted in a gain of two transcription factor–binding sites, indicating that rs1333040 polymorphism might upregulate the expression of *ANRIL*, accelerating the process of the DNA repair and decreasing MN frequency. More functional studies are needed to investigate the possible role of rs1333040 in the process of genetic damage. The lack of statistical significance in MN frequency between persons with CC genotype and those with TT genotype in the present study might be partly due to a low number of individuals with CC genotype.

SNP rs1663689 is located 908 kb downstream of the transcription factor *GATA3*. Minor homozygotes (GG) of rs1663689 have lower MN frequency compared with major allele homozygotes (AA), consistent with its effect on lung cancer risks (3). Previous studies reported that GATA3 was the substrate of human checkpoint kinases 1 and 2, which were the kinases in the cellular DNA-damage response (44), suggesting that *GATA3* might be a fundamental factor contributing to tumorigenesis by regulating DNA-damage response. Bioinformatic analyses indicated that SNP rs1663689 resulted in a loss of three transcription factor–binding sites. However, whether rs1663689 has effect on *GATA3* expression requires further investigation.

SNP rs3813572, located in the 5′ end of *PSMA4*, is a moderate LD with rs2036534 on 15q25 ($D' = 1$, logarithm of odds = 4.5), which was associated with lung cancer risk (31). In this study, SNP rs3813572 exhibited an association with decreased MN frequency. *PSMA4* plays a role in promoting cancer cell proliferation and downregulation of *PSMA4* expression induced apoptosis (45). Bioinformatic analyses showed that rs3813572 A>G substitution resulted in the loss of transcription factor–binding sites but its impact on *PSMA4* expression remains to be elucidated.

We carried out a comprehensive evaluation of potential gene–environment correlations between 25 lung cancer risk–related SNPs and two genetic damage biomarkers. A total of four SNPs could modulate the associations between PAH exposure and genetic damage (one for 8-OHdG levels and three for MN frequency). PAH exposure has a higher correlation with genetic damage in individuals with lung cancer risk alleles (10p14-rs1663689 AA, 5p15.33-rs2736100 GG, 10q25.2-rs7086803 GA+AA, and 17q24.3-rs7216064 AA) than in those with protective alleles (rs1663689 AG/GG, rs2736100 TT/TG, rs7086803GG, and rs7216064 AG+GG), respectively. It was assumed that the genetic variants altered the duration of exposure to PAHs. Thus, the correlations of PAH exposure to genetic damage in this study might partly depend on the genotypes of these SNPs. Our hypothesis is that, given the same exposure, persons with risk alleles have higher damage levels and are more susceptible to the progress of lung cancer, compared with those carrying protective alleles.

In summary, our results suggested that lung cancer risk–related SNPs may influence one's susceptibility to PAH-induced genetic damage. These findings provided some potential mechanisms for genetic heterogeneity of PAH carcinogenesis and lung cancer development. People carrying risk alleles may suffer from more serious genetic damage and more susceptible to the initiation and evolvement of lung cancer even under similar environment. Thus, this research implied that major gains in the prevention of cancer will necessitate health and regulatory policies that protect more susceptible individuals from naturally occurring and man-made environmental carcinogens. Although the data about PAH exposure and

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oxidative DNA damage (8-OHdG) were reported in our previous study (33), the genotype data for SNPs associated with lung cancer risks were original and were first reported in this study, indicating that our job was not just a reanalysis of the existing data. To the best of our knowledge, this is the first study to investigate the relationships of lung cancer risk–related SNPs identified from GWAS to the genetic damage induced by PAHs. Future prospective studies with a larger sample size in populations of different ethnic backgrounds are warranted to validate our findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Dai, S. Deng, G. Qiu, J. Ye, W. Zhang, H. Guo, T. Wu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Dai, S. Deng, J. Li, B. Yang, W. Feng, W. Zhang, H. Guo, T. Wu
Writing, review, and/or revision of the manuscript: X. Dai, S. Deng, W. Zhang, M. He, T. Wu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Dai, S. Deng, T. Wang, G. Qiu, W. Feng, Q. Deng, J. Ye, W. Zhang
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