# The interaction effects of FEN1 rs174538 polymorphism and polycyclic aromatic hydrocarbons exposure on damage in exon 19 and 21 of EGFR gene in coke oven workers

**Siqin Chen**
Guangzhou Medical University
**Yuefeng He**
Kunming Medical University
**Maosheng Yan**
Other Press
**Yun Zhou**
Guangzhou Medical University
**Qinghua He**
Guangzhou Medical University
**Jingwen Tan**
Kunming Medical University
**Binyao Yang** ( yangbinyao@126.com )
Guangzhou Medical University

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# Abstract

Mutagenesis is a multifactor process associated with increased risk of cancer. Polycyclic aromatic hydrocarbons (PAHs) exposure and genetic susceptibility were conductive to genotoxic effects including gene damage, which can increase mutational probability and are potential carcinogenic etiology. We aimed to explore the dose-effect associations of PAHs exposure with damage of exons of epidermal growth factor receptor (EGFR) and breast cancer susceptibility gene 1 (BRCA1), as well as their associations whether modified by Flap endonuclease 1 (FEN1) genotype. 288 coke oven male workers were recruited and we detected the concentration of 1-hydroxypyrene (1-OH-pyr) as PAHs exposure biomarker in urine and examined base modification in exons of EGFR and BRCA1 respectively, and genotyped FEN1 rs174538 polymorphism in plasma by PCR methods. Compared to low exposure group, the high exposed workers had significantly higher urinary concentrations of 1-OH-pyr, after adjustment for multiple covariates ($P < 0.001$). We found that the damage index of exon 19 and 21 of EGFR (EGFR-19 and EGFR-21) were both significantly associated with increased urinary 1-OH-pyr (both $P_{trend} < 0.001$). The multiple linear regression analysis showed that the levels of urinary 1-OH-pyr were both significantly associated with increased EGFR-19 and EGFR-21 in both smokers and nonsmokers (both $P < 0.001$). Additionally, we observed that the urinary 1-OH-pyr concentrations were both linearly associated with EGFR-19 and EGFR-21 only in rs174538 GA + AA genotype carriers (both $P < 0.001$). Moreover, FEN1rs rs174538 showed modifying effects on the associations of urinary 1-OH-pyr with EGFR-19 and EGFR-21 (both $P_{interaction} < 0.05$). Our findings revealed the linear dose-effect association between exon damage of EGFR and PAHs exposure and highlight differences in genetic contributions to exon damage and have the potential to identify at-risk subpopulations who are susceptible to adverse health effects induced by PAH exposure.


# 1. Introduction

The epidermal growth factor receptor (EGFR) is one kind of the HER/ErbB family of receptor tyrosine kinases (RTKs), which includes HER1 (EGFR/ErbB1), HER2 (neu, ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (da Cunha Santos et al. 2011). It exerts critical functions of regulating epithelial tissue development and homeostasis physiologically but also drives tumorigenesis mostly of lung cancer, breast cancer and glioblastoma pathologically (Sigismund et al. 2018). The tyrosine kinases (TK) activity of EGFR may be dysregulated by several oncogenic mechanisms, including EGFR gene mutation, increased gene copy number, and EGFR protein overexpression (da Cunha Santos et al. 2011). Specially, epidemiologic researches have shown that the EGFR mutation is closely correlated with the non-small cell lung cancer (NSCLC) in different area worldwide (Bircan et al. 2014, Cheng et al. 2019). Due to its overexpression and hyperactivation, EGFR has been the rational therapeutic target for human malignancies (da Cunha Santos et al. 2011). According to current research, deletion mutations in exon 19 and point mutations at codon 858 in exon 21 are most prevalent and have been utilized as predictors of therapeutic intervention and sensitivity in lung cancer (Bircan et al. 2014, Krawczyk et al. 2016). DNA damage is associated closely with gene mutation and can increase mutational probability triggering the advent of genetic

mutations (Gedik et al. 2002), and the base damage of EGFR may be a potential marker of EGFR mutation (Scalise et al. 2016). Therefore, it is of necessity to investigate the EGFR gene damage.

The involvement of EGFR in the process of the carcinogenesis is a multifactor and multistage process, which is modified not only by environment factors but also by genetic factors such as single nucleotide polymorphisms (SNPs). A published genome-wide association study (GWAS) had comprehensively reported SNPs associated with EGFR mutations based on the 10,780 never-smoking cases (Seow et al. 2017). These findings provided us with EGFR-related genetic variations. Several studies have shown that the gene polymorphisms have great significance on the EGFR mutation, which can be utilized clinically to predict cancer aggressiveness, metastatic, potential and therapeutic responsiveness of lung cancer patients (Huang et al. 2018, Lin et al. 2020). The related research has reported that the rs2910164 G allele in miR-146a decreased inhibition of the expression of EGFR, which induced the proliferation of human keratinocytes (Zhang et al. 2014). With the further knowledge to EGFR tyrosine kinase inhibitors (EGFR-TKIs), which are the treatment of choice for advanced-stage NSCLC patients with mutations in EGFR, SNPs was considered to be the reason to the different outcomes varying from person to person (Perez-Ramirez et al. 2019, Winther-Larsen et al. 2019). Specially, flap endonuclease 1 (FEN1) plays a crucial role in both DNA replication and damage repair and researches have shown that FEN1 may represent a prognostic biomarker and potential therapeutic target for NSCLC treatment (He et al. 2017, Zhang et al. 2018). Several meta-analyses have revealed that the polymorphism of FEN1 may be associated with the cancer susceptibility (Ren et al. 2015, Rezaei et al. 2016, Ying et al. 2015). Nevertheless, the role of FEN1 polymorphism in DNA damage remains unknown.

Polycyclic aromatic hydrocarbon (PAHs), as a main composition of environment tobacco smoke and $PM_{2.5}$, has been of scientific concern for many years and has attracted public health attention, due to their potential to bioaccumulation and their effects of toxicity, carcinogenesis and mutagenesis on human health (Armstrong et al. 2004, Samanta et al. 2002). In this context, several studies have revealed that urinary 1-Hydroxypyrene (1-OH-Pyr) was used as a suitable indicator to assess exposure to the organic carbon compounds of PAHs from the environment and occupational settings (Jongeneelen 2001, McClean et al. 2012, Sobus et al. 2009). In addition, urinary 1-OH-Pyr was proved to be the most comprehensive carcinogenic biomarker of exposure to PAHs (Yamano et al. 2014). Therefore, 1-OH-Pyr as a biomarker has been widespread used due to its convenience and accessibility to evaluate the early genotoxic effects induced by PAHs exposure. However, the association analysis of urinary PAH exposure and EGFR DNA damage, and whether its association modified by gene polymorphism remain unclear.

Here, we performed a cross-sectional study consisting of 288 male coke-oven workers in an attempt to analyze the associations of urinary 1-OH-Pyr, an indicator of assessing PAHs exposure, with the damage index of exon 19 and 21 of EGFR gene (EGFR-19 and EGFR-21) and their associations whether modified by FEN1 rs174538 polymorphism were further evaluated.

# 2. Materials And Methods

## 2.1 Study subjects

This study included 288 coke-oven workers who were all healthy males, aged between 19 to 32.5 years, and worked in the same coking plant in south China. They had been working at the different workplaces for at least 3 months. We gained the concentrations of external total PAHs in different workplaces from coke-oven plant intermittent monitoring. Briefly, the concentration of external total PAHs (mean ± SD, □/m3) was 6.48 ± 3.89 in the coal preparation recovery workshop, 32.07 ± 23.61 in the coking oven workshop. So, 151 workers from the coal preparation recovery workshop were served as low exposure group and 137 workers from the coking oven workshop were served as high exposure group. After acquiring the written informed consent from each participant, we use occupational health questionnaire to collect personal basic information, occupational history, medical history and lifestyle including working years and smoking history and other data. Those who had smoked < 1 cigarettes per day for less than 1 year were regarded as nonsmokers; otherwise, subjects were viewed as smokers. At the end (post-shift) of each work-shift, we collected 20 mL urine from each participate in 50ml polyethylene tube for biological detection of 1-OH-Pyr and 5 mL peripheral blood was collected with 5ml disposable Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant tube for detection of gene damage index. All biological samples were stored at -80 ℃ after collection until laboratory examinations. The workers who are exposed to known DNA-damaging agents, such as radio-therapy and chemotherapy in the last 3 months, were excluded. The Ethics Committee of Kunming Medical University approved the study.

## 2.2 Measurement of urinary 1-OH-Pyr and urinary creatinine

We measured the concentrations of urinary 1-OH-Pyr using gas chromatography-mass spectrometry following the measurement methods previously reported in detail (Kuang et al. 2013, Yang et al. 2016). Considering the inter-individual variations in urinary metabolites on dilution status, we measured urinary creatinine concentration employing an automated clinical chemistry analyzer according to Jaffe's colorimetric method to calibrate urinary 1-OH-Pyr and expressed as μmol/mmol creatine.

## 2.3 Determination of exon damage index of gene EGFR and BRCA1

DNA was extracted using the DNA fluorescence quantitative kit of Kangwei century company. PCR primers were designed to amplify exon 19 and 21 of EGFR gene and exon 20 of BRCA1, and β-actin sequence which was used as control fragment. The sequence was as following in detail: β-actin (forward: CGGGAAATCGTGCGTGACAT; reverse: GAAGGAAGGCTGGAAGAGTG); exon 19 of EGFR gene (forward: GTGCATCGCTGGTAACATCCA; reverse: AAAGGTGGGCCTGAGGTTCA); exon 21 of EGFR gene (forward: CCTCACAGCAGGGTCTTCTCTG; reverse: TGGCTGACCTAAAGCCACCTC); exon 20 of BRCA1 gene (forward: GAGTGGTGGGGTGAGATTTTTGTC; reverse: CCTGATGGGTTGTGTTTGGTTTCT); The PCR was conducted in a 20 ul final volume containing: 2×UltraSYBR Mixture 10ul, 10μM Forward Primer 0.4ul, 10μM Reverse Primer 0.4ul, DNA 1ul and sterile water was used to supplement up to 10 ul. The real-time PCR protocols began with one cycle at 95°C for 600 seconds followed by 45 cycles of the following: 95℃ for 10s, 60℃ for 10s and 72℃ for 15s.

The amplification process was finished with the Roche LightCycler96 real-time fluorescence quantitative PCR instrument. All PCR reactions were performed in triplicate for each sample. At the end of the reaction, the cycle threshold (Ct value) was automatically read out by the fluorescent PCR tape software. The more cycles needed for each sample to reach a certain amount of products, the greater of the Ct value, and the smaller of the initial template plate is. The average amplification efficiency of PCR is proportional to 2Ct0-Ct1, Ct1 is one specific gene to be measured and Ct0 is β-actin. In this paper, we use Δ Ct (Δ Ct = Ct1-Ct0) to express the damage of gene, according to the previously published measurement methods as described in detail (Wen et al. 2008). The higher the value, the higher the damage.

### 2.4 Genotyping examination and HRM (High Resolution Melting) analysis

We designed the primer of FEN1 to detect the genotype and confirm whether wild type or mutation. Samples were assayed in duplicate using the Roche Light Cycler 96 real-time fluorescence quantitative PCR instrument. Each 20ul reaction contained 4ul Buffer, 10mM dNTP Mix 0.4ul, 10uM Forward Primer 0.4ul, 10uM Reverse Primer 0.4ul, DNA 1ul, Evagreen 1ul (Biotium company number 31000) and 12.8ul water aiming to add the system into 20ul in all. The same touchdown PCR program and melting conditions were used for all amplicons: 95°C for 10 minutes, 45 cycles of 95℃ for 10s, 60℃ for 10s and 72℃ for 15s. Data and melting curve were acquired and analyzed with the accompanying Gene Scanning software.

### 2.5 Statistics

Data analysis was conducted using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA). The normality of continuous variables was examined by Kolmogorov-Smirnov normality test. The level of creatinine-adjusted 1-OH-Pyr, damage index of exon 19 and 21 of gene EGFR, and damage index of exon 20 of gene BRCA1 were natural logarithm (ln) transformed to improve normality and stabilize variance. Considering age was highly correlated with working years ($r = 0.831$, $P < 0.001$), so age was not included in the multiple linear regression models. We considered working years (continuous), workplace (low exposure/high exposure) and smoking status (smokers/nonsmokers) as potential confounders, and included them in our statistical analyses unless otherwise specified. We performed Student's t-tests for continuous variables and chi-squared tests for categorical variables to assess the differences of general characteristics between the low exposure group and high exposure group. We analyzed the between-group differences of exposure levels of urinary 1-OH-Pyr, EGFR-19, EGFR-21, and the damage index of exon 20 of gene BRCA1 (BRCA1-20) by multivariate analysis of covariance with adjustment for all covariates. We further evaluated the associations of urinary 1-OH-Pyr (as independent variables) with the continuous value of exon damage index (as dependent variables) in the multiple linear regression models among the 288 coke-oven workers. Additionally, we also categorized these 288 workers into 3 subgroups by the tertiles of internal exposure biomarker of urinary 1-OH-Pyr (T1 ≤ 33rd percentile, T2 > 33rd percentile but ≤ 67th percentile and T3 > 67th percentile) and the multiple linear regression models were employed to explore the linear trend *P*-value and compute the relative association coefficients (βs) and 95% confidence intervals (95%CIs) with adjustment for above confounders in $T_1$, $T_2$ and $T_3$ subgroups

with T1 as the reference group. The correlation coefficients (Pearson r) between the ln-transformed urinary 1-OH-Pyr and EGFR-19 and EGFR-21 were calculated by simple linear correlation analyses.

We then performed the stratified analyses by FEN1 rs174538 genotypes, considering the sample size, subjects with genotype GA and AA were combined. We conducted Hardy-Weinberg equilibrium (HWE) test with chi-square test for each SNP before the analysis. The associations between urinary 1-OH-Pyr (as independent variables) with the continuous value of exon damage index (as dependent variables) in the multiple linear regression models were estimated by βs and 95% CIs with adjustment of potential confounders. Moreover, gene-environment interaction analysis was examined by introducing the interaction term [SNP∈ ln-transformed urinary 1-OH-Pyr (continuous)] into the confounders-adjusted linear regression model. For all associations, βs and their 95%CIs were reported to represent the estimated difference in ln-transformed dependent variables associated with a 1-SD increment in ln-transformed independent variables. Two-tailed $P < 0.05$ was defined as statistical significance.

# 3. Results

### 3.1 General characteristics of the study participants

As shown in Table 1, 288 participants were divided into low exposure group (n = 151) and high exposure group (n = 137) according to their workplaces and external total PAHs concentrations mentioned above (see “Study subjects” in “Materials and methods”). Among the low exposure group or the high exposure group, no significant differences were found between the observed genotypic frequencies and those expected under the HWE ($\chi^2 = 2.90$, $P < 0.05$ and $\chi^2 = 5.36$, $P < 0.05$, respectively). All the participants were males, and we observed no statistically significant difference between the two groups in age, working years and FEN1 rs174538 genotype (all $P > 0.05$); while, marginal significance was found in smoking status between the low exposure group and the high exposure group ($P = 0.05$). Furthermore, compared to low exposure group, we observed higher urinary 1-OH-Pyr, EGFR-19 and EGFR-21 in the high exposure group with adjustment of potential confounders (all $P < 0.05$) and no significant difference was observed in BRCA1-20 between the two groups ($P > 0.05$). Additionally, no significant differences were found in urinary 1-OH-Pyr levels and EGFR-19, EGFR-21 and BRCA1-20 between non-smokers and smokers (all $P > 0.05$, Table S1).

Table 1
Baseline characteristics of study participants.

| Variables | Low exposure group (n = 151 ) | High exposure group (n = 137 ) | *p* value |
|---|---|---|---|
| General characteristics | | | |
| Age (years) | 25.58 ± 2.68 | 25.19 ± 3.26 | 0.272[a] |
| Working years (years) | 3.19 ± 1.30 | 3.02 ± 1.28 | 0.251[a] |
| Smoking status (smokers/nonsmokers, %smokers) | 110/41 (72.8%) | 85/52 (62.0%) | 0.05[a] |
| PAH internal exposure biomarkers | | | |
| urinary 1-OH-Pyr ( μmol/mmol creatine) | 3.73 (2.57–5.02) | 6.81 (4.10–12.51) | < **0.001**[b] |
| Genotype of FEN1 (rs174538) | | | 0.188[c] |
| GG | 41 (27.2%) | 47 (34.3%) | |
| GA + AA | 110 (72.8%) | 90 (65.7%) | |
| Exon genetic damage index of gene | | | |
| EGFR-19 | 1.98 (1.74–2.33) | 2.09 (1.87–2.32) | **0.009**[b] |
| EGFR-21 | 1.83 (1.62–2.10) | 1.93 (1.68–2.13) | **0.047**[b] |
| BRCA1-20 | 2.77 (2.57–2.99) | 2.84 (2.61–3.27) | 0.100[b] |
| PAHs, polycyclic aromatic hydrocarbons; 1-OH-Pyr, 1-hydroxypyrene; EGFR-19: exon 19 genetic damage index of Epidermal Growth Factor Receptor gene; EGFR-21: exon 21 genetic damage index of Epidermal Growth Factor Receptor gene; BRCA1-19, exon 19 genetic damage index of The Breast Cancer 1 gene. | | | |
| Values shown are mean ± SD, n (%) and median (25th percentile, 75th percentile), or n (%). | | | |
| [a]Student's t-test for continuous variables and Chi-squared test for categorical variables. | | | |
| [b]Multivariate analysis of covariance with adjustment for working years,workplace and smoking status. | | | |

### 3.2 Dose-response relationships of urinary 1-OH-Pyr with EGFR-19 and EGFR-21

The median of the EGFR-19 in the 1st, 2nd and 3rd urinary 1-OH-Pyr tertiles were 1.90, 2.04 and 2.23, respectively; and the median of the EGFR-21 were 1.76, 1.87 and 2.02 in the 1st, 2nd and 3rd urinary 1-OH-Pyr tertiles, respectively. Multiple linear regression analysis was further used to estimate the covariate-adjusted associations of creatinine-standardized 1-OH-Pyr concentrations with EGFR-19 and EGFR-21,

BRCA1-20 (Table 2). We found that estimated differences in the EGFR-19 were gradually increased across tertiles of the concentrations of urinary 1-OH-Pyr after adjustment for working years (model 1) and working years, workplace and smoking status (model 2) (both $P_{trend} < 0.001$). Similarly, the estimated differences in EGFR-21 were also increased with each tertile increase in urinary 1-OH-Pyr levels in adjusted-model 1 and model 2 (both $P_{trend} < 0.001$). Additionally, after multivariable adjustment, differences in both ln-transformed EGFR-19 and EGFR-21 per increment of ln-transformed urinary 1-OH-Pyr were presented as the adjusted beta coefficients (95% CI) were 0.120 (0.083–0.156) and 0.116 (0.076–0.156), respectively. After adjustment for working years (model 1) and working years, workplace and smoking status (model 2), there were no significant changes in BRCA1-20 across urinary 1-OH-Pyr tertile (both $P > 0.05$). Furthermore, we also observed significantly positive correlations between urinary 1-OH-Pyr and EGFR-19 and EGFR-21 in the present study (Pearson r = 0.358 and 0.316, respectively, and both $P < 0.001$; Fig. 1A and 1B).

Table 2
The estimated difference in ln-transformed exon genetic damage index [ (95% CI)] associated with tertiles of urinary 1-OH-Pyr and per increment of ln-transformed urinary 1-OH-Pyr (n = 288)

| β | | | | | | |
|---|---|---|---|---|---|---|
| Variables | Tertile of urinary 1-OH-Pyr (μmol/mmol creatine) | | | *P* trend | Per increment of ln-transformed urinary 1-OH-Pyr | |
| | $T_1$ (< 3.07) | $T_2$ (3.07–6.31) | $T_3$ (> 6.31) | | β (95% CI) | *P* value |
| **EGFR-19** | | | | | | |
| Median | 1.90 | 2.04 | 2.23 | | 2.06 | |
| No. of Participants | 97 | 95 | 96 | | 288 | |
| Model 1[a] | 0 (reference) | 0.071 (-0.007–0.149) | 0.226 (0.148–0.305) | **< 0.001** | 0.120 (0.083–0.156) | **< 0.001** |
| Model 2[b] | 0 (reference) | 0.068 (-0.011–0.148) | 0.219 (0.133–0.305) | **< 0.001** | 0.116 (0.076–0.156) | **< 0.001** |
| **EGFR-21** | | | | | | |
| Median | 1.76 | 1.87 | 2.02 | | 1.90 | |
| No. of Participants | 97 | 95 | 96 | | 288 | |
| Model 1[a] | 0 (reference) | 0.039 (-0.043–0.121) | 0.200 (0.118–0.282) | **< 0.001** | 0.110 (0.071–0.149) | **< 0.001** |
| Model 2[b] | 0 (reference) | 0.040 (-0.044–0.124) | 0.199 (0.109–0.289) | **< 0.001** | 0.110 (0.067–0.152) | **< 0.001** |
| **BRCA1-20** | | | | | | |
| Median | 2.80 | 2.76 | 2.87 | | 2.80 | |
| No. of Participants | 97 | 95 | 96 | | 288 | |
| Model 1[a] | 0 (reference) | -0.014 (-0.077–0.049) | 0.001 (-0.061–0.064) | 0.96 | -0.001 (-0.070–0.068) | 0.97 |
| Model 2[b] | 0 (reference) | -0.019 (-0.083–0.045) | 0.010 (-0.079–0.058) | 0.76 | -0.015 (-0.090–0.059) | 0.68 |

[a] Model 1 was adjusted for working years

| β |
| --- |
| [b] Model 2 was adjusted for working years, workplace and smoking status. |

### 3.3 Associations of urinary 1-OH-Pyr with EGFR-19 and EGFR-21 in non-smokers and smokers

In both non-smokers and smokers, the multiple linear regression analysis revealed that the concentrations of urinary 1-OH-Pyr were significantly associated with EGFR-19 and EGFR-21, respectively, after adjustment for working years (model 1) and working years and workplace (model 2) (Table 3, all $P < 0.001$). However, no such significant association was observed between urinary 1-OH-Pyr levels and BRCA1-20 (all $P > 0.05$).

Table 3
Stratification Analysis of the estimated difference in ln-transformed exon genetic damage index [ (95% CI)] associated with a 1-SD increase in ln-transformed exposure levels of urinary 1-OH-Pyr among 288 coke oven workers by smoking status

| β | | | |
|---|---|---|---|
| rs174538 | Gene variables | β (95% CI) | *P* value |
| Non-smoker (n = 93) | EGFR-19 | | |
| | Model 1[a] | 0.134 (0.083–0.185) | **< 0.001** |
| | Model 2[b] | 0.132(0.076–0.187) | **< 0.001** |
| | EGFR-21 | | |
| | Model 1[a] | 0.103 (0.054–0.151) | **< 0.001** |
| | Model 2[b] | 0.107 (0.055–0.159) | **< 0.001** |
| | BRCA1-20 | | |
| | Model 1[a] | 0.003(-0.036–0.041) | 0.88 |
| | Model 2[b] | 0.003 (-0.039–0.045) | 0.89 |
| Smokers (n = 195) | EGFR-19 | | |
| | Model 1[a] | 0.107 (0.056–0.158) | **< 0.001** |
| | Model 2[b] | 0.104 (0.048–0.160) | **< 0.001** |
| | EGFR-21 | | |
| | Model 1[a] | 0.114(0.058–0.169) | **< 0.001** |
| | Model 2[b] | 0.112 (0.051–0.173) | **< 0.001** |
| | BRCA1-20 | | |
| | Model 1[a] | -0.004 (-0.047–0.039) | 0.86 |
| | Model 2[b] | -0.014 (-0.060–0.033) | 0.56 |
| [a]Model 1 was adjusted for working years. | | | |
| [b]Model 2 was adjusted for working years and workplace . | | | |

### 3.4 Interactive effects of FEN1 rs174538 with urinary 1-OH-Pyr on genetic exon damage index

We further sub-grouped the study subjects into rs174538 GA + AA and rs174538 GG genotype carriers. The multiple linear regression analysis showed that there was a significant association of levels of urinary 1-OH-Pyr with EGFR-19 in rs174538 GA + AA genotype carriers, after adjustment for working years (model 1) and working years, workplace and smoking status (model 2) (Table 4, both $P < 0.001$). Similarly, urinary 1-OH-Pyr concentrations were associated with EGFR-21 with adjustment for working years (model 1) and working years and workplace (model 2) in these subjects with rs174538 GA + AA genotype (both $P < 0.001$). However, no such association was observed between urinary 1-OH-Pyr concentrations and EGFR-19 and EGFR-21 in rs174538 GG genotype carriers, after adjustment for potential confounders (all $P > 0.05$). Additionally, we failed to observe significant associations between urinary 1-OH-Pyr concentrations and BRCA1-21 in both GG genotype carriers and GA + AA genotype carriers (all $P > 0.05$).

Table 4
Stratification Analysis of the estimated difference in ln-transformed exon genetic damage index [ (95% CI)] associated with a 1-SD increase in ln-transformed exposure levels of urinary 1-OH-Pyr among 288 coke oven workers by FEN1 rs174538 polymorphism

| β | | | |
|---|---|---|---|
| rs174538 | Gene variables | β (95% CI) | *P* value |
| GG (n = 88) | EGFR-19 | | |
| | Model 1[a] | 0.007 (-0.069–0.084) | 0.85 |
| | Model 2[b] | 0.009(-0.077–0.094) | 0.84 |
| | EGFR-21 | | |
| | Model 1[a] | 0.038 (-0.057–0.132) | 0.43 |
| | Model 2[b] | 0.047 (-0.059–0.152) | 0.38 |
| | BRCA1-20 | | |
| | Model 1[a] | 0.007(-0.050–0.064) | 0.80 |
| | Model 2[b] | 0.009 (-0.054–0.073) | 0.77 |
| GA + AA (n = 200) | EGFR-19 | | |
| | Model 1[a] | 0.164 (0.124–0.204) | **< 0.001** |
| | Model 2[b] | 0.154 (0.110–0.199) | **< 0.001** |
| | EGFR-21 | | |
| | Model 1[a] | 0.137 (0.099–0.175) | **< 0.001** |
| | Model 2[b] | 0.133 (0.092–0.175) | **< 0.001** |
| | BRCA1-20 | | |
| | Model 1[a] | -0.006 (-0.041–0.030) | 0.75 |
| | Model 2[b] | -0.015 (-0.054–0.023) | 0.43 |
| [a]Model 1 was adjusted for working years. | | | |
| [b]Model 2 was adjusted for working years, workplace and smoking status. | | | |

We subsequently evaluated the interactive effects between FEN1 rs174538 genotype and urinary 1-OH-Pyr on genetic exon damage index by modeling an interaction term of continuous urinary 1-OH-Pyr ∈ FEN1 rs174538 genotype in the covariate-adjusted linear regression models. We found rs174538 were nominally interacted with urinary 1-OH-Pyr on EGFR-19 and EGFR-21 ($P_{interaction}$ < 0.001 and

$P_{interaction}$ =0.031, respectively; Fig. 2). These subjects with rs174538 GA + AA genotype have greater effects of urinary 1-OH-Pyr on EGFR-19 and EGFR-21 compared to those with rs174538 GG genotype (β = 0.154 and β = 0.133 in rs174538 GA + AA genotype carriers vs. β = 0.009 and β = 0.047 in rs174538 GG genotype carriers). However, we failed to detect the interactive effect between rs174538 genotype and urinary 1-OH-Pyr on BRCA1-20.

# 4. Discussion

To our best of knowledge, this is the first study to explore the dose-effect associations of PAHs exposure with damage of exons of EGFR, as well as their associations whether modified by FEN1 rs174538 polymorphism in an occupational population. We observed significantly higher levels of urinary 1-OH-Pyr and significantly higher EGFR-19 and EGFR-21 in these individuals exposed to higher PAHs levels, after adjusting for multiple covariates. Subsequently, we revealed the significant linear associations of the urinary excretion of 1-OH-Pyr with EGFR-19 and EGFR-21, respectively. More importantly, their associations were modified by FEN1 rs174538 polymorphism.

Epidemiologic studies implied that exposure to PAHs from occupational settings was significantly related to the concentrations of PAHs metabolites such as urinary 1-OH-Pyr. As a major metabolite of pyrene, 1-OH-Pyr was reported to be a good biomarker for total PAHs exposure and is thought to reflect PAHs molecular activation (Jeng et al. 2013, McClean et al. 2012, Zhang et al. 2001). Especially, a cross-sectional study of coke oven workers in China reported that urinary 1-OH-Pyr was the most comprehensive carcinogenic indicator of exposure to PAHs (Yamano et al. 2014). In the present study, we also observed a significant difference in urinary 1-OH-Pyr levels according to environmental PAHs exposure among coke oven workers and individuals with higher environmental PAHs exposure had higher concentrations of urinary1-OH-Pyr. This result was consistent with previous studies on individuals including coke oven workers who exposed to PAHs (Bin et al. 2008, Kuang et al. 2013).

During the metabolism process of PAHs, it can lead to formation of ROS, which could lead to carcinogenesis via oxidative DNA damage (Moorthy et al. 2015). After metabolic transformation, DNA adducts are formed, a kind of compounds that carcinogenic substances form with cellular macromolecules. The level of specific DNA adducts is commonly considered to be a biomarker of the biologically effective dose, and if the adducts are able to induce mutations leading to cancer, they may also be recognized as biomarker of effect (Ewa &Danuta 2017). By causing DNA damage directly and forming DNA adducts, PAHs may exert great effect on gene mutation.

Our data showed a significantly higher damage in exon 19 and 21 of EGFR gene in these individuals exposed to higher environmental PAHs, after adjustment for multiple covariates. Moreover, we observed a significantly linear dose-effect relationship between PAHs exposure and EGFR damage in exon 19 and 21, respectively. Hence base modification is one of possible mechanisms led to EGFR mutation by which population exposed to PAHs may be associated with predisposition to lung cancer. To date, there is a lack of epidemiological study to investigate the association between PAHs exposure and exon damage in EGFR gene. It is widely known that PAHs were usually co-exist at high abundance in prevalent sources of pollution such as fine particulate matter (≤ 2.5μm in aerodynamic diameter; $PM_{2.5}$ ) air pollution and cigarette smoking and $PM_{2.5}$ and cigarette smoking were proved to be deleterious to EGFR damage and mutation of exon 19 and 21, and were closely related with the high level of EGFR mRNA expression (Han et al. 2016, Yanagawa et al. 2011). Evidences from epidemiological and laboratory studies have reported that after $PM_{2.5}$ exposure, the EGFR was hypomethylated in the promoter methylation site, resulting in upregulation of gene expression. Mutations in EGFR are linked to pulmonary exposure to $PM_{2.5}$ emitted from coal combustion, such as by inducing lung inflammation, elevating ROS, iNOS, EGF, and CXCL1 (Jin et al. 2017). Cigarette smoking exact also promotes EGFR signaling and ROS generation in non-small cell lung cancer cell lines (Zhang et al. 2017). Tobacco smoking was the most researched environmental factor confirmed to induce EGFR mutations (Hosgood et al. 2013, Soo et al. 2017). All of these above-mentioned scientific evidences suggested the possibility of our current association of PAH exposure with EGFR damage of exon 19 and 21 in EGFR gene.

Besides environment factors, genetic factors such as SNPs were reported to associate with predisposition to EGFR mutation. A meta-analysis based on previous GWAS have confirmed eight known SNPs associated with EGFR mutations such as rs2736100 (TERT), rs4488809 (TP63), rs7086803 (VTI1A), rs7741164 (FOXP4), rs9387478 (ROS1/DCBLD1) and so on (Seow et al. 2017). FEN1, as a DNA repair protein, plays a crucial role in both DNA replication and damage repair and is a key enzyme in maintaining genomic instability and preventing carcinogenesis. What's more, overexpressed FEN1 represents a prognostic biomarker and potential therapeutic target for non-small cell lung cancer treatment (Zhang et al. 2018), and FEN1 is critical for the rapid proliferation of lung cancer cells (He et al. 2017). In recent two decades, polymorphisms of FEN1 gene have gained attention for susceptibility of tumorigenesis or carcinogenesis (Moazeni-Roodi et al. 2019, Ying et al. 2015). Several studies focused on the associations of its major polymorphisms, namely − 69G/A (rs174538) and 4150G/T (rs4246215), and different disease risk, including the lung cancer (Liu et al. 2012, Yang et al. 2009). Interestingly, in the present study, we specifically examined the interaction effects of FEN1 rs174538 polymorphism and PAHs exposure on damage in exon 19 and 21 of EGFR and observed significantly increased trends for damage index of exon 19 and 21 as urinary 1-OH-Pyr concentrations increased only in FEN1 rs174538 GA + AA genotype carriers but not in rs174538 GG genotype carriers. At the same time, we also found rs174538 was significantly interacted with PAHs exposure on damage index of exon 19 and 21 of EGFR, even after adjusting for potential confounders. Our data indicate that the environmental PAHs exposure effects on exon damage in EGFR are stronger in persons with FEN1 rs174538 GA + AA genotype carriers than in those with rs174538 GG genotype carriers, which provides us meaningful information on the role

of air PAHs exposure in triggering EGFR damage even mutation. Furthermore, genetic susceptibility may partly contribute to the diverse response in EGFR mutation induced by PAHs exposure. Evidence especially in lung cancer research, has been reported that genetic polymorphisms in FEN1 confer susceptibility to lung cancer among coke-oven workers. Those with GA and GG genotype had higher transformed value of DNA damage compared with AA genotype in rs174538 (Yang et al. 2009). Therefore, our results indicated that the functions of FEN1 were more impaired or modified with higher PAH exposure.

To our best of knowledge, our study data is among the first to reveal the interaction effect of PAH exposure and FEN1 rs174538 polymorphism on exon damage of EGFR in an occupational male population, which imply the role of the environmental PAHs exposure and genetic polymorphisms in the regulation of exon damage in EGFR and provide scientific evidence for identifying susceptible population as well as putting forward corresponding interventions. Nevertheless, we acknowledge that the present study is exploratory and several limitations should not be neglected when interpreting our results. Firstly, because of the present cross-sectional study design, our data seems difficult to infer the causality. However, besides urinary 1-OH-Pyr was a good exposure marker and the exon damage in EGFR was acceptable to regard as effective variable, the dose-effect associations and gene-environment interaction could be well interpreted by previous evidence to a large extent. Secondly, we just detected urinary 1-OH-Pyr instead of all urinary metabolites of PAHs exposure, while like above-mentioned studies, urinary 1-OH-Pyr was a good indicator for environmental exposure to PAHs and positively correlated to total PAHs exposure. Urinary 1-OH-Pyr is a well biomarker to access the comprehensive toxicity of PAHs. Thus, it can represent the PAHs activation in one degree. Urinary metabolites had relatively short half-lives and were determined by using a spot urine instead of continuous samples and the coke-oven workers are long-termly exposed to PAHs continuously in their occupational environment, but the PAH metabolites in a single time point urine were also widely used as internal exposure biomarkers in the environmental epidemiological studies. Thirdly, insufficient sample size of this study to detect interaction with enough power and the lack of independent replication are also important limitations. Further investigations with larger sample-sized populations and functional studies are needed to verify the current findings and related molecular biological pathways.

# 5. Conclusion

Our present study showed a significant dose-effect relationship between PAHs exposure and EGFR-19 and EGFR-21, respectively. Notably, exposure to PAHs may interact with FEN1 rs174538 polymorphism and further contribute to exon damage of EGFR. These findings implied the potential to identify at-risk subpopulations who are susceptible to adverse health effects attributed to PAH exposure. Further studies are warranted to validate our findings and investigate the underlying molecular mechanisms.

# Declarations

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Kunming Medical University.

### Consent for publication

Not applicable

### Authors Contribution

We thank all individuals who volunteered to participate in this study. M.Y., Q.H., J.T., Y.H., and S.C. collected the samples and established the database and conducted experiments. M.Y, Y.Z.,and Y.B.designed and carried out the study.  S.C., Y.H.,and B.Y. conducted the data analysis and drafted the manuscript. Y.B. revised the manuscript. All the authors had access to the data and reviewed and approved the final submitted manuscript.


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### Conflicts of interest statement

The authors declare no conflicts of financial interest.

### Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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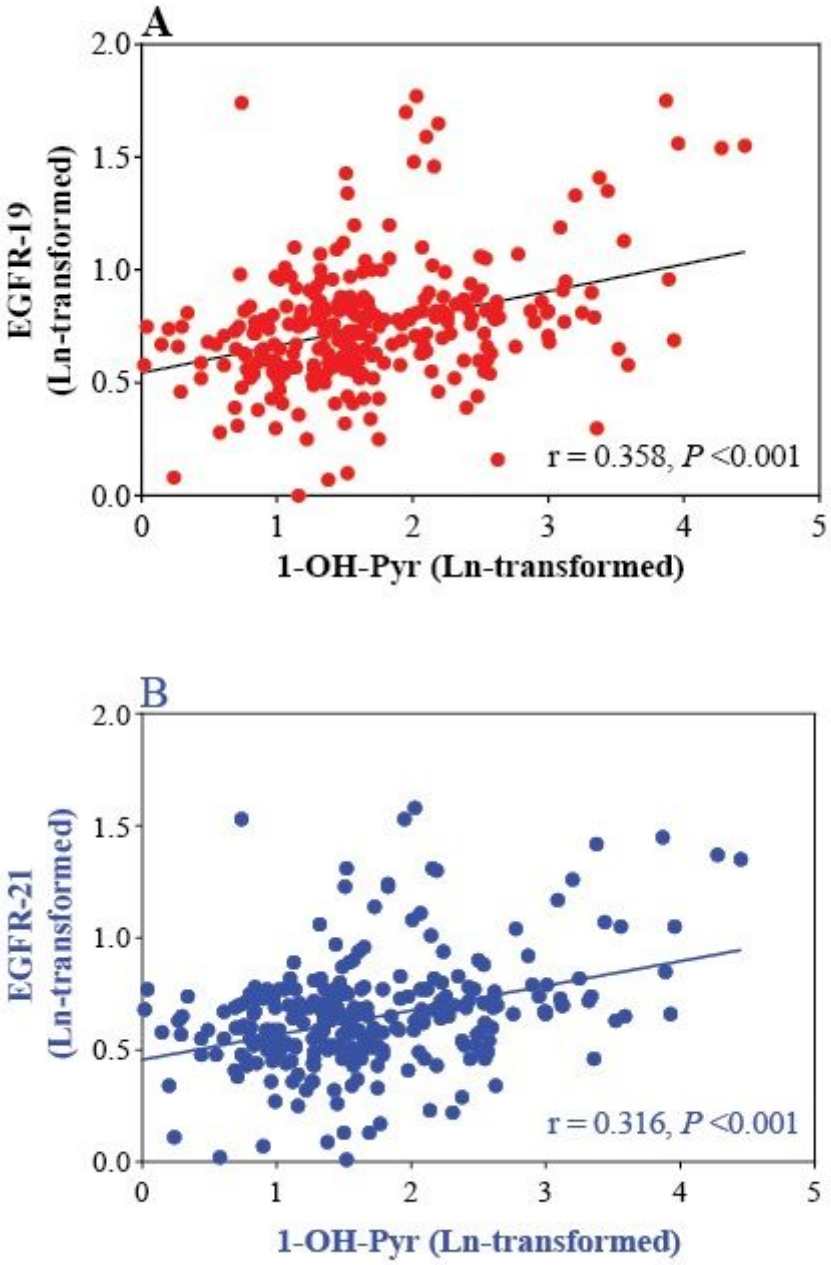
# Figures




## Figure 1

Correlation of the concentrations of urinary 1-OH-pyr metabolites with EGFR-19 (A) and EGFR-21(B) in the coke oven workers (n = 288).

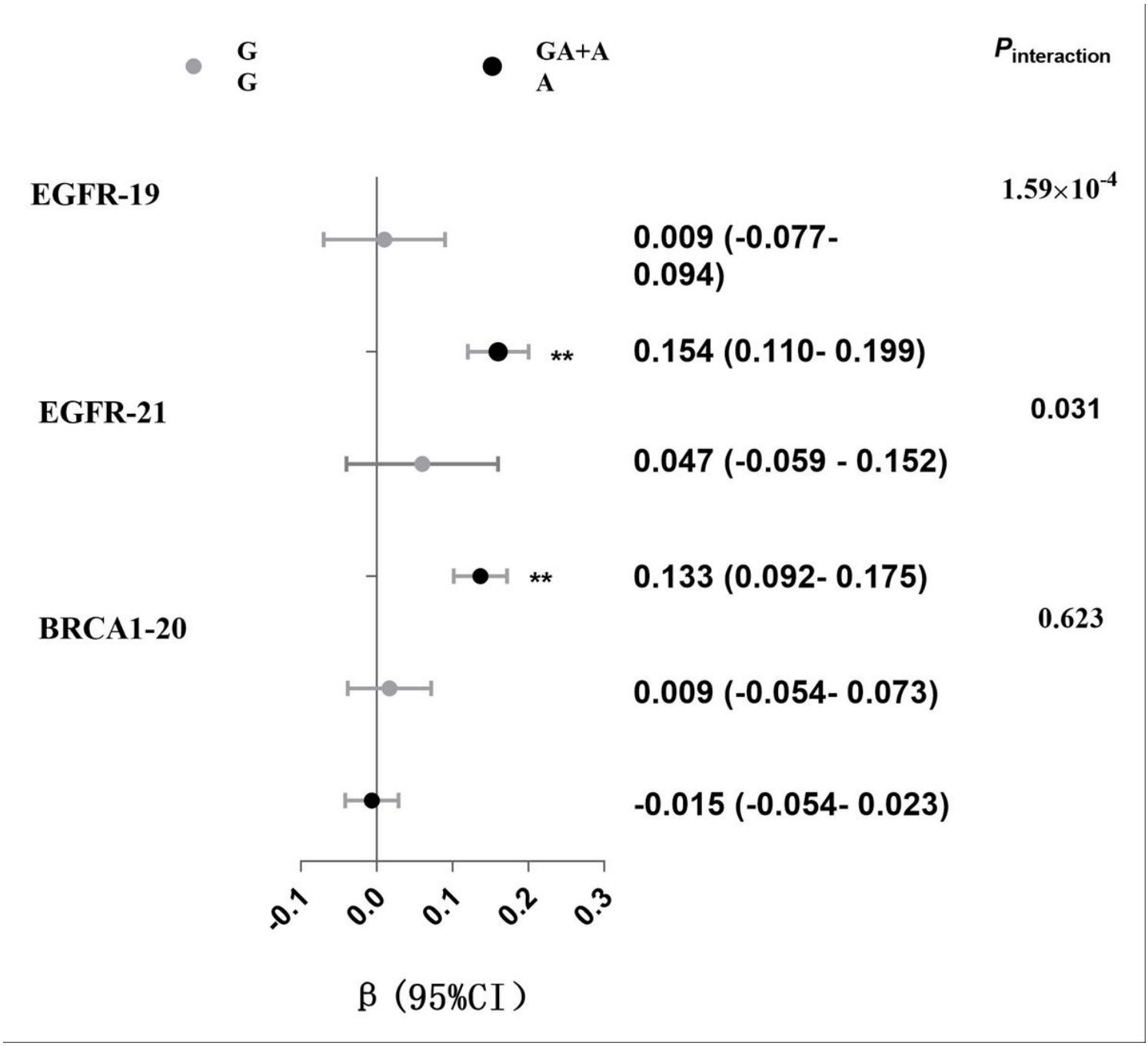



**Figure 2**

The interaction effects of urinary 1-OH-pyr with FEN1 rs174538 polymorphism on EGFR-19 and EGFR-21. The lines in panels represent β (95% CI) based on multivariate linear regression models adjusted for working years, smoking status and workplace, Pinterction was calculated by entering an interaction term between urinary 1-OH-pyr (continuous) and FEN1 rs174538 polymorphism (categorical) into the confounder-adjusted linear regression models.

# Supplementary Files

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- SupplementaryTables.doc