Myeloperoxidase -463G>A Polymorphism dose not Contribute to the Risk of Primary Lung Cancer in a Korean Population

Jae Hyoung Park, M.D.[1], Jung Min Park, M.D.[2], Eun Jin Kim, M.D.[1], Suk Jin Yun, M.D.[1], Sung Jun Lee, M.D.[1], Sung Ick Cha, M.D.[1], Eung Bae Lee, M.D.[3], Chang Ho Kim, M.D.[1], Sin Kam, M.D.[4], Dong Sun Kim, M.D.[5], Tae Hoon Jung, M.D.[1], Jae Yong Park, M.D.[1,2]*

[1]*Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Korea*
[2]*Cancer Research Institute, School of Medicine, Kyungpook National University, Daegu, Korea*
[3]*Department of Thoracic Surgery, Kyungpook National University Hospital, Daegu, Korea*
[4]*Department of Preventive Medicine, School of Medicine, Kyungpook National University, Daegu, Korea*
[5]*Department of Anatomy, School of Medicine, Kyungpook National University, Daegu, Korea*

한국인에서 Myeloperoxidase (MPO) 유전자의 -463G>A 다형성과 원발성 폐암의 위험도

박재형[1], 박정민[2], 김은진[1], 윤석진[1], 이승준[1], 차승익[1], 이응배[3], 김창호[1], 감 신[4], 김동선[5], 정태훈[1], 박재용[1,2]

[1]경북대학교 의과대학 내과학교실, [2]경북대학병원 암연구센터, [3]경북대학교 의과대학 흉부외과학교실,
[4]경북대학교 의과대학 예방의학교실, [5]경북대학교 의과대학 해부학교실

목 적 : Myeloperoxidase (MPO)는 benzo(a)pyrene, aromatic amines과 같은 발암전구물질 활성화를 통해 폐암 발생에 관여한다. MPO 유전자 promoter 부위의 -463G>A다형성은 MPO유전자의 발현량을 감소시킨다고 알려져 있다. 저자들은 MPO 유전자 promoter 부위의 -463G>A 다형성과 폐암 위험도의 상관 관계를 조사하였다.
방 법 : 경북대학교병원에서 폐암으로 진단된 432예를 대상으로 하였으며 대조군은 건강검진센타를 방문한 건강인 가운데 환자군과 연령 및 성을 match하여 무작위로 선택한 432명을 대상으로 하였다.
결 과 : MPO -463G>A의 유전자형은 폐암군의 경우 GG, GA, AA형이 각각 353명(81.7%), 76명(17.6%), 3명(0.7%)이였고 대조군의 경우 각각 356명(82.4%), 72명(16.7%), 4명(0.9%)으로 두 군간에 유의한 차이가 없었다. -463 AA+GA 유전자형은 -463 GG 유전자형에 비해 전체 폐암의 경우 위험도의 유의한 차이가 없었으며 (adjusted OR= 1.03, 95% CI= 0.72-1.47), 연령, 성별, 흡연력, 조직형으로 구분하였을 경우에도 유의한 차이가 없었다.
결 론 : MPO 유전자의 -463G>A 다형성은 한국인에서 폐암의 위험도를 결정하는 주요 인자가 아닌 것으로 생각된다.
(Tuberc Respir Dis 2005; 59: 157-163)

Key words : Myeloperoxidase, Polymorphism, Lung cancer susceptibility

Introduction

Myeloperoxidase (MPO), is a phase I enzyme found in neutrophils and monocytes, and it activates a wide range of tobacco smoke procarcinogens such as benzo(a)pyrene and aromatic amines[1]. The -463G >A polymorphism in the promoter region of the *MPO* gene leads to the loss of a SP1 binding site in an *Alu* hormone-responsive element, and this has been shown to reduce gene expression and activity[2,3]. Therefore, it is possible that carriers of the -463A allele may have a decreased risk of lung cancer because of the decreased metabolic activation of procarcinogens. A number of case-control studies have been conducted to evaluate the potential role of this *MPO* polymorphism on the risk of lung cancer, but the estimates of the nature and extent of the association have shown considerable variation between the different studies[4-6]. Moreover, only a few studies have explored the relationship between this *MPO* polymorphism and lung cancer in Asian populations[7,8]. In the present study, we have conducted a case-control study to evaluate the association

This study was supported by the Korea Research Foundation Grant to D.S. Kim (KRF-2002-013-F00001).

Address for correspondence : **Jae Yong Park, M.D.**
Department of Internal Medicine, Kyungpook National University Hospital, Samduk 2Ga 50, Daegu, 700-412, South Korea
Phone : 82-53-420-5536 Fax : 82-53-426-2046
E-mail : jaeyong@knu.ac.kr
Received : May. 2. 2005
Accepted : Jul. 18. 2005

between the *MPO* -463G>A polymorphism and the risk of lung cancer in a Korean population.

Materials and methods

1. Study population

This case-control study included 432 lung cancer patients and 432 healthy controls. The details of the study population have been described elsewhere[9-11]. In brief, the eligible cases included all the patients who were newly diagnosed with primary lung cancer between January 2001 and February 2002 at Kyungpook National University Hospital, Daegu, Korea. There were no age, gender, histological or stage restrictions, but patients with a prior history of cancers were excluded from this study. The cases included 210 (48.6%) squamous cell carcinomas, 141 (32.6%) adenocarcinomas, 73 (16.9%) small cell carcinomas, and 8 (1.9%) large cell carcinomas. The demographics and clinical characteristics of the cases were consistent with those of a nationwide lung cancer survey conducted by the Korean Academy of Tuberculosis and Respiratory Disease in 1998[12]. The control subjects were randomly selected from a pool of healthy volunteers who visited the general health check-up center at Kyungpook National University Hospital during the same period. The control subjects were frequency matched (1:1) to the cancer cases based on gender and age (± 5 years). All the cases and the controls were ethnic Koreans and they resided in Daegu City or the surrounding regions. A detailed questionnaire was completed for each patient and control by a trained interviewer. The questionnaire included information on the average number of cigarettes smoked daily and the number of years the subjects had been smoking. For smoking status, a person who had smoked at least once a day for > 1 year in his or her lifetime was regarded as a smoker. A former smoker was defined as one who had stopped smoking at least 1 year before diagnosis in the case of patients and 1 year before the date signed on an informed consent for blood sample collection in the case of controls. Cumulative cigarette dose (pack-years) was calculated using the following formula: pack-years = (packs per day) x (years smoked).

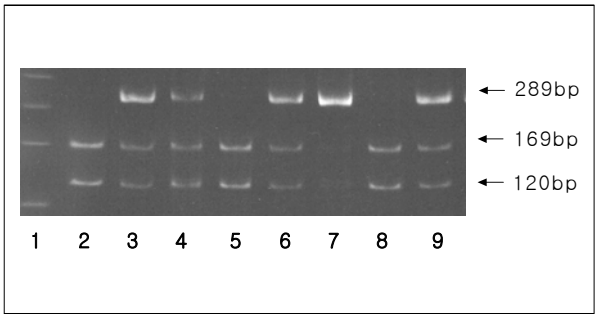

Figure 1. Representative figure of MPO -463G > A genotype
Lane 1: maker
Lane 2, 5, 8: GG genotype
Lane 3, 4, 6, 9: GA genotype
Lane 7: AA genotype

2. *MPO* genotyping

The *MPO* -463G>A genotype was determined using the PCR-RFLP method as was reported previously[13] (Figure 1). For the quality control, genotyping analysis was performed "blind" with respect to the case/control status. About 10% of the samples were then randomly genotyped again by a different author, and the results were 100% concordant. To confirm the genotyping results, selected PCR-amplified DNA samples (n = 2, respectively, for each genotype) were examined by DNA sequencing, and the results were also 100% concordant.

3. Statistical analysis

Cases and controls were compared using the Student's t-test for continuous variables and the x^2

test for categorical variables. Hardy-Weinberg equilibrium was tested with a goodness-of-fit x^2 test with one degree of freedom for the comparing the observed genotype frequencies with the expected genotype frequencies among the subjects. Unconditional logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), with adjustment for the possible confounders (gender as a nominal variable; age and pack-years of smoking, as continuous variables). The ORs were calculated as the combined AA + GA genotype relative to the GG genotype since the number of homozygous variant AA genotype was small among the cases and controls. Multiple logistic regression analyses were performed to analyze the association between the genotypes and risk of lung cancer after stratification into age (median age, ≤62 years/>62 years), gender, smoking status, cigarette consumption (median pack-years, ≤40 pack-years/>40 pack-years) and histological types of lung cancer. All analyses were performed using Statistical Analysis Software for Windows, version 8.12 (SAS institute, Gary, NC, USA).

Results

The demographics of the cases and controls enrolled in this study are shown in Table 1. There were no significant differences between the cases and controls in mean age or sex distribution, suggesting that the matching based on these two variables was adequate. Cases had a higher prevalence of current smokers than controls ($P < 0.001$), and the number of pack-years in smokers was significantly higher in cases than in controls (39.9 ± 17.9 versus 34.4 ± 17.6 pack-years; $P < 0.001$). These differences were controlled for later by multivariate analyses.

The distributions of *MPO* -463G>A genotypes among the cases and controls were in Hardy-Weinberg equilibrium (Table 2). The frequency of the -463A allele among the controls was 0.093 and similar to that previously reported in a Korean population[14]. The genotype distribution and the frequency of -463A allele among the cases were not significantly different from those among the controls. The risk estimate for lung cancer of the combined -463 AA + GA genotype was not significantly different from that of the -463GG genotype (adjusted OR = 1.03, 95% CI = 0.72-1.47). When the analyses were stratified by age, gender, smoking status, and pack-years of smoking, no significant association was found between the genotypes and risk of lung cancer. Moreover, the genotypes exhibited no apparent relationship with any of histological types of lung cancer.

Table 1. Characteristics of the study population

Variable	Cases (n = 432)	Controls (n = 432)
Age (years)	61.6 ± 9.0	34.4 ± 17.6
Gender		105 (24.3)
Male	352 (81.5)[a]	98 (22.7)
Female	80 (18.5)	229 (53.0)
Smoking status[b]		
Current	317 (73.4)	80 (18.5)
Former	39 (9.0)	352 (81.5)
Never	76 (17.6)	
Pack-years[c]	9.9 ± 17.9	60.9 ± 9.3

[a] Numbers in parenthesis, percentage.
[b] $P = 0.001$.
[c] In current and former smokers, $P < 0.001$.

Table 2. MPO -463G>A genotype frequencies, and adjusted ORs (95% CIs) for lung cancer by selected variables

	Cases			Controls			Adjusted OR (95% CI) for AA + GA vs GG[a]
	GG	GA	AA	GG	GA	AA	
All subjects	353 (81.7)	76 (17.6)	3 (0.7)	356 (82.4)	72 (16.7)	4 (0.9)	1.03 (0.72-1.47)[b]
Age (years)							
≤62	163 (81.5)	36 (18.0)	1 (0.5)	194 (82.2)	39 (16.5)	3 (1.3)	1.05 (0.64-1.73)[c]
>62	190 (81.9)	40 (17.2)	2 (0.9)	162 (82.7)	33 (16.8)	1 (0.5)	1.01 (0.61-1.68)[c]
Gender							
Male	285 (81.0)	65 (18.5)	2 (0.6)	285 (81.0)	64 (18.2)	3 (0.8)	0.99 (0.68-1.45)[d]
Female	68 (85.0)	11 (13.8)	1 (1.2)	71 (88.8)	8 (10.0)	1 (1.2)	1.22 (0.46-3.20)[d]
Smoking status							
Never	68 (89.5)	8 (10.5)	0 (0.0)	91 (86.7)	13 (12.4)	1 (0.9)	0.79 (0.31-2.00)[e]
Former	33 (84.6)	5 (12.8)	1 (2.6)	80 (81.6)	17 (17.4)	1 (1.0)	0.85 (0.30-2.37)[e]
Current	252 (79.5)	63 (19.9)	2 (0.6)	185 (80.8)	42 (18.3)	2 (0.9)	1.04 (0.68-1.61)[e]
Pack-yearsf							
≤40 pack-years	176 (78.9	45 (20.2)	2 (0.9)	190 (81.5)	41 (17.6)	2 (0.9)	1.17 (0.74-1.87)[e]
>40 pack-years	109 (82.0)	23 (17.3)	1 (0.7)	75 (79.8)	18 (19.1)	1 (1.1)	0.87 (0.44-1.70)[e]
Histological type							
Squamous	71 (81.4)	38 (18.1)	1 (0.5)	356 (82.4)	72 (16.7)	4 (0.9)	1.02 (0.65-1.58)[b]
Adeno	117 (83.0)	23 (16.3)	1 (0.7)	356 (82.4)	72 (16.7)	4 (0.9)	1.08 (0.64-1.81)[b]
Large	7 (87.5)	1 (12.5)	0 (0.0)	356 (82.4)	72 (16.7)	4 (0.9)	0.71 (0.09-5.91)[b]
Small	58 (79.5)	14 (19.2)	1 (1.3)	356 (82.4)	72 (16.7)	4 (0.9)	1.13 (0.60-2.11)[b]

[a] Dominant model for the variant allele.
[b] Adjusted for age, sex and pack-years of smoking.
[c] Adjusted for sex and pack-years of smoking.
[d] Adjusted for age and pack-years of smoking.
[e] Adjusted for age and sex.
[f] In current and former smokers.

Discussion

In present study, we found no significant association between the *MPO* -463G>A genotype and the risk of lung cancer. In addition, we observed no evidence of effect modification by age, gender, smoking history or tumor histology. These results suggest that this polymorphism does not affect the susceptibility to lung cancer in Koreans. Except for one study of a Chinese population[8], this is the only report to analyze a large number of lung cancer cases for *MPO* genotype and the risk of lung cancer in Asian populations.

The frequency of the -463A allele among the healthy controls in this study was significantly lower ($P < 0.05$) than those of Japanese (0.169; Ref. 7)), Chinese (0.155; Ref. 8), Caucasians (0.204-0.259; Refs. 4-7 and 13) and African-Americans (0.299; Ref. 13).

Several studies of Caucasian populations have indicated that the -463A allele was protective for lung cancer[4,6,13]. A meta-analysis[4] has showed that Caucasians carrying the -463A allele have approximately 20% reduced risk for lung cancer. However, several studies have found no association of *MPO* -463G>A genotype with lung cancer risk in Caucasians[5,15]. A recent French Caucasian study by Chevrier et al.[16], showed that the *MPO* haplotypes of 8 variants, including -463G>A polymorphism, did not have an effect on the risk of lung cancer. The previous studies of Caucasian populations are also not in agreement regarding the histology-specific effect of the *MPO* -463G>A polymorphism. Cascorbi et al.[17] have observed a significant protective effect of this polymorphism for adenocarcinoma and squamous cell carcinoma, while Schabath et al.[18] showed protective effect for adenocarcinoma and small cell carcinoma, but not for squamous cell carcinoma. Dally et al.[6] have recently reported that a significant ass-

ociation was observed only for small cell lung cancer.

Only a few studies have studied the association between the *MPO* -463G>A polymorphism and the risk of lung cancer in Asian populations, but their results were relatively consistent in suggesting an association. In a small Japanese study[7], the -463AA genotype was associated with a reduced risk of lung cancer as compared with the -463 GG genotype, although this difference was not statistically significant. A Chinese study has also shown that the -463A allele was associated with a reduced risk of squamous cell carcinoma[8]. However, in the current study, the *MPO* -463G>A genotype was not associated with the risk of lung cancer. Moreover, the genotypes exhibited no apparent relationship with any of the histological types of lung cancer.

Although it is hard to decipher the reasons for the observation that there is no apparent association of the *MPO* -463G>A polymorphism with lung cancer risk, this may be due to Koreans' specific genetic background and/or environmental factors. Lung cancer is known to be a polygenic disease, and the genetic susceptibility to lung cancer may result from several polymorphisms in the genes that are involved in various cellular processes such as carcinogen metabolism, DNA repair and apoptosis. Therefore, some of the polymorphisms, which exhibit stronger effects on the susceptibility to lung cancer in Koreans, can mask potential influence of the *MPO* polymorphism on lung cancer. Another reason may be due to low prevalence of the variant -463A allele in Koreans. The rarity of the variant -463A allele may possibly attribute to the lack of statistical power in our study. Alternatively, the results may be due to unmeasured contributing factors such as environmental ozone exposure[7,15].

A major strength of the current study is the inclusion criteria for the controls. The *MPO* -463G>A polymorphism has been previously associated with various inflammation related diseases, such as *Helicobacter pylori* infection[19]. Moreover, the *MPO* genotype frequencies may differ in nonmalignant lung diseases[6]. In the present study, we have included in the control group only healthy persons who were free of disease on a health check-up. Therefore, the effect of nonmalignant disease on the genotype distribution in our control group can be excluded. There are a number of possible limitations in this study. Since this study was a hospital-based case-control study, there might be some selection bias. Given that most lung cancer patients are treated at the University Hospital in Korea, the demographics and clinical characteristics of the cancer patients in the current study were compatible those of a nationwide lung cancer survey[12]. Furthermore, since all the lung cancer patients who diagnosed at the University Hospital were included in this study, it is reasonable to assume that the case group represents the lung cancer cases in our community. Another selection bias may be derived from the controls that did not participate in this study. However, because the age and gender distribution of non-participating controls were similar to those of the participating controls in the current study (age; 52.2 ± 11.4 versus 52.1 ± 11.3, respectively; P = 0.80; and % of male: 52.5% versus 52.1%, respectively; P = 0.80), a self-selection bias is unlikely. Moreover, the controls in the current study were extensively evaluated for other polymorphisms[9-11], and the genotype distribution for each polymorphism met the conditions for Hardy-Weinberg equilibrium, this further supports the randomness of our control selection.

For overall lung cancers, this study had 80% power (two-sided test of significance, $\alpha = 0.05$) to detect an OR < 0.58 (assuming a protective effect) or an OR > 1.59 (assuming a risk effect) for the carriers of -463 AA or GA genotype relative to the carriers of -463 GG genotype. Because of the low

prevalence of the -463 AA genotype, we combined this genotype with the heterozygous -463 GA genotype into one group. Therefore, we cannot rule out a small effect of the homozygous -463 AA genotype on lung cancer risk. For the analyses of the specific histological types of lung cancer, the numbers in the subgroups, especially the small cell lung cancer group, were relatively small. Therefore, it should be kept in mind that the analyses for specific histological types might have type II error.

Although there is variability in the results among the published studies, there is relatively consistent epidemiologic evidence of an association between the *MPO* -463G>A genotype and the risk of lung cancer. However, publishing null studies as well as positive studies is certainly necessary to elucidate the range of effects associated with this polymorphism and the risk of lung cancer. In the current study, we found no significant association between the *MPO* -463G>A genotype and the risk of lung cancer, regardless histological type, age, gender, or smoking behavior.

References

1. Mallet WG, Mosebrook DR, Trush MA. *Activation of (±)-trans-7, 8-dihydroxy-7, 8-dihydrobenzo[a]pyrene to diolepoxides by human polymorphonuclear leukocytes or myeloperoxidases. Carcinogenesis 1991;12: 521-4.*
2. Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF. *An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 1996;271:14412-20.*
3. van Schooten FJ, Boots AW, Knaapen AM, Godschalk RW, Maas LM, Borm PJ, et al. *Myeloperoxidase (MPO) -463G→A reduces MPO activity and DNA adduct levels in bronchoalveolar lavages of smokers. Cancer Epidemiol Biomarkers Prev 2004;13:828-33.*
4. Feyler A, Voho A, Bouchardy C, Kuokkanen K, Dayer P, Hiroven A, et al. *Myeloperoxidase -463 G → a polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1550-4.*
5. Xu LL, Liu G, Miller DP, Zhou W, Lynch TJ, Wain JC, et al. *The myeloperoxidase -463 G → a polymorphism does not decrease lung cancer susceptibility in Caucasians. Cancer Epidemiol Biomarkers Prev 2002; 11:1555-9.*
6. Dally H, Gassner K, Jager B, Schmezer P, Spiegelhalder B, Edler L, et al. *Myeloperoxidase (MPO) genotype and lung cancer histologic types: the MPO -463 a allele is associated with reduced risk for small cell lung cancer in smokers. Int J Cancer 2002; 102:530-5.*
7. le Marchand l, Seifried A, Lum A, Wilkens LR. *Association of the myeloperoxidase -463 G → a polymorphism with lung cancer risk. Cancer Epidemiol Biomarkers Prev 2000;9:181-4.*
8. Lu W, Xing D, Qi J, Tan W, Miao X, Lin D. *Genetic polymorphism in myeloperoxidase but not GSTM1 is associated with risk of lung squamous cell carcinoma in a Chinese population. Int J Cancer 2002;102: 275-9.*
9. Lee SJ, Jeon HS, Jang JS, Park SH, Lee GY, Lee BH, et al. *DNMT3B polymorphisms and risk of primary lung cancer. Carcinogenesis 2004;26:403-9.*
10. Lee GY, Jang JS, Lee SY, Jeon HS, Kim KM, Choi JE, et al. *XPC polymorphisms and lung cancer risk. Int J Cancer 2005;15:807-13.*
11. Lee SJ, Lee SY, Jeon HS, Park SH, Jang JS, Lee GY, et al. *Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev 2005;14:571-5.*
12. Lee CT, Kang KH, Koh Y, Chang J, Chung HS, Park SK, et al. *Characteristics of lung cancer in Korea, 1997. Lung Cancer 2000;30:15-22.*
13. London SJ, Lehman TA, Taylor JA. *Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res 1997;57:5001-3.*
14. Hong YC, Lee KH, Yi CH, Ha EH, Christiani DC. *Genetic susceptibility to term pregnant women to oxidative damage. Toxicol Lett 2002;129:255-62.*
15. Misra RR, Tangrea JA, Virtamo J, Ratnasinghe D, Andersen MR, Barrett M, et al. *Variation in the promoter region of the myeloperoxidase gene is not directly related to lung cancer risk among male smokers in Finland. Cancer Lett 2001;164:161-7.*
16. Chevrier I, Stucker I, Houllier AM, Cenee S, Beaune P, Laurent-Puig P, et al. *Myeloperoxidase: new polymorphisms and relation with lung cancer risk. Pharmacogenetics 2003;13:729-39.*
17. Cascorbi I, Henning S, Brockmoller J, Gephart J,

Meisel C, Miller JM, et al. *Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant -463A of the myeloperoxidase gene. Cancer Res 2000;60:644-9.*

18. Schabath MB, Spitz MR, Hong WK, Delclos GL, Reynolds WF, Gunn GB, et al. *A myeloperoxidase polymorphism associated with reduced risk of lung cancer. Lung Cancer 2002;37:35-40.*
19. Hamajima N, Matsuo K, Suzuki T, Nakamura T, Matsuura A, Tajima K, et al. *Low expression myeloperoxidase genotype negatively associated with Helicobacter pylori infection. Jpn J Cancer Res 2001;92: 488-93.*