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Difference in Lung Functions according to Genetic Polymorphism of Tobacco Substance Metabolizing Enzymes of Korean Smokers

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한국인 흡연자들의 담배 물질 대사 효소의 유전자 다형성에 따른 폐기능 차이

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Abstract This study aimed to determine whether there was a difference in lung functions of smokers according to the presence of carcinogenic genetic-metabolizing enzymes by comparing the results of lung functions and the presence of genetic metabolizing enzymes that metabolize tobacco substances. To achieve this, 31 smokers without no illness and no psychiatric history were selected (28 males and 3 females); they were aged 20 to 27 years and were physically and mentally healthy students attending K University. Their lung functions were measured, and gene polymorphisms of cytochrome P-450 1A1 (CYP1A1) related to metabolic activation of tobacco components and gene polymorphism of tumor protein 53 (TP53) related to lung cancer were analyzed. As a result, the mean values of lung function of TT and Arg / Arg without genetic mutations were the highest, and ANOVA analysis of CYP1A1 and lung functions showed that the P-value of FVC was 0.049, which was different between groups. In other words, there is no high mutation in Cytochrome P-450 1A1 (CYP1A1) gene, which is associated with the metabolic activation of tobacco components. In other words, In the absence of the mutant Cytochrome P-450 1A1 (CYP1A1) gene, which is associated with the metabolic activation of tobacco components, the value of FVC was high.

Key Words : CYP1A1, TP53, Genetic Polymorphism, FVC, FEV1

요 약 흡연자들의 흡연 물질 대사효소의 유전적 다형성에 따른 폐기능의 차이를 보기 위하여 질병력과 정신과적 병력이 없는 신체적·정신적으로 건강한 만 20~27세 이하의 흡연자 31명(남 29, 여 3)을 대상으로 연구를 진행하였다. 폐활량 측정기(Wright Respirometer, Ferraris Development and Engineering Co, Ltd, UK)를 이용하여, 노력성 폐활량(Forced vital capacity, FVC), 1초간 노력성 호기량(Forced expiratory volume at one second, FEV 1), 1초간 노력성 호기량의 노력성 폐활량에 대한 비(FEV1 % FVC)을 측정하였으며, 유전자 검사는 DNA로 PCR하여 CYP1A1과 TP53의 유전자 발현 검사를 하였다. 실험결과 유전자 돌연변이형이 없는 TT와 Arg/Arg의 폐기능 평균값이 가장 높았으며, CYP1A1와 lung functions의 ANOVA 분석에서 FVC의 P-값이 0.049로 그룹 간의 차이가 있는 것으로 나타났다. 즉 담배성분의 대사 활성화와 연관이 많은 Cytochrome P-450 1A1 (CYP1A1) 유전자의 돌연변이형이 없을때 FVC의 값이 높게 나타난 것이다.

주제어 : 흡연물질 대사효소, 발암물질 대사효소, 유전적 다형성, 노력성 폐활량, 1초간 노력성 호기량

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1. Introduction

Smoking is the largest risk factor for public health around the world; more than seven million people die every year due to smoking. Among them, six million die from direct smoking, and 890,000 die from second-hand smoking[1]. The World Health Organization (WHO) specifies that the most obvious and preventable cause of the main noninfectious diseases, such as cardiovascular disease, diabetes, cancer, and chronic respiratory disease, is smoking. The smoking population has steadily increased in South Korea up to 1990s since the Korea Tobacco and Ginseng Corporation was founded in 1945, and smoking damage is predicted to be the highest after 2010. Korean cancer prevention study (KCPS) data estimated that the number of deaths due to tobacco in 2003 was 46,207[2]. This number was re-calculated as of 2012 to 58,155 persons who died from smoking-related diseases[3]. Smoking increases the onset rates of many diseases, including cancer, cardiovascular disease, and chronic lung disease. The harmful substances in tobacco causes damage to many organs, and the most common smoke-related disease is lung cancer, followed by heart disease and chronic lung disease[4]. Tobacco contains thousands of chemicals such as carbon monoxide, tar, nicotine, nitrosamines, hydrogen cyanide, and benzopyrene, which are the direct and indirect main sources of chemical oxidants and free radicals[5]. The chemicals generated during tobacco burning have more than 4,000 mixed chemical components of particles and gaseous phase forms. Typically, they are hydrogen cyanide, sulphur dioxide, carbon monoxide, ammonia, and formaldehyde, which are harmful and toxic to the entire body and even contain carcinogens and mutants. In addition, chemicals such as nicotine, cadmium, and carbon monoxide may damage the reproduction process[2]. Smoking lowers lung function. Looking at the previous studies, it was found that the longer the smoking period, the more the non-smoker group, the lower the% FEV1 in the smoking group, and the smoking group with a smoking history of 30 years or more was significantly reduced than the non-smoking group.[6]. As such, smoking is one of the major causes for the increase in the incidence of specific diseases, and it is highly related to the occurrence of lung cancer in particular[7].

Nonetheless, lung cancer does not develop in all smokers, even if they smoke for a long period of time, and not all patients with lung cancer smoke. Apart from indoor air pollution, atmospheric pollution, occupational exposure to toxic substances, drinking, and dietary habits, and genetic factors are related to the development of lung cancer. For lung cancer, genetic effect, shared environmental effect, and nonshared random environmental effect accounted for 26%, 12%, and 62% of the total variation between patients of lung cancer, respectively[8]. During smoking, chemotaxis and viability of polymorphonuclear leukocytes are reduced[9]. In addition, the number of macrophages is reduced, and the reproductive integrity of T lymphocytes is decreased, thereby inhibiting the formation of immune globulin against pathogens[10]. McGuire et al[11] claimed that the changes in lymphoid hyperplasia was due to the inhibition of DNA synthesis. The absorption and distribution of tobacco components are generally dependent on nongenetic factors; however, some of the chemicals that exist in cigarette smoke play a role as substrates of various types of enzymes. Thus, the absorption and distribution of tobacco components are also affected by

already genetically determined cytoplasm and nuclear receptor variation; that is, the effect of cigarette smoking on the human body varies genetically. Although a number of studies have been conducted on the incidence rate of lung cancer due to tobacco smoking according to genetic difference, few studies have examined the differences in lung functions, which is a prior stage.

Accordingly, this study aimed to determine the difference in lung functions of smokers according to the presence of carcinogenic genetic metabolizing enzymes by comparing the results of lung functions and the presence of genetic metabolizing enzymes that metabolize tobacco substances. To this effect, gene polymorphism of cytochrome P-450 1A1 (CYP1A1), which is related to metabolic activation of tobacco components, and gene polymorphism of tumor protein 53 (TP53), which is related to lung cancer, were analyzed. The results revealed that people whose metabolic gene type is wild must reduce or stop smoking to reduce the incidence rate of smoking-related diseases and improve lung functions. In addition, it is expected that further studies on tobacco substance metabolizing enzymes will significantly contribute to the treatment of diseases due to smoking.

This study aimed to examine whether the consideration of not only age and height but also genetic polymorphism was useful when analyzing the examination results of lung functions and to determine the effect of genetic polymorphism of tobacco substance metabolizing enzymes on lung functions.

2. Subjects and method

2.1 Study subjects

To achieve this, 31 smokers without no illness and no psychiatric history were selected (28 males and 3 females); they were aged 20 to 27 years and were physically and mentally healthy students attending K University. Male subjects were selected from the range of age (20-25 years), height (170-180 cm), weight (70-80 kg), and smoking ability (5 years or less). Subjects of female subjects were selected within the range of age (20-25 years), height (150-160 cm), weight (40-50 kg), and smoking ability (less than 5 years). They were recruited and participated voluntarily in the experiment through social network services and survey questionnaires. A questionnaire was used to ensure that subjects had no medical history of lung-related diseases, liver disease, high blood pressure, stroke, heart disease, cancer, and tuberculosis. This study was conducted after obtaining the approval of the Institutional Bioethics Committee of Kyung woon University. In addition to the resear chpurpose, the subjects were in formed tha the confidentiality of their study participation would be ensured and that they had the right to stop and suspend participation in the study at anytime. They were also informed that the survey questionnaires and results would only be used for research purposes and that the data collected would be stored for 3 years after study completion and then deleted completely and permanently.

2.2 Study methodology

2.2.1 Lung function measurement: Vital capacity measurement

The maximum value out of at least three measurements on vital capacity (VC) was selected using the Wright Respirometer (Ferraris Development and Engineering Co., Ltd., UK), and the forced vital capacity (FVC), forced expiratory volume at 1 sec (FEV 1), and a ratio

of FEV 1 to FVC (FEV 1 % FVC) were measured.

• FVC: This refers to the amount of exhaled air that a patient can expel from the lungs after maximum inhalation[12].

• Forced expired volume in 1 sec (FEV 1): This refers to the volume of air that can forcibly be blown out in the first 1 sec after full inspiration. Even if two patients have the same FVC, their FEV 1 may differ because FEV 1 is an indicator of how fast patients can exhale for the first 1 sec. The airway of asthma patients is narrow so that their air blowing speed is very slow. Thus, their amount of air (expiratory volume) for 1 sec is significantly lower than that of normal persons, even if they forcibly exhale as much as possible.

• Ratio of FEV 1 % FVC: Normal persons can exhale 70% or larger of their forced vital capacity for the first 1 sec. That means FEV 1 % FVC 〉 0.7. On the other hand, if FEV 1, which is an exhaled amount of air for the first 1 sec is small compared to the FVC (FEV 1 % FVC 〈 0.7), it implies an impairment of exhalation. Thus, FEV 1 % FVC is a useful indicator that verifies whether the bronchus is blocked or not.

2.2.2 Gene tests

① DNA extraction process

DNA extraction was conducted using plasma after blood collected in the EDTA tube was centrifuged. Twenty ul of Proteinase K and 200 ul of blood were put into the EDTA tube followed by vortexing for 15 sec and then quick spinning at a rate of 8,000 rpm/10 sec. 200 ul of GC buffer was added followed by vortexing for 15 sec; then, the mixture was left for 10 min at 56 ℃. Later, rapid spinning at 8,000 rpm/10 sec was performed, which was followed by adding 200 ul of 100% ethanol, and vortexing for 15 sec and quick spinning at 8,000 rpm/10 sec. The mixed reactant was moved to the column, which was followed by centrifuging at a rate of 8,000 rpm/1 min. Then, the column was moved to the 2 ml tube again, which was followed by the addition of 500 ul of AW1 buffer and centrifuging at a rate of 8,000 rpm/1 min. After this, the column was moved to the 2 ml tube, which was followed by adding 500 ul of AW2 buffer and centrifuging at a rate of 12,000 rpm/2 min. The precipitated solution in the bottom was removed and centrifuged at a rate of 12,000 rpm/1 min, and the column was moved to a new EDTA tube. Then, 70 ul of EL buffer was added to the column that was moved to the new tube; then it was left for 5 min at room temperature and centrifuged at a rate of 8,000 rpm/2 min to extract the DNA of the 30 subjects. The polymerase chain reaction was conducted using the extracted DNA, thereby performing the gene expression testing of CYP1A1 and TP53.

② Polymerase Chain Reaction (PCR)

The entire reaction volume of the reaction mixtures of the PCR was 20 ul after mixing 1 ul of genomic DNA, 1 ul each of sense primer and antisense primer, Taq DNA polymerase 1 unit, 2 ul of 10x buffer, and 1 ul of dNTP. For the PCR machine, Gene-Amp PCR System (Perkin-Elmer) was used. For the PCR of CYP1A1, pre-denaturation was conducted at 94 ℃ for 5 min., and denaturation at 94℃ for 1 min, annealing at 54 ℃ for 1 min, 30 cycles of extension at 72 ℃ for 1 min, and post-extension at 72 ℃ for 5 min. For the PCR of TP53, pre-denaturation was conducted at 94 ℃ for 5 min, and denaturation at 94 ℃ for 1 min, annealing at 54 ℃ for 1 min, 30 cycles of extension at 72 ℃ for 1 min, and post-extension at 72 ℃ for 5 min.

• CYP1A1: This is a metabolizing enzyme that plays a role in in vivo detoxification of toxins

and generating intermediate toxic metabolites[13]. CYP1A1, which is a gene that is involved in generating enzymes that convert carcinogenic precursors such as benzopyrene to carcinogens, has been known to be related to lung cancer, as some genotypes have excessive enzyme activation, although the level of activation varies depending on ethnicity.

• TP53: TP53 gene, which has a very high frequency of variation in various types of cancer such as colorectal or breast cancer, has been known to be highly related to lung cancer as well[14]. Normally, TP53 gene induces cell apoptosis as it plays a role in cell cycle control, thereby inhibiting tumors; however, if it fails to play its normal role, it easily leads to tumor generation[15].

2.3 Analysis

SPSS Win 20.0 program was used to analyze the collected data. The analysis method is as follows. First, descriptive statistical analysis was conducted to see the mean and standard deviation of variables. Second, One-way Analysis of Variance (ANOVA) was conducted to test the mean difference between groups.

3. Results

3.1 Lung capacity results

The analysis results of difference in Table 1 lung functions showed that subjects were divided into 13 subjects in high group and 17 subjects in low group, 19 subjects in the high group and 11 subjects in low group, and 16 subjects in high group and 14 subjects in low group according to the results of FVC, FEV 1, and FEV 1 % FVC, respectively.

Table 1. Pulmonary function test

N	FVC(%)	FEV1(%)	FEV1% FVC
1	110	108	98
2	84	88	106
3	99	108	109
4	95	83	87
5	92	92	100
6	75	82	110
7	96	87	91
8	89	72	81
9	82	43	52
10	93	98	106
11	95	92	97
12	100	104	93
13	100	108	89
14	108	109	101
15	89	80	90
16	102	105	103
17	115	96	84
18	92	94	102
19	79	79	100
20	96	68	70
21	91	92	101
22	81	59	76
23	100	89	92
24	93	104	112
25	82	71	88
26	88	101	116
27	95	95	101
28	83	79	95
29	101	90	89
30	88	78	89
Average	94.2	88.2	94.2

FVC : forced vital capacity, FEV1 : forced expiratory volume in one second

3.2 Genetic results

The genetic test results revealed in Table 2 that TT type (normal/normal), TC type (normal/mutation), and CC type (mutation/mutation) CYP1A1 (Cytochrome P450, family1, subfamily A, polypeptide1) were detected in 11, 15, and four subjects, respectively. In addition, Arg/Arg type (normal/normal) and Arg/Pro type (normal/mutation) TP53 (Tumor protein p53) were detected in 19 and 11 subjects, respectively (Table 2).

Table 2. Genetic diagnosis results

	CYP1A1	TP53		CYP1A1	TP53
1	TT	Arg/Arg	16	TT	Arg/Pro
2	TT	Arg/Arg	17	TT	Arg/Arg
3	TT	Arg/Pro	18	TC	Arg/Arg
4	TC	Arg/Pro	19	TC	Arg/Pro
5	TC	Arg/Arg	20	TC	Arg/Arg
6	TC	Arg/Arg	21	CC	Arg/Pro
7	CC	Arg/Pro	22	TC	Arg/Pro
8	TC	Arg/Pro	23	TT	Arg/Arg
9	TC	Arg/Arg	24	TC	Arg/Arg
10	TC	Arg/Pro	25	TT	Arg/Arg
11	TT	Arg/Arg	26	TC	Arg/Arg
12	TC	Arg/Pro	27	TT	Arg/Arg
13	TC	Arg/Arg	28	TC	Arg/Pro
14	TT	Arg/Arg	29	CC	Arg/Pro
15	TT	Arg/Pro	30	CC	Arg/Pro

CYP1A1 : Cytochrome P450, family1, subfamily A, polypeptide1
TP53 : Tumor protein p53

3.3 Descriptive Statistics by Group

3.3.1 Descriptive statistics of CYP1A1

The TT genotypes were FVC 98.09, FEV 94.64, FEV 1% FVC 97.18. TC genotype has the largest standard deviation of 18.48 in FEV (Table 3).

3.3.2 Descriptive statistics of TP53

The genotypes of Arg / Arg were FVC 94.19, FEV 90.00, FEV 1% FVC 94.88, which showed the highest mean of lung function and the largest standard deviation in Table 3.

3.4 Comparison of lung functions and gene expression degree

3.4.1 ANOVA analysis of lung functions with CYP1A1

The F-statistic value of the FVC is 3.37 and the P-value is 0.049, which is less than the significance level of 0.05. In FEV1 and FEV 1% FVC, each P-value shows 0.258 and 0.672, and there is no difference between groups in Table 4.

3.4.2 ANOVA analysis of lung functions with TP53

FVC has an F-statistic value of 0.46 and a P-value of 0.505, no difference. Even in FEV1 and FEV 1% FVC, each P-value is 0.575 and 0.793, indicating no difference between groups in Table 4.

Table 4. ANOVA analysis of lung functions with CYP1A1 & TP53

		F-value	p-value
CYP1A1	FVC	3.37	0.049
	FEV1	1.43	0.258
	FEV 1% FVC	0.40	0.672
TP53	FVC	0.46	0.505
	FEV1	0.32	0.575
	FEV 1% FVC	0.07	0.793

*p<.05

Table 3. Descriptive Statistics by Group

	CYP1A1	Mean	SD		TP53	Mean	SD
FVC	CC	94.00	5.72	FVC	Arg/Arg	94.19	10.85
	TC	89.20	7.66		Arg/Pro	91.86	7.47
	TT	98.09	10.51				
FEV1	CC	86.75	6.18	FEV1	Arg/Arg	90.00	17.39
	TC	84.40	18.48		Arg/Pro	86.71	13.84
	TT	94.64	12.36				
FEV 1 % FVC	CC	92.50	5.74	FEV 1 % FVC	Arg/Arg	94.88	16.17
	TC	92.60	17.37		Arg/Pro	93.57	9.41
	TT	97.18	7.86				

Table 5. Posthoc all-pairs multiple comparisons result

	Mean difference	SD difference	95% CI	T-value	Modified F-value
TC - CC	-4.80	4.87	(-16.90, 7.30)	-0.98	0.593
TT - CC	4.09	5.06	(-8.46, 16.64)	0.81	0.701
TT - TC	8.89	3.44	(0.36, 17.42)	2.59	0.040

Table 6. Grouping information using 95% confidence intervals

CYP1A1	N	Mean	Grouping	
TT	11	98.09	A	
CC	4	94.00	A	B
TC	15	89.20		B

3.4.3 Posthoc all-pairs multiple comparisons result

Since the F-value is 3.37 and the P-value is 0.049, which is less than the significance level of 0.05, it is considered that there is a difference between groups (Table 4). When there was a difference between the groups, a post-test was performed to confirm which group had a difference in means. As a result of the post-test, the P values of TT and TC are 0.040, which is smaller than the significance level of 0.05, so it is judged that there is a difference in the mean. Since the P values of TC and CC, and TT and CC are 0.593 and 0.701, respectively, they are greater than the significance level of 0.05, so it is judged that there is no average difference between groups in Table 5.

3.4.4 Grouping information using 95% confidence intervals

FVC is considered to have an average difference between groups. It can be seen that the average of TT's lung capacity was the largest in Table 6.

4. Discussion and Conclusion

The mean values of pulmonary function of TT and Arg / Arg genotype without genetic mutation were the highest. ANOVA analysis of CYP1A1 and lung functions showed that the P-value of FVC was 0.049, which was different between groups. In other words, when there is no mutation of the Cytochrome P-450 1A1 (CYP1A1) gene, which is associated with the metabolic activation of tobacco components, the value of FVC is high.

The present study results demonstrated that lung functions were relatively high in persons lacking cancer-related gene mutation types for smokers. In the same context, lung functions were relatively low in the presence of gene mutation types. That is, lung functions may differ according to whether or not cancer-related gene mutations caused by tobacco substances are present.

Smoking accounts for 30% of all cancer -related deaths, and approximately 87% of lung cancer cases has been caused by smoking[16]. Most studies on smoking-related lung cancer occurrence have been concentrated on lung cancer incidence rate due to smoking[7], and difference in lung functions according to smoking frequency[17]. For gene-related studies, there was a study that analyzed gene mutation in patients with lung cancer[15]. however, no studies have examined differences in lung functions according to the degree of cancer-related gene expression of smokers. Thus, this study aimed to determine the effect of the degree of cancer-related gene expression on lung functions. Cytochrome P-450 1A1 (CYP1A1) is located on chromosome 15. It promotes metabolism of aromatic hydrocarbons such as benzopyrene, 6-nitrochrysene, and dimethylbenzanthracene (DMBA). Many CYP1A1 substrates are carcinogens or are metabolized into carcinogens by the action of CYP1A1[18]. Tumor protein 53 (TP53) gene is highly related to lung cancer incidence, and much attention has been paid to TP53 because it has the highest frequency of mutation in cancer or

tumor suppressor genes discovered in human tumors [19]. Normally, the TP53 gene is involved with cell cycle control and inhibits cell proliferation of damaged DNA or induces apoptosis in irrecoverably damaged cells. If the TP53 gene fails to play its role due to any external or internal factors, including tumor virus, the risk of tumorigenesis is significantly increased [20]. A study by Jung et al. reported a high frequency of TP53 mutation in squamous cell carcinoma compared to other carcinomas, and the most frequent change in TP53 gene mutation was found in codon 179 in relation to lung cancer incidences in Korean patients.

In this gene expression study, we predicted that pulmonary function would be highest in TT and Arg / Arg without genetic mutations. In fact, TT without the CYP1A1 gene mutation showed the highest value of FVC. However, in the TP53 gene, the value of pulmonary function for mutations was not significant. And in the results, the level of lung capacity of the subjects was generally above or above the normal range, which is thought to be due to the experiment of healthy and young subjects. Nonetheless, this result cannot be generalized because this study only included a small number of subjects and did not consider smoking frequency, gender, physical condition, and exercise frequency. The experiment results revealed that lung functions were related to metabolizing enzymes to some extent. Thus, if this study in the future includes more study subjects, it would significantly contribute to improvements in medical services and drug development. Diseases are caused by a combination of gene and environmental factors, which play a role in determining the sensitivity to diseases of each individual [21]. The disease susceptibility of individuals is closely related to metabolizing enzymes of disease-causing substances, which are involved with activation or detoxification of disease-causing substances, and the activation and expression degree of these metabolizing enzymes differ individually and has been verified through numerous experiments [22]. This study aimed to determine the effect of the degree of cancer-related gene expression on lung functions. The results demonstrated that there were differences in lung functions according to cancer incidence-related gene mutations that reacted with tobacco substances. Thus, people with wild metabolic gene type must reduce or stop smoking to prevent diseases related to lung functions due to smoking. This study verified that the consideration of age and height as well as gene polymorphism was useful in analyzing lung functions.

The study results will significantly contribute to the development of medicines that improve lung functions and can count as data that supports the development and research of cancer drugs that utilize the genetic differences of metabolizing enzymes.

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