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Gene polymorphism for microsomal epoxide hydrolase and susceptibility to emphysema in a Japanese population

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Gene polymorphism for microsomal epoxide hydrolase and susceptibility to emphysema in a Japanese population. K. Takeyabu, E. Yamaguchi, I. Suzuki, M. Nishimura, N. Hizawa, Y. Kawakami. ©ERS Journals Ltd 2000.

ABSTRACT: Recently, it was reported that gene polymorphism for microsomal epoxide hydrolase (mEPHX), an enzyme involved in the first-pass metabolism of epoxide intermediates, was associated with susceptibility to emphysema.

This association was examined in a Japanese population, performing polymerase chain reaction (PCR)-based direct sequencing and restriction fragment length polymorphism assays for variant forms of mEPHX. The subjects consisted of 79 smokers with moderate to severe emphysema diagnosed by lung computed tomography scans, 58 smokers without emphysema, with a comparable smoking history, and 114 consecutive subjects who undertook annual health checkups. The allele frequency of exon 3 Tyr^{113} to His^{113}, which was reported to confer slow mEPHX activity, was substantially higher in the population control group compared with that of the Caucasian control subjects in a previous study.

However, neither the genotype distribution of exon 3, nor that of exon 4 His^{139} to Arg^{139}, was significantly different between the two groups of smokers. These data indicate that the gene polymorphism for mEPHX is not associated with susceptibility to emphysema in the Japanese population.

The discrepancy between the two studies may be explained either by racial difference or by the selection bias of subjects in the previous study, which examined those who had only mild to moderate emphysema with lung cancer or those who were clinically diagnosed as having chronic obstructive pulmonary disease.

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Although cigarette smoking is a major exogenetic cause of pulmonary emphysema, it is still an open question why only a minority of smokers develop clinically apparent emphysema with a normal level of circulating α_1-antitrypsin. Intensive research has been going on to explore the association of some gene polymorphisms with susceptibility to lung diseases such as bronchial asthma. However, it remains to be elucidated what genetic factors are associated with the individual susceptibility to pulmonary emphysema without α_1-antitrypsin deficiency, since α_1-antitrypsin deficiency is a very minor cause of emphysema and not a susceptibility gene for most subjects with pulmonary emphysema.

Microsomal epoxide hydrolase (mEPHX) is an enzyme involved in the first-pass metabolism of smoking-induced highly reactive epoxide intermediates. Since these xenobiotics directly inhibit antiproteases and increase protease secretion from neutrophils, the association between the activity of mEPHX and the susceptibility to pulmonary emphysema has received attention. HASSETT *et al.* [1] reported that the variable activity of this enzyme was associated with exon-3 and exon-4 polymorphism in the mEPHX gene in a transiently transfected cell line. HASSETT *et al.* [1] demonstrated that an exon-3 T to C mutation (Tyr^{113} to His^{113}) in this gene decreased the activity by ≥50% (slow allele), while an exon-4 A to G mutation (His^{139} to Arg^{139}) increased the activity by ≥25% (fast allele). Subsequently, SMITH and HARRISON [2] reported that the gene polymorphism for mEPHX was associated with the susceptibility to pulmonary emphysema. However, the subjects in their study were clinically diagnosed as having chronic obstructive pulmonary disease (COPD) or had only mild to moderate emphysema with lung cancer. Accordingly, they might have included those who did not necessarily have severe emphysema, but instead had chronic bronchitis or small airway disease.

In this study, the association of the gene polymorphism for mEPHX and the susceptibility to smoking-induced emphysema in a Japanese population was examined. For this purpose, subjects were chosen who had been confirmed to have moderate to severe pulmonary emphysema, not only clinically, but also, by high-resolution computed tomography (HRCT) and examined smokers who had a similar smoking history but did not have pulmonary emphysema. The absence of pulmonary emphysema in these subjects was judged either by HRCT or by conventional chest radiographs. Consecutive community-based subjects were also included as a general population group who visited a health care centre for annual health checkups.

Methods

Study population

The subjects in this study consisted of the two groups of smokers; one with pulmonary emphysema (n=79, 72 males

and seven females) and the other without emphysema (n=58, 55 males and three females). They were recruited from Hokkaido University Hospital, Japan and several community hospitals affiliated with the University Hospital. They were diagnosed as having pulmonary emphysema if they had a smoking history, demonstrated nonreversible airflow obstruction with forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) of <70%, and showed significant degrees of low attenuation areas by HRCT. Smoking subjects who visited the hospitals because of hypertension, diabetes mellitus, and others, but, did not have any evidence of emphysema by pulmonary function tests and conventional radiographs were then chosen. Of 58 subjects, 28 were further confirmed not to have emphysema by HRCT.

There was no significant difference in pack-years of smoking between smokers with emphysema and those without emphysema (54±3 *versus* 48±3 SEM pack-years). Pulmonary function tests revealed that FEV1/FVC and diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) were significantly lower in the smokers with emphysema than in those without emphysema (50±2 *versus* 75±2% SEM; 64±4 *versus* 94±8 SEM % predicted, respectively, $p< 0.05$ for both). There were no subjects who had α_1-antitrypsin deficiency. The study was approved by the Ethics Committee of Hokkaido University School of Medicine, Japan.

Additionally, blood samples from 114 consecutive subjects (58 males and 56 females, age: 46±13 SD yrs) who visited a health care centre, Sapporo Social Insurance Central Hospital, Japan, for annual health checkups were obtained as a general population control group. All of the data were used for later analysis irrespective of the results of health checkups.

High-resolution computed tomography

The HRCT examination was performed with a Yokokawa CT 9000 unit (Yokokawa Co., Tokyo, Japan), which was set at a window of 1,000 Hounsfield units (HU) and a level of -700 HU. Serial horizontal slices with a width of 2 mm were obtained at 10 mm intervals from the apex to below the diaphragm. The subject was defined as having emphysema if both a pulmonary physician and a radiologist independently agreed that low attenuation areas were present in >25% of all of the lung fields.

Direct sequencing

Deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes. Fifty nanograms of genomic DNA was amplified by polymerase chain reaction (PCR) with a thermal cycler (GeneAmp PCR system 9600; Perkin Elmer Applied Biosystems, Norwalk, CT, USA) in 20 μL of reaction mixture containing 1.5 mM $MgCl_2$, 100 ng of primers, 200 μM deoxynucleotide triphosphate (dNTP), and 1 unit of AmpliTaq Gold (Roche Molecular Systems Inc., Branchburg, NJ, USA). The PCR conditions consisted of an initial single cycle of 95°C, 10 min and 38 cycles of 56°C for 20 s, 72°C for 20 s, and 94°C for 30 s.

Before direct sequencing to determine the polymorphism in exon-3, PCR restriction-fragment length polymorphism was performed according to the method by SMITH and HARRISON [2]. In this method, a base in the downstream primer was changed artificially near the mutation site so as to produce an *EcoR* V restriction enzyme site (GATATC) in the wild type. However, it was found that the results by this method were not consistent with those by the direct sequencing and did not conform to the Hardy-Weinberg equilibrium in the control population (data not shown). Therefore direct sequencing was performed by using newly adopted primer pair EPO1 (5′-GATCGATAAGTTCCGTTTCACC, starting at base pair (bp) 321 in mEPHX complimentary ((c)DNA) and EPO2 (5′-TCATGACATACATCCCTCTCTG, starting at bp 544). To detect the mutation for exon 3, the PCR-products were sequenced by the dye terminator method using a sequence kit (BigDye™ Terminator Cycle Sequencing Ready Reaction; Perkin Elmer Applied Biosystems, Foster City, CA, USA) and an automated capillary-type sequencer (ABI PRISM 310; Perkin Elmer Applied Biosystems, Norwalk, CT, USA).

Polymerase chain reaction restriction fragment length polymorphism

PCR was performed for the determination of polymorphisms in exon-4 as described above by using a primer pair EPO-3 (5′-ACATCCACTTCATCCACGT, starting at bp 494) and EPO4 (5′-ATGCCTCTGAGAAGCCAT, bp 685). The products were then digested with 15 U of Rsa-I (Toyobo Co., Osaka, Japan) for 2 h and visualized by electrophoresis on 4% Nusieve agarose (FMC Bioproducts, Rockland, ME, USA).

Statistical analysis

Associations between disease groups and specific genotypes and phenotypes were analysed for significance by the two-tailed Chi-squared test. The logistic regression model was used to calculate odds ratios (ORs) adjusted for age, sex, and pack-years of smoking between smokers with and without emphysema (Prophet 5.0; BBN Systems and Technologies, Cambridge, MA, USA). In each analysis, differences with a p-value of <0.05 were accepted as significant.

Results

There was no significant difference either in the mutant allele frequency or the OR for carriage of the mutant allele between the smokers with and without emphysema for either exon 3 or exon 4 (table 1). A general population group showed a similar distribution in the mutant allele frequency. Compared with the previous report by SMITH and HARRISON [2], the mutant allele frequency of exon 3 Tyr^{113} to His^{113} was substantially higher in the general population in the present study compared with that of the control subjects in the study by SMITH and HARRISON [2] (0.52 *versus* 0.31), whereas the mutant allele frequency of exon 4 His^{139} to Arg^{139} was similar in the two studies (0.19 *versus* 0.15).

Table 1. – Distribution of microsomal epoxide hydrolase genotypes in control and disease groups

	Number of individuals				
	Homozygous wild	Heterozygotes	Homozygous mutant	Mutant allele frequency	Odds ratio for carriage of mutant allele
Exon 3 polymorphism (slow)					
Control subjects n=114	30 (26)	51 (45)	33 (29)	0.52	
Smokers without emphysema n=58	13 (22)	26 (45)	19 (33)	0.56	1.0
Smokers with emphysema n=79	20 (25)	34 (43)	25 (32)	0.53	0.92 (0.40–2.16)
Exon 4 polymorphism (fast)					
Control subjects n=114	76 (67)	32 (28)	6 (5)	0.19	
Smokers without emphysema n=58	39 (67)	17 (29)	2 (4)	0.18	1.0
Smokers with emphysema n=79	53 (67)	22 (28)	4 (5)	0.19	1.17 (0.54–2.53)

Data are presented as absolute numbers with either percentage or 95% confidence interval in parenthesis.

On the basis of the report by SMITH and HARRISON [2], the current subjects were classified into four groups of the putative mEPHX phenotypes; normal, fast, slow and very slow (normal: no mutation in the gene, or heterozygotes for both exon-3 and exon-4 mutations; fast: at least one fast mutation (exon-4) and no exon-3 mutations; slow: one slow (exon-3) allele; very slow: two slow alleles). There was no significant difference in these putative mEPHX phenotypes either between the two groups of smokers or between all three groups (table 2).

Comparison of the observed distributions of mEPHX genotypes and those predicted by allele frequencies of exon 3 or 4 polymorphisms (His^{139} to Arg^{139}) by the Chi-squared test showed that all the study groups were in Hardy-Weinberg equilibrium, indicating that they were sufficiently random and representative.

Discussion

In this study, it was found that there was no significant difference in the genotype distribution and allele frequency of exon 3 or exon 4 polymorphism in mEPHX gene among the two groups of smokers with and without emphysema who have similar smoking history, and a general population group. These results indicate that the gene polymorphism for mEPHX does not confer susceptibility to smoking-induced pulmonary emphysema in a Japanese population.

SMITH and HARRISON [2] first reported that the gene polymorphism for mEPHX was associated with susceptibility to pulmonary emphysema. In the study by SMITH and HARRISON [2] it was found that the allele frequency of exon 3 Tyr^{113} to His^{113} was significantly higher in subjects who had pulmonary emphysema than in control subjects, while there was no significant difference in the genotype distribution and allele frequency of exon 4 polymorphism (His^{139} to Arg^{139}). The discrepancy between this study and the present study may be explained by the different backgrounds of the subjects. First of all, racial difference should be considered. There have been a number of examples showing that the allele frequencies of polymorphisms are quite different among races so that the association of such gene polymorphisms and a given disease varies across races as well [4–6]. Indeed, in the present study it was found that the allele frequency of exon 3 Tyr^{113} to His^{113} was substantially higher in a Japanese population compared with the control subjects in a previous study [2]. Another possible explanation for the discrepancy between the two studies is the difference in subject selection. In the present study, the authors have confirmed the presence of emphysema by HRCT for all of the subjects who had moderate to severe airway obstruction. The control subjects were further selected to have a comparable smoking history but no evidence of pulmonary emphysema. Although the authors did not confirm the absence of emphysema by HRCT for all of the subjects in this group, thus leading to the possibility that a small number of subjects with subclinical emphysema might be included, there was no significant difference in the allele frequency between the HRCT-confirmed subjects and those who were diagnosed only by pulmonary function tests and conventional radiographs (data not shown). On the other hand, one group of subjects in the study by SMITH and HARRISON [2] had only mild to moderate emphysema, because they had lung cancer that was surgically resectable. The diagnosis was made using surgically-resected specimens. Surprisingly, as many as 94 of 144 consecutive subjects with lung cancer were diagnosed as having emphysema. Another group of subjects in the study were clinically diagnosed as having COPD. Accordingly, SMITH and HARRISON [2] might have included those who did not necessarily have severe emphysema but instead had chronic bronchitis or small airway disease as causes for irreversible airflow obstruction. The current authors suspect that what SMITH and HARRISON [2] found in their study might be the association of the gene polymorphism for mEPHX with COPD rather than emphysema itself.

Finally, two recent reports by PIRMOHAMED and coworkers [6, 7] should be considered. The study did not find

Table 2. – Distribution of putative microsomal epoxide hydrolase phenotypes

	Number of individuals			
	Normal	Fast	Slow	Very slow
Control subjects n=114	24 (21)	17 (15)	40 (35)	33 (29)
Smokers without emphysema n=58	17 (30)	5 (9)	17 (29)	19 (33)
Smokers with emphysema n=79	20 (25)	10 (13)	23 (29)	26 (33)

Data are presented as absolute numbers with percentages in parenthesis.

any significant association of the mEPHX activity and exon-3 polymorphism in a genotyped human liver bank. The reports by PIRMOHAMED and coworkers [6, 7] did not agree with the report by HASSETT *et al.* [1] who demonstrated such an association in a transiently transfected cell line. There is also another report by OMIECINSKI *et al.* [8] that demonstrated a poor correlation between the mEPHX activity in the lung and that of lymphocytes. If mEPHX activity is in one way or another involved in the pathogenesis of pulmonary emphysema and interindividual differences in its activities are responsible for variable susceptibility to emphysema, the gene polymorphism for mEPHX should reflect its enzymatic activity in the lung. Thus, a compelling biological hypothesis may be lacking for the association between mEPHX gene polymorphism and the development of emphysema.

In conclusion, the gene polymorphism for microsomal epoxide hydrolase was not associated with the susceptibility to smoking-induced pulmonary emphysema in a Japanese population. The authors believe that further studies are needed to confirm the association of this gene polymorphism with susceptibility to pulmonary emphysema or chronic obstructive pulmonary disease.

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