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GSTM1, *GSTT1*, and *GSTP1* Gene Polymorphisms Modify the Effect of Smoking on Serum Immunoglobulin E Level

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Abstract

Immunoglobulin E (IgE) plays an important role in the development of allergic disorders including asthma. Cigarette smoking was reported to elevate serum IgE level and air pollutants such as NO_2 have been reported to modulate the immune system including inflammation. Moreover, genetic polymorphisms of glutathione S-transferases (GSTs) were reported to affect inflammatory diseases including asthma. Therefore, in the present study we tried to investigate whether tobacco smoke or NO_2 exposure increases the level of IgE and the GST gene polymorphisms are associated with change of IgE level due to tobacco smoke or NO_2 exposure. We measured urinary cotinine, personal NO_2 exposure, and serum IgE levels in 300 healthy university students without allergic disorders. Allelic loss of the *GSTM1* and *GSTT1* and the GSTP1 (Ile105Val) polymorphism were determined by PCR and RFLP. Total serum IgE levels were significantly different according to urinary cotinine levels (P=0.046), while NO_2 passive dosimeter level and genetic polymorphisms of three GSTs were not associated with total IgE level. Moreover, subjects with cotinine 500 μg/g creatinine or more showed the highest level of total IgE when they had null type of *GSTM1*, null type of *GSTT1*, or variant type of *GSTP1* (P<0.05). When we considered IgE level according to urinary cotinine levels in strata with the combinations of *GSTM1*, *GSTT1*, and *GSTP1* genetic polymorphisms, the subjects with *GSTM1* null, *GSTT1* null, and *GSTP1* variant types showed the largest difference between IgE levels of subpopulations according to cotinine levels (P=0.030). However, there was no significant difference between IgE levels of subpopulations according to NO_2 passive dosimeter levels in any group with combinations of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms. This result suggests that smoking increases allergic response measured as IgE level and combinations of the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms modify the effect of smoking on serum IgE level.

Keywords: IgE, Smoking, NO_2, *GSTM1*, *GSTT1*, *GSTP1*, polymorphism

Immunoglobulin E (IgE) has important functions in the development of allergic disorders including asthma. High serum IgE level has been reported to be correlated with the clinical expression of allergy and asthma[1]. Epidemiologic studies have shown that higher IgE level is associated with bronchial hyperresponsiveness (BHR), a major component of the asthma phenotype. Even in children with no history or symptoms of atopy or asthma, BHR is strongly associated with elevated serum IgE level[2]. Therefore, increase of IgE is regarded as an important underlying condition for the development of allergic disorders.

A variety of environmental factors have been reported to increase IgE level[3-5]. Among them, cigarette smoking was reported to elevate serum IgE level[6,7], although inconsistent results have been reported on relationship between serum IgE level and passive smoking[8,9]. Nitrogen dioxide (NO_2) is an environmental air pollutant. Air pollutants such as NO_2 have been reported to modulate the immune system including inflammation[10,11]. Therefore, it is believed that smoking or NO_2 increases IgE level which could be associated with increased risk of allergic disorders.

The glutathione S-transferases (GSTs), M1, T1, and P1, are detoxification enzymes, which have a pivotal role in catalyzing the conjugation of glutathione

(GSH) to electrophilic substrates[12,13]. Because of this function of GSTs, they may protect against immune dysfunction including inflammation and oxidative stress through detoxification of various toxic substances[14,15]. Several studies have demonstrated that the GSTs affect the inflammatory diseases including asthma[16,17]. In addition, GST polymorphisms have been reported to affect the risk of tobacco-related cancers including lung, bladder, and head and neck[18,19].

The human *GSTM1* and *GSTT1* are polymorphic and their deletions are responsible for the existence of null types lacking the enzyme function[20]. Furthermore, a polymorphic site at codon 105 (A to G substitution replacing isoleucine with valine) of *GSTP1* is known to change the kinetic property of the enzyme in binding with a few of electrophilic substrates including PAH diolepoxides[21]. For this reason, *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms may contribute to susceptibility to allergic disorders including asthma.

We hypothesized that the genetic polymorphisms of *GSTM1*, *GSTT1* and *GSTP1* may modify allergic response induced by smoking and NO_2. Therefore, in the present study we tried to investigate whether tobacco smoke or NO_2 exposure increases the level of IgE and the GST gene polymorphisms are associated with allergic response due to tobacco smoke or NO_2 exposure.

The characteristics of the study participants were described in Table 1. Total serum IgE levels were significantly different according to urinary cotinine levels (P=0.046), while age, sex, BMI, and NO_2 passive dosimeter levels were not associated with the levels of total IgE. The mean of IgE levels for subjects with cotinine 500 μg/g creatinine or more was 133.7 IU/mL, whereas that for subjects with cotinine 500 μg/g creatinine or less was 94.2 IU/mL.

The frequencies for *GSTM1*, *GSTT1*, and *GSTP1* wild genotypes were 46.7%, 45.3% and 62.0%, respectively (Table 2). The homozygous and heterozygous allelic variants of *GSTP1* were combined in the analysis because of small number of homozygous variant genotype. All three genetic polymorphisms were not associated with total IgE level significantly (P>0.05).

Figure 1 shows the relationships between urinary cotinine and total IgE levels according to the genetic polymorphisms of *GSTM1*, *GSTT1*, and *GSTP1* after controlling for age, sex and BMI. The subjects with cotinine 500 μg/g creatinine or more showed the highest level of total IgE when they had null type of *GSTM1*, null type of *GSTT1*, or variant type of *GSTP1* (P<0.05). However, total serum IgE level did not show difference between NO_2 passive dosimeter levels according to *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms.

When we considered IgE level according to urinary cotinine levels with the combinations of *GSTM1*, *GSTT1*, and *GSTP1* genetic polymorphisms, the subjects with *GSTM1* null, *GSTT1* null, and *GSTP1* variant types showed the largest difference between IgE levels of subpopulations according to cotinine levels (P=0.03) (Table 3). However, there was no significant difference between IgE levels of subpopulations according to NO_2 passive dosimeter levels in any group with combinations of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms (Table 4).

IgE protein has been known to play an important role in allergic disorders including asthma, and tobacco smoke and NO_2 have been known to modulate immune system including IgE[1,10,11]. Therefore, allergic response caused by smoking or NO_2 may be

Table 1. Total serum IgE levels according to subject characteristics and smoking and NO_2 exposure.

Variables	N (%)	Serum IgE level (IU/mL)	
		GM (GSD)	*P*-value*
Age (years)			
<25	172 (57.3)	110.2 (4.1)	
≥25	128 (42.7)	99.3 (4.3)	0.532
Sex			
Male	279 (93.0)	108.6 (4.2)	
Female	21 (7.0)	71.6 (3.7)	0.197
Body mass index (kg/m^2)			
<25	128 (42.7)	98.6 (4.5)	
≥25	172 (57.3)	110.9 (3.9)	0.481
Urinary cotinine (μg/g creatinine)			
<500	203 (67.7)	94.2 (3.9)	
≥500	97 (32.3)	133.7 (4.5)	0.046
NO_2 exposure level (ppb)			
<33.1	151 (50.3)	101.9 (4.2)	
≥33.1	149 (49.7)	109.1 (4.1)	0.669

*P-value from t-test

Table 2. Serum IgE levels according to *GSTM1*, *GSTT1*, and *GSTP1* genotype.

Genotypes	N (%)	Serum IgE level (IU/mL)	
		GM (GSD)	*P*-value*
GSTM1			
Wild	140 (46.7)	95.5 (4.2)	
Null	160 (53.3)	114.8 (4.2)	0.248
GSTT1			
Wild	136 (45.3)	117.5 (3.8)	
Null	164 (54.7)	95.5 (4.4)	0.208
GSTP1			
I/I	186 (62.0)	95.5 (3.9)	
I/V, V/V	114 (38.0)	123.0 (4.5)	0.143

*P-value from t-test

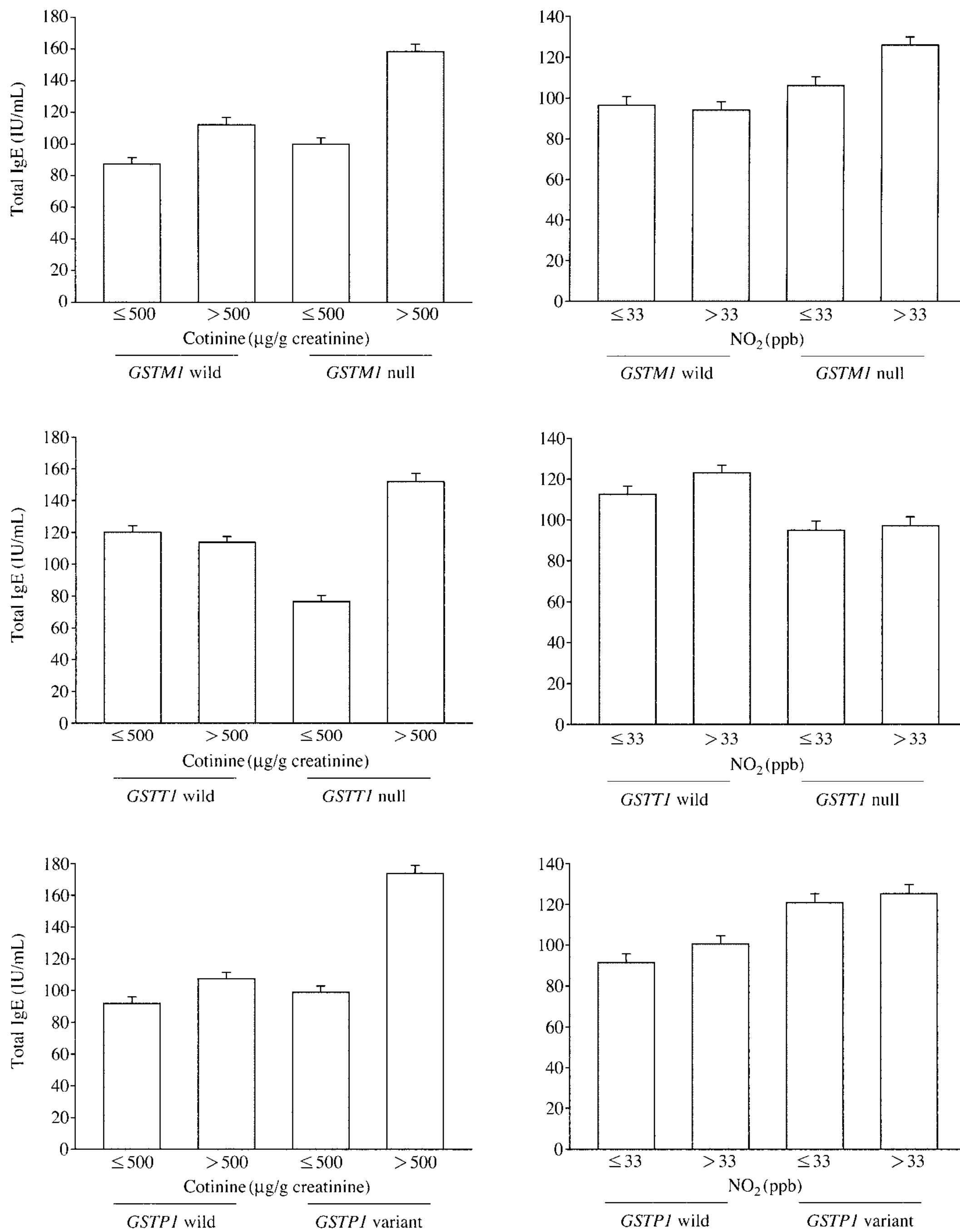

Fig. 1. Serum total IgE concentrations according to urinary cotinine and NO_2 exposure levels by *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms. Error bars indicate GSD.

promoted through increase of IgE level. In this study, significant association between smoking and IgE level was found, which support previous reports that smoking affects IgE level. In addition, subjects exposed to NO_2 33.1 ppb or more showed increased IgE level than those exposed to NO_2 33.1 ppb or less, although it did not show the statistical significance in regard that NO_2 increases IgE level. Therefore, both smoking and NO_2 may have a possibility inducing allergic response through the increase of IgE level.

Genetic polymorphisms of GST enzymes have been known to be very important in the development

Table 3. Serum IgE levels according to urinary cotinine levels in strata of combinations of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms.

GSTM1	*GSTT1*	*GSTP1*	Serum IgE level (IU/mL)				(j-i)	*P*-value*
			Cotinine < 500		Cotinine ≥ 500			
			N	GM (GSD) (i)	N	GM (GSD) (j)		
Wild	Wild	I/I	22	75.7 (5.7)	10	31.3 (3.1)	−44.4	0.118
Null	Wild	I/I	32	126.8 (3.2)	13	163.5 (3.7)	36.7	0.548
Wild	Null	I/I	35	96.0 (2.7)	15	117.6 (4.2)	21.6	0.433
Null	Null	I/I	44	77.0 (4.4)	15	155.0 (3.5)	78.0	0.073
Wild	Wild	I/V, V/V	16	145.1 (3.9)	12	212.0 (2.4)	66.9	0.430
Null	Wild	I/V, V/V	23	152.6 (3.3)	8	124.6 (2.9)	−28.0	0.701
Wild	Null	I/V, V/V	19	56.8 (4.4)	11	167.9 (6.3)	111.1	0.111
Null	Null	I/V, V/V	12	62.0 (4.7)	13	183.4 (8.8)	121.4	0.030

**P*-value from Kruskal-Wallis test

Table 4. Serum IgE levels according to personal NO_2 exposure levels in strata of combinations of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms.

GSTM1	*GSTT1*	*GSTP1*	Serum IgE level (IU/mL)				(j-i)	*P*-value*
			$NO_2 < 33.1$		$NO_2 \geq 33.1$			
			N	GM (GSD) (i)	N	GM (GSD) (j)		
Wild	Wild	I/I	14	56.6 (6.1)	18	58.1 (4.5)	1.6	0.849
Null	Wild	I/I	21	112.2 (4.0)	24	161.9 (2.7)	49.7	0.406
Wild	Null	I/I	25	110.9 (3.0)	25	93.9 (3.3)	−17.0	0.621
Null	Null	I/I	33	85.2 (4.2)	26	101.4 (4.4)	16.2	0.760
Wild	Wild	I/V, V/V	11	168.2 (2.5)	17	172.4 (3.8)	4.2	0.796
Null	Wild	I/V, V/V	16	156.4 (2.9)	15	133.4 (3.5)	−23.0	0.707
Wild	Null	I/V, V/V	17	85.3 (5.8)	13	83.5 (5.2)	−1.8	0.900
Null	Null	I/V, V/V	14	105.9 (6.8)	11	113.0 (8.0)	7.1	0.869

**P*-value from Kruskal-Wallis test

of a variety of inflammatory diseases including asthma[16,17,19]. In this study, we showed that combination of *GSTM1* null, *GSTT1* null, and *GSTP1* variants increases IgE level by smoking, although three genes did not modify IgE level separately. Our data that combinations of *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms modify IgE level by tobacco smoke exposure supports previous reports that *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms play a role in the development of a variety of inflammatory diseases including asthma. Therefore, our results suggest that *GSTM1*, *GSTT1*, and *GSTP1* gene polymorphisms identify genetically susceptible people for enhanced allergic response due to tobacco smoke. However, we did not show the effects of genotypes on increase of IgE by NO_2 exposure, although NO_2 exposure showed similar trend in the effect of smoking on IgE level according to *GSTM1* and *GSTP1* gene polymorphisms. Non-significance of NO_2 on IgE level may be due to small sample size of population. Therefore, this study results should be confirmed through studies of larger subjects.

Discussion

In conclusion, our study findings suggest that smoking induces allergic response measured as IgE level and combinations of the *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms modify the effect of smoking on serum IgE level, indicating genetic susceptibility to smoking-related diseases.

Methods

Study Population and Sampling

A total of 300 students at Inha University, Incheon, Korea, were recruited during September 2002 after exclusion of students having conditions affecting allergic response such as allergic disorder or medication of anti-inflammatory drugs. Information on demographic characteristics and lifestyle habits including smoking and alcohol consumption were collected by trained interviewers using a structured ques-

tionnaire. We controlled fasting state by asking them for overnight fasting to participate in this study and samplings of blood and urine were done in the morning. After collecting venous blood samples from subjects, serum was separated by centrifugation within 4 h and frozen at −70°C for the analysis of IgE level. In addition, urine was kept frozen at −20° C for the analysis of cotinine level. The study protocol was approved by the institutional review board at Inha University Hospital and written informed consent was provided by all study subjects.

Measurement of Urinary Cotinine Levels

Urinary cotinine levels were determined for monitoring tobacco exposure. After urine samples were centrifuged to remove particulate matter, cotinine level was analyzed by a liquid phase radioimmunoassay method using the fundamental principles of a competitive reaction between antibodies and ^{125}I-labeled cotinine and urinary cotinine (DPC, Los Angeles, CA). After allowing antigen-antibody reaction for 30 min, the fraction bound to antibody was precipitated and its radioactivity was measured for 1 min using Cobra D5010 Quantum, a gamma-counter (Packard, Meriden, CT). Urinary cotinine level was adjusted to urine creatinine.

Measurements of Personal NO_2 Exposure

Personal NO_2 exposure levels were determined for assessment of air pollution exposure. Polyester housed badge-type NO_2 passive sampler (Envors Co., Korea) was used for the measurement. One of the investigators gave instructions on how to wear and care for the sampler. Unsealed passive sampling badge was attached with a pin in a site between chest and head in the morning, and was sealed in following day. Wearers were instructed to keep the badges near the bedside during sleeping. The collected samplers were sealed with a gas-tight polyester lid and kept in a zippered plastic bag before and after sampling until analysis. The ambient NO_2 concentration was determined spectrophotometrically by the Saltzmann method and calculated by using NO_2 standard solution curve (Hangartner. 1998).

Measurement of Serum IgE Levels

Serum IgE levels were analyzed by a liquid phase radioimmunoassay method using the fundamental principles of a competitive reaction between antibodies and ^{125}I-labeled IgE and serum IgE (Coat-A-Count Total IgE IRMA Kit, DPC, Los Angeles, CA). After allowing antigen-antibody reaction for 30 min, the fraction bound to antibody was precipitated and its radioactivity was measured for 1 min using Cobra D5010 Quantum, a gamma-counter (Packard, Meriden, CT).

Genotyping Assays

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), and analyzed using PCR and RFLP method for *GSTM1, GSTT1,* and *GSTP1* gene polymorphisms. The 20 μL PCR reaction mixture used for *GSTM1, GSTT1*, and *GSTP1* genotyping contained 10 mM of Tris-HCl (pH 9.0), 40 mM of KCl, 1.5 mM of $MgCl_2$, 0.25 mM of each dNTP, 1 unit of Taq polymerase (Bioneer, Seoul, Korea), 20 pmole of the forward and reverse primers, and 50 ng of genomic DNA as a template. Amplifications were performed using the following conditions; initial denaturation at 94°C for 5 min, 35 cycles with denaturation at 94°C for 1 min, annealing at 65 °C for 1 min, and extension at 72°C for 1 min, and then completion at 72°C for 7 min. PCR amplification of reaction mixtures was carried out using a thermal cycler, PTC-200 (MJ Research, Watertown, MA, USA).

GSTM1, GSTT1, and *GSTP1* were determined using a multiplex PCR method. Primer sets for *GSTM1* and *GSTP1* genes were used for the amplification reaction. The primers designed were: *GSTM1*-forward, 5′-GAACTCCCTGAAAAGCTAAAGC-3′, *GSTM1*-reverse, 5′-GTTGGGCTCAAATATACGGTGG-3′; *GSTT1*-forward, 5′-TTCCTTACTGGTCCTCACATCTC-3′, *GSTT1*-reverse, 5′-TCACCGGATCATGGCCAGCA-3′; *GSTP1*-forward, 5′-CCAGTGACTGTGTGTTGATC-3′, *GSTP1*-reverse, 5′-CAACCCTGGTGCAGATGCTC-3′. The PCR products were digested with restriction enzyme *BsmA* I at 55°C for 3 h for *GSTP1* genotyping and detected by electrophoresis on 3% 3 : 1 NuSieve/agarose gel (Cambrex Bio Science, Rockland, ME, USA). Genotypings of the *GSTM1* and *GSTT1* genes were determined based on the presence of 215-bp and 480-bp products, respectively. For the genotyping of the *GSTP1* gene, the 189-bp PCR product remained intact for the A allele, but was cleaved into smaller fragments of 149-bp and 40-bp in the case of the G allele. For confirming the analyses, ten percent of the samples were randomly chosen and genotyped again, producing identical results.

Statistical Analysis

Urinary concentration of cotinine was adjusted to creatinine level to control variations in urine flow. We log-transformed IgE value to normalize the distribution. Student's t-test and Kruskal-Wallis test were used to investigate the relationships between exposure

to smoking or NO_2 and total IgE level according to GST genotypes or combinations of the genotypes. A probability level of 0.05 was used as the criterion of statistical significance based on two-sided test. SAS, version 6.12 (SAS institute Inc., Cary, NC, USA) was used for the statistical analysis.

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