

## Research Article



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### \*Correspondence:

#### Surabhi Shah

Department of Occupational and Environmental Medicine, College of Medicine, Ewha Womans University, 25 Magokdong-ro 2-gil, Gangseo-gu, Seoul 07804, Korea.  
E-mail: surabhi.3007@gmail.com

#### Eunhee Ha

Department of Occupational and Environmental Medicine, College of Medicine, Ewha Womans University, 25 Magokdong-ro 2-gil, Gangseo-gu, Seoul 07804, Korea.  
E-mail: eunheeha@ewha.ac.kr

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### ORCID iDs

Tai Kyung Koh   
<https://orcid.org/0000-0002-9027-9708>  
Hyesook Park   
<https://orcid.org/0000-0002-9359-6522>  
Yun-Chul Hong   
<https://orcid.org/0000-0001-9010-7271>  
Mina Ha   
<https://orcid.org/0000-0003-1011-9446>  
Yangho Kim   
<https://orcid.org/0000-0002-6462-0829>  
Bo-Eun Lee   
<https://orcid.org/0000-0003-1278-4191>

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# Association between prenatal polycyclic aromatic hydrocarbons and infantile allergic diseases modified by maternal glutathione S-transferase polymorphisms: results from the MOCEH birth cohort

Tai Kyung Koh <sup>1,2</sup>, Hyesook Park <sup>2,3</sup>, Yun-Chul Hong <sup>4</sup>, Mina Ha <sup>5</sup>, Yangho Kim <sup>6</sup>, Bo-Eun Lee <sup>7</sup>, Surabhi Shah <sup>8\*</sup>, and Eunhee Ha <sup>2,8\*</sup>

<sup>1</sup>Department of Occupational and Environmental Medicine, Ewha Womans University Mokdong Hospital, Seoul, Korea

<sup>2</sup>Graduate Program in System Health Science and Engineering, Ewha Womans University, Seoul, Korea

<sup>3</sup>Department of Preventive Medicine, College of Medicine, Ewha Womans University, Seoul, Korea

<sup>4</sup>Department of Preventive Medicine, College of Medicine, Seoul National University, Seoul, Korea

<sup>5</sup>Department of Preventive Medicine, Dankook University College of Medicine, Cheonan, Korea

<sup>6</sup>Department of Occupational and Environmental Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Korea

<sup>7</sup>Environmental Health Research Department, National Institute of Environmental Research, Incheon, Korea


<sup>8</sup>Department of Occupational and Environmental Medicine, College of Medicine, Ewha Womans University, Seoul, Korea

## ABSTRACT

**Background:** Prenatal exposure to polycyclic aromatic hydrocarbons (PAH) has been linked to allergic disease onset. Variations in the glutathione S-transferase (GST) gene family can impact the progression of allergic diseases. We sought to examine the association between prenatal PAH exposure and infantile allergic diseases in 6-month-old infants, and how maternal glutathione S-transferase M1 (*GSTM1*) or T1 (*GSTT1*) polymorphisms affects the association between prenatal PAH exposure and allergic diseases in the Mothers and Children's Environmental Health (MOCEH) study.

**Methods:** The study sample comprised 349 infants and their mothers from the MOCEH study, for whom 1-hydroxypyrene (1-OHP) and 2-naphthol were measured in both the early period of pregnancy and late period of pregnancy. An infant was deemed to be affected by an allergic disease if diagnosed with or if developed at least one of the allergic diseases. A logistic regression analysis was performed to study the association between urinary 1-OHP and 2-naphthol levels during pregnancy and allergic diseases in 6-month-old infants. Furthermore, analyses stratified by maternal *GSTM1* or *GSTT1* present/null polymorphisms were performed.

**Results:** The risk of allergic diseases in 6-month-old infants was significantly increased in accordance with an increase in urinary 1-OHP during the early period of pregnancy (odds ratio [OR]: 1.84; 95% confidence interval [CI]: 1.05, 3.23; by one log-transformed unit of 1-OHP  $\mu\text{g/g}$  creatinine). The increased risk of infantile allergic diseases associated with urinary 1-OHP during the early period of pregnancy was limited to the maternal *GSTT1* null

Surabhi Shah <https://orcid.org/0000-0003-3652-2823>Eunhee Ha <https://orcid.org/0000-0002-4224-3858>

### Abbreviations

BMI: body mass index; CI: confidence interval; Cr: creatinine; DEP: diesel exhaust particle; ETS: environmental tobacco smoke; FFQ: food frequency questionnaire; GM: geometric mean; GSD: geometric standard deviation; GSH: glutathione; GST: glutathione S-transferase; GSTM1: glutathione S-transferase M1; GSTT1: glutathione S-transferase T1; IFN: interferon; IgE: immunoglobulin E; IL: interleukin; ISAAC: International Study of Asthma and Allergies in Childhood; LOD: limit of detection; Min.: Minimum; Max.: Maximum; MOCEH: Mothers and Children's Environmental Health; OR: odds ratio; PAH: polycyclic aromatic hydrocarbons; PCR: polymerase chain reaction; RERI: Relative Excess Risk due to Interaction; 1-OHP: 1-hydroxypyrene.

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### Competing interests

The authors declare that they have no competing interests.

### Author Contributions

Conceptualization: Koh TK, Shah S, Ha E; Data curation: Koh TK, Shah S; Formal analysis: Koh TK, Shah S; Methodology: Koh TK, Shah S, Ha E; Project administration: Koh TK, Shah S, Ha E; Software: Koh TK, Shah S; Writing - original draft: Koh TK, Shah S; Writing - review & editing: Koh TK, Shah S, Ha E, Park H, Hong YC, Ha M, Kim Y, Lee BE.

type (OR: 2.69; 95% CI: 1.17, 6.21, by one log-transformed unit of 1-OHP  $\mu\text{g/g}$  creatinine); however, the Relative Excess Risk due to Interaction was not statistically significant.

**Conclusions:** The present study found that infantile allergic diseases could be affected by intrauterine PAH exposure, particularly in the early prenatal period and the risk was limited to the maternal *GSTT1* null type.

**Keywords:** Allergic diseases in infants; Polycyclic aromatic hydrocarbons; *GSTT1*; *GSTM1*; GST polymorphisms

## BACKGROUND

Over the last 2 decades, there has been an increase in the prevalence of childhood allergic diseases such as asthma, atopic dermatitis, food allergies, and allergic rhinitis. Allergic diseases are among the most common chronic diseases in both children and adults [1]. Based on the 2007 National Survey of Children's Health, the prevalence of atopic dermatitis in US children was 13.0% [2]. According to data from multicenter international studies, geographical variations of the prevalence of asthma range between 1.0% and 18.0%. Moreover, although epidemiologic studies on rhinitis are scarce, a nearly 30-fold increase (i.e., from 1.4% to 39.7%) was observed in the prevalence of rhinitis among 13–14-year-old children across 56 countries [3]. In the Republic of Korea, the prevalence of atopic dermatitis, asthma, and allergic rhinitis in 2014 was 19.0, 36.3, and 133.1 per 1,000 people, respectively [4], and the prevalence of atopic dermatitis in 6–7-year-old children increased from 8.8% in 1995 to 12.7% in 2015 [5].

According to “a study on research methodology and long-term planning regarding estimation of economic burden of major diseases in Korea (2009),” asthma was ranked fifth by occurrence among all examined chronic diseases [6]. The “developmental origins of health and disease” hypothesis proposes that in utero and early childhood exposure to environmental pollutants may increase susceptibility to allergic diseases [7]. Allergic diseases that are not properly managed in childhood and adolescence may become severe diseases in adulthood, thereby increasing economic burden and losses. Several risk factors are known to be associated with allergic diseases in epidemiologic studies, including early-life sensitization, traffic-related air pollution (black carbon,  $\text{SO}_2$ ,  $\text{NO}_2$ ,  $\text{PM}_{2.5}$ ,  $\text{PM}_{10}$ , ozone), pre- or postnatal tobacco smoke exposure, viral infections (human rhinovirus, respiratory syncytial virus), mold, microbial exposure, and pets [8].

Polycyclic aromatic hydrocarbons (PAH) are fused aromatic ring-environmental pollutants, produced by the incomplete combustion of carbon-containing materials, such as coal, crude oil, wood, gasoline, food, and cigarettes [9]. Major sources of indoor PAH include emissions from residential heating, gas appliances, environmental tobacco smoke (ETS), and fumes from cooking, grilling, and frying. Previous studies have suggested a link between exposure to environmental pollutants such as PAH in concert with ETS postnatally and the onset of childhood respiratory symptoms or asthma [10,11]. Therefore, it is necessary to further explore and evaluate the association between prenatal PAH exposure and childhood-onset allergic diseases.

Allergic diseases are multifactorial, meaning that they result from a combination of environmental factors and genetic predisposition. This is illustrated by the rapid increase

in the prevalence of allergic diseases in recent decades, which has accelerated too rapidly to be explained by genetic changes alone. The interaction between genetic predisposition and environmental exposure critically affects the developing immune system of neonates and children [8,12].

Genetic variations in glutathione S-transferases (GSTs) with different enzymatic activities can have a significant effect on children's susceptibility to atopic dermatitis [13]. The GST family comprises 3 superfamilies (cytosolic, mitochondrial, and microsomal) [14]. Human cytosolic GSTs belong to the alpha (GSTA1, GSTA2, GSTA3, GSTA4, and GSTA5), zeta (GSTZ1), theta (GSTT1, GSTT2, and GSTT4), mu (GSTM1, GSTM1L, GSTM2, GSTM3, GSTM4, and GSTM5), pi (GSTP1), sigma, and omega (GSTO1 and GSTO2) classes [15]. GSTs catalyze the conjugation of glutathione (GSH) to xenobiotic substrates to make them more water-soluble [14]. Their cellular defense activity against oxidative stress consists of detoxifying endogenous compounds such as peroxidized lipids and breaking down xenobiotics. GSTs may also bind with toxins and serve as transport proteins [16]. The general reaction of GST enzymes is the addition of GSH to electrophiles. There is a wide diversity of endogenous and exogenous electrophilic substrates, among which PAH are notable examples of exogenous electrophilic substrates [17]. GSTs catalyze the conjugation of GSH with PAH-derived free radicals. Previous studies have suggested the detoxifying properties of GSTs on ETS exposure [18,19], and have reported that children with prenatal exposure to ETS and *GST* deficiencies had a higher likelihood of asthma or decreased lung function.

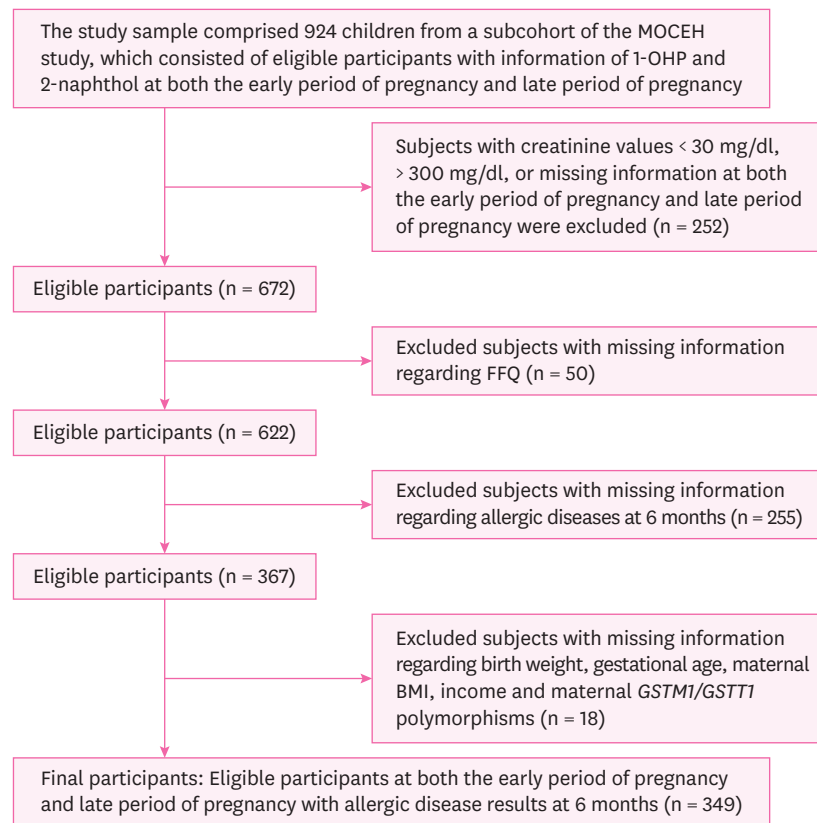
It is difficult to identify the exact origin of allergic diseases because exposure factors interact with one another, and, in turn, these interactions affect allergic disease susceptibility. Therefore, this study sought to examine the effect of prenatal PAH exposure on allergic disease onset in 6-month-old infants. Among the various known GST isoenzymes, glutathione S-transferase M1 (*GSTM1*) and glutathione S-transferase T1 (*GSTT1*) are each encoded by different genes, and, if they are null allele variants, the null genotypes for both enzymes result in a lack of enzyme activity [20,21]. Therefore, we further assessed the association between prenatal PAH exposure and allergic diseases and how these relate to maternal *GSTM1/GSTT1* polymorphisms to evaluate gene-environment interactions.

## METHODS

### Study participants

This study was conducted as part of the Mothers and Children's Environmental Health (MOCEH) study. The MOCEH study is a multi-center birth cohort study with research centers in Seoul, Cheonan, and Ulsan in the Republic of Korea. Each center had a community-based network comprised of a university hospital and several public health centers. The MOCEH study was designed to investigate the effects of prenatal and postnatal pollutant exposures on growth, development, and health from fetal development to childhood. The pollutants assessed in the MOCEH cohort study were evaluated for endocrine disruptors, heavy metals, and PAH. The endocrine disruptors included bisphenol A and phthalate metabolites, and the harmful heavy metals included lead, mercury and cadmium, and the PAH included 1-hydroxypyrene (1-OHP) and 2-naphthol.

From 2006 to 2011, pregnant women living in the vicinity of the aforementioned locations, over the age of 18, and less than 20 weeks of gestational age were enrolled in the study along



**Fig. 1.** Flow diagram of the study design.

MOCEH: Mothers and Children's Environmental Health; 1-OHP: 1-hydroxypyrene; FFQ: food frequency questionnaire; BMI: body mass index; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1.

with their spouses (n = 1,751). In our study, pregnant women were selected based on whether information on both 1-OHP and 2-naphthol (n = 924) exposures were available. Furthermore, we excluded participants with < 30 mg/dL, > 300 mg/dL, or missing data on urinary creatinine (Cr) concentrations (n = 252). Those who did not respond to the self-administered food frequency questionnaire (FFQ) were also excluded (n = 50), and subjects with missing information regarding birth weight, gestational age, maternal body mass index (BMI), income, and maternal *GSTM1/GSTT1* polymorphisms were excluded as per the complete case analysis (n = 18) [22]. Lastly, 6-month-old infants who had missing information on allergic diseases were also excluded (n = 255). In the final analysis, eligible participants for both the early period of pregnancy and late period of pregnancy (n = 349) were included in the study (Fig. 1).

### Urinary 1-OHP and 2-naphthol level measurements

Urinary 1-OHP and 2-naphthol levels were used as biomarkers of airborne PAH exposure [23]. Specifically, 1-OHP and 2-naphthol were measured in pregnant women via spot urine collections during their visits to the hospital throughout the early period of pregnancy (< 20 weeks of gestational age) and late period of pregnancy (> 28 weeks of gestational age). Maternal urine samples were collected in the morning and sent to a specialized laboratory for analysis. The 1-OHP and 2-naphthol urine concentrations were analyzed via high-performance liquid chromatography with fluorescence detection [24] and the levels measured during the early period of pregnancy and during late period of pregnancy were examined in the present study.

The limit of detection (LOD) was 0.02 µg/L for 1-OHP and 0.057 µg/L for 2-naphthol, and samples below the LOD were divided by the square root of 2 [25].

### Assessment of allergic disease occurrence in 6-month-old infants

Mothers/caregivers were asked about infantile symptoms, medical history, and the diagnosis of atopic dermatitis, asthma, food allergies, and any other allergic diseases or symptoms through an International Study of Asthma and Allergies in Childhood (ISAAC)-based questionnaire when the infants were 6 months old. ISAAC was established in 1991 and is the largest worldwide collaborative research project that focuses on asthma, rhinitis, and eczema in children. If an infant had symptoms, visited hospitals, or was diagnosed with either food allergies, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, asthma, or other allergic symptoms, the infant was deemed to have an allergic disease [26].

### Maternal *GSTM1* and *GSTT1* polymorphism assessment

Maternal DNA was extracted from maternal whole blood samples using the QIAamp DNA blood kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed to identify maternal *GSTM1* and *GSTT1* polymorphisms. Moreover, a 268-bp β-globin gene fragment was amplified as a positive control. *GSTM1* and *GSTT1* genotyping were conducted with a PCR mixture containing 10 mM Tris-HCl, 40 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.25 mM of each dNTP, 1 unit of *Taq* polymerase (Bioneer, Seoul, Korea), 20 pmol of forward and reverse primers, and 50–100 ng of the genomic DNA as a template. The following *GSTM1* and *GSTT1* primer sets were used for PCR amplification: 5'-GAA CTC CCT GAA AAG CTA AAG C-3' (forward) and 5'-GTT GGG CTC AAA TAT ACG GTG G-3' (reverse) for *GSTM1*, and 5'-TCA CCG GAT CAT GGC CAG CA-3' (forward) and 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' (reverse) for *GSTT1*. PCR amplification was performed with a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with an initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 1 minutes, annealing at 65°C for 1 minutes, and extension at 72°C for 1 minutes; and a final extension at 72°C for 7 minutes. To examine the PCR-amplified fragments, electrophoresis was performed on a 3% 3:1 NuSieve/agarose gel (Cambrex Bio Science, Rockland, ME, USA). *GSTM1* and *GSTT1* genotyping were conducted based on the presence/absence of a 215-bp product and a 459-bp product, respectively. The GST null genotype was identified as a homozygous deletion of both of the examined genes. To confirm the results, 10% of the samples were randomly selected and analyzed once again to verify that the results were identical.

### Covariates

Trained nurses interviewed the study participants to collect information on maternal age, socioeconomic status, maternal education level, and parental allergic history using a structured questionnaire. Nutrition and dietary information were collected from the pregnant women during their prenatal visits using a semi-quantitative self-administered FFQ. The FFQ method has been previously validated in the Korean population [27]. The FFQ includes questions on the consumption frequency of beef, pork, and chicken products prepared by grilling, roasting, or smoking. The potential consumption frequency responses were divided into nine categories: 'never or rare,' 'once monthly,' '2 or 3 times a month,' 'once or twice a week,' '3 or 4 times a week,' '5 or 6 times a week,' 'daily,' 'twice a day,' and '3 times a day.' For downstream analyses, the maternal patterns of barbecued, fried, roasted, or grilled beef, pork, and chicken product consumption were further classified into 3 categories: (1) '1 time a month,' (2) '2–3 times a month,' and (3) '1–6 times a week'. The pre-pregnancy BMI was calculated as the body weight in kilograms divided by the height in meters squared. The

participants were then divided into 3 groups: underweight (BMI < 18 kg/m<sup>2</sup>), normal weight (BMI 18–25 kg/m<sup>2</sup>), and overweight (BMI ≥ 25 kg/m<sup>2</sup>). Information related to the infant's sex, birth weight, and gestational age was obtained from medical records.

### Statistical analyses

Differences in study participant characteristics were determined using the t-test for continuous variables and the  $\chi^2$  test for categorical variables. The maternal urinary 1-OHP and 2-naphthol levels were corrected for Cr concentrations and were converted to a logarithmic base e scale to obtain a normal distribution. Logistic regression analysis was conducted to study the association between urinary 1-OHP and 2-naphthol levels during the early period of pregnancy and during late period of pregnancy and allergic diseases in 6-month-old infants. The covariates used in this study were the following: infant's sex (female versus male; categorical variable), gestational age (completed weeks; continuous variable), birth weight (grams; continuous variable), maternal age at delivery (< 30, ≥ 30 years old; ordinal variable), pre-pregnancy BMI (< 18, 18–25, ≥ 25; ordinal variable), family income (< 2, ≥ 2 million KRW; ordinal variable), maternal urinary cotinine concentration (ng/mL; < 18: non-smoker, 18–50: secondhand smoker, ≥ 50: active smoker; ordinal variable), maternal allergic history (yes or no; categorical variable), paternal allergic history (yes or no; categorical variable), and intake frequency of barbecued, fried, roasted or grilled beef, chicken, and pork (3 categories, as described above; ordinal variable).

Furthermore, to identify the interactive effect of GSTs and the metabolites of maternal PAH on infantile allergic diseases, an interaction between 1-OHP or 2-naphthol levels and GSTs was estimated. Interaction on an additive scale was estimated via the Relative Excess Risk due to Interaction (RERI) [28]. An RERI value larger than 0 suggested an additive interaction. The following formula was used to calculate the RERI:  $RERI = OR_A \times OR_B \times OR_{AB} - OR_A - OR_B + 1$ , where  $OR_A$  is the odds ratio (OR) of exposure to prenatal PAH,  $OR_B$  is the OR of carrying different GST polymorphisms, and  $OR_{AB}$  is the OR of the interaction of prenatal PAH and GSTs. After identifying any interaction effects, the association between prenatal PAH exposure and infantile allergic diseases in relation to the maternal *GSTM1/GSTT1* polymorphisms was assessed. Stratified logistic regression analysis based on the GST null/present genotypes was performed to evaluate the effect of prenatal PAH exposure on allergic diseases in 6-month-old infants. Statistical significance was defined as *p*-value < 0.05. Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

### Ethics statement

Before enrollment, written informed consent was obtained from the pregnant women, both for themselves and on behalf of their children. All protocols were approved by the Institutional Review Board of Ewha Womans University Hospital (approval No. 12-07B-15), Dankook University Hospital (approval No. 2011-09-0340), and Ulsan University Hospital (approval No.06-29).

## RESULTS

The general characteristics of the 6-month-old infants with/without allergic diseases and those of their parents are summarized in **Table 1**. Of the 349 infants studied herein, 141 (40.4%) were affected by at least one allergic disease. There were no significant differences in maternal age among infants with/without allergic diseases. However, significant differences

**Table 1.** Comparison of general characteristics of participant 6-month-old infants by allergic diseases

Variables	Total	Not having allergic diseases	Having allergic diseases	p-value <sup>a</sup>
Total	349 (100.0)	208 (59.6)	141 (40.4)	< 0.01 <sup>c</sup>
Sex of the child				0.86
Male	191 (54.7)	113 (54.3)	78 (55.3)	
Female	158 (45.3)	95 (45.7)	63 (44.7)	
Maternal age (year)				0.06
< 30	164 (47.0)	89 (42.8)	75 (53.2)	
≥ 30	185 (53.0)	119 (57.2)	66 (46.8)	
Maternal BMI (kg/m <sup>2</sup> )				0.05 <sup>c</sup>
0 < BMI < 18	5 (1.4)	3 (1.4)	2 (1.4)	
18.0 ≤ BMI < 25.0	259 (74.2)	145 (69.7)	114 (80.9)	
BMI ≥ 25.0	85 (24.4)	60 (28.9)	25 (17.7)	
Income (million KRW/month)				0.79
< 2	89 (25.5)	52 (25.0)	37 (26.2)	
≥ 2	260 (74.5)	156 (75.0)	104 (73.8)	
Maternal allergic disease history				0.01 <sup>c</sup>
No	234 (67.1)	150 (72.1)	84 (59.6)	
Yes	115 (32.9)	58 (27.9)	57 (40.4)	
Paternal allergic disease history				0.04 <sup>c</sup>
No	255 (73.1)	160 (76.9)	95 (67.4)	
Yes	94 (26.9)	48 (23.1)	46 (32.6)	
Barbecued/fried beef <sup>b</sup>				0.96
1 time/month	181 (51.9)	108 (51.9)	73 (51.8)	
2–3 times/month	106 (30.4)	64 (30.8)	42 (29.8)	
1–6 times/week	62 (17.8)	36 (17.3)	26 (18.4)	
Barbecued/fried pork <sup>b</sup>				0.93
1 time/month	37 (10.6)	21 (10.1)	16 (11.4)	
2–3 times/month	120 (34.4)	72 (34.6)	48 (34.0)	
1–6 times/week	192 (55.0)	115 (55.3)	77 (54.6)	
Barbecued/fried chicken <sup>b</sup>				0.35
1 time/month	122 (35.0)	79 (38.0)	43 (30.5)	
2–3 times/month	138 (39.5)	79 (38.0)	59 (41.8)	
1–6 times/week	89 (25.5)	50 (24.0)	39 (27.7)	
Cotinine at early period of pregnancy (ng/mL)				0.75
0 < Cotinine < 18	333 (95.4)	197 (94.7)	136 (96.5)	
18 ≤ Cotinine < 50	3 (0.9)	2 (1.0)	1 (0.7)	
Cotinine ≥ 50	13 (3.7)	9 (4.3)	4 (2.8)	
Cotinine at late period of pregnancy (ng/mL)				0.41
0 < Cotinine < 18	334 (95.7)	197 (94.7)	137 (97.2)	
18 ≤ Cotinine < 50	5 (1.4)	3 (1.4)	2 (1.4)	
Cotinine ≥ 50	10 (2.9)	8 (3.9)	2 (1.4)	
GSTM1				0.57
Null	192 (55.0)	117 (56.3)	75 (53.2)	
Positive	157 (45.0)	91 (43.7)	66 (46.8)	
GSTT1				0.13
Null	176 (50.4)	98 (47.1)	78 (55.3)	
Positive	173 (49.6)	110 (52.9)	63 (44.7)	

Values are presented as number (%).

BMI: body mass index; GSTM1: glutathione S-transferase M1; GSTT1: glutathione S-transferase T1.

<sup>a</sup>Tested by  $\chi^2$  or Fisher's exact test; <sup>b</sup>Obtained from the food frequency questionnaire; <sup>c</sup>The p-value < 0.05.

were observed in maternal BMI, indicating that mothers of non-allergic infants tended to weigh more. Moreover, parents of infants with allergic diseases tended to have a history of allergic diseases themselves. Interestingly, there was no significant difference in barbecued, fried, roasted, or grilled meat intake and cotinine concentration between the tested groups.

The geometric means of 1-OHP and 2-naphthol levels were 0.53  $\mu\text{g/g Cr}$  and 2.12  $\mu\text{g/g Cr}$  at the early period of pregnancy and 0.54  $\mu\text{g/g Cr}$  and 2.09  $\mu\text{g/g Cr}$  at late period of pregnancy,

## Prenatal PAH and allergic diseases modified by GST

**Table 2.** Distribution of 1-OHP and 2-naphthol levels in the maternal urine with correction for creatinine concentration at the early period of pregnancy and late period of pregnancy

PAH metabolites	Gestation (n = 349)	GM	GSD	Min	25th percentiles	50th percentiles	75th percentiles	Max
1-OHP ( $\mu\text{g/g}$ creatinine)	Early <sup>a</sup>	0.53	1.52	0.13	0.39	0.54	0.73	1.39
	Late <sup>b</sup>	0.54	1.49	0.16	0.42	0.55	0.73	1.94
2-naphthol ( $\mu\text{g/g}$ creatinine)	Early <sup>a</sup>	2.12	1.63	0.28	1.56	2.21	2.85	7.35
	Late <sup>b</sup>	2.09	1.59	0.23	1.54	2.15	2.77	9.23

PAH: polycyclic aromatic hydrocarbons; 1-OHP: 1-hydroxypyrene; GM: geometric mean; GSD: geometric standard deviation; Min.: Minimum; Max.: Maximum. <sup>a</sup>Gestational age < 20 weeks; <sup>b</sup>Gestational age > 28 weeks.

respectively (Table 2). The 2017 Korean National Environmental Health Survey (KoNEHS) reported urinary 1-OHP and 2-naphthol geometric means of 0.15  $\mu\text{g/g}$  Cr and 3.16  $\mu\text{g/g}$  Cr, respectively. The 2013–2014 National Health and Nutrition Examination Survey reported urinary 1-OHP and 2-naphthol geometric means of 0.15  $\mu\text{g/g}$  Cr and 4.98  $\mu\text{g/g}$  Cr, respectively.

Logistic regression analysis indicated that an increase in urinary 1-OHP during the early period of pregnancy was associated with an increased risk of allergic disease in 6-month-old infants (Table 3). When the 1-OHP concentration in the early period of pregnancy increased by one log-transformed unit of 1-OHP/Cr, the risk of allergic disease in 6-month-old infants increased by 1.53 times in the crude model, albeit not significantly. However, in the adjusted model, when the 1-OHP concentration in the early period of pregnancy increased by one log-transformed unit of 1-OHP/Cr, the risk of allergic disease in 6-month-old infants increased significantly by 1.84 times. We did not find a significant association between prenatal 2-naphthol and allergic diseases in 6-month-old infants.

The RERI results provided evidence of a positive additive interaction between 1-OHP in the early period of pregnancy and the *GSTT1* null genotype but it was not statistically significant (RERI = 2.61; 95% confidence interval [CI]: -6.02, 11.24) (Table 4). Nevertheless, stratification analysis of maternal *GSTT1* (null/present) supported the association between allergic diseases and 1-OHP in the early period of pregnancy (Table 4). In the adjusted model, when the 1-OHP concentration in the early period of pregnancy increased by one log-transformed unit of 1-OHP/Cr, the risk of allergic disease in 6-month-old infants with a maternal *GSTT1* null genotype increased significantly by 2.69 times. However, no significant association was observed in the stratified analysis of maternal *GSTM1*. Additionally, the association between maternal GST genotype and allergic disease in 6-month-old infants, except for PAH exposure is described in Supplementary Table 1. The OR of allergic disease increased when *GSTT1* was null compared to when *GSTT1* was present but it was not statistically significant.

**Table 3.** Association between maternal urinary PAH metabolites and allergic diseases in 6-month-old infants

PAH metabolites	Risk of allergic diseases in 6-month-old infants (n = 349)	
	Crude	Adjusted <sup>a</sup>
Early period of pregnancy <sup>b</sup>		
1-OHP	1.53 (0.91, 2.58)	1.84 (1.05, 3.22) <sup>d</sup>
2-naphthol	1.08 (0.70, 1.68)	1.03 (0.64, 1.65)
Late period of pregnancy <sup>c</sup>		
1-OHP	1.40 (0.81, 2.40)	1.50 (0.83, 2.71)
2-naphthol	0.98 (0.62, 1.56)	0.97 (0.59, 1.61)

Values are presented as odds ratio (95% confidence interval). Unit of the PAH metabolites, log-transformed  $\mu\text{g/g}$  creatinine.

PAH: polycyclic aromatic hydrocarbons; 1-OHP: 1-hydroxypyrene.

<sup>a</sup>Model adjusted for maternal age, maternal body mass index, birth weight, gestational age, infant's sex, family income, maternal allergic history, paternal allergic history, frequency of barbecued, fried, roasted or grilling beef, pork, and chicken, cotinine level (ng/mL); <sup>b</sup>Gestational age < 20 weeks; <sup>c</sup>Gestational age > 28 weeks; <sup>d</sup>The *p*-value < 0.05.



**Table 4.** Association between maternal urinary PAH metabolites and allergic diseases in 6-month-old infants in relation to maternal GST genotypes

Genotype	Number (%)	Risk of allergic diseases in 6-month-old infants <sup>a</sup>							
		Early period of pregnancy <sup>b</sup>				Late period of pregnancy <sup>c</sup>			
		1-OHP		2-naphthol		1-OHP		2-naphthol	
	OR (95% CI) <sup>a</sup>	RERI (95% CI)	OR (95% CI) <sup>a</sup>	RERI (95% CI)	OR (95% CI) <sup>a</sup>	RERI (95% CI)	OR (95% CI) <sup>a</sup>	RERI (95% CI)	
<i>GSTT1</i>									
Null	176 (50.4)	2.69 (1.17, 6.21) <sup>d</sup>	2.61 (-6.02, 11.24)	0.68 (0.33, 1.39)	-0.25 (-0.53, 0.04)	2.37 (0.96, 5.83)	3.62 (-7.79, 15.04)	1.53 (0.66, 3.52)	0.44 (-0.99, 1.88)
Present	173 (49.6)	1.36 (0.58, 3.20)		1.50 (0.70, 3.21)		0.93 (0.40, 2.16)		0.64 (0.31, 1.33)	
<i>GSTM1</i>									
Null	192 (55.0)	1.16 (0.50, 2.67)	-0.78 (-1.67, 0.11)	0.92 (0.48, 1.76)	-0.12 (-0.52, 0.28)	1.49 (0.65, 3.42)	0.23 (-1.98, 2.44)	0.97 (0.43, 2.20)	0.01 (-0.45, 0.46)
Present	157 (45.0)	2.23 (0.83, 5.20)		1.31 (0.59, 2.91)		1.35 (0.52, 3.48)		1.09 (0.54, 2.22)	

Unit of the PAH metabolites, log-transformed  $\mu\text{g/g}$  creatinine.

PAH: polycyclic aromatic hydrocarbons; RERI: Relative Excess Risk due to Interaction; 1-OHP: 1-hydroxypyrene; OR: odds ratio; CI: confidence interval; GST: glutathione S-transferase; *GSTM1*: glutathione S-transferase M1; *GSTT1*: glutathione S-transferase T1.

<sup>a</sup>Model adjusted for maternal age, maternal BMI, birth weight, gestational age, infant's sex, family income, maternal allergic history, paternal allergic history, frequency of barbecued, fried, roasted or grilling beef, pork, and chicken, cotinine level (ng/mL); <sup>b</sup>Gestational age < 20 weeks; <sup>c</sup>Gestational age > 28 weeks; <sup>d</sup>The *p*-value < 0.05.

## DISCUSSION

The present study showed that 1-OHP exposure during the early period of pregnancy was associated with a significantly increased risk of allergic diseases in 6-month-old infants. Particularly, the association was limited to infants with a maternal *GSTT1* null genotype; however, the RERI was not statistically significant.

PAH may contribute to increased prevalence and morbidity of allergic diseases. PAH on diesel exhaust particles (DEPs) can promote the mediated detoxification of the cytochrome P450 family 1 A1 [8]. In vitro and in vivo studies have demonstrated that inhalation of PAH-DEP upregulated interleukin (IL)-4 and IL-8 production [29-31]. A study by Takenaka et al. [32] showed that the PAH extract (obtained from PAH-DEP) enhanced the immunoglobulin E (IgE) production in the B cells due to IL-4. PAH can also inhibit the differentiation of human monocytes into macrophages, and this inhibitory effect may contribute to the potent immunotoxicity of PAH [33].

The developing immune functions in fetuses may be susceptible to prenatal PAH exposure, which can lead to adverse effects later in life [34]. A study from Spain showed that prenatal exposure to PAH was associated with the risk of respiratory infections in children during their first year of life [35]. Zhang et al. [36] reported that PAH were detected in the umbilical cord and placentas; they suggested the possibility of PAH transfer from the mother to the fetus via the placenta. Maternal exposure to PAH and placental transport can lead to the formation of DNA adducts easily, thereby confirming the heightened fetal susceptibility to prenatal PAH exposure [37,38]. Fetal T cells begin to differentiate between 8 and 12 weeks of gestational age [39,40]. Mature T helper cells or cytotoxic T cells appear at approximately 12 weeks of gestational age; subsequently, by 16 weeks of gestational age, CD4 and CD8 are expressed in the fetal thymus in patterns similar to the postnatal thymus, suggesting that human T cell repertoire is established by the end of the first trimester. At a similar time (approximately 11 weeks of gestational age), the fetus starts to produce IgE [41].

Various studies have suggested that the risk of allergic disease is determined by prenatal exposure to pollutants or chemicals that modify the T helper cell phenotype in postnatal life [42,43]. Polarization of naive CD4<sup>+</sup> T cells into T<sub>H</sub>2 cells that induce IL-4 tends to inhibit interferon (IFN)- $\gamma$  transcription by methylation of the IFN- $\gamma$  promoter [44,45]. A birth cohort

study in the Czech Republic examined short-term associations of air pollution, including PAH exposure with lymphocyte immunophenotypes in cord blood, thereby indicating that average ambient PAH levels before birth were associated with reductions in T-lymphocyte phenotype fractions [46]. A study by Tang et al. [47] reported that prenatal exposure to PAH was associated with promoter methylation of an asthma-related gene, *IFN- $\gamma$* , but not with the promoter methylation of *IL-4*. In addition, a study by Kim et al. [48] found that maternal cytokine levels started to polarize toward T<sub>H</sub>2-dominant immune response beginning in the first trimester and that a marked T<sub>H</sub>2-biased responsiveness increased susceptibility to childhood wheezing and atopy at 3 years of age. This suggests that any maternal cytokine dysregulation may start during early pregnancy, and marked T<sub>H</sub>2-biased responsiveness may induce the development of wheezing and atopic dermatitis in the offspring. Hence, exposure to PAH, which may cross the placenta, in the early period of pregnancy can lead to both maternal and fetal cytokine dysregulation and cause postnatal allergic diseases in infants and children. The mechanisms of PAH on fetal immune development between the first and second trimester, and how they are affected by epigenetics, remain to be elucidated; however, these findings support the potential effect of prenatal exposure to pollutants including PAH in the early period of pregnancy on fetal immune development.

The PAH metabolism pathway involves phase I drug metabolism enzymes (cytochrome P450) and phase II enzymes (primarily GSTs). GSTs catalyze the conjugation of PAH or their reactive metabolites to GSH, thereby making them easily excretable. The GSTs are polymorphic, and genetic polymorphisms in GSTs have been associated with abnormal PAH metabolism. A study by Chen et al. [49] reported a decreased 1-OHP excretion rate in Chinese coke oven workers with the *GSTT1* null genotype, indicating that GSTT1 might be an important enzyme in the metabolism of PAH. Human GSTT1 is predominantly expressed in kidney and liver [50] with some expression in the placenta [51]. The exact role of GSTT1 in fetal and maternal detoxification is little known, but a higher proportion of GSTT1 compared to other GSTs suggests that GSTT1 is important in placental detoxification. Xenobiotics in the maternal blood can cross the placenta and accumulate in fetal organs [52]. Furthermore, deficiencies in maternal GSTT1, which is needed to detoxify xenobiotics, may lead to higher levels of toxicant exposure in their offspring. Summarizing the results of previous studies, fetuses with maternal *GSTT1* null genotype may be exposed to more PAH in utero. Particularly, exposure in the early period of pregnancy may increase the likelihood of allergic disease after birth. Therefore, we could conclude that the results of our study may be explained by the aforementioned mechanisms and supported by the results of previous studies. However, the RERI was not statistically significant between allergic diseases in 6-month-old infants and maternal urinary PAH metabolites in relation to maternal GST genotypes. Thus, we could not firmly conclude that there was a significant additive interaction between 1-OHP exposure in the early period of pregnancy and maternal *GSTT1* null genotype. In addition, more research on biological plausibility is needed.

To our knowledge, this is the first study to investigate the link between in utero exposure to PAH, maternal *GSTT1* genotypes, and allergic disease onset in infants. Many studies have demonstrated direct links between childhood allergic diseases and the children's own GST genes; however, no studies have identified the association between childhood allergic disorders and maternal *GSTT1* genes.

Another strength of this study is that, it was based on a cohort design with well-collected clinical and environmental exposure data. Such a multi-generational study design has the

potential to provide the required resolution for the examination of early life disease risk factors. Furthermore, since this study was based on a prospective cohort study, it enabled us to limit recall bias. The main advantage of this study was that the effects of prenatal PAH exposure were studied during both the early period of pregnancy and late period of pregnancy. Therefore, our study identified the critical window of prenatal PAH exposure associated with allergic disease risk in 6-month-old infants.

The main limitation of the present study was that the effect of prenatal PAH exposure could not be clearly distinguished from postnatal environmental exposure. However, we addressed this limitation by adjusting the frequency of barbecued, fried, roasted, or grilled beef, pork, and chicken and the concentration of maternal cotinine. Given the early age of the children involved in this study (6 months), neither skin prick tests nor pulmonary function tests could be performed.

Another limitation was that urine measurement of 1-OHP and 2-naphthol at the early period of pregnancy or late period of pregnancy might not reflect the whole exposure during the early period of pregnancy or late period of pregnancy due to the short half-life of 1-OHP and 2-naphthol. In humans, the urinary elimination rate of 1-OHP varies considerably depending on the exposure routes. The half-life of urinary 1-OHP varies from 3.0 to 5.7 hours, 3.7 to 9.9 hours, and 11.5 to 15.0 hours after oral, inhalation, and dermal absorption, respectively [53]. It could have been influenced by smoking, exposure to exhaust gas, or the diet on the day before the measurement. To compensate for this limitation, the cotinine concentration was adjusted in the analysis; however, this method could not have completely ruled out the effects of dietary intake or exhaust gas exposure on the previous day. In addition, when the maternal urine samples were collected, they were not collected at defined weeks of pregnancy and instead they were sampled at various times over the period of the early or late pregnancy. This could affect the interpretation of the results.

Also, we did not find a significant additive interaction between 1-OHP exposure in the early period of pregnancy and a maternal *GSTT1* null genotype. It is acknowledged that studies on gene-environmental interaction require a sample size at least 4 times larger than one required to detect the main effect of the same magnitude [54]. Thus, due to the small sample size of our study, we could not get significant interaction effects in relation to maternal GST genotypes.

Lastly, the final study participants accounted for only 37.8% ( $n = 349$ ) of the initial study population, and this might have led to selection bias. Therefore, we compared the general characteristics of the participants that were included in the analysis versus those of the excluded participants (**Supplementary Table 2**). We found that there were no statistically significant differences between the included and excluded participants and therefore, selection bias could be ruled out.

## CONCLUSION

Our study showed the effect of early prenatal exposure to PAH on the likelihood of allergic disease onset in infants and how this relates to maternal *GSTT1* polymorphisms. However, the underlying mechanisms of these associations still require further exploration in future studies.

## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

Association between maternal GST genotypes and allergic diseases in 6-month-old infants

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### Supplementary Table 2

Characteristics of study participants who were included in our analyses versus excluded

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