이학박사 학위논문

면역계에 미치는 교통소음 영향과 유아기 천식에 미치는 주산기 인자에 관한 연구

Effects of road traffic noise on the immune system and effects of perinatal factor on childhood asthma

울 산 대 학 교 대 학 원

생 명 과 학 과

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Effects of road traffic noise on the immune system and effects of perinatal factor on childhood asthma

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ABSTRACT

Environmental factors affect human health, and in particular, the prevalence of asthma worldwide with a steadily increasing trend. Road traffic noise, passive smoking, and air pollution are among the most important environmental factors.

In the first part, research was performed to investigate the effect of road traffic noise and the noise sensitivity on the immune system. A survey was conducted through questionnaire (ISO/TS 15666) in 172 female subjects in Korea, including 128 from Ulsan and 44 from Seoul. The average noise level was calculated, and blood samples were collected for measurements of cortisol levels, natural killer (NK)/natural killer T (NKT) cell populations, and NK cell activity [through measurements of interleukin-12 (IL-12) and interferon-gamma (INF-γ) concentrations]. Multivariate linear regression analysis of the measured biomarkers according to the road traffic noise level and self-reported noise sensitivity were conducted adjusting for the effects of age, alcohol status, smoking status, regular exercise, and residence period. IL-12 levels increased, whereas the NKT cell population decreased with increasing noise levels. The results further suggested that cortisol levels are more influenced by the subject's sensitivity to noise than to the level of chronic road traffic noise. Therefore, noise appears to have the largest effect on IL-12 levels as well as the population and activity of NKT cells.

In the second part, the effects of perinatal factors and environmental factors on asthma were studied. The study involved 3,770 children (mean age 9.1 years [range 5.68–12.16 years] years; male 51.9%) who were enrolled in the "Elementary School Student Cohort (2009–2014) for Identifying Environmental Factors of Allergic Disease" in the Atopy Environmental Health Center, Ulsan University Hospital (Ulsan, Korea). Subjects were

divided into an asthma group (n=514) and a non-asthma group (n=3256) and examined using questionnaires and laboratory tests. To identify independent/combined risk factors, multivariate and subgroup analyses were performed. Multivariate analyses revealed that early life (< first week) oxygen therapy (adjusted odds ratio [aOR] 1.864 [95% confidence interval [CI] 1.156–3.004) and breastfeeding (aOR 0.763 [95% CI 0.606–0.960]) were two significant perinatal risk factors influencing the development of asthma. Environmental tobacco smoke (ETS) (aOR 1.634 [95% CI 1.298–2.058]) and parental allergic disease (aOR 1.882 [95% CI 1.521–2.328]) were also identified as potent risk factors. Using subgroup analyses, there were combined effects on asthma development between perinatal risk factors (early life oxygen therapy and breastfeeding) and other risk factors (distance to major roadway [traffic-related air pollution], ETS, parental allergic disease, and atopy]). Early life oxygen therapy and breastfeeding were identified as two important perinatal risk factors influencing the development of asthma. Through these studies, it was confirmed that road traffic noise and secondhand smoke are not only a strong independent risk factor for human immune response but also a risk factor for immune response when combined with other influencing factors.

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CHAPTER I

Effects of self-reported sensitivity and road traffic noise levels on the immune system

ABSTRACT

Sensitivity to noise, particularly road traffic noise, can increase cortisol levels and result in changes in immune system biomarkers. Therefore, continuous exposure to noise can have an effect on immune function, hormonal levels, and cardiovascular function, leading to hypertension and stress. The purpose of this study was to investigate the changes in stress- and immune system-related biomarkers according to the self-reported sensitivity to noise and exposure to road traffic noise, to ultimately determine the potential effects of noise on health. A survey was conducted through questionnaire (ISO/TS 15666) sent to 172 female subjects in Korea, including 128 from Ulsan and 44 from Seoul. The average noise level was calculated, and blood samples were collected for measurements of cortisol levels, Natural killer (NK) / Natural killer T (NKT) cell populations, and NK cell activity (through measurements of interleukin-12 (IL-12) and interferon-gamma (INF-γ) concentrations). Multivariate linear regression analysis of the measured biomarkers according to the road traffic noise level and self-reported noise sensitivity was conducted adjusting for the effects of age, alcohol status, smoking status, regular exercise, and residence period. IL-12 levels increased, whereas the NKT cell population decreased with increasing noise levels. The results further suggested that cortisol levels are more influenced by the subject's sensitivity to noise than to the level of chronic road traffic noise. Therefore, noise appears to have the largest effect on IL-12 levels as well as the population and activity of NKT cells. In conclusion, our results suggest that low-level road traffic noise and sensitivity to noise can affect health by causing changes in the immune response through mechanisms other than increased cortisol.

INTRODUCTION

Environmental noise can be defined as the noise emitted from all sources, other than noise at an industrial workplace (1). Several studies have shown that environmental noise such as that due to traffic, aircrafts, and construction can have physiological and psychological consequences (2, 3) such annoyance, sleep disturbance (4), cardiovascular disease (5), hypertension (6) and stress (7, 8). Therefore, there has been much research interest on the potential health effects of exposure to environmental noise.

Through meta- analysis, a small number of studies have shown that noise-induced cardiovascular disease is associated with a higher risk for men than for women and a higher risk for people aged older than 65 years (9).

Recent studies have also reported that sleep quality and noise sensitivity are not related to vascular function or noise sensitivity, but rather that night noise increases the risk of cardiovascular disease due to increased blood pressure in patients and controls (10). Road traffic noise is a particular noise source that affects a large portion of urban populations. Indeed, more than 40% of the populations in European Union countries are exposed to noise of 55 dB or above; 20% are exposed to daytime noise of 65 dB and above, and more than 30% are exposed to nighttime noise of 55 dB or above (1). The World Health Organization stated that noise has negative effects on health by triggering physiological changes such as impairment of hearing function and increases in stress hormones and sensitivity (11, 12).

The most well-known mechanism mediating the response to such stress is the hypothalamic-pituitary- adrenal axis (HPA axis). When the HPA axis receives a signal of a

stress response, corticotropin releasing factor is secreted from the hypothalamus, releasing adrenocorticotropic hormone from the pituitary gland. Adrenocorticotropic hormone then promotes the secretion of cortisol from the adrenal cortex through the blood, which triggers responses to various kinds of stress. The secretion of cortisol in response to stress inhibits the function of the HPA axis to disrupt the secretion of neurohormones and neurotransmitters as well as influencing the endocrine system, thereby disturbing homeostasis of the body, which can induce the development of various stress-related diseases (13). Recently Meyer et al. (14) reported that mental stress and noise exposure could activate inflammatory cytokines such as interleukin (IL)-6 and IL-1β. These cytokines also interact with each other. Extracllular IL-6 induces cortisol from the zona fasciculate of the adrenal cortex, and has been reported to affect the synthesis of cortisol even when the HPA axis is inhibited. Thereby, the immune, endocrine and nervous systems are related to each other.

Thus, environmental noise has direct effects on health as well as indirect effects through the release of stress hormones such as cortisol (15, 16), dopamine (17, 18) and changes of alpha-amylase levels (19).Increased cortisol can further cause changes in the immune system such as cellular proliferation, cytokine secretion, antibody production and cytotoxicity (20, 21). Indeed, one study showed that the activity of natural killer (NK) cells was decreased by increased cortisol (22). NK cells are a leukocyte subset and important components of innate immunity. The innate immune system provides a rapid, non-specific host response against foreign agents such as bacteria, viruses, or tumors before triggering the adoptive immune system (23-25). Innate immunity includes the antigen-presenting cells,

monocytes/macrophages, dendritic cells (DCs), NK cells, and NKT cells.

Moreover, cytokine signaling is essential for intercellular communication in the immune system to mediate and control immune functions. IL-12 is an important cytokine mediating immune responses, which is mainly produced by monocytes, macrophages, and DCs in response to bacterial products, intracellular pathogens, or upon interaction with activated T cells. IL-12 has been shown to play a critical role in the pathogenesis of a variety of immune-related diseases. This cytokine can induce interferon-gamma (IFN-γ) production (26), cell proliferation, and cytotoxicity mediated by NK cells and T cells.

Hence, exposure to environmental noise affects immunity by inducing stress and increasing the secretion of stress hormones. As sensitivity to noise itself will affect the stress response and such sensitivity varies among individuals, the effects and response of exposure to identical environmental noise can show high individual variation.

However, studies on the negative effects of environmental noise, including road traffic noise, on health are still lacking in Korea. Therefore, the aim of our study was to analyze the immune response against noise generated from road traffic as well as the self-reported sensitivity to noise in two metropolitan cities in Korea. Specifically, the level of exposure to road traffic noise was estimated using the noise map generated for Ulsan Nam-gu and Seoul Yangchun-gu, and the changes of immune response parameters according to road traffic noise and sensitivity were statistically analyzed.

MATERIALS AND METHODS

1. Participants and noise measurement

All subjects participated voluntarily and provided written informed consent.

We recruited 1,000 people in Yangcheon-gu (Seoul) and Nam-gu (Ulsan), respectively from July 2015 to December 2015. Participants were stratified according to noise exposure level based on noise map data. Although the residents of Yangcheon-gu are exposed to aircraft and road traffic noise, those of Nam-gu are exposed only to road traffic noise. A total of 336 participants each in Nam-gu, Ulsan and Yangcheon-gu, Seoul finally agreed to the blood test and included in the study for analysis (Fig 1). The questionnaires and blood test results of 336 participants were reviewed. Questionnaires were missing for four subjects, 93 subjects were excluded because of underlying chronic diseases, and 67 subjects were men; thus, 172 female subjects were finally selected. The male participants were excluded because they tend to have more activity in environments outside of the residence target area, which could influence the sensitivity to the environmental noise around the residence. Subjects with any chronic illness (hypertension, hyperlipidemia, stroke, myocardial infarction, angina, arrhythmia, diabetes, and other diseases) were also excluded, because of a potential interference on the influence of noise on the immune response. To examine the level of environmental noise that the participants were exposed to, a three-dimensional noise map generated in 2014 based on the participants' addresses confirmed from the survey was used to calculate the mean noise levels of the buildings the participants reside in as a road traffic noise index. The noise index used in this study was

the day-night average sound level (Ldn), which is a qualitative index of the weighted equivalent day/night noise level that divides a 24h period into 6 am–10 pm and nighttime. Actual noise exposure levels were assessed using a sound level meter (Ulsan: NA-28, Rion, Japan; Seoul: B&K 2250, Brüel & Kjær, Denmark).

2. Questionnaires

The questionnaires included age, residence period, education level, monthly income, alcohol status, smoking status, and exercise status. Education level was divided into a high school degree or below and a community college degree or above. Monthly income was divided into less than 3 million Won and above. Smoking status was divided into current smokers and current non-smokers (past smokers and non-smokers). Alcohol status was divided into current drinker and current non-drinker (past drinker and non-drinker). Exercise status was divided into participants currently exercising regularly and those who were not. To assess the sensitivity to noise, an 11-point visual analogue scale was generated and used in this study based on ISO/TS 15666 (27). All research procedures were approved by the Ulsan University Hospital Institutional Review Board (UUh 2014-08-008-012).

3. Blood sampling and NK cell preparation and flow cytometry

After the subjects were selected, 7 mL of venous whole blood was taken from the respondents between 9.00 am to 12.00 pm. Peripheral blood mononuclear cells (PBMCs) were isolated from all 172 subjects. The NK/NKT cell population was analyzed by

fluorescence-activated cell sorting (FACs) at Green Cross LabCells Corporation (Seoul, Korea) to identify the proportion of $CD45^+CD16^+CD56^+$ cells/$CD45^+$ $CD3^+CD16^+CD56^+$ cells. Cortisol levels were measured by Quantikine enzyme-linked immunosorbent (ELISA) analysis using serum from Green Cross LabCells Corporation. The concentrations of IL-12 and INF-γ in the sera were measured using the Quantikine enzyme-linked immunosorbent (ELISA) kit, according to the manufacturer's instructions (Bio Legend San Diego, CA, USA).

4. Statistical analysis

The statistical analysis was performed with SPSS v.21 for Window (IBM SPSS Inc., Chicago, IL, USA). The data regarding immune response parameters such as cortisol, NK cell population, and cytokines showed positively skewed distributions; thus, logarithmic transformations were performed for these variables to facilitate further statistical analyses under normal data assumptions. Statistical analyses were performed using multiple linear regression and Pearson's correlation analysis. The results were considered statistically significant when $P < 0.05$.

RESULTS

1. General characteristics of the participants

The sociodemographic variables and sensitivity to noise for 128 participants in Ulsan Nam-gu and the 44 participants in Seoul Yangchun-gu are summarized in Table 1.

In both Ulsan and Seoul, the distribution rate of participants was highest at 31.3% and 31.8%, respectively. The average age of all participants in Ulsan was 42.9 years and the average age of all participants in Seoul was 45.6 years. The age group with the lowest participation rate was more than 60 years in Ulsan and Seoul. In the case of Seoul, the subjects in their 20s also showed the lowest participation rate.

The mean age of the participants was 45.1 years and the mean residence period at the current residence was 9.2 years. The mean sensitivity to noise was 5.72. With respect to education level, 49.4% of the participants had a community college degree or below and 50.6% had a community college degree or above. For monthly income, 79.7% of the participants reported earning 3,000,000 Won or more and 20.3% reported earning less than 3,000,000 Won. The frequency of current and non-drinkers was 55.8% and 44.2% respectively, and the frequency of current and non-smokers was 1.7% and 98.3%, respectively. In addition, 52.3% of the participants reported exercising regularly and 47.7% reported that they did not exercise regularly.

2. Correlation among noise levels, noise sensitivity and biomarker

As shown in Table 2, road traffic noise and IL-12 were significantly positively correlated

(r=0.2333). Road traffic noise and the NKT cell distribution rate showed a significant negative correlation (r= 0.214; $P < 0.01$). IL-12 and the NKT distribution were also negatively correlated (r= 0.622), whereas IL-12 and INF-γ showed a significant positive correlation ($P < 0.01$). The change in biomarker for Ldn and noise sensitivity are shown using correlation curves in Fig 2.

3. Immune responses to road traffic noise, noise sensitivity, and Cortisol.

Multivariate linear regression analysis was performed to analyze the relationships among road traffic noise, individual sensitivity, and age-dependent biomarkers, adjusting for the effects of alcohol status, smoking status, regular exercise, and residence period. As shown in Table 3, as the sensitivity level increased by 1 step, the cortisol level increased by 0.032 μg/dL. Moreover, as the road traffic noise increased by 1 dB, the percentage of NKT cells decreased by - 0.038 %, whereas the IL-12 level increased by 0.006 pg/mL. The percentage of NK cells and INF-γ levels were not significantly associated with road traffic noise or sensitivity ($P > 0.05$), and therefore were meaningless as regression coefficients. As shown in Fig 3, the mean value of Ldn and the noise sensitivity interval are shown as correlation curves through the adjusted predicted value of each biomarker.

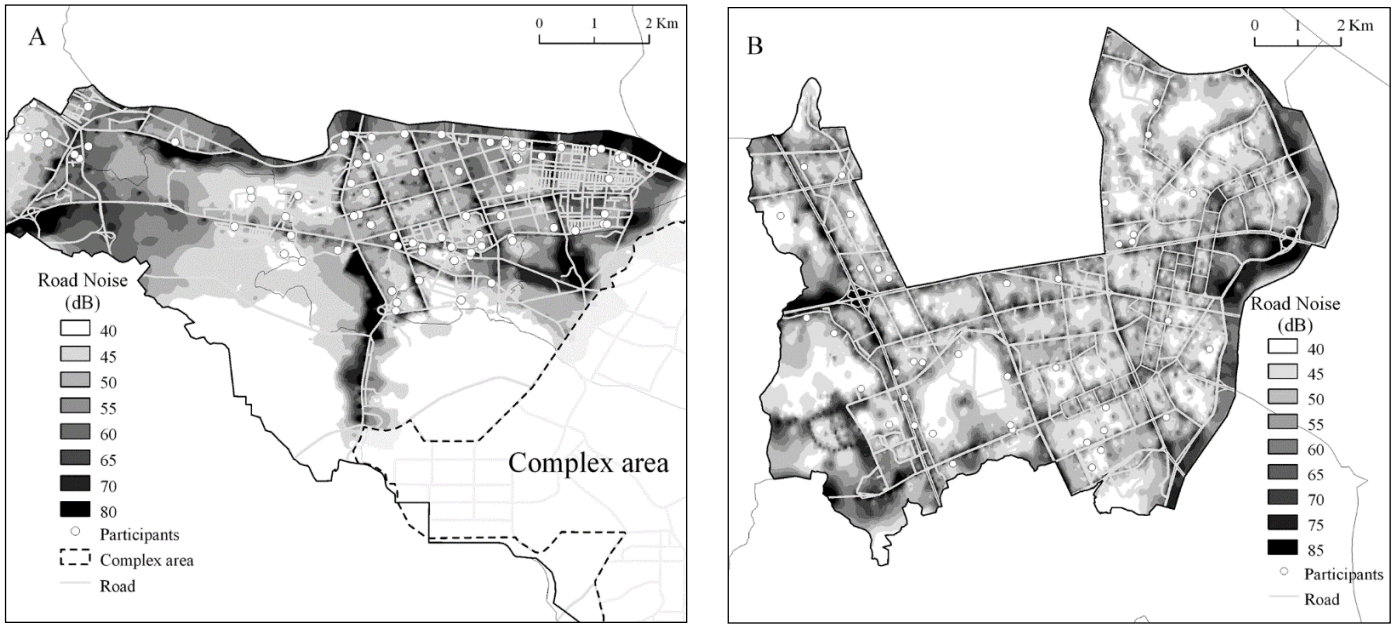


Figure 1. Location of residents in Ulsan (A, Nam-gu), Seoul (B, Yangcheon-gu) in noise map

It shows the noise level of each area and the location of participants in Nam-gu of Ulsan (A) and Yancheon-gu of Seoul (B).

Table 1. General characteristics of the subjects

		Ulsan			Seoul			Total		
		N	**Mean**	**95% CI**	**N**	**Mean**	**95% CI**	**N**	**Mean**	**95% CI**
Age (years)		128	44.9	42.9-46.8	44	45.6	41.8-49.5	172	45.1	43.3-46.8
	20 – 29 years	11 (8.6)			5 (11.4)			16 (9.3)		
	30 – 39 years	29 (22.7)			7 (15.9)			36 (20.9)		
	40 – 49 years	40 (31.3)			14 (31.8)			54 (31.4)		
	50 – 59 years	39 (30.5)			13 (29.5)			52 (30.2)		
	Over 60 years	9 (7.0)			5 (11.4)			14 (8.1)		
Residence period (years)		128	9.6	8.3-10.9	42	7.9	5.5-10.3	170	9.2	8.0-10.3
Noise sensitivity		128	5.4	5.1-5.8	44	6.5	5.9-7.2	172	5.72	5.38-6.05
Education level	High school and less	62			23			85 (49.4)		
	College and more	66			21			87 (50.6)		
Income	Under 3,000,000 (KWR)	16			19			35 (20.3)		
	Over 3,000,000 (KWR)	112			25			137 (79.7)		
Alcohol status	No	48			28			76 (44.2)		
	Yes	80			16			96 (55.8)		
Smoking status	No	125			44			169 (98.3)		
	Yes	3			0			3 (1.7)		
Regular exercise	No	70			12			82 (47.7)		
	Yes	58			32			90 (52.3)		

Unit: Number (percentage)

Table 2. Correlation among noise levels, noise sensitivity and immune response

Variables	Ldn	Noise sensitivity	Cortisol	NK cells	NKT cells	IL-12
Noise sensitivity	-0.103					
Cortisol	0.065	0.138				
NK cells	0.147	0.074	0.057			
NKT cells	-0.214**	0.106	-0.138	-0.120		
IL-12	0.233**	-0.134	0.149	0.007	-0.620**	
INF-γ	0.127	0.017	0.041	-0.167	-0.148	0.251**

**. Correlation is significant at the 0.01 level (2-tailed), Immunologic profiles were transformed by natural log.

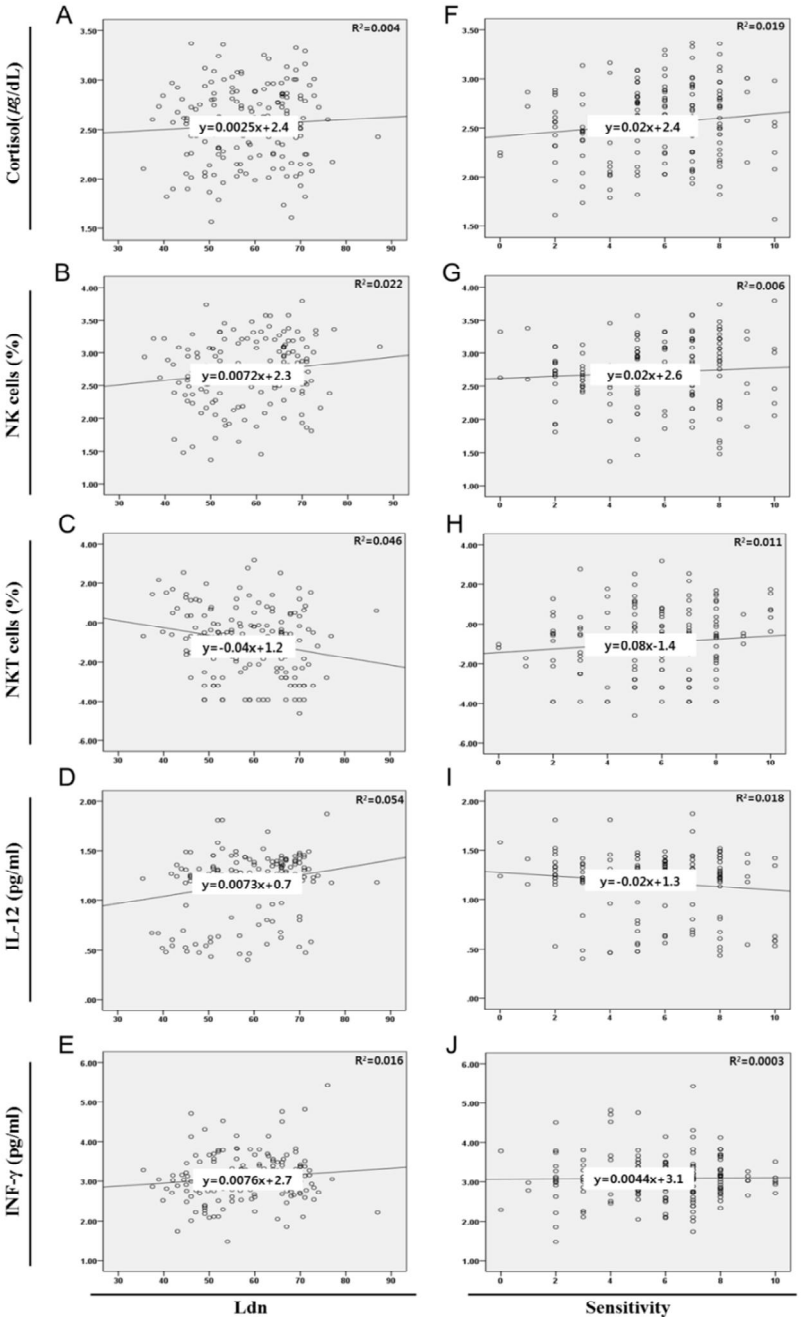


Figure 2. Each real correlation curve for biomarker, Ldn and noise sensitivity

(A-E) It shows each the real correlation curve for Ldn and biomarker such as cortisol (A), NK(B) and NKT cells (C), cytokines (D and E)) and it also shows each the real correlation curve for noise sensitivity and biomarker(F-J).

Table 3. Immune responses to road traffic noise, noise sensitivity, and stress response

Variables			Multivariate analysis		
Dependent	Independent		β±SE	*p-value*	R^2
ln_Cortisol (㎍/dL)	Ldn		0.003±0.003	0.319	0.057
	Sensitivity		0.032±0.014	0.020	
ln_NK cells (%)	Ldn		0.006±0.004	0.111	0.193
	Sensitivity		0.018±0.016	0.263	
ln_NKT cells (%)	Ldn		-0.033±0.014	0.021	0.079
	Sensitivity		0.046±0.063	0.464	
ln_IL-12 (pg/mL)	Ldn		0.006±0.002	0.010	0.097
	Sensitivity		-0.010±0.011	0.365	
ln_INF-γ(pg/mL)	Ldn		0.007±0.005	0.118	0.105
	Sensitivity		0.007±0.020	0.750	

SE: standard error, Immunologic profiles were transformed by natural log. Linear regression models adjusted for age, alcohol consumption, smoking status, regular exercise, residence period.

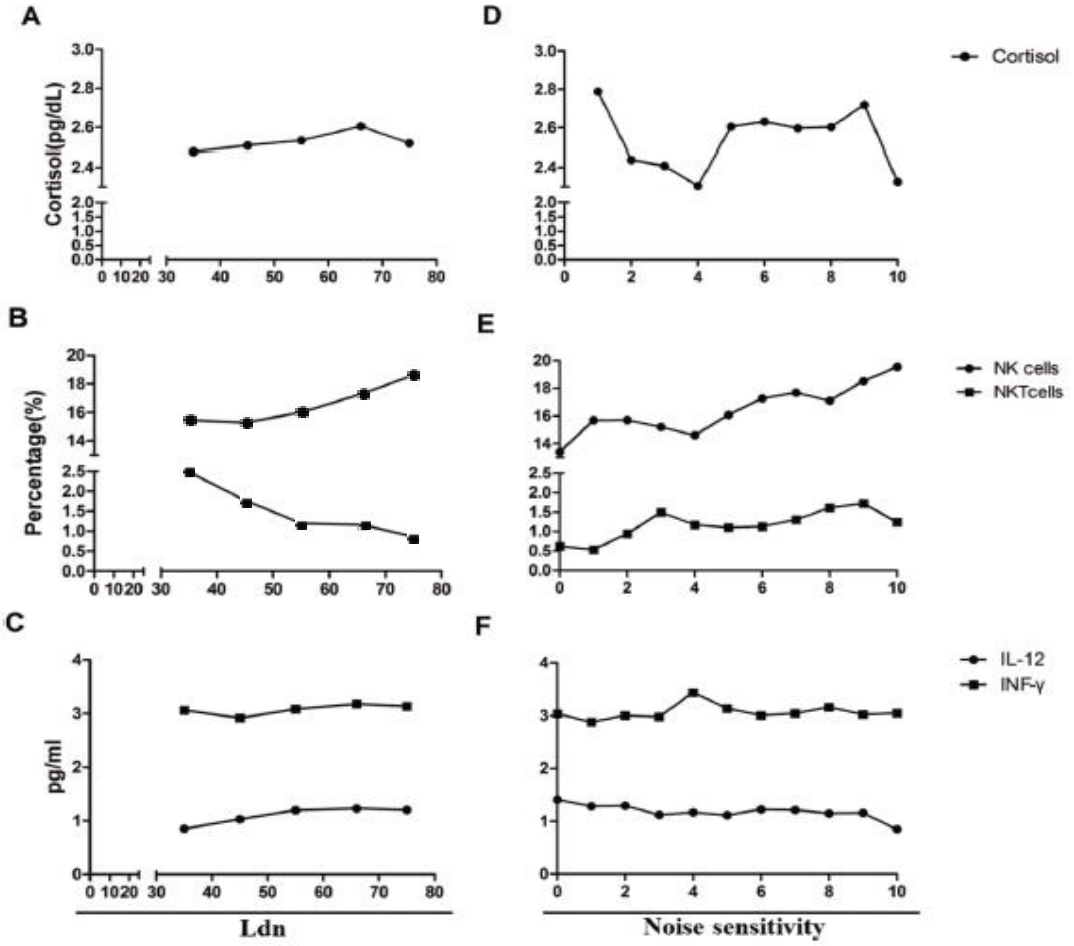


Figure 3. Adjusted predicted value change of biomarker for Ldn and noise sensitivity interval

The values of cortisol (pg/dL), NK and NKT cells (%), cytokines (pg/ml) were adjusted for age, alcohol consumption, smoking status, regular exercise, residence period. The mean values of cortisol (A), NK/NKT cells (B), cytokines (C) for each interval for Ldn are shown, and D-F shows the mean value of each biomarker for the noise sensitivity interval.

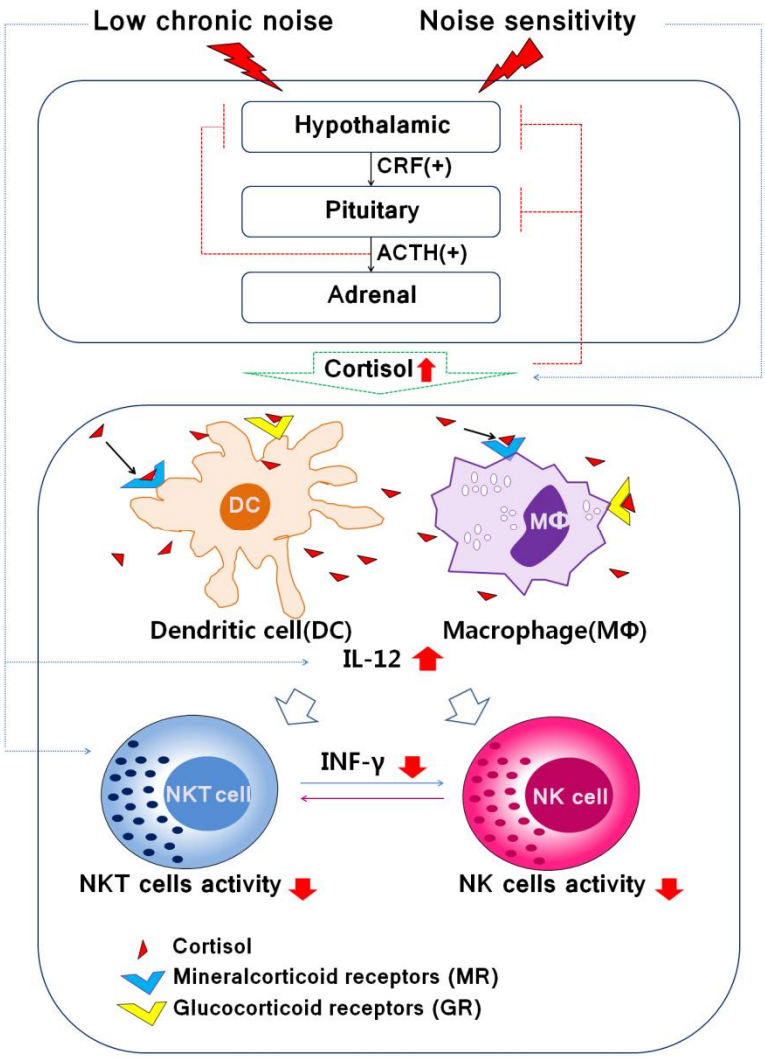


Figure 4. Schematic representation of the potential immune response by exposure noise levels or noise sensitivity

Low chronic noise affects DCs and Macrophages and increases IL-12 concentration, but noise sensitivity increases the concentration of cortisol in the body. Therefore, we expect to decrease INF-γ activity, NKT and NK cells population by Ldn and noise sensitivity, which are two influencing factors.

DISCUSSION

In this study, the correlations among road traffic noise, sensitivity to noise, stress hormones, and immunity-associated factors were investigated.

Previous studies have shown that a high level of noise or sensitivity to noise induces sleep disorders (28, 29), hypertension (30-32), and cardiovascular disease (33, 34).

The result of traffic noise, depression, and anxiety are somewhat limited and somewhat controversial; however, Beutel et al. (35) found that strong noise discomfort was related to higher depression and anxiety in the general population. Participants with a mental health problem may also have higher noise sensitivity and report higher discomfort levels (36).

When exposed to heavy nighttime aircraft, patients with coronary heart disease reported chest pain caused by a typical heart disease (37).

Meover, these consequent increases in the levels of the stress hormone cortisol from noise or sensitivity can have negative effects on health by reducing the activity of NK cells (38, 39).

Duggal et al. (40) suggested that the observed association between NK cell immunesenescence and cortisol was more strongly affected by mental stress rather than by physical stress.

Munzel et al. (41) suggested a mechanism of the noise-triggered activation of the immune system using a mouse model, indicating that noise exposure not only increased levels of noradrenalin (NA), adrenalin (A) and angiotensin II (Ang II), but also increased cortisol thereafter. Increased Ang II has been reported to activate endothelial NADPH oxidase, which causes oxidative stress that can lead to direct scavenging of nitric oxide and enhanced nitric oxide synthatse uncoupling. Since then, the increase in the NADPH oxidase subunit NOX-2 has been reported to increase immune cell activation and infiltration, such as NK cells, myelomonocytic cells, leukocytes, and macrophages/monocytes. Therefore, noise stress

causes blood pressure and vascular dysfunction associated with oxidative stress.

In the present study, the IL-12 and INF-γ levels were positively correlated, suggesting that an increase of cortisol decreases the IL-12 level with a consequent decrease of NK cells and INF-γ, a marker of NKT cell activation. Hence, it is predicted that an increase in cortisol levels would reduce immune function by decreasing the activity of NK cells and NKT cells.

Although there are few studies on the relationship between NKT cells and noise, these cells can be activated in both antigen-dependent and independent manners. Furthermore, because they have pro-inflammatory and immunoregulatory characteristics, they are known to play an important role in autoimmune diseases (42, 43), viral infections (44, 45) and cancer (46).

In this study, the level of road traffic noise showed a positive correlation with IL-12 levels but did not affect the NK cell population. However, the proportion of NKT cells was negatively correlated with road traffic noise.

In addition, multivariate linear regression analysis on the relationships between road traffic noise level or noise sensitivity to immune and stress parameters, controlling for the potentially confounding variables of age (47-49), alcohol status, smoking status, regular exercise, residence period, showed that the increase of noise level increased the IL-12 level but decreased the NKT cell distribution rate, similar to results of the correlation analysis. Because cortisol is considered to be more strongly affected by sensitivity than chronic road traffic noise and road traffic noise does not largely affect cortisol unlike the biological response to > 80 dB noise exposure (11), it appears that the IL-12 level and NKT cell frequency and activity are likely regulated by different mechanisms (11). That is, cortisol increases through the HPA-axis, which is stimulated by a sensitivity response to activate DCs and macrophages, and the chronic noise itself can increase the levels of IL-12, a pro-inflammatory cytokine, as a synergistic effect. However, noise stress will decrease the NKT

cell population to induce a pro-inflammatory response, with negative consequences for health (15).

The limitations of this study are as follows. First, as a cross-sectional study, although correlations could be determined, the cause-and-effect relationship cannot be established from these data. Second, the effects of extreme noise levels such as occupational noise were not considered. Finally, not all potentially confounding variables were controlled in the analysis.

Nevertheless, the clear associations detected in the present study and previous work suggest the importance of carrying out longitudinal studies on the effects of chronic noise on health and to determine the mechanism underlying the immune response to chronic loud noise. Although previous health effect evaluations associated with noise were mostly based on survey results, heart rate variability, stress hormones (e.g., cortisol, norepinephrine, epinephrine), or studies of the activation of NK cells in response to loud noise and noise sensitivity through *in vitro* experiments, clinical studies on a large number of subjects of similar ages should be performed to more objectively analyze the *in vivo* effects of exposure to a low level of environmental noise with respect to the involvement of NK cells, and NKT cells population and activation. Such investigations are expected to provide better indices for future health effect evaluations on the immune response against noise and sensitivity.

Low-level environmental noise and sensitivity to noise are likely to have negative effects on health by triggering changes in the immune response through a mechanism distinct from an increase in cortisol (Fig 4).

CHAPTER II

Perinatal factors and the development of childhood asthma

ABSTRACT

Asthma is caused by a combination of environmental factors and genetic predisposition and is the most common chronic disease in children. However, the rapid increase in the prevalence of asthma over the past 30 years suggests that environmental factors play an even more important role in its development than initially believed. Perinatal environmental risk factors are also suspected to have a significant impact on the development of asthma, sufficiently powered studies have not yet been performed. The aim of the present study was to evaluate whether perinatal factors and other risk factors have an independent or combined effect on the development of asthma. This study involved 3,770 children (mean age 9.1 years [range 5.68–12.16 years] years; male 51.9%) who were enrolled in the "Elementary School Student Cohort (2009–2014) for Identifying Environmental Factors of Allergic Disease" in the Atopy Environmental Health Center, Ulsan University Hospital (Ulsan, Korea). Subjects were divided into an asthma group (n=514) and a non-asthma group (n=3256) and examined using questionnaires and laboratory tests. To identify independent/combined risk factors, multivariate and subgroup analyses were performed. Multivariate analyses revealed that early life (< first week) oxygen therapy (adjusted odds ratio [aOR] 1.864 [95% confidence interval [CI] 1.156–3.004) and breastfeeding (aOR 0.763 [95% CI 0.606–0.960]) were two significant perinatal risk factors influencing the development of asthma. Environmental tobacco smoke (ETS) (aOR 1.634 [95% CI 1.298–2.058]) and parental allergic disease (aOR 1.882 [95% CI 1.521–2.328]) were also identified as potent risk factors. Using subgroup analyses, there were combined effects on asthma development between perinatal risk factors (early life oxygen therapy and breastfeeding) and other risk factors (distance to major roadway [traffic-related air pollution], ETS, parental allergic disease, and atopy]). Early life oxygen therapy and breastfeeding were identified as two important perinatal risk factors influencing the development of asthma. Furthermore, these perinatal factors have combined effects with

other risk factors (ETS, traffic-related air pollution, parental allergic disease, and atopy) on the development of asthma.

INTRODUCTION

Asthma is a serious health problem worldwide. There are about 334 million patients with asthma globally, and its prevalence ranges from 1% to 16% (1). Further, asthma is the most common chronic disease in children, and the prevalence of childhood asthma has increased over the past 30 years [1, 2] (1, 2).

Asthma is caused by a combination of genetic predisposition and environmental factors. Although there is clear evidence that genetic predisposition is involved in the onset of asthma, the rapid increase in its prevalence over the past 30 years suggests that environmental factors play a more crucial role[2, 3](2, 3). Previously known environmental risk factors for asthma development include environmental tobacco smoke (ETS), air pollution, westernized living conditions, dietary habits, allergen exposure, obesity, pet breeding, and the use of antibiotics in the first year of life [2, 4](2, 4).

To date, however, most studies evaluating risk factors for the development of asthma have been limited to life events occurring long after birth. In fact, from the moment of conception, humans are affected by various environmental factors. Fortunately, active researches are now underway investigating various environmental risk factors for asthma development that are present in utero or during the perinatal period [5-7](5-7). The present study focused on perinatal factors.

The roles of perinatal risk factors in the occurrence of asthma have yet to be firmly established. Although preterm birth (8-10), low birth weight (11, 12), cesarean section (11, 12), transient tachypnea of the newborn(13) and meconium aspiration syndrome (14) have been reported to be associated with development of asthma, results have not been consistent and further studies are needed. Recently, interesting studies investigating synergistic mechanisms of perinatal factors in the development of asthma have been published (8-10). According to the studies, perinatal factors alone have little to no impact on the development

of asthma, or confer only a slight increase in risk. However, in the presence of associated risk factors, such as “prematurity + chorioamnionitis” / “prematurity + atopy” / “prematurity + maternal smoking during pregnancy”, the risk for asthma was dramatically increased (8-10). In other words, perinatal factors alone (e.g., prematurity) were not potent risk factors for asthma development. However, simultaneous exposure to other risk factors (e.g., atopy) could effect a potent synergism in asthma development.

With the above considerations, we hypothesized that perinatal factors are associated with the development of asthma; however, this association would depend on exposure to other risk factors. The objective of the present study was to evaluate whether perinatal factors and other risk factors have an independent or combined effect on the development of asthma.

METHODS

1. Study participants

Subjects included 3,770 children (mean age 9.1 years [range 5.68–12.16 years]; male, 51.9%) who were enrolled in the "Elementary School Students Cohort (2009-2014) for Identifying Environmental Factors of Allergic Disease" in the Atopy Environmental Health Center, Ulsan University Hospital (15, 16). The study participants were recruited from 3 elementary schools in Ulsan. Parental consent was obtained for enrollment of study participants. This study was approved by the Institutional Ethics Review Committee of Ulsan University Hospital. At the time of enrollment, the subjects were examined with detailed questionnaires and laboratory tests. The questionnaires used in this study included ISAAC (International society of asthma and allergy of children) survey items (17). Various asthma-related outcomes were gathered using following questions: asthma ever diagnosis by: "Has your child ever been diagnosed with asthma by doctor?"; ever wheezing by: "Has your child ever experienced wheezing or whistling in chest?"; and current (<1 year) wheezing by: "Has your child experienced wheezing or whistling in chest in the recent 12 months?". In the present study, asthma was defined as physician-diagnosed asthma (i.e., ever diagnosis) OR wheeze in the prior year (i.e., current wheezing) (18-21). Accordingly, subjects were divided into an asthma group (n=514) and a non-asthma group (n=3,256). Demographic information, including sex, age, height, and weight, was collected. Socioeconomic parental data, including monthly household income, parental education, birth order, and parental asthma and allergic diseases, were also gathered. As perinatal factors, we gathered birth weight (low birth weight [< 2.5 kg] or not), delivery method (cesarean section or vaginal delivery), gestational age (preterm [<37 weeks] or term), oxygen therapy in the first week after birth, breastfeeding (any or not at all), and history of general anesthesia before 1 year of age. These data were collected using the following questions: Was your child low birth weight at birth?; What is the delivery method of your

child?; Was your child premature?; Did your child undergo oxygen therapy in first week after birth?; Did your child consume breast milk (including colostrum)?; Has your child ever undergone general anesthesia before one year of age? Any current exposure to ETS, distance to a major roadway, and exposure to traffic exhaust were obtained as environmental factors. Any current exposure to ETS was identified using the following question: "Is there any chance that your child is exposed to someone else's cigarette smoke?". Distance to major roadway and exposure to traffic exhaust were identified as parameters of traffic-related air pollution. Each of the above items was examined using the following questions: "How far is the nearest major roadway for city bus from the house in which you live?"; "How much traffic do you have on the adjacent roads described above?" Blood levels of eosinophils and immunoglobulin E (IgE) were examined. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) data were collected using forced spirometry (22) and percent of predicted values were calculated (23). The presence of atopy was confirmed using the allergic skin reaction test. The allergens examined included *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, tree pollen mixtures, grass pollen mixtures, ragweed, mugwort, cat fur, dog fur, cockroach, aspergillus, alternaris, and other fungus mixtures (Allergopharma®, Darmstadt, Germany). If any positive response was found in the skin test, the subject was defined as having atopy (24).

2. Statistical analysis

SPSS version 21 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. The Independent t-test and chi-squared test was used to analyze basal differences. Univariate and multivariate analyses were performed to determine possible relationships between the variables and asthma development. Multivariate logistic regression analyses were performed using variables found to be significant (i.e., $P < 0.05$) in the univariate analysis, and those

reported to be associated with asthma development in previous studies. Age, sex, body mass index (BMI), ETS, air pollution factor (distance to major roadway), monthly household income, parental education level, birth order, presence of parental allergic disease, delivery method, low birth weight, prematurity, oxygen therapy in first week after birth, breastfeeding, eosinophil and IgE levels, FEV_1, and atopy were adopted for adjustment (2, 4). In addition, the significant perinatal factors, identified as independent risk factors in multivariate analyses, were further investigated by subgroup analyses to identify combined effect with other risk factors on various asthma-related outcomes (such as overall asthma development, ever diagnosis of asthma, ever wheezing, ever diagnosis AND current wheezing, and current wheezing). In subgroup analyses, adjustments were made to the same variables in the above multivariate analysis except variables needed to differentiate subgroups. $P < 0.05$ was considered to be statistically significant in all analyses.

RESULTS

1. Characteristics of the study participants

Table 1 shows the characteristics of the study participants. Demographically, the asthma group shows that the mean age was lower; male sex was more common; and BMI was higher. Among perinatal factors, there were no significant differences in cesarean section, low birth weight, prematurity, breastfeeding, and general anesthesia before one year of age between the two groups. However, oxygen therapy in the first week after birth was significantly higher in the asthma group. Among environmental factors, the asthma group had significantly higher exposure to ETS. There were no differences in distance to major roadways and exposure to traffic exhaust between the asthma and non-asthma groups. Among socioeconomic/parental factors, parental asthma and parental allergic disease were significantly associated with asthma. Additionally, there was a higher prevalence of asthma in first-born children. Monthly household income and parental education did not differ between the two groups. Laboratory findings revealed that the asthma group had higher IgE and eosinophil levels. In addition, the asthma group had more atopy and lower lung function.

2. Independent risk factors for asthma development

Table 2 is the results of multivariate analysis for the presence of asthma. Younger age and higher BMI were independently associated with the development of asthma. Among perinatal factors, oxygen therapy in the first week after birth demonstrated a high independent association with asthma development, and breastfeeding had a significant preventive effect for asthma development. Among environmental factors, exposure to ETS demonstrated a high independent association with asthma development. Among socioeconomic / parental factors, parental allergic disease and the first-born child were independently associated with

asthma development. Among laboratory factors, atopy, eosinophil levels, and FEV_1 were significantly associated with asthma. Male sex, distance to major roadway, monthly household income, parental education, cesarean section, prematurity, low birth weight, and elevated IgE levels, which have been suggested as risk factors for asthma in previous studies, were not identified as independent risk factors for asthma in this study.

3. Combined effects of perinatal factors and other risk factors on various asthma-related outcomes

In addition, subgroup analyses were performed to know the combined effects on asthma-related outcomes between two independent perinatal factors (early life oxygen therapy [oxygen therapy in the first week after birth] and breastfeeding) and other risk factors (such as ETS, distance to major roadway, parental allergic disease, and atopy).

Early life oxygen therapy (+) showed significant combined effects with ETS (+) on "overall asthma development"," ever wheezing", "ever diagnosis AND current wheezing", and "current wheezing": when combined, in particular, the OR of "current wheezing" showed the most prominent increase. Early life oxygen therapy (+) AND living < 100 m from major roadway also increased "current wheezing". Early life oxygen therapy/ parental allergic disease and early life oxygen therapy/ atopy also showed combined effects on various asthma-related outcomes (Table 3 and Figure 1).

Breastfeeding (–) also showed combined effects with ETS (+) on "overall asthma development"," ever wheezing", "ever diagnosis AND current wheezing", and "current wheezing": when combined, in particular, the OR of "current wheezing" showed the most prominent increase. Breastfeeding/ living < 100 m from major roadway, breastfeeding/ parental allergic disease and breastfeeding/ atopy also showed combined effects on various asthma-related outcomes (Table 4 and Figure 2).

Table 4. Characteristics of the study participants

Characteristic	Non-asthma (n=3256)	Asthma (n=514)	*P-value*
Demographic factors			
Age, year	9.1 ± 1.7	8.7 ± 1.6	0.001
Male	1663 (51.1)	295 (57.4)	0.007
Height, cm	135.3 ± 11.3	134.6 ± 11.1	0.228
Weight, kg	33.4 ± 9.9	34.0 ± 11.0	0.199
Body mass index, kg/m^2	17.9 ± 3.0	18.3 ± 3.4	0.006
Perinatal factors			
Delivery by cesarean section	1283 (39.6)	212 (41.5)	0.430
Low birth weight (<2.5kg)	160 (5.0)	30 (5.9)	0.374
Prematurity (<37weeks)	154 (4.8)	31 (6.1)	0.205
Oxygen therapy in first week after birth	139 (4.3)	37 (7.2)	0.003
Breastfeeding	2396 (74.1)	364 (71.5)	0.211
General anesthesia before 1 year of age	90 (2.8)	17 (3.3)	0.483
Environmental factors			
Environmental tobacco smoking (ETS)	660 (22.1)	221 (29.3)	< 0.001
Distance to major roadway <100 meters	1947 (60.7)	312 (61.1)	0.882
Exposure to traffic exhaust	1682 (52.4)	265 (51.9)	0.826
Parental and socioeconomic factors			
Household income < KRW 3,000,000/month	1052 (33.0)	167 (32.8)	0.940
Maternal education level ≤ High school	1394 (43.7)	211 (41.4)	0.333
Paternal education level ≤ High school	1221 (38.0)	185 (36.2)	0.442
Parental education level ≤ High school	985 (30.8)	145 (28.3)	0.280
First child	1618 (49.7)	289 (56.3)	0.016
Maternal asthma	71 (2.3)	35 (7.6)	< 0.001
Any maternal allergic disease	1051 (33.1)	218 (44.9)	< 0.001
Paternal asthma	41 (1.3)	19 (4.2)	< 0.001
Any paternal allergic disease	897 (28.2)	210 (42.6)	< 0.001
Parental asthma	107 (3.5)	53 (11.8)	< 0.001
Any parental allergic disease	1549 (48.4)	324 (65.1)	< 0.001
Laboratory factors			
Eosinophil, %	3.08 ± 2.41	3.92 ± 2.92	< 0.001
Eosinophil, count/μL	204.8 ± 170.5	271.6 ± 214.4	< 0.001
LogIgE (IU/mL)	1.9 ± 0.6	2.1 ± 0.6	< 0.001
FEV1 (L)	1.54 ± 0.41	1.49 ± 0.41	0.035
FEV1 (% of predicted value)	83.3 ± 12.0	81.7 ± 11.6	0.006
FVC (L)	1.75 ± 0.48	1.72 ± 0.48	0.167
FVC (% of predicted value)	83.1 ± 11.6	82.3 ± 11.3	0.138
FEV1(L)/FVC(L)	0.88 ± 0.08	0.87 ± 0.78	0.043
Atopy	1604 (50.3)	317 (63.3)	< 0.001

Data were presented as n (%) or mean ± standard deviation.

FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; KRW, South Korean Won

Table 5. Multivariate analysis for independent risk factors on asthma development

Variable	Unadjusted				Adjusted			
	OR	**95% CI**		***P-value***	**OR**	**95% CI**		***P-value***
Age (year)	0.906	0.856	0.959	0.001	0.852	0.796	0.913	< 0.001
Male	1.290	1.069	1.557	0.008	1.172	0.950	1.446	0.138
Body mass index (kg/m^2)	1.046	1.016	1.076	0.003	1.077	1.039	1.115	< 0.001
Delivery by cesarean section	1.079	0.893	1.305	0.430	1.051	0.850	1.300	0.643
Low birth weight (<2.5kg)	1.200	0.803	1.793	0.374	0.716	0.379	1.352	0.303
Prematurity (<37weeks)	1.292	0.868	1.923	0.206	0.919	0.484	1.747	0.797
Oxygen therapy in first week after birth	1.740	1.195	2.532	0.004	1.864	1.156	3.004	0.011
Breastfeeding	0.876	0.711	1.078	0.212	0.763	0.606	0.960	0.021
Environmental tobacco smoking	1.575	1.228	1.933	< 0.001	1.634	1.298	2.058	< 0.001
Distance to major roadway <100 meters	1.015	0.838	1.229	0.882	0.940	0.763	1.157	0.559
Household income < KRW 3,000,000/month	0.992	0.813	1.211	0.940	0.944	0.752	1.185	0.620
Parental education level ≤ High school	1.120	0.911	1.377	0.281	1.147	0.903	1.458	0.262
First child	1.292	1.071	1.558	0.007	1.260	1.025	1.549	0.028
Any parental allergic disease	1.986	1.631	2.418	< 0.001	1.882	1.521	2.328	< 0.001
Eosinophil (per %)	1.002	1.001	1.002	< 0.001	1.079	1.036	1.124	< 0.001
LogIgE (IU/mL)	1.650	1.412	1.928	< 0.001	1.221	0.994	1.500	0.057
FEV_1 (% of predicted value)	0.989	0.982	0.997	0.006	0.989	0.980	0.998	0.013
Atopy*	1.706	1.404	2.072	< 0.001	1.271	1.001	1.615	0.049

FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; KRW, South Korean Won; OR, odds ratio; CI, confidence interval

Table 6. Subgroup analysis for identifying various asthma-related outcomes: combined association between oxygen therapy in first week after birth and other risk factors

Combined factors		Asthma [ever diagnosis OR current wheezing]	Asthma ever diagnosis	Ever wheezing	Ever diagnosis AND current wheezing	Current wheezing
Oxygen	ETS					
(–)	(–)	1	1	1	1	1
(–)	(+)	1.681 (1.326–2.131)*	1.479 (1.128–1.940)*	1.570 (1.262–1.953)*	1.684 (1.328–2.134)*	2.150 (1.556–2.970)*
(+)	(–)	2.107 (1.233–3.600)*	1.663 (0.902–3.065)	2.021 (1.232–3.314)*	2.006 (1.177–3.418)*	3.150 (1.602–6.191)*
(+)	(+)	2.324 (1.043–5.181)*	1.685 (0.644–4.411)	2.084 (1.002–4.338)*	2.238 (1.005–4.982)*	3.696 (1.396–9.786)*
	<100m from road					
(–)	(–)	1	1	1	1	1
(–)	(+)	0.945 (0.762–1.172)	0.883 (0.694–1.123)	0.913 (0.751–1.109)	1.128 (0.712–1.785)	1.180 (0.860–1.620)
(+)	(–)	1.964 (0.970–3.974)	1.786 (0.824–3.872)	2.055 (1.086–3.892)*	3.863 (1.263–11.818)*	2.579 (1.031–6.448)*
(+)	(+)	1.704 (0.944–3.076)	1.167 (0.576–2.363)	1.487 (0.857–2.578)	2.497 (0.841–7.416)	3.087 (1.475–6.459)*
	Parental allergic disease					
(–)	(–)	1	1	1	1	1
(–)	(+)	1.911 (1.534–2.382)*	1.954(1.522–2.510)*	1.676 (1.376–2.040)*	1.828 (1.140–2.930)*	1.666 (1.214–2.286)*
(+)	(–)	2.194 (1.055–4.563)*	1.051(0.382–2.892)	2.369 (1.248–4.494)*	1.406 (0.277–7.152)	3.407 (1.412–8.219)*
(+)	(+)	3.274 (1.841–5.824)*	3.339(1.783–6.252)*	2.519 (1.461–4.341)*	6.372 (2.496–16.263)*	3.717 (1.764-7.831)*
	Atopy					
(–)	(–)	1	1	1	1	1
(–)	(+)	1.213 (0.948–1.551)	1.096 (0.831–1.446)	1.251 (1.001–1.562)*	0.254(0.099–0.650)	1.364 (0.950–1.959)
(+)	(–)	1.166 (0.532–2.553)	0.864 (0.332–2.250)	1.754 (0.941–3.271)	–	1.674 (0.569–4.924)
(+)	(+)	3.042 (1.663–5.564)*	2.260 (1.146–4.455)*	2.269 (1.279–4.025)*	0.952(0.123–7.381)	4.379 (2.082–9.210)*

Multiple logistic regression analyses were performed, and adjusted ORs (95% CIs) are presented.

**P* value < 0.05. A relation is significant if the CI does not include 1.

ETS, environmental tobacco smoking; oxygen, oxygen therapy in first week after birth

Figure 5. Subgroup analysis for combined associations between oxygen therapy in the first week after birth and other risk factors: asthma (ever diagnosis or current wheezing; left) and current wheezing (right). aOR, adjusted odds ratio; CI, confidence interval; ETS, environmental tabacco smoke.

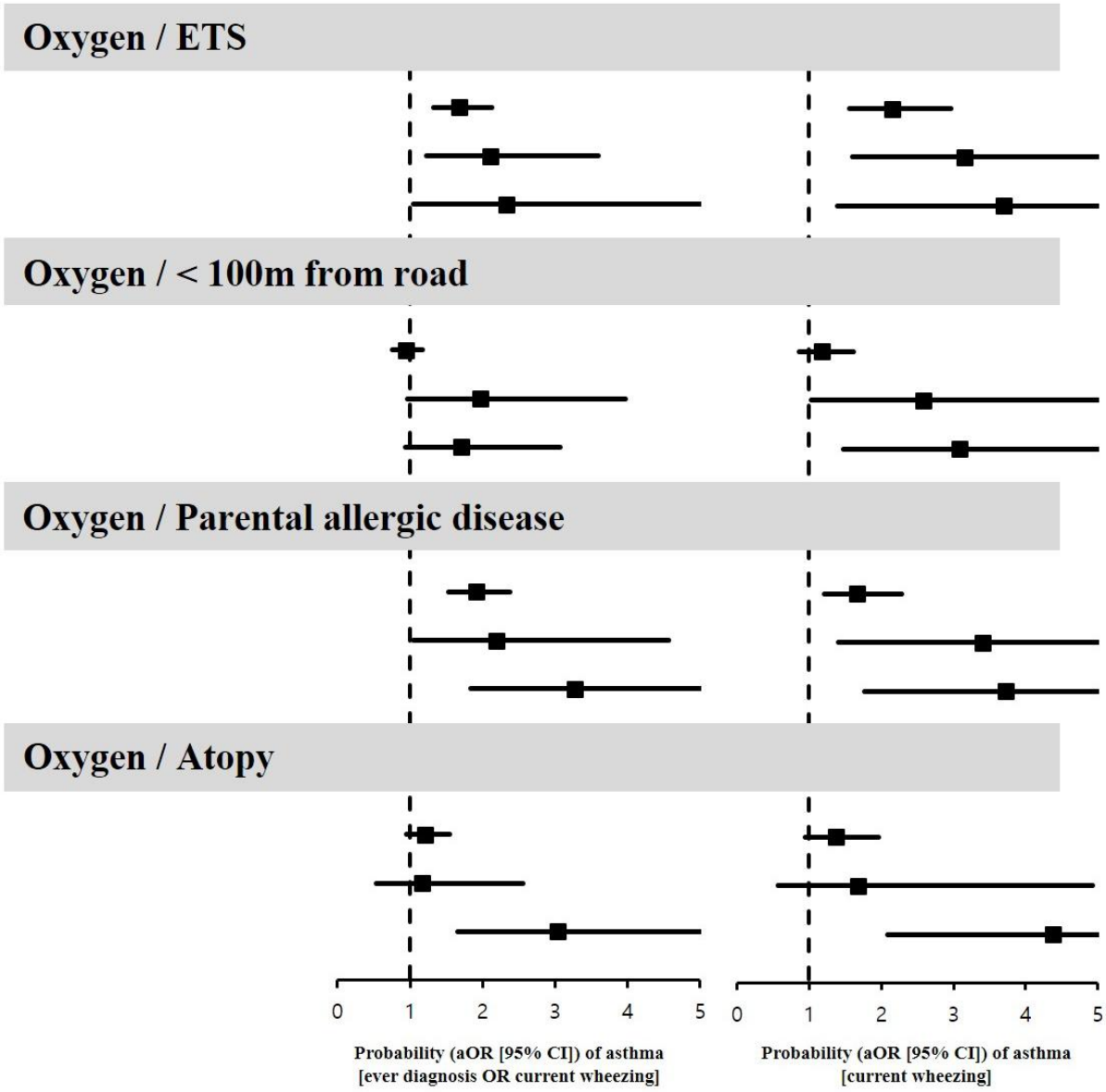


Table 7. Subgroup analysis for identifying various asthma-related outcomes: combined association between breastfeeding and other risk factors

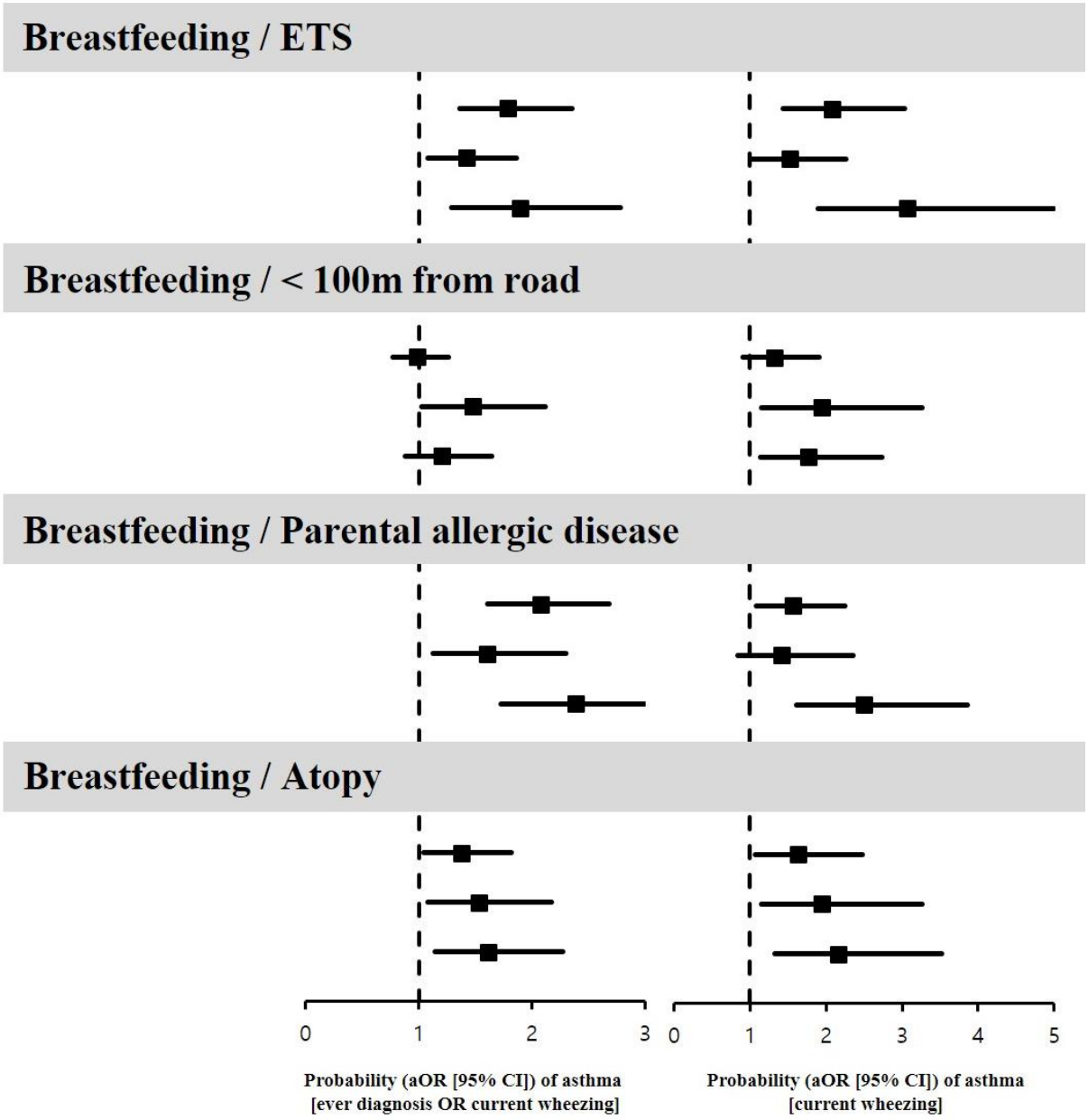
Combined factors		Asthma [ever diagnosis OR current wheezing]	Asthma ever diagnosis	Ever wheezing	Ever diagnosis AND current wheezing	Current wheezing
Breastfeeding	ETS					
(+)	(–)	1	1	1	1	1
(+)	(+)	1.791 (1.364–2.351)*	1.647 (1.213–2.236)*	1.846 (1.439–2.367)*	2.036 (1.191–3.480)*	2.083 (1.429–3.038)*
(–)	(–)	1.423 (1.086–1.864)*	1.297 (0.955–1.760)	1.545 (1.213–1.969)*	1.324 (0.738–2.376)	1.528 (1.032–2.260)*
(–)	(+)	1.895 (1.291–2.781)*	1.349 (0.845–2.151)	1.578 (1.097–2.270)*	2.662 (1.288–5.502)*	3.073 (1.890–4.995)*
	<100m from road					
(+)	(–)	1	1	1	1	1
(+)	(+)	0.990 (0.775–1.266)	0.899 (0.685–1.179)	0.843 (0.676–1.053)	1.175 (0.703–1.963)	1.317 (0.912–1.902)
(–)	(–)	1.473 (1.023–2.121)*	1.244 (0.825–1.877)	1.151 (0.820–1.616)	1.643 (0.777–3.477)	1.937 (1.149–3.263)*
(–)	(+)	1.208 (0.885–1.649)	0.972 (0.681–1.390)	1.182 (0.897–1.558)	1.360 (0.780–2.611)	1.760 (1.130–2.741)*
	Parental allergic disease					
(+)	(–)	1	1	1	1	1
(+)	(+)	2.079 (1.611–2.683)*	2.424 (1.808–3.250)*	1.514 (1.210–1.894)*	2.300 (1.326–3.988)*	1.565 (1.088–2.252)*
(–)	(–)	1.609 (1.124–2.304)*	1.722 (1.140–2.603)*	1.096 (0.789–1.524)	1.817 (0.833–3.965)	1.409 (0.841–2.361)
(–)	(+)	2.389 (1.723–3.314)*	2.154 (1.462–3.173)*	2.213 (1.664–2.944)*	2.568 (1.287–5.122)*	2.494 (1.610–3.863)*
	Atopy					
(+)	(–)	1	1	1	1	1
(+)	(+)	1.378 (1.044–1.820)*	1.234 (0.905–1.683)	1.291 (1.005–1.658)*	1.338 (0.726–2.465)	1.626 (1.066–2.480)*
(–)	(–)	1.534 (1.081–2.175)*	1.321 (0.886–1.969)	1.394 (1.017–1.912)*	1.466 (0.643–3.343)	1.936 (1.150–3.261)*
(–)	(+)	1.614 (1.146–2.273)*	1.271 (0.858–1.882)	1.593 (1.170–2.169)*	1.681 (0.827–3.419)	2.162 (1.328–3.519)*

Multiple logistic regression analyses were performed, and adjusted ORs (95% CIs) are presented.

**P* value < 0.05. A relation is significant if the CI does not include 1.

ETS, environmental tobacco smoking

Figure 6. Subgroup analysis for combined association between breastfeeding and other risk factors: asthma (ever diagnosis or current wheezing; *left*) and current wheezing (*right*). aOR, adjusted odds ratio; CI, confidence interval; ETS, environmental tobacco smoke.



DISCUSSION

In the present study, early life (< first week) oxygen therapy and breastfeeding were found to be two significant perinatal risk factors influencing the development of asthma. ETS and parental allergic disease were also identified as other potent risk factors. In addition, we found the asthma-developing combined associations between perinatal risk factors (early life oxygen therapy and breastfeeding) and other risk factors (distance to major roadway [traffic-related air pollution], ETS, parental allergic disease, and atopy).

Oxygen therapy in the first week after birth is a newly identified risk factor for asthma development that has not been previously reported, suggesting that lung damage in early life sufficiently severe to require oxygen therapy is associated with future asthma. Childhood asthma associated with early life oxygen therapy may be explained by two mechanisms. First, it is a deterioration of lung function due to underlying diseases that require oxygen therapy. Typical conditions that require oxygen therapy in the first week after birth are respiratory distress syndrome, transient tachypnea of newborn, and meconium aspiration syndrome. There have been reports of decreased lung function in adulthood in these diseases (13, 14). Second is lung injury caused by oxygen itself, given that it is well established that oxygen could be toxic, especially to the lung and retina (25, 26). It has been suggested that external oxygen induces the generation of mitochondrial reactive oxygen species (ROS), which is a key component in the mechanism of oxygen toxicity, ROS could induce alveolar and airway injury and inflammation (27-29).

In the prese Probability (aOR [95% CI]) of asthma [ever diagnosis OR current wheezing] Probability (aOR [95% CI]) of asthma [current wheezing]
development, a resu

to reduce the number of clinically significant respiratory tract infections in infants and, would therefore, be expected to reduce wheezing associated with infections. In a systematic review and meta-analysis, the strongest protective effect of breastfeeding was observed in the 0 to 2

years of age group, for both "asthma ever" and "recent asthma", and was consistent regardless of duration or exclusivity of breastfeeding (34).

Other perinatal factors, such as cesarean section, low birth weight, and prematurity, were also evaluated for their effects on asthma development. However, we could not find independent associations with these factors. These perinatal factors have also shown conflicting results in previous studies (8-12). In our opinion, it is thought that rather than the perinatal diseases (or conditions) themselves, the severity of the disease caused by those (which enough to receive oxygen therapy) is associated with the development of future asthma.

In the current study, we found that asthma develops through the combined effects of perinatal risk factors (i.e., early life oxygen therapy and breastfeeding) and other risk factors (such as distance to a major roadway [traffic-related air pollution], ETS, parental allergic disease, and atopy). Traffic-related air pollution was identified by the distance from a major roadway. Road traffic is a major cause of particulate matter and noxious gases (nitrogen dioxide and sulfur dioxide) (35-37). Several studies have found increased risks for asthma or asthma symptoms in children who live near roadways with high traffic counts (38-43). The mechanisms by which traffic emissions cause asthmatic airway inflammation are only partially understood; however, it is known that both gases and particulates from traffic exhaust can induce a lower airway inflammatory response (44-46). Further, we found that the presence or absence of air pollution factor (living close to a busy major roadway) enhance early life oxygen therapy related asthma developing risk and breastfeeding related asthma preventive effect.

ETS is a leading cause of indoor air pollution (47). Exposure to ETS is a particularly important factor because it is common in children (48). Recently, the regulation of smoking in public places has reduced the prevalence of ETS in society as a whole. However, ETS in

the household continues, and almost one-half of children are exposed to secondhand smoke in the home (48). ETS reduces pulmonary function and increases airway hyper-responsiveness in children, leading to asthma occurrence and aggravation (1, 2). In the current study, we found that exposure to ETS was a very potent risk factor for asthma development. In addition, similar to traffic-related air pollution factors, ETS also showed combined effects on early life oxygen therapy-related asthma development risk and the preventive effect of breastfeeding. With this study, we also evaluated various asthma-related outcomes by subgroup analyses. In the data of ETS/early life oxygen, ETS/breastfeeding, 100m from road/early life oxygen, and 100m from road/breastfeeding, ETS (+) and living <100m from road significantly increased the aORs of current wheezing rather than ever wheezing in terms of perinatal factors (early life oxygen and breastfeeding). Likewise, ETS (-) and living ≥ 100m from road significantly decreased the aORs of current wheezing rather than ever wheezing. This tendencies were less evident in subgroups of parental allergic disease/early life oxygen, parental allergic disease/breastfeeding, atopy/early life oxygen, and atopy/breastfeeding. This might be because ETS and traffic-related air pollution (<100m from road) are the present risk factors, whereas parental allergic disease and atopy are the risk factors from past to present.

The present study has many limitations. First, our study is the result of retrospective analyses of the prospective cohort data. Therefore, there could be many biases in selections of case/control group, measurement of various variables and information (recall bias) in perinatal and other environmental risk factors. Secondly, our asthma definition could overestimate asthma prevalence since we included wheeze in the prior year. The cause of wheezing could be other disease such as bronchiolitis (the frequency might not be high since subjects were school age). Thirdly, we could not identify the precise perinatal disease or disease severity of subjects who received oxygen therapy. Lastly, since ETS and air pollution are current risk factors, there is a limitation in explaining interactions with past risk factors

(i.e., perinatal factors) in the development of asthma.

We found two important perinatal factors influencing the development of asthma (early life oxygen therapy and breastfeeding). Furthermore, we demonstrated that these perinatal factors have combined effects with other risk factors (including ETS, traffic-related air pollution, parental allergic disease, and atopy).

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