International Archives of Allergy and Immunology

Clinical Allergy – Research Article

Int Arch Allergy Immunol
DOI: 10.1159/000516057

Received: 12 17, 2020
Accepted: 03 22, 2021
Published online: May 6, 2021

Dog Ownership in Early Life Increased the Risk of Nonatopic Asthma in Children

Min Jee Park[a] So-Yeon Lee[a] Kun Baek Song[a] Si Hyeon Lee[b] Kil yong Choi[c] Ki Won Lee[b] Sungsu Jung[d] Dong In Suh[e] Youn Ho Sheen[f] Kyung Won Kim[g] Kangmo Ahn[h] Soo-Jong Hong[a]

[a]Department of Pediatrics, Childhood Asthma Atopy Center, Humidifier Disinfectant Health Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [b]Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [c]Department of Environmental Energy Engineering, Anyang University, Anyang, South Korea; [d]Department of Pediatrics, Pusan National University Yangsan Hospital, Yangsan, South Korea; [e]Department of Pediatrics, Seoul National University College of Medicine, Seoul, South Korea; [f]Department of Pediatrics, CHA University School of Medicine, Seoul, South Korea; [g]Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, South Korea; [h]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Keywords
Dogs · Bronchial hyperresponsiveness · Asthma

Abstract
Background: It is still debatable whether dog ownership during early childhood is a risk factor for the development of allergic diseases. ***Objective:*** We investigated the association of dog ownership in early life with sensitization and asthma in childhood. ***Methods:*** Data from the Cohort for Childhood Origin of Asthma and Allergic diseases were used to investigate the association between dog ownership at any time from pregnancy to 1 year of age and sensitization to aeroallergens at 3 and 7 years old, bronchial hyperresponsiveness (BHR), and asthma at 7 years old. We analyzed the cytokine levels in cord blood (CB) and indoor environmental measurement concentrations in the mother's residence obtained at 36 weeks of pregnancy. ***Results:*** Sensitization to dogs at age 3 and 7 did not differ between dog ownership and nonownership, but dog ownership during early life decreased the risk of sensitization to aeroallergens at age 7 (aOR = 0.44, 95% CI 0.21–0.90). Dog ownership significantly increased the risk of nonatopic BHR (aOR = 2.86; 95% CI 1.32–6.21). In addition, dog ownership was associated with asthma, especially nonatopic asthma at 7 years old (aOR = 2.73, 95% CI 1.02–7.32; aOR = 7.05, 95% CI 1.85–26.90, respectively). There were no significant differences in the concentrations of IL-13 or interferon-γ in CB or indoor environmental measurements according to dog ownership during pregnancy. ***Conclusion:*** Early-life dog exposure in this birth cohort has been shown to reduce atopy but increase the risk of nonatopic BHR and nonatopic asthma at 7 years old.

© 2021 S. Karger AG, Basel

Introduction

The prevalence of asthma continued to increase in South Korea until the late 1990s, but it appeared to decline slightly or remained stable after 2000 [1]. Asthma is a persistent respiratory disease and the burden of asthma

Edited by: D.Y. Wang, Singapore.

karger@karger.com
www.karger.com/iaa

© 2021 S. Karger AG, Basel

Correspondence to:
So-Yeon Lee, imipenem@hanmail.net
Soo-Jong Hong, sjhong@amc.seoul.kr

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

on children and their families is substantial, so asthma in children is still one of the major concerns around the world.

While many factors appear to be involved in the development of childhood asthma, environmental factors are likely to be the most suitable to explain the increasing prevalence over the past few decades rather than genetic factors alone [2]. Modern westernized lifestyles are characterized by reductions of outdoor exposures (soil, green space, and microorganisms), a lack of contact with nature, and the keeping of pets indoors, which might all have an association with the rise of allergic diseases. In addition, dog ownership in South Korea has surged in recent years, in line with the country's lower birth rate and aging population, and in 2017, 25.5% of all households in the country owned dogs, up 9.2 percentage points compared to 7 years ago [3].

In tandem with the increase in dog ownership, attention to the possible effects of dog ownership on allergic diseases has been growing. Although several studies and meta-analyses about exposures (time, duration, contact type, and pet type) and outcomes (atopy, bronchial hyperresponsiveness [BHR], and symptoms of allergic diseases) have been conducted to clarify the role of dog ownership in the development of asthma [4–14], they reached different conclusions, making it difficult to provide recommendations regarding the exposure to dogs in early life. Several studies showed no association between dog exposure in early life and the risk of asthma [8, 15]. Other previous studies have shown a protective effect against allergic disease associated with early dog exposure [12–14], while conversely, a meta-analysis showed that exposure to dogs increases the risk of asthma [4], and the prevalence of asthma attacks due to dog allergen increases among dog allergen-sensitized individuals [16].

The prenatal period [17], postnatal period [18], and infant period [12, 14] are all known to have an important impact on the innate immune system through gene expression and critical to early-life immune programming. Therefore, we investigated how early dog exposure, especially at any time from pregnancy to age 1 year, affects sensitization and development of asthma to answer unresolved questions about primary prevention of asthma related to dog ownership. In addition, we investigated the relationship between cytokine levels in cord blood (CB) and indoor environmental measurements concentrations with dog ownership in early life to explain the mechanisms of the subsequent development of sensitization, BHR, and asthma in a prospective birth cohort study.

Materials and Methods

Study Design

The COCOA, which is short for COhort for Childhood Origin of Asthma and Allergic diseases, is a general prospective population-based birth cohort study designed to reveal the impact of environmental exposure [19]. Starting in 2007, 3,004 mothers have been registered and the research is intended to follow up the children for 20 years after birth. Regular follow-up visits for physician's examinations and self-report questionnaires about their indoor environment were conducted as scheduled starting at 36 weeks of gestation, at birth, at the ages of 6 months, 1 year, and then yearly thereafter. The questionnaire that the mother completed was a modified version of the International Study of Asthma and Allergies in Childhood questionnaire [20]. From the annual follow-ups of pediatric allergists, we collected information on the children's allergic symptoms, diagnosis, and any treatments.

Of 3,102 children, 1,163 children (37.49%) were analyzed (shown in Fig. 1) in this study. A total of 744 children have been lost to follow-up and 583 children were under the age of 3, thus unable to provide the necessary outcome information. Children with incomplete questionnaires or missing data and children with early exposure to cats were excluded. A total of 1,136 and 554 children were followed up until the age of 3 and 7, respectively.

Data Collection

Measurement of Atopy

Skin prick tests (SPTs) were conducted at age 3 and 7 with a total of 18 allergens (i.e., *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog epithelium, cat epithelium, German cockroaches, *Alternaria alternata*, *Aspergillus fumigatus*, grass mixture, ragweed, mugwort, alder, birch, oak, Japanese hop, peanuts, milk, egg whites, and soybean), including histamine and saline as a positive and negative control, respectively. When an allergen wheal was >3 mm or larger than the negative control, we defined the subject as sensitized to this allergen. Atopy was defined as exhibiting a positive response to at least 1 aeroallergen on SPTs [19].

BHR and Pulmonary Function Tests

Subjects underwent pulmonary function tests at the age of 7 via conventional spirometry according to the European Respiratory Society and American Thoracic Society guidelines [21]. The forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), maximal mid-expiratory flow, and peak expiratory flow were measured.

Bronchial provocation tests were conducted in children who were followed up until the age of 7 (n = 370, 66.8%) in accordance with the internationally standardized methacholine guidelines [22]. Fresh solutions of Provocholine® (Methacholine chloride USP, Methapharm Inc., Brantford, ON, Canada) were prepared in buffered saline solution at 5 concentrations (0.625, 0.25, 1, 4, and 16 mg/mL). A KoKo dosimeter (Pulmonary Data Services; Doylestown, PA, USA), triggered by a solenoid valve set to remain open for 0.6 s, was used to generate the aerosol from a DeVilbiss 646 nebulizer (DeVilbiss Health Care, Somerset, PA, USA), with pressurized air at 20 pounds per square inch. Each subject inhaled 5 inspiratory capacity breaths of buffered saline solution and increasing concentrations of methacholine at 5-min intervals, resulting in a mean ± standard deviation output of 0.009 ± 0.0014 mL per inhalation. The FEV_1 was measured at 30 and 90 s after the nebulization was completed, and

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

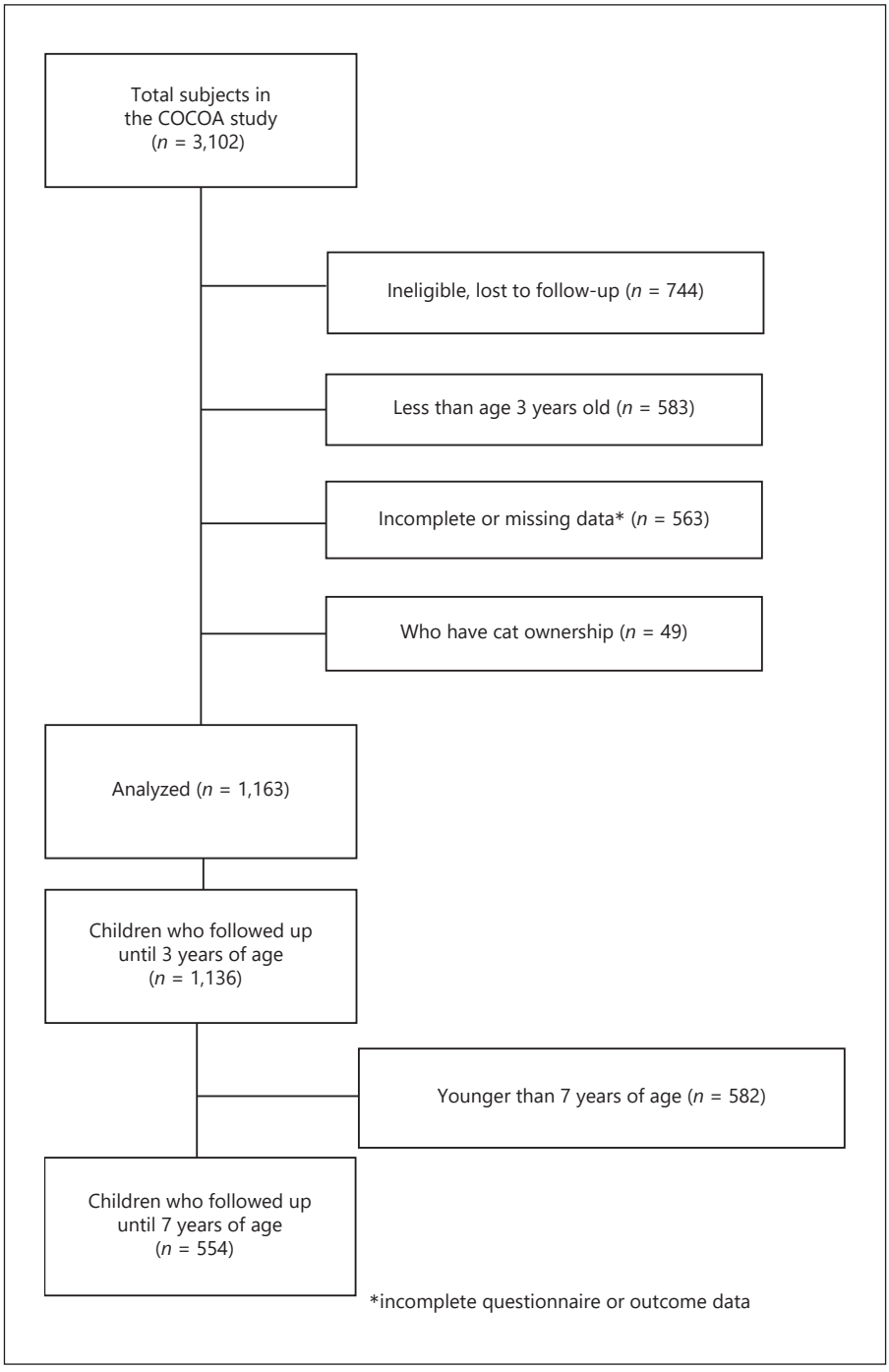


Fig. 1. Study flow and reasons for exclusion between total subjects and study referral are provided.

the next dosing schedule proceeded within 5 min. The provocative concentrations of Provocholine® that caused a 20% (PC20) decrease in FEV_1 from the baseline were calculated by linear interpolation. BHR was determined as PC20 <8 mg/mL. We defined atopic BHR as the presence of BHR along with a positive SPT.

Analysis of CB

The heparinized CB samples were obtained at birth according to a standard protocol [23]. Eosinophils were counted and total IgE levels were measured using an Immuno-CAP (Thermo Fisher Scientific, Waltham, MA, USA) system. For the measurement of cytokine level, cord blood mononuclear cells (CBMCs) were separated on a Histopaque (Sigma Chemical Co., St Louis, MO, USA) gradient within 24 h of sample collection, and the collected cells were washed with phosphate-buffered saline according to the method described in previous studies [19, 24]. CBMCs were cultured in Iscove's modified Dulbecco's medium supplemented with 1% antibiotic-mycotic and 10% fetal bovine serum (all from GIBCO BRL, Eggenstein, Ger-

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

Table 1. General characteristics of the study population*

	Dog ownership		
	nonownership (n = 1,065), n/N (%)	ownership (n = 98), n/N (%)	p value†
Parent's history of allergic disease (yes)	536/1,061 (50.5)	57/98 (58.2)	0.15
AD at 6 months (yes)	174/988 (17.6)	16/92 (17.4)	0.96
Sex (boys)	539/1,065 (50.6)	58/98 (59.2)	0.10
ETS during pregnancy (yes)	604/1,013 (59.6)	54/96 (56.3)	0.52
Delivery mode, C/s	387/1,061 (36.5)	36/97 (37.1)	0.90
Use of antibiotics during pregnancy (yes)	122/1,016 (12.0)	11/96 (11.5)	0.87
Use of antibiotics within 1 year of age (yes)	472/793 (59.5)	40/70 (57.1)	0.70
Maternal age at birth	33.04±3.44	33.02±3.69	0.95
Maternal education level††			
Low	60/1,062 (5.6)	8/98 (8.2)	
Medium	767/1,062 (72.2)	73/98 (74.5)	0.35
High	235/1,062 (22.1)	17/98 (17.3)	
Breastfeeding (yes)	756/1,017 (74.3)	71/90 (78.9)	0.37

Values are frequency (%). ETS, environmental tobacco smoke; AD, atopic dermatitis. * The denominator of each variable was different due to missing responses in the questionnaires. † χ^2 test for proportions, Student's t test for mean. †† Categorized according to the Korean educational system: high school graduation, university/college, and graduate school graduation for low, medium, and high, respectively; the highest education level from the parents was recorded.

many). CBMCs were re-suspended to 1×10^5 cells/200 μL/well in 96-well plates and incubated with 100 μg/mL ovalbumin (OVA, chicken egg albumin grade V; Sigma Chemical Co.) or 10 μL/mL phytohemagglutinin-M (GIBCO BRL) for 48 h, with 1 μCi of 3H-thymidine added to each well for the final 12 h. The cells were harvested onto microfiber filters (Simport, Beloeil, Canada) and the radioactivity on the dried filters was measured in a liquid scintillation counter. The supernatants of the phytohemagglutinin-stimulated cells were obtained after 48 h in culture and stored at −70°C until assayed for IL-13 and IFN-γ by enzyme-linked immunosorbent assays using commercially available kits (R&D Systems, Minneapolis, MN, USA). All supernatants were assayed in duplicate. The limits of detection were 62.5 pg/mL for IL-13 and 15.6 pg/mL for IFN-γ.

Measurement of Indoor Environment

Concentrations of indoor air pollutants (i.e., indoor aeroallergens [HDM], bacterial endotoxin, tobacco smoking, airborne bacteria and fungi, and particulate matters [PMs] 2.5 and 10) in the mother's residence were measured at 36 weeks of pregnancy. A Mini-volume Air Sampler Model 4.1 (AirMetrics Co., Springfield, OR, USA) was used to sample the particulate matters. Each sample collection was conducted in the mother's bedroom for 24 h at a constant sampling flow rate of 10 L/min. The samplers were positioned at least 1 m away from the walls, ceiling, and door and 1.2–1.5 m above the floor. The PMs were measured by using a particle discriminator (Model GT-331, SIBATA Co., Saitama, Japan) with a laser light-scattering optical particle counter.

Microbial sampling was performed by using a single-stage air sampler at the same place the PMs were sampled. Bacterial aerosols and airborne fungi were cultivated in tryptic soy agar and Sabouraud dextrose agar for 24 h at 24°C and room temperature, respectively. The colony-forming units per m^3 were calculated by dividing the colony-forming unit value by the indoor air volume.

Dust samples were collected from a 1×1 m^2 bedding surface for 2 min by using a vacuum cleaner in which the dust filter was fixed to allow even dust collection. After being put into ziplock bags to prevent the dust from being brushed off, the collected dust was kept cold (below −4°C) until analysis. Thereafter, 100 and 50 g of each dust sample were used to measure HDMs and endotoxin, respectively, as described elsewhere [25]. Briefly, Der f 1 and Der p 1 were measured by calorimetric analysis using an enzyme-linked immunosorbent assay reader (Molecular Device, VersaMax). Endotoxin is determined by kinetic chromogenic analysis where a spectrophotometer is employed at a wavelength of 405 nm according to the standard method described by the American Society for Testing and Materials [19].

Definition of Dog Ownership and Asthma

We defined dog ownership in early childhood as the history of dog ownership at any time between conception and 1 year of age, but no longer at age 1 or any time thereafter therefore excluding the children who raised a dog after age 1. Dog ownership was reported by caregivers at each annual interview from 36 weeks of gestational age, at the age of 6 months, 1 year, and then yearly thereafter. A diagnosis of asthma at 7 years of age was determined if at least one of the following conditions by medical records was met: (1) physician diagnosis of asthma at 4–7 years combined with asthma symptoms or the use of asthma controller medication in last 12 months; (2) a methacholine PC_{20} value of 8 mg/mL or less combined with asthma symptoms or the use of asthma controller medication in the last 12 months; or (3) 1 or more of the following occurred in the last 12 months: ≥2 wheezing episodes, ≥2 doctor visits for asthma/wheezing, or ≥1 hospitalization for asthma/wheezing (definition modified from the URECA study [26]).

Downloaded by: C. Alessandri - 87218 94.38.70.80 - 5/16/2021 11:17:03 AM

Table 2. Association between dog ownership in early life and sensitization at ages 3 and 7

Sensitization	Atopy		HDM	
	n/*N* (%)	aOR* (95% CI)	*n*/*N* (%)	aOR* (95% CI)
3 years				
Nonownership (*n* = 1,038)	206/999 (20.6)	1.00 (ref)	169/999 (16.9)	1.00 (ref)
Ownership (*n* = 98)	23/95 (24.2)	1.31 (0.79–2.19)	19/95 (20.0)	1.31 (0.76–2.27)
7 years				
Nonownership (*n* = 508)	193/409 (47.2)	1.00 (ref)	165/409 (40.3)	1.00 (ref)
Ownership (*n* = 46)	12/42 (28.6)†	0.44† (0.21–0.90)	11/42 (26.2)	0.57 (0.28–1.18)

Values are frequency (%). Atopy: exhibiting a positive response against at least 1 aeroallergen on SPTs. HDM, house dust mite; SPT, skin prick test. * Adjusted for the level of education of mother, parent's history of allergic disease, and breastfeeding. † *p* value <0.05.

Table 3. Association between dog ownership in early life and BHR at age 7

	BHR		Atopic BHR		Nonatopic BHR	
	n/*N* (%)	aOR* (95% CI)	*n*/*N* (%)	aOR* (95% CI)	*n*/*N* (%)	aOR* (95% CI)
Nonownership	121/317 (38.2)	1.00 (ref)	64/317 (20.3)	1.00 (ref)	57/317 (18.0)	1.00 (ref)
Ownership	19/34 (55.9)	1.70 (0.83–3.46)	6/34 (16.7)	0.67 (0.25–1.84)	13/34 (38.2)	2.86† (1.32–6.21)

BHR was determined as PC_{20} < 8 mg/L. Atopic BHR: presence of BHR along with a positive skin prick test. Nonatopic BHR: presence of BHR along with a negative skin prick test. BHR, bronchial hyperresponsiveness. * Adjusted for level of education of mother, parent's history of allergic disease, and breastfeeding. † *p* value <0.05.

Statistical Analysis

To evaluate the associations between the 2 groups according to dog ownership (the group that had dog ownership and the group that did not) and sensitization, χ^2 tests and Fisher's exact tests were applied. To calculate the crude and adjusted ORs and 95% confidence intervals (CI) for the BHR and sensitization, multivariate logistic regression analyses were used after adjustment for possible confounding variables such as a parent's history of allergic disease, breastfeeding, and maternal educational level. Associations of dog ownership with atopic and nonatopic asthma versus no asthma were also compared using multivariate logistic regression models.

In these analyses, cytokine values, endotoxin, and other indoor environmental measurements were $\log_{10}$-transformed to improve the normality of their distribution and their associations with dog ownership were estimated using Student's *t* test.

All statistical analyses were performed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA), and a level of 0.05 was considered as statistically significant.

Results

Of the 1,163 children, children with complete data did not differ significantly from those with missing data, but the children with complete data had less frequent breastfeeding (see online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000516057). A comparison of dog ownership and nonownership with respect to their background characteristics is summarized in Table 1. No differences were found for the characteristics of participants between dog ownership and nonownership, including the parents' having a history of allergic diseases.

Association of Dog Ownership and Sensitization

There was no statistically significant difference in atopy between dog ownership and nonownership at 3 years old. However, at 7 years old, children with dog ownership during early life had a lower risk of atopy (Table 2; aOR = 0.44, 95% CI 0.21–0.90). There was no difference in sensitization to dog allergens at 3 and 7 years of life irrespective of dog ownership (online suppl. Table 2).

Association of Dog Ownership with BHR, Lung Function, and Asthma

Dog ownership during early life increased the risk of BHR in nonatopic children at 7 years of age (Table 3; aOR = 2.86; 95% CI 1.32–6.21), whereas there was no as-

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

Table 4. Association between dog ownership in early life and asthma at age 7

	Asthma		Atopic asthma		Nonatopic asthma	
	n/*N* (%)	aOR* (95% CI)	*n*/*N* (%)	aOR* (95% CI)	*n*/*N* (%)	aOR* (95% CI)
Nonownership	25/377 (6.6)	1.00 (ref)	18/377 (4.8)	1.00 (ref)	7/377 (1.9)	1.00 (ref)
Ownership	6/39 (15.4)	2.73† (1.02–7.32)	2/39 (5.1)	1.08 (0.24–4.93)	4/39 (10.3)	7.05† (1.85–26.90)

Asthma: considered to be present at age 7 years if at least one of the following: physician diagnosis at sometime between ages 4–7 or BHR with symptom or treatment of asthma in last 12 months; one or more of the following occurred in the last 12 months: ≥2 wheezing episodes or ≥2 doctor visits for asthma/wheeze or ≥1 hospitalization for asthma/wheeze. * Adjusted for the level of education of mother, parent's history of allergic disease, and breastfeeding. † *p* value <0.05.

Table 5. Association between CB parameters with and without dog ownership during pregnancy

Variable	Dog exposure at pregnancy but not after child's birth		
	nonownership (*n* = 670) mean ± SD	ownership (*n* = 43) mean ± SD	*p* value
Total IgE, kUA/L	0.25±3.03	0.26±2.85	0.76
Eosinophil, %	2.79±2.32	2.40±3.02	0.31
IL-13, pg/mL	1,410.65±3.22	1,179.34±2.54	0.45
IFN-γ, pg/mL	224.53±4.37	166.15±3.80	0.31
IL-13/IFN-γ	6.82±4.63	7.10±4.80	0.90

Values are mean ± SD. CB, cord blood; IL, interleukin; IFN, interferon.

Table 6. Association between indoor environment in the third trimester of pregnancy and dog ownership during pregnancy

Variable	Dog exposure at pregnancy but not after pregnancy		
	nonownership (*n* = 554) mean ± SD	ownership (*n* = 29) mean ± SD	*p* value
Der f 1, ng/g	4.52±1.40	4.24±1.54	0.30
Der p 1, ng/g	3.17±1.46	4.26±1.11	0.15
Endotoxin, EU/mg	8.22±1.82	8.05±1.89	0.62
Airborne bacteria	2.21±1.30	2.11±1.23	0.72
Airborne fungi	2.97±1.31	2.70±1.33	0.31
$PM_{2.5}$, μg/m^3	1.47±0.81	1.49±0.81	0.88
PM_{10}, μg/m^3	3.09±0.69	3.05±0.49	0.76

Data are all log-transformed. Values are mean ± SD. *p* value for the difference in indoor air pollution in the third trimester of pregnancy geometric mean calculated from two-sample *t* test for differences in log-transformed indoor air pollution. *Der f 1*, *Dermatophagoides farinae*; *Der p 1*, *Dermatophagoides pteronyssinus*; EU, endotoxin units; PM, particulate matters.

sociation in atopic children (aOR = 1.70; 95% CI 0.83–3.46). Children with dog ownership had lower mean values for percent-predicted FVC and FEV_1 (FVC, $p = 0.05$; FEV_1, $p = 0.03$) than that of children with nonownership and they had an increased risk of FEV_1 <80% (aOR = 4.38; 95% CI, 1.35–14.14) (online suppl. Table 3).

Dog ownership increased the risk of asthma at 7 years old (aOR = 2.73, 95% CI 1.02–7.32, Table 4). The different effect of dog ownership for atopic and nonatopic asthma was also evident; a significant association was observed between dog ownership and nonatopic asthma at 7 years of age (aOR = 7.05, 95% CI 1.85–26.90).

Associations between Dog Ownership and CB Parameters

There were no statistically significant differences in the concentrations of total IgE, eosinophils, IL-13, IFN- γ, or IFN-γ/IL-13 ratio by dog ownership during pregnancy (Table 5).

Dog Ownership and Indoor Environmental Measurements

Concentrations of indoor environment in the mother's residence at 36 weeks of pregnancy were analyzed. Ownership of a dog during early life was not found to have any association with indoor HDM allergen including *Dermatophagoides farinae,* and *Dermatophagoides pteronyssinus* levels ($p = 0.30$, and 0.15, respectively). The measured values of endotoxin were slightly higher in homes without a dog, but it was not statistically significant ($p = 0.62$). In addition, dog ownership had no association with airborne bacteria and fungi, $PM_{2.5}$, and PM_{10} (Table 6).

Downloaded by: C. Alessandri - 87218 94.38.70.80 - 5/16/2021 11:17:03 AM

Discussion

In this birth cohort study, we provide evidence that dog exposure from pregnancy to child's age of 1 year has a protective effect toward atopy at 7 years of age, whereas there was no association with sensitization to dog allergens. For BHR and asthma, an increased risk was evident mainly among nonatopic children. The relationships were independent of cytokine levels of CB and indoor endotoxin level at 36th weeks of pregnancy. These results suggest that early-life exposure of children to dogs might reduce atopy, but there might be differences in the effect on asthma depending on the presence of atopy, which should be a step toward developing individual clinical advice about dog-keeping based on asthma phenotype.

Dog exposure in early life has no association with sensitization to dog allergens at 3 and 7 years of age, presumably because of the ubiquity of dog allergens in a variety of indoor environments with a high proportion of dog owners [27]. Contrary to our results, 2 previous reports that analyzed the effects of pet ownership during the first year of life found significant associations between pet ownership and sensitization; we assume that such difference in the results between those studies and ours is due to the fact that the previous studies included cat ownership in addition to dog ownership [11, 28].

In relation to a protective effect against atopy, our findings are further supported by the results of another analysis that suggested an underlying "mini-farm" mechanism [14]. The present study focused on the association between dogs and childhood allergy in a metropolitan city, where there are relatively close relationships with pets due to the increase in households of 1 or 2 people following urbanization and in favor of the nuclear family. Therefore, we can expect that exposure to dogs can be considered higher [27] and that there will be the same dose-dependent protective effect against atopy as shown in other studies [5, 14]. This would have resulted in the same effect as providing a "mini-farm" environment in early life, leading to tolerance of inhalant allergens.

There are many studies that have dealt with an association between dog ownership and asthma; however, they have shown contradictory results. A meta-analysis of European birth cohort studies found no association between dog exposure in early life and asthma in children [8], and other studies have concluded that dog exposure in the first year of life reduced the risk of asthma [12–14]. The difference from our findings that dog exposure led to an increased risk of asthma could be a consequence of discrepancies in the duration of dog ownership, the analyzed pet types, exposure environment, and the specific outcome definitions and outcome timing among studies.

Data from the multicultural and population-based European Community Respiratory Health Survey also showed dog ownership was associated with increased risk of asthma among nonatopic subjects [29]; however, the measures of exposure and time of exposure were crude due to the cross-sectional design of the study and were different from our study. An ALSPAC birth cohort study also indicated evidence of an association of dog ownership with atopic or with nonatopic asthma. It has been shown that continuous dog ownership (owned before and after age 3 years) was associated with a reduced risk of sensitization to aeroallergen and atopic asthma, but later dog ownership (owned at age 3 years or later, not before) was associated with increased risk of nonatopic asthma [15]. While it is difficult to compare outcomes because the definition of exposure time in the aforementioned study and our study is different, it is important to note that the period of dog ownership in our study is more strictly defined from pregnancy to child's age of 1 year and not after 1 year, thus placing more emphasis on the effect of dog ownership during early life on allergic diseases.

BHR is often regarded as a hallmark of asthma, and BHR testing is frequently performed to support a diagnosis of asthma. There is only one previous study investigating the association between BHR and dog ownership, and the bronchial response to methacholine challenge was not associated with pet ownership, regardless of atopic status [15]. In our study, we have shown consistent findings of an increased risk of BHR and a diagnosis of asthma in nonatopic children, which supports our results of a relationship between dog ownership and asthma.

We can explain that 1 or more mechanisms are involved in the pathophysiology of an increased risk of nonatopic asthma but decreased atopy due to dog ownership in early life: dog exposure early in life has been demonstrated to induce diverse microbiota, which allows the developing immune system to tolerate various environmental antigens, thereby protecting against allergic conditions, indicating a reduction in atopy [7, 18]. Another probable mechanism responsible for the protective effect against atopy is alterations in pet-specific tolerance through the production of IgG_4 antibodies by high IL-10 cytokine secretion [30, 31]. In this study, IL-13 concentrations in the serum of CB of the dog ownership group were lower than those in the nonownership group, although the strength of the association was insignificant ($p = 0.45$). We can postulate from our findings that exposure to dogs in

Downloaded by: C. Alessandri - 87218 94.38.70.80 - 5/16/2021 11:17:03 AM

early life modifies immune development by decreasing IL-13 responses, thereby reducing atopy. A more informative study could have been done if the actual objective indicators, such as levels of dog-specific IgG_4 and IL-10, were measured as evidence of modification of the Th2-like antibody responses, so further research is necessary.

As for the mechanisms that increase the risk of asthma, and especially nonatopic asthma, dog ownership has been demonstrated to transport outdoor allergens indoors, promoting airway hypersensitivity and wheezing by amplifying lipopolysaccharide/toll-like receptor signals in macrophage-like cells [32]. In this analysis, we also showed that dog ownership was associated with an increased risk of a percent-predicted FEV_1 <80% at age 7, which may be related to an increased risk of asthma diagnosis in children with lower lung function [33, 34]. These findings should be a step toward individual clinical advice on raising dogs in children at the highest risk of asthma.

This study had several limitations of note. First, there is a possibility that dog ownership may be affected depending on whether the parents or children have allergic diseases. However, not only did the parents have a greater tendency to have a dog if they had allergic diseases (online suppl. Table 4), but we also limited the exposure period from pregnancy to age 1 before the child had asthma or aeroallergen sensitization to reduce these errors. Moreover, there is no statistically significant difference in a diagnosis of AD at 6 months of age between nonownership and ownership, which means reverse causality can be excluded. Second, there was a lack of adjustment for other confounder variables that could affect the concentration of allergens (i.e., time spent outdoors, measures for avoiding pet allergens such as the frequency of washing the pet, air purifying systems, and type and number of owned pets). Another limitation of the study is the small sample size. Since this study is an ongoing general population-based cohort study, some of the study subjects have not reached 7 years of age during follow-up. Considering these limitations, the results of this study need to be verified using more subjects in the future.

The main strength of this study was the prospective evaluation of environmental exposures and availability of outcomes at several time points, from pregnancy through to 7 years of age. We attempted to include as much clinic-derived data on sensitization, BHR, and lung function as possible along with data of potential confounders. The general population-based prospective design of this study minimized the risk of recall bias, which complicates the assessment of causality in both cross-sectional and retrospective cohort studies. In addition, to avoid overestimation of the prevalence of asthma, it was defined by a single variable such as parent-reported symptoms or a doctor's diagnosis, and we used a more convincing definition of asthma based on doctor's diagnosis or BHR with parent-reported asthma symptoms or the use of medications for asthma before 12 months. Although there was no clear association between the IL-13 and IFN-γ concentrations in the serum of CB and indoor endotoxin levels, attempts have been made to demonstrate the mechanisms causing the effects of dog ownership. Further research of mechanisms explaining these findings is necessary.

Dog ownership early in life has been shown to protect against allergic sensitization, while increasing the risk of nonatopic asthma and BHR at 7 years old. Taken together, our findings raise the possibility that dog exposure in early life is likely to stimulate immunologic maturation but at the same time promote nonatopic BHR through alternative inflammatory mechanisms [29]. The relationship between asthma and dog ownership in early life in this study might offer clues relevant to primary prevention strategies of asthma. When people are faced with having to choose whether to keep a dog or not because of pregnancy or a history of allergic disease, these choices might have a significant impact on allergic disease.

Statement of Ethics

The study was approved by the Institutional Review Boards of Asan Medical Center (IRB No. 2008-0616), the Samsung Medical Center (IRB No. 2009-02-021), the Severance Hospital (IRB No. 4-2008-0588), and the CHA Medical Center (IRB No. 2010-010). Written informed consent was obtained from the parents of each infant before the study-related interview was performed. The obtainment of consent was confirmed by the IRB.

Conflict of Interest Statement

The authors have no conflicts of interest to declare

Funding Sources

This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI17C1603), and supported by a fund (2008-E33030-00, 2009-E33033-00, 2011-E33021-00, 2012-E33012-00, 2013-E51003-00, 2014-E51004-00, 2014-E51004-01, 2014-E51004-02, 2017-E67002-00, 2017-E67002-01, 2017-E67002-02) from the Research of Korea Centers for Disease Control and Prevention.

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

Author Contributions

Park M.J., Lee S.Y., and Hong S.J. designed the study and wrote the manuscript and performed the analyses. Kun Baek Song, Sungsu Jung, Dong In Suh, Youn Ho Shin, Kyung Won Kim, and Kangmo Ahn participated in the collection of the data. Lee Ki Won, Si Hyeon Lee, and Kil-yong Choi participated in the analysis and interpretation of the study. Lee S.Y. critically reviewed and supervised the execution of the study, and Hong S.J. has given final approval of the version to be published. All authors agree to be held responsible for all aspects of the work related to its accuracy or integrity.

References

1 Hwang G, Ham S, Yoon C. Prevalence of asthma in children in worldwide and Korea. KJPH. 2018;55(1):1–12.
2 Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. J Allergy Clin Immunol. 2017;140(1):1–12.
3 Chi IB, Kim HJ, Kim WT, Seo GC. Development strategies for the companion animal industry. Naju, South Korea: Korea Rural Economic Institute; 2017. p. 34.
4 Takkouche B, González-Barcala FJ, Etminan M, Fitzgerald M. Exposure to furry pets and the risk of asthma and allergic rhinitis: a meta-analysis. Allergy. 2008;63(7):857–64.
5 Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA. 2002;288(8):963–72.
6 Anyo G, Brunekreef B, de Meer G, Aarts F, Janssen NA, van Vliet P. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. Clin Exp Allergy. 2002;32(3):361–6.
7 Sitarik AR, Havstad S, Levin AM, Lynch SV, Fujimura KE, Ownby DR, et al. Dog introduction alters the home dust microbiota. Indoor Air. 2018;28(4):539–47.
8 Lodrup Carlsen KC, Roll S, Carlsen KH, Mowinckel P, Wijga AH, Brunekreef B, et al. Does pet ownership in infancy lead to asthma or allergy at school age? Pooled analysis of individual participant data from 11 european birth cohorts. PLoS One. 2012;7:e43214.
9 Hesselmar B, Aberg N, Aberg B, Eriksson B, Björkstén B. Does early exposure to cat or dog protect against later allergy development? Clin Exp Allergy. 1999;29(5):611–7.
10 Collin SM, Granell R, Westgarth C, Murray J, Paul ES, Sterne JA, et al. Associations of pet ownership with wheezing and lung function in childhood: findings from a uk birth cohort. PLoS One. 2015;10(6):e0127756.
11 Jung S, Noh SR, Lee S-Y, Yoon J, Cho H-J, Kim Y-H, et al. Indoor pet ownership in infancy is a risk factor for the development of sensitization to pets and asthma in childhood. Allergy Asthma Respir Dis. 2019;7(2):99–105.
12 Ojwang V, Nwaru BI, Takkinen HM, Kaila M, Niemelä O, Haapala AM, et al. Early exposure to cats, dogs and farm animals and the risk of childhood asthma and allergy. Pediatr Allergy Immunol. 2020;31(3):265–72.
13 Fall T, Lundholm C, Örtqvist AK, Fall K, Fang F, Hedhammar Å, et al. Early exposure to dogs and farm animals and the risk of childhood asthma. JAMA Pediatr. 2015;169(11):e153219.
14 Hesselmar B, Hicke-Roberts A, Lundell AC, Adlerberth I, Rudin A, Saalman R, et al. Pet-keeping in early life reduces the risk of allergy in a dose-dependent fashion. PLoS One. 2018;13(12):e0208472.
15 Collin SM, Granell R, Westgarth C, Murray J, Paul E, Sterne JA, et al. Pet ownership is associated with increased risk of non-atopic asthma and reduced risk of atopy in childhood: findings from a uk birth cohort. Clin Exp Allergy. 2015;45(1):200–10.
16 Gergen PJ, Mitchell HE, Calatroni A, Sever ML, Cohn RD, Salo PM, et al. Sensitization and exposure to pets: the effect on asthma morbidity in the us population. J Allergy Clin Immunol Pract. 2018 Jan–Feb;6(1):101–e2.
17 Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S, et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. J Allergy Clin Immunol. 2011;127(1):179–e1.
18 Tun HM, Konya T, Takaro TK, Brook JR, Chari R, Field CJ, et al. Exposure to household furry pets influences the gut microbiota of infant at 3–4 months following various birth scenarios. Microbiome. 2017;5(1):40.
19 Yang HJ, Lee SY, Suh DI, Shin YH, Kim BJ, Seo JH, et al. The cohort for childhood origin of asthma and allergic diseases (cocoa) study: design, rationale and methods. BMC Pulm Med. 2014;14:109.
20 Beasley R. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: Isaac. Lancet. 1998;351:1225–32.
21 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319–38.
22 American Thoracic S. Guidelines for methacholine and exercise challenge testing 1999. Am J Respir Crit Care Med. 2000;161:309–239.
23 Kim J, Kim S, Woo S-Y, Chung J-Y, Hong Y-S, Oh S-Y, et al. Prenatal exposure to lead and chromium is associated with il-13 levels in umbilical cord blood and severity of atopic dermatitis: Cocoa study. Immune Netw. 2019;19(6):e42.
24 Kim HB, Ahn KM, Kim KW, Shin YH, Yu J, Seo JH, et al. Cord blood cellular proliferative response as a predictive factor for atopic dermatitis at 12 months. J Korean Med Sci. 2012;27(11):1320–6.
25 Lee CM, Hong SJ, Kim YS, Park GY, Nam Goung SJ, Kim KH. Distribution features of biological hazardous pollutants in residential environments in korea. Environ Sci Pollut Res Int. 2014;21(2):1146–52.
26 O'Connor GT, Lynch SV, Bloomberg GR, Kattan M, Wood RA, Gergen PJ, et al. Early-life home environment and risk of asthma among inner-city children. J Allergy Clin Immunol. 2018;141(4):1468–75.
27 Zahradnik E, Raulf M. Animal allergens and their presence in the environment. Front Immunol. 2014;5:76.
28 Sandin A, Björkstén B, Bråbäck L. Development of atopy and wheezing symptoms in relation to heredity and early pet keeping in a swedish birth cohort. Pediatr Allergy Immunol. 2004;15(4):316–22.
29 Svanes C, Heinrich J, Jarvis D, Chinn S, Omenaas E, Gulsvik A, et al. Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey. J Allergy Clin Immunol. 2003;112(2):289–300.
30 Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet. 2001;357(9258):752–6.
31 Gern JE, Reardon CL, Hoffjan S, Nicolae D, Li Z, Roberg KA, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. J Allergy Clin Immunol. 2004;113(2):307–14.
32 Gómez-Casado C, Díaz-Perales A. Allergen-associated immunomodulators: Modifying allergy outcome. Arch Immunol Ther Exp. 2016;64(5):339–47.
33 Lowe LA, Woodcock A, Murray CS, Morris J, Simpson A, Custovic A. Lung function at age 3 years: effect of pet ownership and exposure to indoor allergens. Arch Pediatr Adolesc Med. 2004;158(10):996–1001.
34 Hu LW, Qian Z, Dharmage SC, Liu E, Howard SW, Vaughn MG, et al. Pre-natal and post-natal exposure to pet ownership and lung function in children: the seven northeastern cities study. Indoor Air. 2017;27(6):1177–89.

Downloaded by: C. Alessandri - 87218
94.38.70.80 - 5/16/2021 11:17:03 AM

eTable 1. Baseline characteristics in our analysis compared with children who had missing data on pet ownership

		Dog ownership		
		Subjects excluded in the study (n = 1939 unless otherwise shown)	Subjects included in the study (n = 1163 unless otherwise shown)	p-value*
Parent's history of allergic disease (Yes)		869/1691 (51.4%)	593/1159 (51.2%)	0.91
AD at 6 months (Yes)		196/1219 (16.1%)	190/1080 (17.6%)	0.33
Sex (Boys)		931/1268 (54.3%)	597/1163 (51.3%)	0.12
ETS during pregnancy (Yes)		811/1454 (55.8%)	658/1109 (59.3%)	0.07
Delivery mode (C/sec)		632/1709 (37%)	423/1158 (36.5%)	0.09
Use of antibiotics during pregnancy (Yes)		204/1461 (14.0%)	133/1112 (12.0%)	0.14
Use of antibiotics within 1 year of age (Yes)		507/887 (57.2%)	512/863 (59.3%)	0.36
Maternal age at birth		33.04 ± 3.46	33.08 ± 3.61	0.77
Maternal education level†	low	83/1752 (4.7%)	68/1160 (5.9%)	0.36
	medium	1296/1752 (74.0%)	840/1160 (72.4%)	
	high	373/1752 (21.3%)	252/1160 (21.7%)	
Breastfeeding		1002/1254 (79.9%)	827/1107 (74.7%)	<0.01
Physician's diagnosis of AD at 7 years of age		8/83 (9.6%)	48/410 (11.7%)	0.59

Values are frequency (%) or mean ± SD

ETS, Environmental tobacco smoke; AD, Atopic dermatitis

* Chi-squared test for proportions, Student's *t*-test for means

† Categorized according to the Korean educational system: high school graduation, university/college, and graduate school graduation for low, medium, and high, respectively; the highest education level from the parents was recorded.

eTable 2. Association between dog ownership in early life and sensitization at ages 3 and 7

Sensitization	3 years		7 years	
	Dog		Dog	
	n/N (%)	aOR* (95% CI)	n/N (%)	aOR* (95% CI)
Non-ownership	9/999 (0.90)	1.00 (ref)	18/409 (4.4)	1.00 (ref)
Ownership	1/95 (1.1)	1.50 (0.18–12.70)	3/42 (7.10)	1.06 (0.24–4.80)

Values are frequency (%)

*Adjusted for the level of education of the mother, parent's history of allergic disease, and breastfeeding

†P value < 0.05

eTable 3. Relationship between dog exposure in early life and lung function at age 7

		Non-ownership		Ownership		p-value
		N = 229	%	N = 18	%	
FVC (% of pred)	<80	14	6.1	3	16..7	0.12
	≥80	215	93.9	15	83.3	
	mean ± SD	95.80±14.56		88.46±24.79		0.05
	aOR (95% CI)	1.00 (ref)		3.70 (0.90–15.21)		0.07
FEV_1 (% of pred)	<80	20	8.7	5	27.8	0.02†
	≥80	209	91.3	13	72.2	
	mean ± SD	94.99 ± 15.36		86.40±24.43		0.03†
	aOR (95% CI)	1.00 (ref)		4.38 (1.35–14.14)		0.02†
MMEF (% of pred)	<80	2	1.2	0	0	1
	≥80	162	98.8	15	100	
	mean ± SD	103.30 ± 10.49		103.33 ± 9.45		0.99
	aOR (95% CI)	1.00 (ref)		.		0.99
PEF (% of pred)	<80	50	26.2	4	26.7	1
	≥80	141	73.8	11	73.3	
	mean ± SD	96.01±26.56		83.13±30.21		0.08
	aOR (95% CI)	1.00 (ref)		0.94 (0.28–3.13)		0.92

pred, predicted; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; PEF, peak expiratory flow; MMEF, maximal midexpiratory flow

Values are mean ± SD

†P value < 0.05

eTable 4. Association between dog ownership and sensitization at age 3 and 7 according to the parental history of allergic disease.

Sensitization		Parental history of allergic disease			No parental history of allergic disease		
		Non-ownership	Ownership	p-value	Non-ownership	Ownership	p-value
3 year	Atopy*	91/494 (18.6%)	8/39 (20.5%)	0.75	113/501(22.6%)	14/56 (25.0%)	0.48
	Dog	5/494 (1.0%)	0/39 (0%)	1.00	4/501 (0.8%)	1/56 (1.8%)	0.42
	HDM	77/494 (15.6%)	5/39 (12.8%)	0.65	90/501(18.0%)	14/56 (25.0%)	0.20
7 year	Atopy*	94/209 (45.0%)	6/22 (27.3%)	0.11	98/198 (49.5%)	6/20 (30.0%)	0.10
	Dog	9/209 (4.3%)	2/22 (9.1%)	0.32	9/198 (4.5%)	1/20 (5.0%)	1.00
	HDM	82/209 (39.2%)	6/22 (27.3%)	0.27	82/198 (41.4%)	5/20 (25.0%)	0.15

Values are frequency (%)

HDM, House dust mites

*Atopy: exhibiting a positive response against at least one inhalant allergen on the skin prick test