Genetic and Environmental Risk Factors for Childhood Eczema Development and Allergic Sensitization in the CCAAPS Cohort

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Eczema is very common and increasing in prevalence. Prospective studies investigating environmental and genetic risk factors for eczema in a birth cohort are lacking. We evaluated risk factors that may promote development of childhood eczema in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) birth cohort ($n = 762$) of infants with at least one atopic parent. Objective environmental exposure data were available for each participant. At annual physical examinations, children underwent skin prick tests (SPTs), eczema was diagnosed by a clinician, and DNA was collected. Among Caucasian children, 39% developed eczema by age 3. Children with a pet dog were significantly less likely to have eczema at age one (odds ratio (OR) = 0.62, 95% confidence interval (CI): 0.40–0.97) or at both ages 2 and 3 (OR = 0.54, 95% CI: 0.30–0.97). This finding was most significant among children carrying the CD14–159C/T CC genotype. Carriers of the *CD14*–159C/T and *IL4Rα* I75V single-nucleotide polymorphisms (SNPs) had an increased risk of eczema at both ages 2 and 3 (OR = 3.44, 95% CI: 1.56–7.57), especially among children who were SPT+. These results provide new insights into the pathogenesis of eczema in high-risk children and support a protective role for early exposure to dog, especially among those carrying the *CD14*–159C/T SNP. The results also demonstrate a susceptibility effect of the combination of *CD14* and *IL4Rα* SNPs with eczema.

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INTRODUCTION

The prevalence of eczema has nearly tripled in industrialized countries during the past three decades, and recent prevalence estimates demonstrate that 15–30% of children are affected (Williams and Flohr, 2006). In the United States, the projected health care costs due to eczema range from $0.9 to $3.8 billion (Ellis *et al.*, 2002). The hallmarks of eczema are a chronic, relapsing form of skin inflammation, a disturbance of epidermal-barrier function, and IgE-mediated sensitization to food and environmental allergens (Bieber, 2008). It is often the first step of the ''atopic march'' that may lead to the development of allergic rhinitis and asthma (Leung *et al.*, 2004). Thus, early identification of risk factors and possible intervention strategies may lead to the discovery of measures that attenuate later expression of allergic diseases.

Eczema results from complex interactions between host (genetic susceptibility and skin barrier dysfunction) and environmental factors (allergens, irritants, and infectious agents). Genome-wide scans have identified several possible eczema loci on chromosomes 3q21.11, 16q, 17q25, 20p,12, and 3p26.13, and most notably 1q21, which harbors a family of epithelium-related genes called the epidermal differentiation complex (Cookson, 2004; Palmer and Cardon, 2005). Thus, there are two predominant groups of genes that have been associated with eczema: genes encoding epidermal structural proteins and genes encoding major elements of the immune system, especially those important in allergic sensitization (Bieber, 2008).

In patients with early-onset eczema, IgE-mediated sensitization often occurs several weeks or months after the lesions appear, suggesting that the skin is the site of sensitization (Spergel and Paller, 2003). Indeed, in animal models, repeated epidermal challenge with antigen induces specific IgE and eczematous lesions at the application site (Spergel *et al.*, 1998). Recently, a unifying hypothesis (Bieber, 2008)

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Abbreviations: 95% CI, 95% confidence interval; CCAAPS, Cincinnati Childhood Allergy and Air Pollution Study; DEP, diesel exhaust particles; OR, odds ratio; SNP, single-nucleotide polymorphism; SPT, skin prick test

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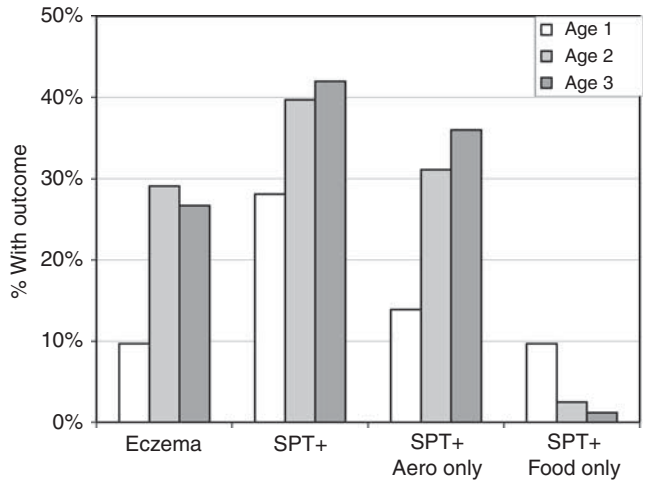

Figure 1. Summary of eczema and SPT results of Caucasians in the CCAAPS cohort at ages 1–3.

has been proposed and suggests that the natural history of eczema has three phases including (1) an initial non-atopic phase in early infancy, when sensitization has not yet occurred; (2) in 60–80% of patients, genetic predisposition results in the induction of IgE-mediated sensitization to foods and/or environmental allergens leading to eczema; and (3) scratching damages skin cells releasing autoantigens and leading to IgE autoantibodies.

Food and inhalant allergens, as well as microbial agents, contribute to eczema (Leung *et al.*, 2004); however, the environmental and genetic factors that protect/contribute remain largely unclear. In order to identify environmental and genetic factors that contribute to the development of eczema in early childhood, we studied a birth cohort of over 762 high-risk infants born to at least one atopic parent followed prospectively as part of Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS).

RESULTS

Participant characteristics and environmental exposures

The Cincinnati Childhood Allergy and Air Pollution Study is comprised of 54.3% males, 76.6% Caucasians, 19.8% African-Americans, and 3.8% are bi-racial or other. Since the genotype frequencies of several studied single-nucleotide polymorphisms (SNPs) were significantly different between Caucasians and African-Americans, only Caucasians were evaluated as power was too low for other racial groups.

Figure 1 describes the eczema status and skin prick test (SPT) results among Caucasian children at ages 1–3. Eczema was present in 9.7% of children and increased to 29.1 and 26.7% at ages 2 and 3, respectively. The proportion that was SPT + for one or more allergens were 28.1, 39.7, and 42.0% at ages 1, 2 and 3, respectively. The percentage of children who were SPT + to aeroallergens alone increased over time, 13.9, 31.1, and 36.0%, while those who were SPT + to food antigens alone decreased, 9.7, 2.5, and 1.2%, respectively. The percentage did not change appreciably for children who were SPT + to both aero- and food allergens, with 4.5, 6.1, and 4.8% positive at ages 1, 2 and 3, respectively (data not shown).

Table 1. Summary of environmental exposure status among CCAAPS cohort Caucasians

Exposure	No.	(%)
ETS		
None	425	73.4
1–19 cigarettes per day	112	19.3
⩾20 cigarettes per day	42	7.3
Dog	203	37.8
Cat	158	28.4
Visible mold		
No mold	222	40.8
Low mold	290	53.3
High mold	32	5.9
High DEP ($\geqslant 0.44\,\mu g\,m^{-3}$)	144	24.9

Endotoxin	Measure	Unit (EU mg^{-1})
	Range	6–800
	Geometric mean	73.2
	25% Quartile	38.5
	50% Quartile	79.0
	75% Quartile	164.7

CCAAPS, Cincinnati Childhood Allergy and Air Pollution Study; DEP, diesel exhaust particle; ETS, environmental tobacco smoke.

A summary of environmental exposures is displayed in Table 1. At enrollment, almost 30% were exposed to environmental tobacco smoke in the home. Dog(s) were present in 38% of the homes, while 28% reported having at least one cat. During the first year of life, almost 60% of homes had visible mold, with 6% in the high mold category. Diesel exhaust particles (DEPs) exposure ranged from $0.23\,\mu g\,m^{-3}$ to $0.88\,\mu g\,m^{-3}$; high DEP was defined as the top quartile. Endotoxin levels obtained from home dust samples ranged from 6.0 to 800.0 EU mg^{-1}, and the geometric mean was 73.2 EU mg^{-1}.

Environmental exposures, SPT results, and eczema

The associations between SPT results, environmental exposures, and eczema outcomes are displayed in Table 2. Children who were SPT + to at least one aeroallergen during the first three years of life were over two times more likely to have eczema by age 3 (OR = 2.69, 95% confidence interval (CI): 1.80–4.01, $P<0.001$) and almost nine times more likely to have eczema at both ages 2 and 3 (OR = 8.78, 95% CI: 3.87–19.92, $P<0.001$). Children with persistent aeroallergen sensitization were over five times more likely to have eczema at both ages 2 and 3 (OR = 5.25, 95% CI: 3.00–9.19, $P<0.001$). A sub-analysis of children who were persistently

Table 2. Unadjusted associations between eczema, SPT testing, and environmental exposures among CCAAPS Caucasians

	Eczema at age 1		Eczema by age 3		Eczema at ages 2 and 3	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
SPT+	1.89 (0.93–3.81)	0.07	**2.69 (1.80–4.01)**	**0.01**	**8.78 (3.87–19.92)**	**<0.001**
Persistent aeroallergen sensitization	1.51 (0.84–2.93)	0.22	**2.38 (1.62–3.48)**	**<0.001**	**5.25 (3.00–9.19)**	**<0.001**
SPT+ to food	**3.58 (2.00–6.44)**	**<0.001**	**3.38 (2.20–5.19)**	**<0.001**	**7.0 (3.37–14.48)**	**<0.001**
Dog exposure	**0.49 (0.25–0.95)**	**0.04**	0.75 (0.51–1.06)	0.10	**0.54 (0.30–0.97)**	**0.04**
Cat exposure	0.58 (0.26–1.26)	0.17	0.87 (0.57–1.31)	0.49	1.04 (0.55–1.97)	0.89
High DEP	**1.89 (1.05–3.04)**	**0.03**	1.05 (0.71–1.55)	0.80	0.62 (0.31–1.22)	0.17
Mold exposure						
None	Ref.		Ref.		Ref.	
Low	0.98 (0.54–1.79)	0.95	1.34 (0.93–1.92)	0.12	1.43 (0.83–2.48)	0.20
High	1.48 (0.47–4.66)	0.50	1.31 (0.61–2.81)	0.48	0.29 (0.04–2.28)	0.24
ETS						
None	Ref.		Ref.		Ref.	
1–19 cigarettes per day	0.80 (0.38–1.7)	0.56	1.10 (0.72–1.69)	0.65	1.42 (0.73–2.80)	0.31
20+ cigarettes per day	0.77 (0.23–2.61)	0.67	0.82 (0.42–1.61)	0.56	0.86 (0.28–2.65)	0.79

CCAAPS, Cincinnati Childhood Allergy and Air Pollution Study; CI, confidence interval; DEP, diesel exhaust particle; ETS, environmental tobacco smoke; OR, odds ratio; SPT, skin prick test.
Bold indicates $P<0.05$.

positive to aeroallergens only and negative to food ($n=470$) yielded similar results (OR = 5.50, 95% CI: 2.73–11.05; data not shown). Children sensitized to foods were significantly more likely to develop eczema at age 1, by age 3, and at both ages 2 and 3 ($P<0.001$).

Children whose parents reported having a pet dog at enrollment were over 50% less likely to have eczema at age one (OR = 0.49, 95% CI: 0.25–0.95, $P=0.04$). Children who had a pet dog had nearly a two-fold decreased risk of eczema at both ages 2 and 3 (OR = 0.54, 95% CI: 0.30–0.97, $P=0.04$). To ensure there was no bias in dog exposures related to parental history of eczema, we examined this relationship and found no effect. There was an increased risk for eczema at age 1 with high DEP exposure (OR = 1.89, 95% CI: 1.05–3.04, $P=0.03$). For all other environmental exposures, no significant association was observed with eczema during the first three years of life.

Association of *CD14* and *IL-4Rα* with eczema

Seven SNPs were genotyped in four atopy-related genes. These genes were chosen because more than 10 independent studies (Ober and Hoffjan, 2006) have shown associations with atopy or asthma phenotypes, and are biologically relevant by altering gene expression or function.

The associations of the *CD14* C-159T and *IL4Rα* I75V SNPs with eczema and SPT+ are summarized in Table 3. The genotype CC of the SNP *CD14* C-159T significantly increased the risk of eczema at both ages 2 and 3 (OR = 3.01 95% CI: 1.04–8.68, $P=0.01$), and this trend was also observed for eczema at age 1 and eczema development by age 3. An increased risk of eczema by age 3 was also observed with the IV genotype of *IL4Rα* I75V (OR = 1.77, 95% CI: 1.07–2.96, $P=0.03$). This same trend was observed for all three eczema outcomes for both the IV and VV genotypes of the *IL4Rα* SNP, so these two genotypes were then combined. Children who carried both the CC and IV/VV variants ($n=64$) were over two times more likely to develop eczema by age 3 (OR = 2.24, 95% CI: 1.27–3.94, $P<0.01$), and were over three times more likely to have eczema at both ages 2 and 3 (OR = 3.44 95% CI: 1.56–7.57, $P<0.01$). The association of the combination of these two SNPs with eczema remained significant even after Bonferroni correction. There were no significant associations observed with any of the other SNPs and eczema. The association of these SNPs with a positive SPT response was also examined. The combination of the two SNPs (*CD14*-159C/T and *IL4Rα* I75V) was not significantly associated with SPT+, but showed a similar trend as observed with eczema (26.1% *vs* 17.3%, $P=0.10$; data not shown).

Since allergen sensitization plays a critical role in eczema development, we evaluated the subgroup of children who were SPT+ and had eczema. Strikingly, children with SPT+ and eczema by age 3 were over three times more likely to have the genotype combination of *CD14* C-159T CC and *IL4Rα* (IV+VV) compared with children without eczema who were SPT− (OR = 3.01, 95% CI: 1.37–6.63, $P<0.01$;

Table 3. Associations of *CD14* C-159T and *IL4Rα* I75V SNPs with Eczema among CCAAPS Caucasians

	Eczema at age 1		Eczema by age 3		Eczema at ages 2 and 3	
SNP	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
CD14 C-158T						
CC	2.25 (0.61–8.31)	0.36	1.47 (0.79–2.75)	0.06	**3.01 (1.04–8.68)**	**0.01**
CT	2.27 (0.66–8.01)	0.32	0.88 (0.48–1.60)	0.15	1.4 (0.49–4.00)	0.55
TT	Ref.		Ref.		Ref.	
IL4Rα I75V						
II	Ref.		Ref.		Ref.	
IV	1.47 (0.60–3.58)	0.40	**1.77 (1.07–2.96)**	**0.03**	1.87 (0.82–4.26)	0.13
VV	1.13 (0.31–4.07)	0.85	1.72 (0.85–3.47)	0.13	2.60 (0.94–7.19)	0.07
Combination of both SNPs						
CC and IV/VV genotype	1.21 (0.49–3.01)	0.68	**2.24 (1.27–3.94)**	**0.01**	**3.44 (1.56–7.57)**	**<0.01**

CCAAPS, Cincinnati Childhood Allergy and Air Pollution Study; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
Bold indicates $P<0.05$.

data not shown). The increased prevalence of this genotype combination between *CD14* and *IL4Rα* among the SPT+ children with eczema supports the potential of combined genetic susceptibility to allergic disease and eczema.

CD14-159C/T and dog exposure association with eczema and SPT+

The associations of *CD14* C-159T and dog exposure are displayed in Figure 2a-b. Of the 148 Caucasian children who had eczema at age 2, about half, 72 (48.6%), also had eczema at age 3. We evaluated the individual and combined effects of the CT and TT genotypes with dog exposure since they both confer protection in our data (see Table 1 and Table 3). The CT/TT genotype of *CD14*-159C/T was significantly associated with a 38% reduction in risk of eczema by age 3 (OR = 0.62, 95% CI: 0.40–0.97, $P=0.03$) and an even stronger reduced risk (57%) of eczema at both ages 2 and 3 (OR = 0.43, 95% CI: 0.23–0.82, $P=0.01$). Dog exposure also reduced the risk of eczema at age 1 (OR = 0.49, 95% CI: 0.25–0.95, $P=0.04$) as well as eczema at both ages 2 and 3 (OR = 0.54, 95% CI: 0.30–0.97), $P=0.04$). This same protective trend was observed with eczema development by age 3 ($P=0.10$). When genetic and environmental factors that conferred decreased eczema risk were combined, an even stronger effect was observed. Children with the *CD14* C-159T CT or TT genotype who lived in homes with dog(s) were almost 60% less likely to have eczema by age 3 (OR = 0.56, 95% CI: 0.33–0.96, $P=0.04$) and eczema at both ages 2 and 3 (OR = 0.36, 95% CI: 0.14–0.89, $P=0.03$) compared with all other children (children with the CC genotype and/or no dog exposure; data not shown). This association was even stronger for atopic eczema at both ages 2 and 3 (OR = 0.33, 95% CI 0.12–0.89, $P=0.03$; data not shown). Thus, dog exposure in early life and the CT or TT genotype of *CD14*-159C/T SNP both provide protection from the development of eczema in young children. There were no significant associations between genetics and DEP exposure with eczema risk.

Similarly, an analysis with persistent SPT+ as the outcome was evaluated. Of the 207 Caucasian children who were SPT+ at age 2, 126 (60.9%) remained SPT+ at age 3. There were no significant associations found between *CD14* C-589T and dog exposure with persistent allergen sensitization.

Adjusted models of eczema

We also evaluated models of the three eczema outcomes including dog exposure, persistent aeroallergen sensitization, food sensitization, and the *CD14* C-159T and *IL4Rα* I75V SNPs, adjusted for gender and parental history of eczema (data not shown). Persistent aeroallergen sensitization was the most significant predictor of eczema at both ages 2 and 3 (OR = 14.39, 95% CI 4.40–47.10, $P<0.001$), and there was a significant interaction between persistent aeroallergen sensitization and the *CD14*-159T CC genotype (OR = 4.66, 95% CI 1.41–15.38, $P=0.01$). This indicates that children who carry the SNP and are persistently sensitized to aeroallergens are at the greatest risk for persistent eczema, suggesting a role for innate immunity. We observed the same significant associations in the model of eczema development by age 3. When stratified by pet dog ownership, the interaction between persistent aeroallergen sensitization and the *CD14* CC genotype remains significant only for children without a pet dog presumably because this was a protective factor. Food sensitization was the most significant predictor of eczema at age 1 (OR = 2.73, 95% CI: 1.03–7.23, $P=0.04$).

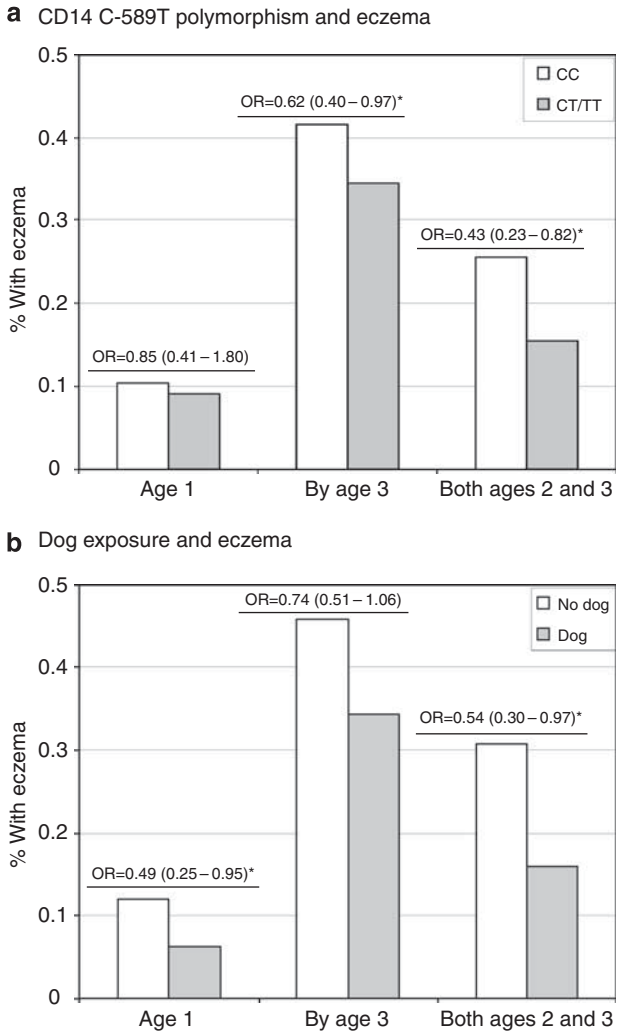

Figure 2. C-589T, dog exposure and eczema. Unadjusted associations between eczema and CD14 C-589T **(a)** and dog exposure **(b)**, among CCAAPS Caucasians. *$P<0.05$

DISCUSSION

In this study, we determined genetic and environmental factors that contribute to the development of eczema in a birth cohort. Our main findings are (1) a combination of the *CD14*C-159T CC and *IL4Rα*I75V IV/VV genotypes increase the risk of eczema up to 3.5-fold; (2) dog exposure significantly decreases eczema risk, even after adjustment for parental eczema and gender; and (3) the protective effect of dog ownership is most evident among carriers of the *CD14* CT or TT genotype. Sensitization to food allergens in early childhood is associated with the development of eczema, the first step in the so-called ''atopic march'' that gives rise to asthma and allergic rhinitis (Leung *et al.*, 2004). Our data support this as sensitization to a food allergen was observed to be the greatest risk factor of eczema development by age 1.

The combination of *CD14* and *IL4Rα* SNPs was strongly associated with eczema, and the effect was strongest among children who were SPT+. This finding further highlights the association of early-life aeroallergen as well as food sensitization and eczema. Recently, it has been suggested that sensitization to allergens in children with eczema is occurring through the skin and that this may predispose children to the future development of asthma. Studies evaluating the importance of the cornified envelope suggest that damage to this barrier by genetic, physical, chemical, and enzymatic compounds may allow allergens to cross and interact with antigen-presenting cells, leading to allergic sensitization and possible secondary development of asthma (Hudson, 2006; McGrath and Uitto, 2008). Hence, sensitization through the skin (especially in the context of an abnormal skin barrier) may promote the development of asthma in later childhood. Our data support a strong association between SPT and eczema. Children who were SPT+ to at least one aeroallergen during the first three years of life were over two times more likely to have eczema by age 3 and almost nine times more likely to have eczema at both ages 2 and 3. This association supports the possibility that children with eczema may initially be sensitized through their skin, and cutaneous sensitization could then predispose development of airway allergic disease.

Our data demonstrate that early-life exposure to dog may be protective for eczema, as reported previously (Nafstad *et al.*, 2001; Holscher *et al.*, 2002; Zirngibl *et al.*, 2002; Gern *et al.*, 2004; Bufford *et al.*, 2008). A pooled analysis of all cohort studies in 2007 showed a 36% decrease in eczema risk with dog exposure (Langan *et al.*, 2007). Out of 11 cohort and cross-sectional studies, five reported significant decreases in eczema risk, yet no significant increases were observed, suggesting that dog exposure may be protective against childhood eczema (Langan *et al.*, 2007). Further, we found that children who were exposed to dog and carried the CT or TT genotype at *CD14*–159C/T had the lowest risk for eczema at both ages 2 and 3. We also observed a borderline gene–environment interaction between the CT genotype and dog exposure in children aged 1 ($P=0.06$; data not shown).

The literature on CD14 polymorphism and dog exposure with respect to eczema has conflicting results. Our data agree with the findings of Gern *et al.* (2004) who reported a significant gene-by-environment interaction between the CD14–589T TT genotype and dog exposure in children aged 1 (Gern *et al.*, 2004). However, another study by Sengler *et al.* (2003) found no association. The inconsistent findings may be due to the presence of other environmental factors that modify the relationship. *CD14* is a pattern-recognition receptor and binds bacterial lipopolysaccharide (Petersen *et al.*, 2007), lipoteichoic acid from Gram-positive bacteria (Cleveland *et al.*, 1996), peptidoglycans (Gupta *et al.*, 1996), mycobacteria, and viruses (Dobrovolskaia and Vogel, 2002; Jiang *et al.*, 2005), and mediates phagocytosis of apoptotic cells without initiating inflammation (Devitt *et al.*, 1998). The protective effect of the *CD14* C-159T allele against eczema may be due to a protective effect conferred by *CD14* ligands such as endotoxin or other bacterial components. Dogs are one of many sources of endotoxin in the house, and has been reported with both dog presence and allergen level (Platts-Mills *et al.*, 2005). While there was a strong correlation between dog exposure and endotoxin level ($P<0.001$) in

our cohort, we did not observe an independent association between endotoxin and eczema (data not shown). Nevertheless, we did observe that the *CD14* C-159T allele combined with dog exposure was protective for eczema. This incongruity might be due to a higher endotoxin level surrounding the dog, producing an exposure ''cloud'' near small children. Therefore, endotoxin levels measured from home dust samples may not reliably reflect an individual child's exposure in their breathing zone. Alternatively, other pathogen-associated molecular patterns acting through CD14 may be more relevant than endotoxin; thus, the protective effects from dogs and endotoxin may work through different mechanisms (Litonjua *et al.*, 2002). We also observed a significant interaction between the *CD14*-158C/T CC genotype and persistent aeroallergen sensitization among children without dog exposure. These findings suggest that environmental exposures (specifically dogs) can potentially block the commencement of the atopic march in even very-high-risk populations.

There are some important limitations of this study. One of the limitations of birth cohort studies in general is that the sample size may be underpowered. Furthermore, these findings are not generalizable to the general population as this cohort is comprised of infants born to at least one atopic parent. However, the clinical outcomes of this study were enriched because it is a high-risk cohort. Approximately 65% of the children had a positive SPT reaction and 39% had eczema during the first three years of life, enabling sufficient sample size and power. Another limitation is the lack of filaggrin data, the strongest known genetic predictor of eczema. While these data are not available for the cohort at this time, we plan to examine the impact of filaggrin in the future. Finally, as with all parental reports of disease, eczema history was subject to parental recall bias, which was minimized by including questions from validated questionnaires and physician diagnosis in the outcome definition.

A major advantage of this study is that it was conducted in a longitudinal birth cohort enabling comparisons in the same individual at different time points in early childhood. Thus, we were able to examine the development and recurrence of eczema through age 3. Results obtained from birth cohort studies are more reliable because they are less subject to sampling selection and recall bias (Hunter, 2005; Manolio *et al.*, 2006). Birth cohort studies provide a more valid basis for investigating genetics and environment since relevant environmental exposures may occur in early infancy prior to disease onset. Another unique advantage in CCAAPS is the availability of quantified measures of exposure.

To the best of our knowledge, this is the first report dissecting the effect of gene and environment on longitudinal eczema and longitudinal allergen sensitization status in a birth cohort. Larger population studies are warranted to determine the potential clinical utility and predictive value of our results.

MATERIALS AND METHODS

Study participants

A cohort of 762 high-risk infants born to at least one atopic parent from the Greater Cincinnati area was followed prospectively from birth (Ryan *et al.*, 2005; LeMasters *et al.*, 2006). The recruitment strategies for the CCAAPS has been described in detail elsewhere (LeMasters *et al.*, 2006). Briefly, birth certificates were used to identify children born between 2001 and 2003. The families were invited to participate based on the proximity of their home to traffic sources. This study was approved by the Institutional Review Board committees of Cincinnati Children's Hospital Medical Center and the University of Cincinnati. Written informed consent was obtained from each participant's parent or legal guardian prior to enrollment. The investigations were conducted according to the Declaration of Helsinki Principles.

When infants were approximately 6–7 months of age, parents were screened for allergy symptoms and underwent SPTs for 15 aeroallergens (meadow fescue, timothy, white oak, maple mix, American elm, red cedar, short ragweed, *Alternaria*, *Aspergillus*, *Penicillium*, *Cladosporium*, dust mite mix, German cockroach, cat, and dog), as well as positive histamine and negative saline controls. All SPTs were performed with a bifurcated device (Accusets ALK America, Round Rock, TX). A positive SPT was defined as having $\geqslant 3$ mm wheal with erythema. Parents with one or more allergy symptoms and a positive (+) SPT were enrolled (LeMasters *et al.*, 2006).

Outcome definitions

At ages 1–3, each participating child was examined by a clinician. At each clinical exam, parent(s) provided a detailed medical history and the child received full physical examination, including an SPT to the same 15 aeroallergens and milk and egg. Children were defined as SPT+ if they had at least one positive reaction to any allergen at age 1, 2, or 3. Children that were positive to any allergen at both ages 24 and 36 months were defined as having persistent SPT+.

Eczema was defined by either a positive medical history or physical examination. A positive medical history was defined as parental report to the clinician that the child experienced ''frequent skin scratching'' (question adapted from the well validated ISAAC core questionnaire for eczema; Asher *et al.*, 1995) and had redness or red spots, raised bumps, and/or rough dry skin for >6 months. A positive physical examination was defined as one of the following: (1) the child's skin showed evidence of atopic eczema (erythema, papulation, exoriations, lichenification, and flexural distribution); (2) the clinician reported that atopic dermatitis was probable or definite; or (3) the clinician's global assessment of skin disease was rated as mild to very severe eczema.

Eczema by age 3 was defined as a positive medical history or physician's assessment at any time between birth and age 3. Eczema at both ages 2 and 3 was defined as medical history or diagnosis of eczema at both ages 2 and 3. Subjects with eczema and SPT+ to aeroallergens at both ages 2 and 3 were defined as having atopic eczema at both ages 2 and 3.

DNA collection and SNP genotyping

Genomic DNA was isolated from buccal swabs using the ZR Genomic DNA II Kit from Zymo Research (Orange, CA). Seven SNPs in four genes: *CD14*-159C/T (rs2569190), *IL4Rα* I75V (rs1805010), *IL4Rα* E400A (rs1805011), *IL4Rα* C431R (rs1805012), *IL4* C-589T (rs2243250), *IL13* C-1112T (rs1800925), and *IL-13* R130Q (rs20541) were genotyped using the Roche LightCycler. Primers and probes were designed using the LightCycler software. Of these SNPs, the

genotype frequencies of three (rs1805010, rs1805012, and rs1800925) were not in Hardy–Weinberg equilibrium in Caucasians. This finding was not unexpected because the CCAAPS birth cohort was at high risk for atopy. Quality control was examined through re-genotyping a subset (20%) of DNA samples; our genotyping error rate was below 5%.

Environmental exposure assessments

The extensive environmental exposure assessment for the CCAAPS cohort has been described previously (Ryan *et al.*, 2005; Biagini *et al.*, 2006; Cho *et al.*, 2006a). Briefly, parents provided detailed smoking habits for each person living in the child's home at the time of the parent SPT. In addition, parental report of cat and/or dog ownership was collected. House dust samples were collected from the floor of the infant's primary activity room during home visits performed by trained personnel before the child's age one clinical exam. Endotoxin levels were quantified from the dust samples using the Limulus Amebocyte lysate assay kit (Cambrex Corporation, Walkersville, MD; Campo *et al.*, 2006). Prevalence of visible mold was indexed based on history of water damage, signs of visible mold/water damage and its size, and moldy odor (Meklin *et al.*, 2004; Cho *et al.*, 2006b). Children living in homes having none of these were considered unexposed to mold. Homes with high mold exposure had visible mold in one room or a combined area of visible mold and water damage $\geqslant 0.2\,m^2$ (Meklin *et al.*, 2004). All other homes were categorized as having low mold.

Each child's average daily exposure estimates to elemental carbon attributable to traffic, hereafter referred to as DEPs, was measured from ambient levels of fine particulate matter with aerodynamic diameter $<2.5\,\mu m$ ($PM_{2.5}$) collected at 27 sampling sites in the greater Cincinnati, OH, area. Source apportionment methodology was used to develop a DEP signature profile and estimates of DEP exposure were calculated using a land-use regression (LUR) model (Kolluri *et al.*, 1995; Hu *et al.*, 2006; Ryan *et al.*, 2008). High estimates were defined as the top quartile ($\geqslant 0.44\,\mu g\,m^{-3}$).

Statistical analysis

Genotypes for each of the seven studied SNPs were examined for deviation from Hardy–Weinberg equilibrium using the χ^2-test in both the Caucasian and African-American groups. Effects of the SNP genotype and environmental exposures OR and 95% CI on clinical outcomes of eczema and positive SPT were estimated by logistic regression. Multivariate models were implemented for the eczema outcomes, adjusted for gender and parental history of eczema. Backward selection was used with a conservative significance level of 0.2 to stay in the model. The analyses were implemented in SAS 9.1 (SAS Institute, Cary, NC). Overall, a *P*-value of 0.05 was considered statistically significant; Bonferroni correction was used to adjust for multiple testing in results interpretation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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