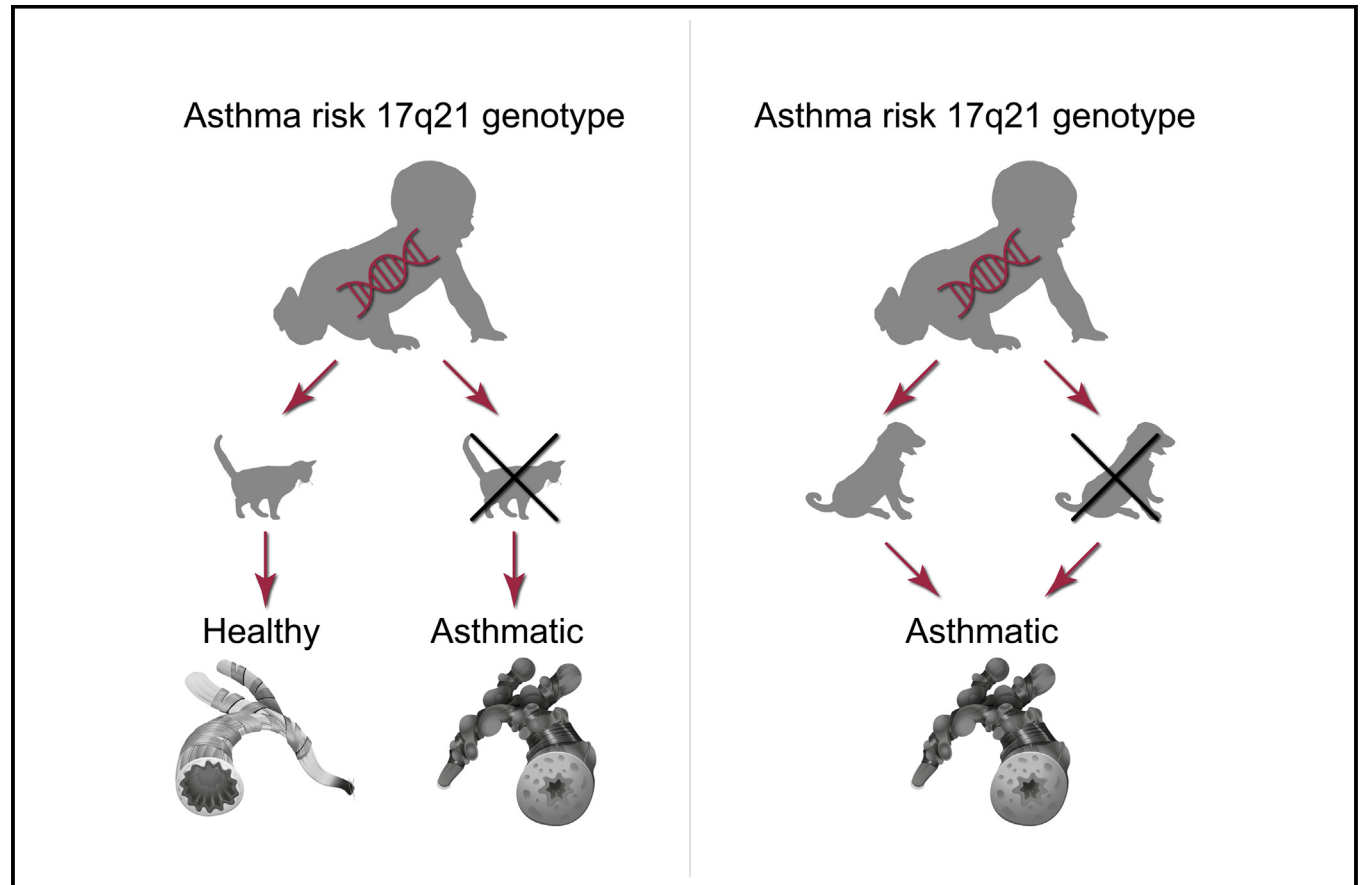


Cat exposure in early life decreases asthma risk from the 17q21 high-risk variant

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GRAPHICAL ABSTRACT



Background: Early-life exposure to cats and dogs has shown diverging associations with childhood asthma risk, and gene-environment interaction is one possible explanation. **Objectives:** We investigated interactions between cat and dog exposure and single nucleotide polymorphism rs7216389 variants in the chromosome 17q21 locus, the strongest known genetic risk factor for childhood asthma. **Methods:** Genotyping was performed in 377 children from the at-risk Copenhagen Prospective Studies on Asthma in

Childhood₂₀₀₀. The primary end point was the development of asthma until age 12 years. The secondary end point was the number of episodes with pneumonia and bronchiolitis from 0 to 3 years of age. Exposures included cat and dog ownership from birth and cat and dog allergen levels in bedding at age 1 year. Replication was performed in the unselected COPSAC₂₀₁₀ cohort with follow-up until 5 years of age. **Results:** Cat and/or dog exposure from birth was associated with a lower prevalence of asthma among children with the

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rs7216389 high-risk TT genotype (adjusted hazard ratio, 0.16; 95% CI, 0.04-0.71; $P = .015$), with no effect in those with the CC/CT genotype (adjusted $P = .283$), demonstrating interaction between cat and dog exposure and the rs7216389 genotype (adjusted $P = .044$). Cat allergen levels were inversely associated with asthma development in children with the TT genotype (adjusted hazard ratio, 0.83; 95% CI, 0.71-0.97; $P = .022$), supporting the cat-rs7216389 genotype interaction (adjusted $P = .008$). Dog allergen exposure did not show such interaction. Furthermore, the TT genotype was associated with higher risk of pneumonia and bronchiolitis, and this increased risk was likewise decreased in children exposed to cat. Replication showed similar effects on asthma risk.

Conclusion: The observed gene-environment interaction suggests a role of early-life exposure, especially to cat, for attenuating the risk of childhood asthma, pneumonia, and bronchiolitis in genetically susceptible subjects. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: MeSH, asthma, cats, dogs, human orosomucoid-like 3 gene protein, gene-environment interaction

Asthma represents the most common chronic disorder in children and is a major cause of hospitalizations and school absence worldwide.^{1,2} Asthma development represents a complex mechanism with a disease trajectory originating from both genetic and environmental effects in early life,³ but known risk factors only explain a minor proportion of disease variance.

Cat and dog exposure has generally been suspected to increase the risk of childhood asthma, but studies have been inconsistent, showing both increased risk, decreased risk, or no effect of cat and dog exposure on asthma development.⁴⁻⁸ The type and timing of these exposures might be critical, and the possible effects in early life might be different from those later in life, when the same exposure can lead to different effects. Similarly, we have reported previously a gene-environment interaction between cat exposure from birth and risk of eczema in carriers of the filaggrin nonfunctional mutation, with cat exposure further increasing the risk in carriers.⁹

In the current study we investigated a possible interaction between a genetic variant (single nucleotide polymorphism [SNP] rs7216389) in the chromosome 17q21 locus, the strongest genetic risk factor for early-onset childhood asthma, and cat and dog exposure from birth with respect to development of childhood asthma, pneumonia, and bronchiolitis. Because asthmatic disease related to 17q21 variants develops within the first years of life, any modifying environmental exposures must precede this. We used data from the Copenhagen Prospective Studies on Asthma in Childhood₂₀₀₀ (COPSAC₂₀₀₀) high-risk birth cohort analyzing the longitudinal development of asthma from age 0 to 12 years and replicated the findings in the unselected COPSAC₂₀₁₀ cohort from age 0 to 5 years.

METHODS

Governance

We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice as defined in the European Union's Directive on Good Clinical Practice, the International Conference on Harmonisation's good clinical practice guidelines, and the

Abbreviations used

aHR:	Adjusted hazard ratio
aIRR:	Adjusted incidence rate ratio
COPSAC ₂₀₀₀ :	Copenhagen Prospective Studies on Asthma in Childhood ₂₀₀₀
HR:	Hazard ratio
IRR:	Incidence rate ratio
LRTI:	Lower respiratory tract infection
ORMDL3:	Orosomucoid-like 3 gene
SNP:	Single nucleotide polymorphism

Helsinki Declaration. We follow national and international rules on the processing of personal data, including the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

Study population

The COPSAC₂₀₀₀ study included 411 infants born to mothers with a history of asthma enrolled at 1 month of age and followed prospectively for 12 years.^{3,10,11} Exclusion criteria were any respiratory symptoms before inclusion, gestational age of less than 36 weeks, and any congenital abnormality or systemic illness. The study was performed according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2015-41-3696). Written informed consent was obtained from both parents.

Clinical surveillance

Medical doctors at the COPSAC clinical research unit were responsible for the diagnosis and treatment of all respiratory and skin-related symptoms. Participants were assessed until age 12 years at the research unit every 6 months for the first 7 years and at additional visits immediately on symptom onset. At every visit, a full physical examination was performed, and personal interviews were guided by structured questions and closed response categories focusing on the child's lung and skin symptoms, medications, health care use, lifestyle, and home environment.

Data validation and quality control followed the guidelines for good clinical practice. Data were stored in a dedicated online database. The data were double-checked against source data and subsequently locked.

Primary end point: asthma

Troublesome lung symptoms were defined to the parents as wheezing or whistling sounds, breathlessness, or persistent troublesome cough severely affecting the child's well-being. The presence or absence of troublesome lung symptoms was recorded as a composite dichotomized (yes or no) score in daily diaries, as previously described.¹² Asthma was solely diagnosed by the COPSAC physicians according to a strict algorithm based on repeated episodes of troublesome lung symptoms, need of intermittent inhaled β_2 -agonist, and positive effects of inhaled corticosteroids relapsing after a 3-month test period.¹¹ Age at asthma diagnosis from 0 to 12 years was used for analysis.

Secondary end point: lower respiratory tract infection

The children were examined, diagnosed, and treated for pneumonia and bronchiolitis by the COPSAC pediatricians in accordance with predefined standard procedures. Clinical pneumonia was defined by troublesome cough accompanied by tachypnea, fever, and abnormal auscultation,¹² whereas bronchiolitis was defined by symptoms of coryza progressing over a few days to cough, tachypnea, chest retractions, and auscultative widespread crepitation and/or rhonchi in a child younger than 2 years of age.¹³ Analyses included the number of lower respiratory tract infection (LRTI) episodes from 0 to 3 years of age.

TABLE I. Baseline characteristics for the entire population and grouped according to living with a cat or dog from birth

	All	Cat and/or dog from birth		P value
		Yes	No	
All, % (no.)	100 (377)	25 (95)	75 (282)	—
Male sex, % (no.)	49 (184)	49 (47)	49 (137)	.880
White, % (no.)	97 (367)	98 (93)	97 (274)	.988
Maternal age at birth (y), mean (SD)	30.1 (4.5)	29.7 (4.8)	30.3 (4.4)	.250
Maternal smoking in pregnancy, % (no.)	23 (88)	38 (36)	18 (52)	<.001
Antibiotic use in pregnancy, % (no.)	31 (115)	32 (30)	30 (85)	.793
Cesarean section, % (no.)	22 (83)	21 (20)	22 (63)	.830
Older children in the home, % (no.)	39 (143)	32 (30)	41 (113)	.121
Maternal educational level, % (no.)				<.001
Low*	60 (220)	77 (71)	55 (149)	
Medium*	26 (96)	17 (16)	29 (80)	
High*	13 (49)	5 (5)	16 (44)	
rs7216389 genotype, % (no.)				.193
CC	23 (86)	29 (28)	21 (58)	
CT	48 (182)	45 (43)	49 (139)	
TT	29 (109)	25 (24)	30 (85)	
Maternal sensitization, % (no.)	62 (208)	48 (41)	67 (167)	.004

*Low is defined as primary school, secondary school or college graduate, medium is defined as tradesman or bachelor's degree, and high is defined as master's degree. Boldface values indicate statistical significance.

Cat and dog exposure

Information on household cats and dogs at birth was obtained during the scheduled 1-month clinical visit and was defined as a cat or dog living in the home at birth. Furthermore, allergen exposure was measured in dust samples collected from the child's bed at age 1 year. Parents vacuumed the bedding (the pillow and mattress) for 5 minutes with a dust trap attached (ALK-Abello, Hørsholm, Denmark). Cat and dog allergen concentrations were measured by using the Sandwich ELISA.¹⁴ Allergen exposure was analyzed as a log₂-transformed continuous variable and dichotomized as high and low (greater than and less than the median level).

Genotyping

Allelic discrimination of rs7216389 was extracted from genome-wide genotyping by using the Infinium HumanOmniExpressExome BeadChip Kit (Illumina, San Diego, Calif), and sample and marker quality control were performed, as previously reported.¹⁵ We chose the rs7216389 SNP as representative of the 17q21 locus¹⁶ because it has been used in previous COPSAC studies^{17,18} and is strongly associated with childhood-onset asthma. Furthermore, the rs7216389 SNP was used in a previous study on pets and 17q21 locus interaction.¹⁹

In these analyses children were dichotomized according to rs7216389 SNP alleles: asthma risk genotype (TT) and nonrisk genotype (CC/CT).

Covariates

Covariates included sex, maternal age at childbirth, maternal smoking during pregnancy, antibiotic use in pregnancy, alcohol intake in pregnancy, delivery by means of cesarean section, older children in the home at birth, and maternal educational level. Serum specific IgE levels against 7 inhalant allergens (dog, cat, birch, timothy grass, mugwort, *Dermatophagoides pteronyssinus*, and molds) were characterized in the mothers after the child's birth (ImmunoCAP, Phadiatop; Pharmacia Diagnostics AB, Uppsala, Sweden). Maternal sensitization was defined as a specific IgE level of greater than 0.35 kU/mL.

Replication cohort

COPSAC₂₀₁₀ is an ongoing unselected Danish cohort study of 738 pregnant women including 700 children followed prospectively from pregnancy and was designed from the COPSAC₂₀₀₀ cohort.²⁰ Exclusion criteria were chronic cardiac, endocrinological, nephrological, or lung disease other than asthma.

The study was performed according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Copenhagen (H-B-2008-093) and the Danish Data Protection Agency (2015-41-3696). Written informed consent was obtained from both parents. Genotyping, information on cat ownership (requiring ≥14 days of exposure after birth), asthma diagnosis, and treatment were performed the same way as in COPSAC₂₀₀₀.¹¹ Age at asthma diagnosis from 0 to 5 years of age was used for analysis.

Statistical analysis

The χ^2 or Student *t* test was used for simple associations in the demographic characteristics of cat and/or dog owners. The effect of the rs7216389 SNP on age of asthma onset and associations between household cats and dogs and age of asthma onset were assessed by using Kaplan-Meier curves and quantified in terms of hazard ratios (HRs) by using Cox proportional hazards regression (*P* values correspond to Wald tests). The children were retained in the analysis from birth until the age of asthma diagnosis, dropout, or age 12 years, whichever came first. The interaction analyses between rs7216389 SNP variants and household cats and dogs with regard to asthma risk were performed by using multiple Cox regression analysis, including genotype and cat and dog exposure as an interaction term (*P* values correspond to Wald tests). Results were adjusted for confounders associated with cat and dog ownership. The association between cat and dog exposure and cumulative incidence of LRTIs was assessed by using quasi-Poisson regression estimates of incidence rate ratios (IRRs). For replication, we analyzed the risk of asthma from 0 to 5 years of age in COPSAC₂₀₁₀. A significance level of .05 was used in all types of analyses. All estimates were reported with 95% CIs. Observations with missing data for covariates were excluded from analyses. All data analysis was performed in the statistical software package R, version 3.3.0.²¹

RESULTS

Information on cat and dog ownership and rs7216389 SNP genotype was available in 92% (377/411) of the children from COPSAC₂₀₀₀. The genotype distribution was as follows: CC, 23% (86 children); CT, 48% (182 children); and TT, 29% (109 children). The clinical follow-up rate among children with the rs7216389 SNP genotype was 86% (325 children) at 12 years of age. Among these children, asthma was diagnosed in 26% (85 children) before age 12 years, and 13% (41 children) had current asthma at age 12 years. Eleven percent (*n* = 44) only

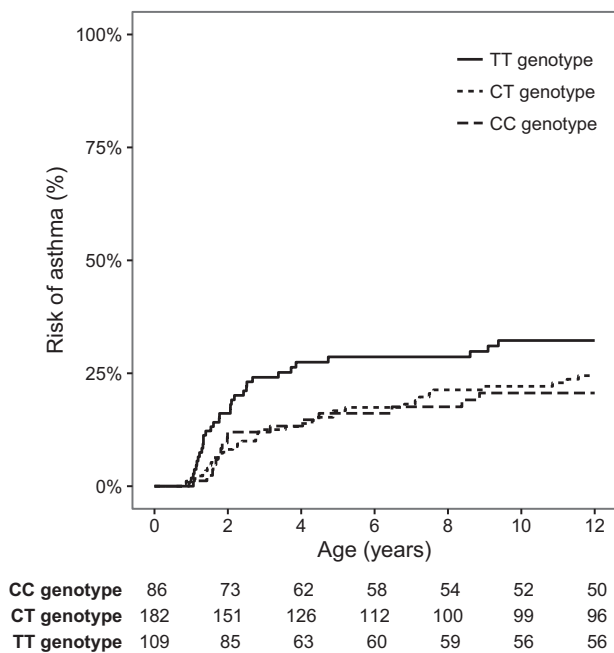


FIG 1. Kaplan-Meier estimates of the cumulative risk of asthma during childhood according to the rs7216389 genotype from age 0 to 12 years. Numbers at risk are presented below the graph.

had a cat, 10% ($n = 38$) only had a dog, 3% ($n = 12$) had both, and 75% ($n = 282$) had neither a cat nor a dog in the home at birth.

Determinants of cat and dog ownership

The prevalence of the rs7216389 SNP genotype did not differ significantly with cat and dog ownership (cat and/or dog: CC, 29%; CT, 45%; and TT, 25%; no cat or dog: CC, 21%; CT, 49%; and TT, 30%). Both cat and dog ownership were positively associated with maternal smoking in pregnancy and inversely associated with higher maternal educational level and maternal sensitization (Table I). Therefore cat and dog exposure analyses were adjusted for these covariates.

rs7216389 and risk of asthma

Children with the TT genotype were at increased risk of asthma, as shown by Kaplan-Meier curves (Fig 1). The effect was mainly established within the first 3 years of life, and we found a significantly increased risk of asthma in children with the TT genotype compared with those with the CC ($P = .039$) and CT ($P = .012$) genotypes, whereas we found no significant differences between children with the CC or CT genotypes ($P = .932$). HRs for asthma development were significantly increased for the TT genotype compared with the CC/CT genotype from age 0 to 3 years (HR, 2.13; 95% CI, 1.26-3.62; $P = .005$) and from age 0 to 7 years (HR, 1.82; 95% CI, 1.14-2.92; $P = .013$), with decreasing effect to age 12 years (HR, 1.62; 95% CI, 1.05-2.52; $P = .030$). Therefore the TT genotype was compared with the CC/CT genotype in the gene-environment interaction analyses.

Cat and dog exposure, rs7216389 genotype, and asthma risk

Cat and/or dog exposure from birth was associated with a nonsignificant lower risk of asthma from 0 to 12 years (HR, 0.58;

95% CI, 0.33-1.01; $P = .053$). This decreased risk was only apparent in children with the high-risk TT genotype (HR, 0.19; 95% CI, 0.04-0.78; $P = .021$), with no changes after covariate adjustment (adjusted hazard ratio [aHR], 0.16; 95% CI, 0.04-0.71; $P = .015$). Cat and/or dog exposure showed no associations with asthma among children with the CC/CT genotype ($P = .678$, adjusted $P = .283$; Fig 2). A multiple Cox regression analysis on asthma risk from age 0 to 12 years, including rs7216389 genotype, cat and/or dog exposure, and the interaction between the 2, demonstrated a significant interaction between cat and/or dog exposure and rs7216389 genotype ($P = .045$), which remained significant after covariate adjustment ($P = .044$).

Cat and dog allergen levels were measured in dust samples from the children's beds at age 1 year, and levels were highly correlated with ownership of the specific pet ($P < .00001$). Levels of the 2 allergens were comparable: $\log_2$ Fel d 1 in homes with a cat (median, 15.87; interquartile range, 14.77-16.55) and $\log_2$ Can f 1 in homes with a dog (median, 15.80; interquartile range, 13.94-17.70). High cat allergen levels were inversely associated with asthma in children with the high-risk TT genotype but not those with the CC/CT genotype (Fig 3). When using allergen levels as continuous $\log_2$ -transformed variables, an inverse association with cat allergen levels and development of asthma was observed for the TT genotype (aHR, 0.83; 95% CI, 0.71-0.97; $P = .022$) but with no association for the CC/CT genotype (Table II). This corresponded to a 17% reduction in asthma risk among children with the TT genotype for every doubling of cat allergen levels in the home. Cox regression analysis showed a significant interaction between rs7216389 genotype and cat allergen levels in the home ($P = .013$), which remained significant after covariate adjustment ($P = .008$). There were no significant associations with dog allergen levels (Table II).

Asthma prevalence by rs7216389 and cat and dog exposure

Fig 4, A, shows temporal development in asthma prevalence from birth until 12 years of age according to the rs7216389 genotype among the 325 children with full follow-up. Asthma prevalence was increased especially in children with the TT genotype in the first 3 years of life compared with children with the CC/CT genotype. This gap diminished until age 12 years, where no differences were observed between genotypes. Stratification by exposure to cat or dog (Fig 4, B) and cat (Fig 4, D) or dog (Fig 4, E) from birth demonstrated a lower prevalence of asthma in children with the TT genotype compared with those with the CC/CT genotype over time when they were exposed to either cat or dog from birth.

Cat and dog exposure, rs7216389 genotype, and risk of LRTI

A total of 185 (57%) of the 325 children with this information experienced at least 1 episode of pneumonia or bronchiolitis in the first 3 years of life. The mean incidence was 1.2 episodes per child (SD, 1.6) throughout these 3 years. Children with the TT genotype were at increased risk of LRTIs compared with children with the CC/CT genotype (IRR, 1.49; 95% CI, 1.12-1.97; $P = .007$). Cat and/or dog exposure from birth was borderline significantly

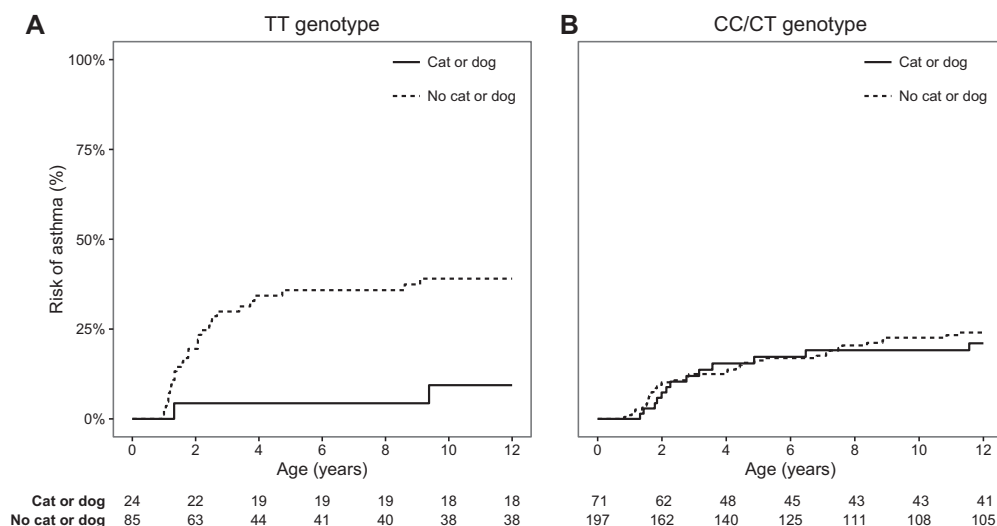


FIG 2. Kaplan-Meier estimates of the cumulative risk of asthma in the first 12 years of life according to cat or dog status at birth. Stratification was by rs7216389 genotype: **A**, TT genotype; **B**, CC/CT genotype. Numbers at risk are presented below the graph.

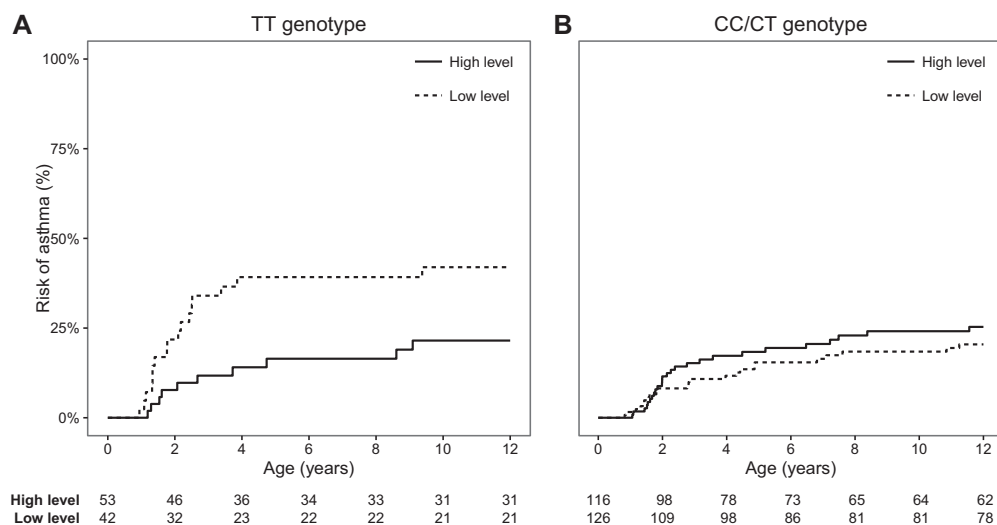


FIG 3. Kaplan-Meier estimates of the cumulative risk of asthma in the first 12 years of life according to cat allergen median level (high vs low) in the home at age 1 year. Stratification was by rs7216389 genotype: **A**, TT genotype; **B**, CC/CT genotype. Numbers at risk are presented below the graph.

associated with a decreased risk of LRTIs in children with the high-risk TT genotype (adjusted incidence rate ratio [aIRR], 0.56; 95% CI, 0.30-1.07; $P = .084$), without any associations with the CC/CT genotype (aIRR, 1.05; 95% CI, 0.70-1.58; $P = .811$; interaction $P = .074$). This effect was driven only by cat exposure (TT genotype: aIRR, 0.47; 95% CI, 0.22-1.00; $P = .054$; CC/CT genotype: aIRR, 1.20; 95% CI, 0.73-1.99; $P = .471$), demonstrating significant interaction after covariate adjustment ($P = .036$). There were no significant associations from dog exposure on risk of LRTI ($P > .86$).

Replication

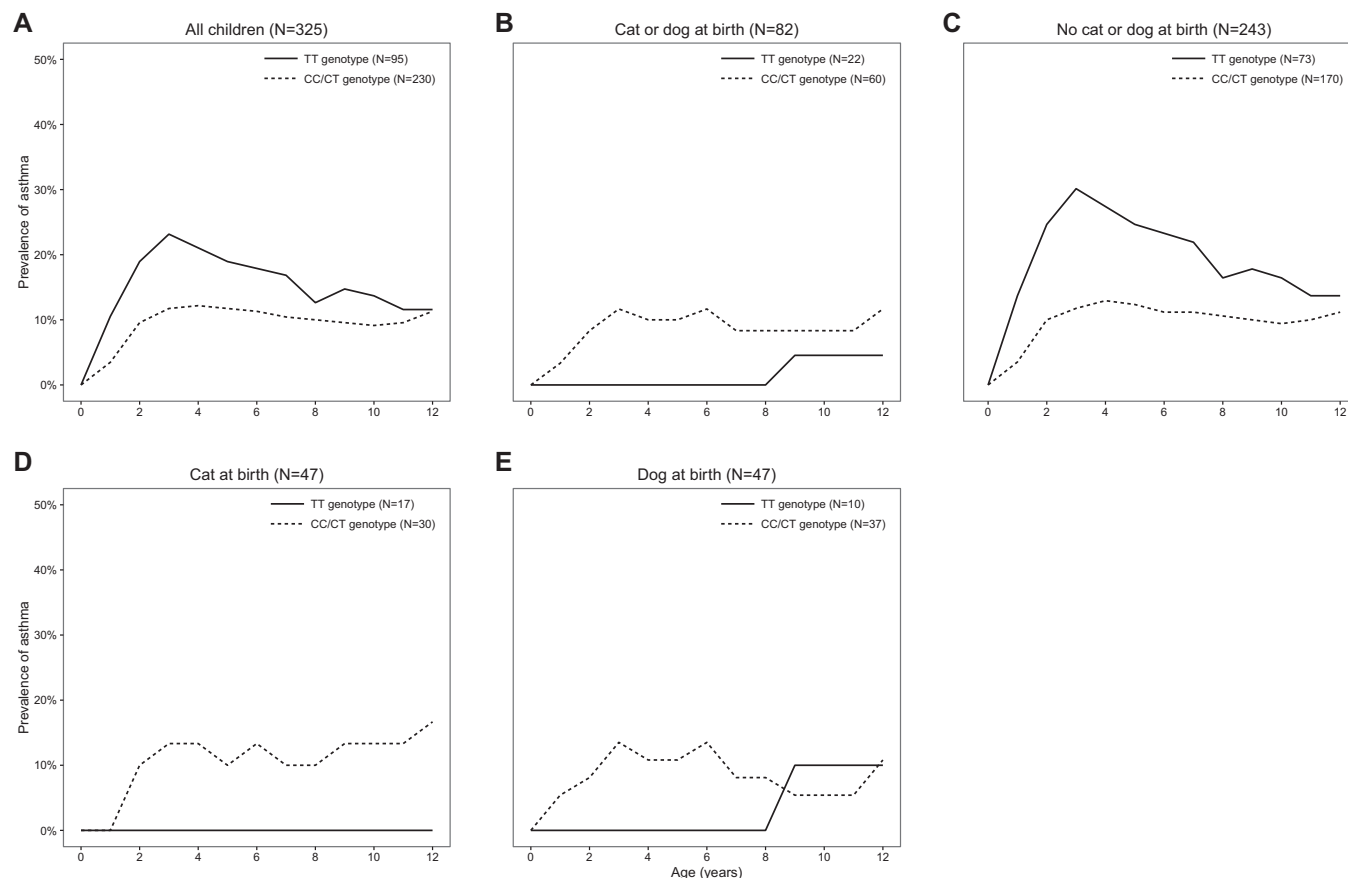
Based on the findings from COPAC₂₀₀₀ that especially cat exposure in the first year of life interacted with the high-risk

TT genotype on asthma risk, we used the unselected COPSAC₂₀₁₀ cohort for replication. Information on cat ownership and rs7216389 SNP genotype was available in 86% (604/700 children). The genotype distribution was as follows: CC, 24% (144 children); CT, 50% (302 children); and TT, 26% (158 children). Twenty-two percent (132 children) owned a cat in the first year of life. Cat exposure was associated with a lower risk of asthma in the first 5 years of life in the TT genotype (aHR, 0.54; 95% CI, 0.22-1.28; $P = .159$) but did not reach statistical significance, with weaker association observed in those with the CC/CT genotype (aHR, 0.86; 95% CI, 0.49-1.52; $P = .608$; adjusted interaction $P = .239$; Fig 5, A and B). Similar tendencies were observed among children born to mothers with asthma, although lower in numbers (Fig 5, C and D).

TABLE II. Cox regression estimates of cumulative risk of asthma by allergen level (log₂-transformed continuous variable) in the home at age 1 year

Allergen	Crude HR (95% CI)	P value	aHR (95% CI)	P value
Cat (Fel d 1)				
TT stratum	0.86 (0.75-0.98)	.023	0.83 (0.71-0.97)	.022
CC/CT stratum	1.04 (0.96-1.13)	.323	1.02 (0.93-1.11)	.730
Dog (Can f 1)				
TT stratum	0.95 (0.83-1.08)	.411	0.97 (0.84-1.13)	.702
CC/CT stratum	0.97 (0.89-1.07)	.575	0.94 (0.86-1.04)	.242

HRs correspond to change for every doubling of the specific allergen level. Stratification was by rs7216389 genotype (TT genotype and CC/CT genotype). Results are adjusted for maternal smoking in pregnancy, maternal educational level, and maternal sensitization. Boldface values indicate statistical significance.

**FIG 4.** Prevalence of asthma during the first 12 years of life according to rs7216389 genotype. Prevalence among all children (**A**) and by cat or dog (**B**), no cat or dog (**C**), cat (**D**), and dog (**E**) status at birth.

DISCUSSION

Principal findings

We found a gene-environment interaction between the rs7216389 genotype and cat and/or dog exposure from birth, attenuating the risk of asthma development during the first 12 years of life in children with the high-risk TT genotype but without any effect in children with the CC/CT genotype. This association seemed driven mainly by cat exposure. Similar observations were found for cat exposure and number of episodes with pneumonia and bronchiolitis from age 0 to 3 years. These findings might explain the previously reported divergent associations between cat and dog exposure and asthma susceptibility ignoring this gene-environment interaction.

Strengths and limitations

The main strength of this study is the design of the COPSAC₂₀₀₀ birth cohort. Children from the cohort were subjected to meticulous prospective clinical monitoring, diagnosis, and treatment of lung symptoms based on standardized operating procedures by clinical researchers from the single research site through 12 years of life at regular 6-month intervals for the first 7 years, as well as for acute lung manifestations. The longitudinal assessments from birth ensured strong clinical end points and improved statistical power. Genetics contribute to the age of onset of asthma, and because the chromosome 17q21 locus is associated with early onset of childhood asthma, the subject's age of onset carries more information about disease etiology than case-control status at a specific time point.

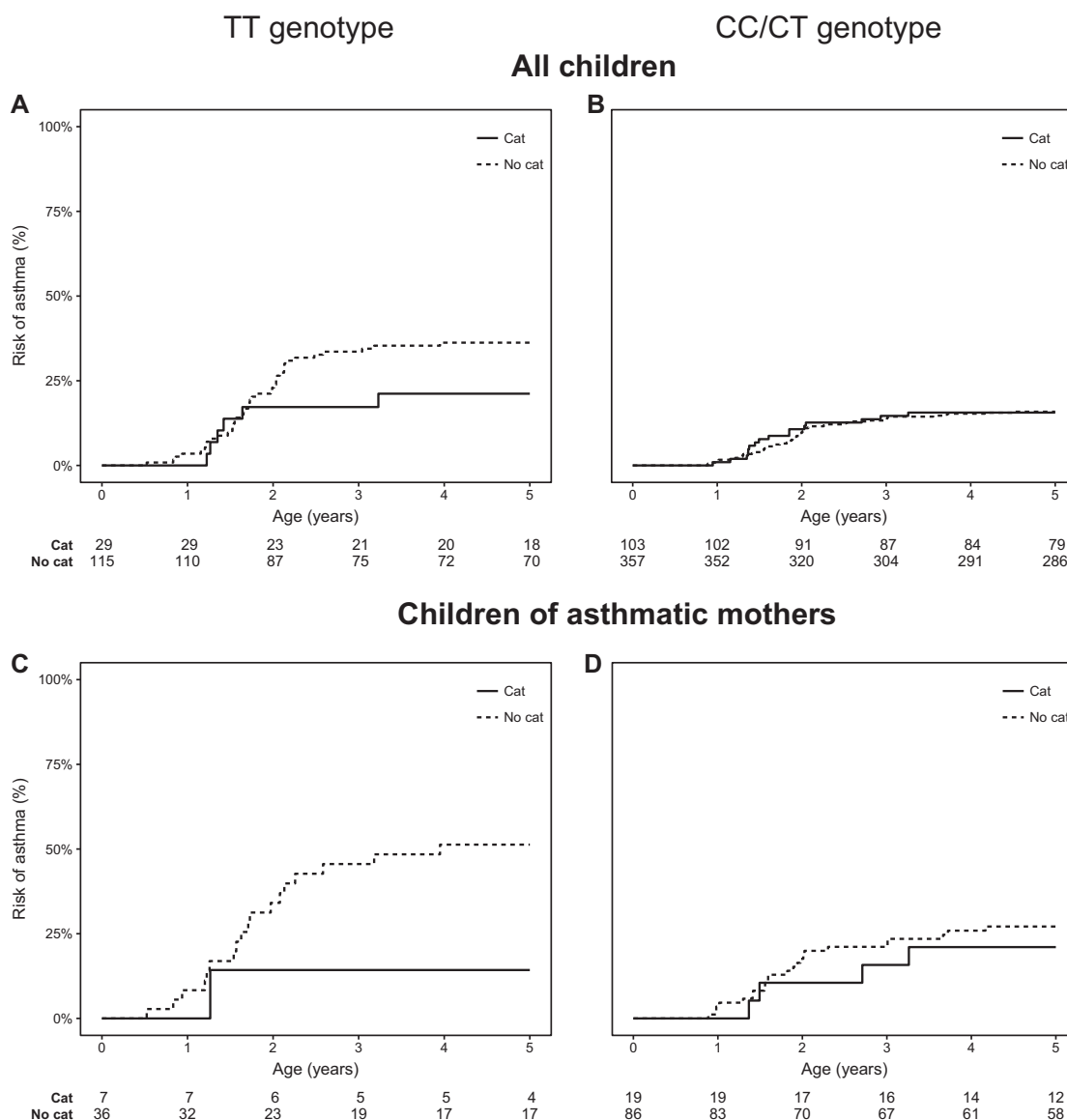


FIG 5. Replication cohort: COPSAC₂₀₁₀. Kaplan-Meier estimates of cumulative risk of asthma in the first 5 years of life according to cat status are shown. Stratification was by rs7216389 genotype: **A**, all children: TT genotype; **B**, all children: CC/CT genotype; **C**, children of asthmatic mothers: TT genotype; **D**, children of asthmatic mothers: CC/CT genotype. Numbers at risk are presented below the graph.

The high reliability of the data obtained on cat and dog exposure in the home from birth is another strength of the study. Data were obtained prospectively by COPSAC research personnel, who interviewed the families during scheduled visits to the research unit during the first month of life. In addition, we complemented analysis of the presence of cats and dogs in the home at birth with allergen levels from bedding at age 1 year, allowing for an objective dose-response analysis. It is a further strength that we were able to adjust the results for lifestyle-related covariates associated with cat and dog ownership. Furthermore, COPSAC₂₀₀₀ is ethnically homogenous. Only 10 (2.7%) nonwhite subjects existed in the population examined, and if these were removed from the analysis, estimates remained unaffected.

It is a limitation that the external validity of this study might be affected by the high-risk nature of the COPSAC₂₀₀₀ birth cohort. However, it is a strength that we were able to observe similar tendencies in an unselected birth cohort (COPSAC₂₀₁₀) after cat

exposure in the first year of life, both among all children and when stratifying for maternal asthma.

We found no differences in cat or dog ownership between the children's genotypes. The observed associations remained significant after adjusting for maternal smoking in pregnancy and maternal educational level as surrogate markers of lifestyle related to cat and dog ownership. Furthermore, the presence of asthma or allergy in the parents could have selected for pet ownership. We found a lower prevalence of sensitized mothers owning a pet, but adjustment for this did not affect the results, suggesting that avoidance behavior was not an important confounder in our study. Still, having a cat and dog is clearly lifestyle related yet unrelated to genetics and therefore unlikely to be confounding our observations.

It could represent a bias that cats might have more access to children's bed than dogs, thereby exposing the child more and driving our results toward a bigger effect for cats than dogs. However, we found comparable levels of cat and dog allergens in

our dust samples and, despite this, only a rs7216389 genotype interaction with cat allergen levels.

Interpretation

Genetic variations in the chromosome 17q21 locus are strongly associated with childhood asthma, and the high-risk genotype defines an early-onset asthma phenotype with recurrent wheeze, asthma, and acute severe exacerbations but no increased risk for eczema, rhinitis, or sensitization.¹⁷ Here we demonstrate that this increased asthma risk is merely a childhood phenomenon equalizing within the first 12 years of life, which is in accordance with a previous study.²² Furthermore, our results suggest that the rs7216389 genotype is also associated with an infection-prone phenotype because the TT genotype also associated with increased risk of pneumonia and bronchiolitis in the children. This could explain why the effects of the rs7216389 genotype decrease with age.

We are still far from understanding which mechanisms determine whether a certain genotype leads to disease.²³ Gene-environment interactions between 17q21 variants and early tobacco smoke exposure have been reported, with tobacco smoke exposure increasing the asthma risk from 17q21 risk variants even further,^{24,25} although this could not be confirmed in a subsequent study.¹⁹ However, the latter study demonstrated an interaction between furred household pets and rs7216389 genotype. They reported an increased prevalence of recurrent wheeze at 18 months in children with the low-risk rs7216389 CC genotype after exposure to furred household pets and a nonsignificant decreased risk in the TT genotype but without analyzing the effect of either cat or dog exposure.¹⁹ This association was only found among children at high risk of asthma, which is comparable with our COPSAC₂₀₀₀ population. We show that early-life cat exposure attenuates the increased risk of asthma otherwise associated with the strongest known genetic risk factor for early-onset childhood asthma to a risk equal to or even lower than that in those with the low-risk genotype.

Various studies have suggested that cat and dog exposure in early life can influence asthma development.²⁶ However, the results have been inconsistent, with some studies showing increased risk after cat and dog exposure^{4,5} and other studies showing decreased risk⁶ or no effect on asthma risk.⁷ The time from exposure to assessment of asthma outcomes differs vastly across studies, which can be critical because the immune modulation affecting the long-term outcome can only take place in a certain time period. Pre- and perinatal life is a period of immune programming during which environmental exposures can affect the maturation of the immune system,²⁷ whereas the same exposure later in life might have a different effect. These complex gene-environment interactions have not been considered in most previous risk analyses of pet exposure.³ We found a consistently lower risk of asthma after cat exposure (both cat in the home and with increasing cat allergens) among children with the high-risk TT genotype, whereas we found no individual effect from dog exposure in the allergen analyses. The same was found for LRTIs, only showing interaction between the rs7216389 genotype and cat exposure, with no effects from dog exposure. The lacking interaction between 17q21 genotype and dog exposure has been reported previously.²⁸

The amount of cat allergen in children's bedding was inversely associated with asthma development in children with the rs7216389 high-risk TT genotype, suggesting an association between the quantity of cat exposure and decreased asthma risk. The cat

allergens could be causal or alternatively mediated through gene-microbial interactions from increased microbial load in early life introduced by the cat. Household cats and dogs represent a massive microbial exposure for the family²⁹ and might be responsible for transmission of bacteria to the owner, leading to an affected microbial ecology. We have previously demonstrated that cat and dog ownership associated with bacterial composition in pregnant women.³⁰ Microbial transmission from cats and dogs to children might be the causal link in the observed modified asthma risk.

Furthermore, we demonstrate another interaction between cat exposure, rs7216389 genotype, and the number of episodes of pneumonia and bronchiolitis in the first 3 years of life. Cat exposure was associated with a lower number of these infections in children with the high-risk TT genotype compared with those without cat exposure, again without any association in the CC/CT genotype. This could point toward a mechanism of reducing the number of infections. An interaction between 17q21 and human rhinovirus illness has been reported to modify asthma risk in childhood,¹⁸ pointing toward immune effects that could also be present in our study.

Orosomucoid-like 3 gene (ORMDL3) expression is highly correlated with asthma-associated SNPs, especially rs7216389.¹⁶ Our findings might reflect modulation of ORMDL3 expression from exposure to cat and dog. The gene-environment interaction on disease penetrance might be mediated through epigenetic modification of gene expression in early life.³¹ DNA methylation in loci related to ORMDL3 regions has been shown to be affected in cord blood of children from farming environments and patients with early asthma, pointing to a possible epigenetic programming effect of the earliest environmental exposures.³²

Conclusion

Our observations suggest a role of especially early cat exposure for attenuating the risk of childhood asthma, pneumonia, and bronchiolitis in otherwise genetically susceptible subjects carrying the rs7216389 high-risk TT genotype. This gene-environment interaction could partly explain the divergent associations between cat and dog exposure and asthma susceptibility and might guide preventive measures in children with this genotype.

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Key messages

- Early-life exposure to cat and dog has shown diverging effects on the risk of childhood asthma, and this might be due to host genotype-specific effects. Genetic variations in the chromosome 17q21 locus are strongly associated with childhood asthma, and the high-risk genotype determines an early-onset asthma phenotype with recurrent wheeze, asthma, and acute severe exacerbations.
- We describe a gene-environment interaction between the rs7216389 genotype and especially cat exposure from birth, attenuating the risk of asthma development during the first 12 years of life in children with the high-risk TT genotype but without any effect in children with the CC/CT genotype.

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