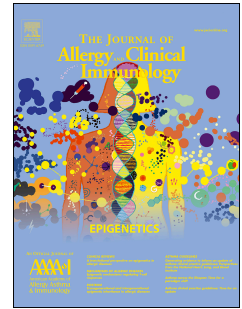


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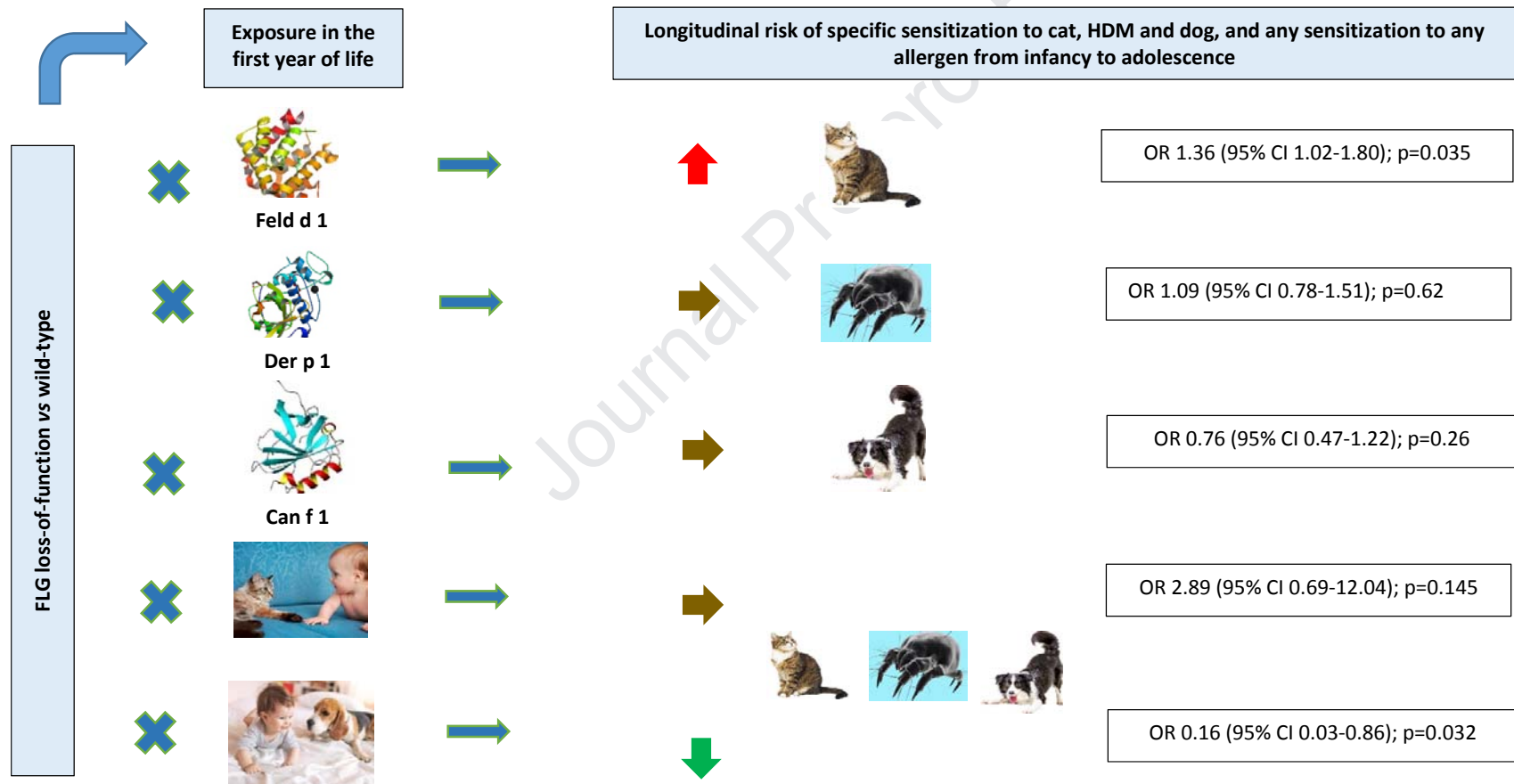
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Early-life inhalant allergen exposure, filaggrin genotype and the development of sensitization from infancy to adolescence



Early-life inhalant allergen exposure, filaggrin genotype and the development of sensitization from infancy to adolescence

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ABSTRACT

Background: We hypothesized that filaggrin loss-of-function mutations modify the impact of allergen exposure on the development of allergic sensitization.

Objective: To determine whether early-life exposure to inhalant allergens increases the risk of specific sensitization, and whether filaggrin mutations modulate these odds.

Methods: In a population-based birth cohort, we measured mite, cat and dog allergen levels in dust samples collected from homes within the first year of life. Sensitization was assessed at 6 time-points between infancy and age 16 years. Genotyping was performed for six filaggrin mutations.

Results: In the longitudinal multivariable model (age 1-16 years), we observed a significant interaction between *filaggrin* and Fel d 1 exposure on cat sensitization, with the effect of exposure being significantly greater among children with *filaggrin* mutations compared to those without (OR 1.36, 95% CI 1.02-1.80, $p=0.035$). The increase in risk of mite sensitization with increasing Der p 1 exposure was consistently higher among children with *filaggrin* mutations, but the interaction did not reach statistical significance. Different associations were observed for dog: there was a significant interaction between *filaggrin* and dog ownership, but the risk of sensitization to any allergen was significantly lower among children with *filaggrin* mutations who were exposed to dog in infancy (OR 0.16, 95% CI 0.03–0.86, $p=0.03$).

Conclusions: Filaggrin loss-of function mutations modify the relationship between allergen exposure and sensitization, but effects differ at different ages and between different allergens.

Clinical Implications: Children with *filaggrin* mutations may benefit from mite and cat avoidance, but may gain from having a dog in early life.

Key words: allergen exposure, house dust mite, cat, dog, sensitization, birth cohort, filaggrin, childhood, Fel d 1, Der p 1, Can f 1

Capsule summary

Filaggrin loss-of-function mutations modify the relationship between allergen exposure and sensitization, but effects differ at different ages. Transcutaneous exposure may be important for allergens that are considered as primarily inhaled.

Abbreviations

Der p 1:	<i>Dermatophagoides pteronyssinus</i> group 1 allergen
Fel d 1:	<i>Felis domesticus</i> group 1 allergen
Can f 1:	<i>Canis familiaris</i> group 1 allergen
FLG:	Filaggrin
GEE:	Generalized Estimating Equations
HDM:	House dust mite
MAAS:	Manchester Asthma and Allergy Study
OR:	Odds ratio
95% CI:	95% Confidence intervals
SPT:	Skin prick test
AD:	Atopic dermatitis

INTRODUCTION

Although being exposed to allergen(s) is a prerequisite for the development of sensitization, the nature of the relationship between the level of exposure and the risk of sensitization is unclear.(1, 2) For example, in some studies exposure to house dust mite (HDM) allergens has been shown to increase the risk of HDM sensitization and asthma,(3-6) particularly in children with parental atopy.(7, 8) However other studies have not confirmed this association(9) (reviewed in(1)). Similarly, conflicting data has been reported on the impact of cat ownership and Fel d 1 exposure on cat sensitization, which has been shown in different studies to be either a risk(6, 9-11) or a protective factor.(12, 13) Early-life exposure to dogs in the home has been shown to reduce subsequent risk of allergic sensitization to multiple allergens,(14) but no studies have assessed the impact of objectively measured dog allergen levels in homes on specific sensitization. A recent study has reported different associations between early-life cat exposure and sensitization to cat at different ages in a birth cohort, pointing at the importance of life-course perspective.(15) In the first three years of life, sensitization was more common amongst cat owners, but after this the increase in sensitization rate was higher among children without a cat, so by adolescence the prevalence of sensitization was numerically higher in this group (although the difference was not statistically significant).(15) Hence, apparently contradictory results may be a consequence of different life-course sensitization trajectories between exposed and non-exposed individuals. Therefore, to understand the complex relationship between early-life exposures and later clinical outcomes, one should not rely only on cross-sectional analyses, as more useful information can be gained through the analysis of longitudinal trajectories.(1, 16)

The impact of early allergen exposure on sensitization is modified by parental atopy and birth order,(17) alluding to the importance of both genetic and environmental factors.(1) The concept that the same environmental exposure may have different effects among individuals with different genetic predisposition has been tested in studies which assessed the interaction between genes and the susceptibility to environmental factors.(18-20) Variability in response to HDM exposure in relation to mite-specific sensitization has been attributed to the *IL4* gene

promoter polymorphism C-590T.(21) Filaggrin (*FLG*) loss-of-function mutations contribute to an impaired skin barrier, and are associated with eczema and a range of allergic conditions(22-24) as well as allergic sensitization.(24, 25) Children with *FLG* mutations were found to have an increased risk of eczema if they were exposed to cat in early life, with no effect of exposure amongst those without *FLG* mutations.(26) In a study in food allergy, we have shown that early-life exposure to peanut allergens measured in dust collected from homes is associated with an increased risk of peanut sensitization and allergy in children who carry *FLG* mutations, with no significant effect of exposure in those without *FLG* mutations.(27) In the current study, we hypothesized that *FLG* loss-of-function mutations would modify the impact of exposure to inhalant allergen (HDM, cat and dog) on the development of sensitization. To test this, we used both cross-sectional and longitudinal analyses to investigate the impact of early-life domestic allergen exposure on subsequent sensitization, and whether these relationships were altered by *FLG* genotype and modified over time.

METHODS

Study design, setting, participants, data sources and definition of outcomes

The Manchester Asthma and Allergy Study is an unselected birth cohort described in detail elsewhere.(28) Detailed description is provided in the Online Repository. Briefly, 1184 subjects were recruited prenatally and followed prospectively. For this study, we used data from 1051 children in the observational cohort, excluding 133 children who took part in the environmental intervention arm.(29, 30) The study was approved by the Local Ethics Committee and parents gave written informed consent.

Participants attended follow-ups at ages 1, 3, 5, 8, 11, and 16 years. We assessed sensitization by skin prick tests (SPT). Mite, cat and dog sensitization was defined as a wheal diameter at least 3 mm greater than the negative control. Allergic sensitization was defined as a positive SPT to at least one of the allergens tested. Cat and dog ownership in the first year of life was ascertained using questionnaires administered at home visit in the first year of life.

Quantification of allergen exposure

Dust samples were collected in the first year of life from the living room and child's floor.(31) Der p 1, Fel d 1, and Can f 1 levels were measured using monoclonal antibody based ELISA (Indoor Biotechnologies, Cardiff, UK) with a detection limit of 0.2 µg/g, as previously described.(32, 33) To determine individual child's allergen exposure, we averaged allergen concentration in samples taken from the living room and child's bedroom.

Genotyping

FLG genotyping was performed using probes and primers as previously described.(26, 27, 34) Genotyping for R501X, S3247X and R2447X mutations were performed using a TaqMan based allelic discrimination assay (Applied Biosystems, Cheshire, UK). Mutation 2282del4 was genotyped by sizing of a fluorescent-labelled PCR fragment on a 3100 or 3730 DNA sequencer. *FLG* mutations 3673delC and 3702delG were assessed by GeneScan analysis of fluorescently labelled polymerase chain reaction products. Data was analyzed as combined carriage of a *FLG* null allele, i.e., children carrying one or more of the six genetic variations were considered as

having a *FLG* loss-of-function mutation.(27) In cases with incomplete *FLG* data, the presence of one *FLG* mutation defined that case as a carrier; participants with incomplete genotyping data in whom all alleles successfully tested were wild-type were excluded from further analysis, as it was not possible to determine their *FLG* genotype status.(27)

Statistical analysis

Allergen levels (expressed in $\mu\text{g/g}$) underwent natural log (\ln) transformation. The effects of *FLG* loss-of-function mutations and allergen exposure on allergen-specific sensitization at each age were investigated using logistic regression. We first analyzed the associations between sensitization and allergen exposure in each *FLG* genotype group. We then modelled the effect of the interaction between exposure and *FLG*, controlling for the main effects. Longitudinal analyses were performed by generalized estimating equations (GEE). Population average GEE models were developed to investigate whether the effect of allergen exposure, *FLG* loss-of-function mutation and their interactions on the development of sensitization changed over time. The coefficients represent the increased/decreased odds of sensitization per log-unit increase in allergen exposure. We also investigated the effect of cat and dog ownership in early life on sensitization. We adjusted all models with confounding variables including sex, socio-economic status and breast-feeding.

We tested the assumption of a linear relationship between allergen exposure and sensitization by conducting likelihood ratio tests to compare the fit of nested models with the inclusion and exclusion of a quadratic term for each exposure at all time points. Furthermore, link tests were carried out to check for model misspecification of the dependent variable when only a linear term for exposure was included.(35)

Given a smaller sample size at age 1, we assessed the sensitivity of our findings from longitudinal analyses with the exclusion of age 1 data. All analyses were conducted in Stata 15.

RESULTS

The flow of children through the study is summarized in Figure E1. Complete *FLG* genotyping was available for 712/933 (76.3%) Caucasian participants, of whom 131 did not have dust samples. We analyzed data from 581 children, of whom 51 (8.8%) had *FLG* loss-of-function mutations; 276 had complete sensitization data from age 3 to 16 years. Excluded participants were more likely to be male and have paternal asthma, but there were no differences in other risk factors and exposures, including *FLG* genotype and pet ownership (Table E1).

Table E2 shows the prevalence of sensitization from age 1 to 16 years in the whole population and stratified by *FLG* genotype. For all allergens, children with *FLG* mutations had significantly higher point prevalence of sensitization in pre-school (1-5 years), and for cat and HDM by mid-school age (11 years), but there were no differences in sensitization between genotype groups in adolescence (age 16) (Figure E2).

Early-life allergen exposure, FLG genotype, and allergen-specific sensitization

Cat: Cross-sectional analyses in each *FLG* genotype group suggested that the effect of early-life Fel d 1 exposure on cat sensitization differed between children with and without *FLG* mutations (Figure 1). Among children with mutations, increase in exposure significantly increased the risk of sensitization at ages 1, 3, 5 and 8; this association was no longer significant thereafter (Table E3). Among children with wild-type genotype, there was a significant association between Fel d 1 exposure and cat sensitization at age one year, with no significant association at later ages. The effect of early-life exposure on sensitization diminished over time in both genotype groups. In longitudinal adjusted GEE models, Fel d 1 exposure significantly increased the risk of sensitization among children with *FLG* mutations (OR 1.26, 95% CI 1.04-1.52, $p=0.017$), with no significant effect of exposure in those without mutations (OR 0.94, 95% CI 0.84-1.06, $p=0.34$). Similar results were obtained in 230 children with complete data from age 3 to 16 (Table E3).

These observations suggesting an interaction between *FLG* genotype and Fel d 1 exposure were formally tested in models which included interaction term controlling for main effects (Table 1). The effect of early-life exposure on cat sensitization was significantly higher in the *FLG* mutation

group at age 5 (OR 1.99, 95% CI 1.05-3.79, $p=0.035$) and 8 (OR=1.59, 95% CI 1.07-2.37, $p=0.02$). In the longitudinal GEE model, we observed a significant interaction between *FLG* genotype and Fel d 1 exposure, in that the effect of early-life exposure on the development of cat sensitization from infancy to age 16 years was significantly greater among children with *FLG* mutations compared to those without (OR 1.36, 95% CI 1.02-1.80, $p=0.035$, Table 1). The interaction effect remained robust to sensitivity testing among participants with complete data.

House dust mite: Analyses in each *FLG* genotype group suggested a broad pattern similar to that observed for cat allergen (Figure 2). At age 1 year, the impact of Der p 1 exposure was markedly higher in children with *FLG* mutations (OR 6.66, 95% CI 1.15-38.58, $p=0.03$); from age 3 onwards, the odds ratios for the effect of Der p 1 exposure were numerically higher among children with *FLG* mutations, but this failed to reach statistical significance (Table E3). In longitudinal models, we observed non-significant trends for the increase in risk of sensitization with increasing Der p 1 in both genotype groups (OR, 95% CI: 1.11, 0.99-1.25, $p=0.06$ and 1.31, 0.96-1.80, $p=0.09$, wild-type and *FLG* mutations respectively; Table E3). Although the increase in risk per increase in unit of Der p 1 exposure was consistently higher among children with *FLG* mutations except at 16 years (Table E3), in multivariable models the interaction between *FLG* and exposure did not reach statistical significance (Table 1).

Dog: The relationship between Can f 1 exposure and dog sensitization differed to that observed for cat and HDM (Figure 3). Analyses in each genotype group showed that Can f 1 exposure in children without *FLG* mutations increased the risk of sensitization, with the effect being significant at age 16 (OR 1.26, 95% CI 1.06-1.50, $p=0.001$), with no significant effect of exposure among children with *FLG* mutations (Table E3). In GEE models, the effect of Can f 1 exposure differed between genotype groups, with a significant increase in the risk of sensitization among children without *FLG* mutations (OR 1.20, 95% CI 1.06-1.37, $p=0.004$), but not among those with *FLG* mutations. The formal interaction analyses showed that the effect of Can f 1 exposure on dog sensitization was consistently lower at all ages in children with *FLG* mutations, but the interaction between *FLG* and dog allergen exposure did not reach significance (Table 1).

For all allergens and models, a quadratic term did not improve the explanatory power of the relationship between exposure and sensitization (results available on request).

Pet ownership in the first year of life, FLG genotype and sensitization during childhood

Figure E3A shows the proportions of cat sensitized children by *FLG* genotype and cat ownership in the first year of life. From infancy to age 11 years, children with *FLG* mutations and cat at home had the highest risk of sensitization; the probability of sensitization converged to a similar level by age 16 in all four groups (Figure E4). Adjusted cross-sectional analyses (Table E4) consistently showed that from age 3 to 8 years, children with *FLG* mutations and cat at home had the highest risk of cat sensitization (~4-fold higher risk compared to those without mutations and no cat). Longitudinal analyses demonstrated that children with *FLG* mutations and cat at home had the highest probability of cat sensitization during childhood, significantly higher compared to children with no cat and *FLG* wild-type (OR 3.02, 95% CI 1.26–7.21, $p=0.013$), Table E4. The results of the adjusted longitudinal model are presented in Table E5.

These relationships were different for dog ownership. Children with *FLG* mutations and dog at home had the lowest point prevalence of dog sensitization at all ages apart from 5 (Figure E3B). In this group, there were no dog-sensitized individuals at 5 follow-ups, rendering results of cross-sectional analyses uncertain (Table E6). Models indicated that dog ownership amongst children with *FLG* mutations was protective (Figure E5), but formal statistical significance of the interaction in GEE models was not achieved (OR 0.06, 95% CI 0.00–1.72, $p=0.10$, Table E7).

To investigate whether dog ownership may offer protection which is not allergen-specific, we proceeded to analyze sensitization to any allergen. Figure E6 shows the proportions of sensitized children according to *FLG* genotype and dog ownership. A consistent finding was decreased risk of sensitization among dog owners with *FLG* mutations, with the opposite effect in those without mutations (Figure 4). In the longitudinal GEE model, there was a significant interaction between *FLG* genotype and dog ownership, in that the risk of sensitization was markedly and significantly lower among children with *FLG* mutations who had a dog in home in infancy (OR 0.16, 95% CI 0.03–0.86, $p=0.03$) (Table 2). There was no effect of cat ownership on sensitization to any allergen, and no interaction with *FLG* (Table E8).

DISCUSSION

In this population-based birth cohort, children with *FLG* mutations were more likely to be sensitized to inhalant allergens from infancy to school-age, but there were no differences in sensitization between those with and without *FLG* mutations in adolescence. Longitudinal sensitization trajectories differed between children exposed to allergen(s) in the first year of life compared to those not exposed, and between genotype groups. In general, the impact of cat and mite allergen exposure on allergen-specific sensitization was higher among children with *FLG* loss-of-function mutations compared to those without. We have shown a significant interaction between early-life cat allergen exposure and *FLG* genotype on the development of cat sensitization during childhood, and the effect of early-life exposure was significantly greater among children with *FLG* mutations (~36% increase in risk per log-unit increase in Fel d 1 in children with *FLG* mutations compared to those without). *FLG* mutations significantly increased the impact of early-life Der p 1 exposure on mite sensitization at age 1 year, but this modifying effect was gradually reduced over time. Markedly different patterns of the relationship between *FLG* genotype and exposure to dog on sensitization were observed, in that the risk of dog sensitization appeared lower, and the risk of sensitization to any allergen was significantly lower among children with *FLG* mutations who were exposed to dog in infancy (on average, more than 5-fold reduction in the risk of sensitization during childhood).

Limitations and strengths

The main limitation of our study is the lack of replication population. However, there are very few birth cohorts which have objective measures of exposure to multiple allergens in early life, and which have assessed sensitization on multiple occasions from early childhood to adolescence, both of which are key to interpreting our findings. Also, we were unable to include all cohort participants because of the availability of early-life dust samples and *FLG* genotyping.

The six *FLG* mutations we assessed have been consistently associated with eczema in Caucasian populations;(36) however, as some of these mutations are not found in non-Caucasians, all non-Caucasian participants were excluded from our analyses, and the results are not applicable to other ethnicities. Our definition of loss-of-function mutations within the *FLG* gene included

carrying one or more of the six genetic variations. As a result, among participants with incomplete genotyping data in whom all alleles successfully tested were wild-type it was not possible to determine their *FLG* status, and these individuals were excluded from further analysis, with a consequent reduction in sample size. We repeated our analyses using *FLG* variants 2282del4 and R501X only, and the results were entirely consistent with findings when *FLG* status was defined using all six mutations, with no material change in any of the reported significant interactions (data available on request). Another limitation is the smaller sample size at age one year and in some of the subgroups (e.g., sensitized dog owners with *FLG* mutations), and our findings need to be interpreted with caution. We could not address the question about the relative importance of exposure in infancy compared to that in later childhood.

Strengths of this study include comprehensive measurements of early-life allergen exposure, and objective evaluation of sensitization from infancy to adolescence. Sensitization was assessed at 6 time-points, which allowed the analysis of the impact of allergen exposure and genotype over time. We used data on both pet allergen exposure and pet ownership, and similar findings in these two measures of exposure strengthen our findings.

Interpretation

To our knowledge, this is the first study to investigate the relationship between objectively measured exposure to inhalant allergens and *FLG* mutations with longitudinal trajectories of allergic sensitization. We have previously shown that *FLG* loss-of-function mutations modify the impact of environmental peanut exposure on the development of peanut sensitization and allergy.(27) Our current study extends this to inhalant allergens, and suggests that transcutaneous route via impaired skin barrier may be important for sensitization. However, although there is currently no consensus about the presence of filaggrin in respiratory tissues(37), we cannot exclude the possibility that the effects observed in this study are mediated via inhaled route and exposure in nose, as filaggrin may be expressed in human nasal mucosa.(38)

Two birth cohorts in the UK and Denmark have shown a significant interaction between *FLG* loss-of-function mutations and early-life cat ownership on the development of infantile atopic

dermatitis (AD).(26) In the birth cohort from the Netherlands, early-life cat ownership enhanced the effect of *FLG* mutations on AD at ages 4 and 8 years, but, similar to our results, not on sensitization to any allergen.(39) A significant association has been reported between severity of AD and cat sensitization in *FLG*-related AD, but not in non-*FLG* related AD,(40) and one mechanism by which cat exposure could drive the development of AD is by enhancing cat-specific sensitization facilitated through an impaired skin barrier. In the current study, in children with *FLG* wild-type, Fel d 1 exposure increased the risk of cat sensitization at age one year, but this association diminished as children got older. In contrast, in children with *FLG* mutation, the increased risk of cat sensitization related to high allergen exposure in infancy persisted over time, with different trajectories of sensitization during childhood in children with different genotypes in relation to the same environmental exposure. By age 16 years, the point prevalence of cat-specific sensitization was the same in children with and without cat in both genotype groups. This may be in part due to exposure to cat allergen outside the home, as previous studies have shown that cat allergen is transported on clothing and can be measured in high levels in homes without cats and in public places.(33, 41)

FLG mutations significantly increased the impact of Der p 1 exposure on mite sensitization at age one. After this, the interaction between *FLG* and exposure decreased considerably. It has been shown that early sensitization (including that to mite and cat) is crucially important for the development of asthma,(42-45) and our finding that the interactions between *FLG* and cat and mite exposure in relation to specific sensitizations are stronger in early life may be one of the mechanisms by which *FLG* loss-of-function mutations increase the risk of asthma.(24)

Mite allergens have proteolytic activity,(46) which can disrupt skin barrier by cleaving tight junction proteins.(47) Mite allergens may thus disrupt the skin barrier without the increased susceptibility of *FLG* loss-of-function mutations. In support of this, in BALB/c mice recombinant Der p 1 was able to induce eczema without skin stripping or addition of an adjuvant.(48) However, it is unclear whether the magnitude of exposure in the animal model would resemble skin exposure in infants in real life, there are no definitive studies to confirm this.

The effect of exposure to dog differed to that observed for cat and mite, in that dog ownership (and high exposure to dog allergen) were protective among children with *FLG* loss-of function mutations, and that protective effect extended to sensitization to any allergen. Dog ownership may offer protection via the increase in microbial exposure,(1, 49) and our finding of a significantly stronger protective effect amongst individuals with *FLG* mutations can be explained by comparatively higher personal exposure to microbial products consequent to the impaired skin barrier. The fact that significant effects of Fel d 1 exposure and cat ownership were confined to cat-specific sensitization (but not sensitization to other allergens), suggests that the observed effects are related to allergen exposure. Taken together, our data support the notion that differences in the effects of cat and dog ownership may be a consequence of a cat being a marker of high allergen exposure, while the protective effect of dog may be mediated via changes in skin microbiome.(50)

Findings of the current study confirm our previous observation of the changing nature of the association between early-life exposures and sensitization with time, and the crucial importance of longitudinal analyses.(15) Furthermore, they raise fundamental questions about the current approach to replication in genetic and gene*environment studies, as the timing of the assessment of outcomes may critically impact upon the results of different studies investigating genes, environment and their interactions. Where there are inconsistencies, we should move from direct replication towards triangulation (i.e. integration of evidence from several approaches with differing and unrelated sources of bias) to improve causal inference.(51)

In conclusion, filaggrin loss-of function mutations modify the relationship between early-life allergen exposure and sensitization, but effects differ at different ages and between different allergens. Children with filaggrin mutations may benefit from mite and cat avoidance but may gain from having a dog in early life, but this will have to be confirmed in prospective studies.

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LEGENDS FOR FIGURES

Figure 1: The effect of exposure to cat allergen Fel d 1 on the predicted probability of cat-specific sensitization among children with and without *FLG* loss of function mutations: ages 1, 3, 5, 8, 11 and 16 years.

Figure 2: The effect of exposure to mite allergen Der p 1 on the predicted probability of mite-specific sensitization among children with and without *FLG* loss of function mutations: ages 1, 3, 5, 8, 11 and 16 years.

Figure 3. The effect of exposure to dog allergen Can f 1 on the predicted probability of dog-specific sensitization among children with and without *FLG* loss of function mutations: ages 1, 3, 5, 8, 11 and 16 years.

Figure 4. Proportion of children with allergic sensitization (assessed by SPT) by *FLG* genotype and dog ownership in early childhood from age 1 to age 16 years.

Table 1: Multivariable analysis indicating the effect of the interaction between *Filaggrin* loss-of-function mutations and Fel d 1, Der p 1, and Can f 1 exposure on the risk of cat, mite, and dog allergen-specific sensitization. *FLG* genotype, allergen exposure, sex, breastfeeding and socio-economic status are included as covariates.

Age	<u>Odds Ratio</u>	<u>(95% CI)</u>	<u>p-value</u>
<i>Interaction: FLG loss-off-function*Fel d 1 exposure</i>			
<i>Cat sensitization</i>			
1 year	12.57	(0.00 - 489810.02)	0.64
3 years	1.48	(0.81 - 2.73)	0.20
5 years	1.99	(1.05 - 3.79)	0.035
8 years	1.59	(1.07 - 2.37)	0.021
11 years	1.34	(0.85 - 2.12)	0.20
16 years	1.07	(0.72 - 1.61)	0.73
GEE: age 1-16	1.36	(1.02 - 1.80)	0.035
GEE: complete age 3-16	1.76	(1.09 - 2.83)	0.021
<i>Interaction: FLG loss-off-function*Der p 1 exposure</i>			
<i>Dust mite sensitization</i>			
1 year	0.00	(0.00 - .)	0.99
3 years	1.00	(0.60 - 1.65)	0.99
5 years	1.25	(0.78 - 2.00)	0.36
8 years	0.97	(0.64 - 1.46)	0.87
11 years	1.23	(0.74 - 2.04)	0.42
16 years	0.93	(0.54 - 1.60)	0.79
GEE: age 1-16	1.09	(0.78 - 1.51)	0.62
GEE: complete age 3-16	0.91	(0.53 - 1.56)	0.72

	<i>Interaction: FLG loss-off-function*Can f 1 exposure</i> <i>Dog sensitization</i>		
1 year	0.55	(0.15 - 2.05)	0.37
3 years	0.79	(0.42 - 1.49)	0.47
5 years	0.94	(0.51 - 1.70)	0.83
8 years	0.75	(0.24 - 2.41)	0.63
11 years	0.45	(0.06 - 3.34)	0.43
16 years	0.77	(0.32 - 1.88)	0.57
GEE: age 1-16	0.76	(0.47 - 1.22)	0.26
GEE: complete age 3-16	1.08	(0.50 - 2.36)	0.84

Table 2: Adjusted GEE analyses showing the interaction effect of dog ownership and *FLG* loss-of function mutation on the development of allergic sensitisation from age 3 to 16.

Dog ownership refers to the presence of a dog in the first year of life. Sensitization is defined as at least one positive test result to *Dermatophagoides pteronyssinus*, cat, dog, grass pollen, molds, milk, and egg (ages 1-5), birch and peanut (ages 8-16; i.e. a total of 9 allergens)

	Allergic sensitization (n=483)	
	OR (95% CI)	p-value
Dog present (1st year of life)	1.56 (0.98 - 2.47)	0.061
<i>FLG</i> loss-of function mutation	2.27 (1.20 - 4.32)	0.012
Dog present * <i>FLG</i> mutations	0.16 (0.03 - 0.86)	0.032
Age	1.08 (1.06 - 1.10)	0.000
Male	1.81 (1.24 - 2.63)	0.002
Breast-feeding	1.00 (0.65 - 1.54)	0.994
Socio-economic status (managerial level)	1.23 (0.83 - 1.83)	0.306

