

Original article

Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure

Background: Filaggrin gene (*FLG*) mutations contribute to the development of eczema and asthma, but their contribution to sensitization and hay fever remains unclear.

Methods: *FLG* mutations R501X, 2282del4 and R2447X were genotyped in the Prevention and Incidence of Asthma and Mite Allergy birth cohort ($n = 934$) to evaluate longitudinally, for up to 8 years, their association with eczema, sensitization, asthma, hay fever and their interaction with cat exposure.

Results: The combined *FLG* mutations were significantly associated with eczema at all ages when occurring in the first year of life (OR = 2.0; 95% CI: 1.4–2.8). Combined *FLG* mutations were associated with both atopic and nonatopic eczema, as well as asthma (OR = 3.7; 95% CI: 1.8–7.5). When the *FLG* 2282del4 mutation was analysed separately, it was significantly associated with the development of eczema during the first year, having eczema up to 8 years and sensitization at the age of 8 years, which was enhanced by early-life cat exposure (ORs being 8.2; 95% CI: 2.6–25.9, 6.0; 95% CI: 3.2–11.3 and 5.4; 95% CI: 1.2–23.6 respectively). *FLG* 2282del4 was significantly associated with hay fever from the age 5 years onwards (OR = 3.9; 95% CI: 1.5–10.5).

Conclusions: *FLG* mutations are associated both with atopic and nonatopic eczema starting in the first year of life. *FLG* mutations combined with eczema in the first year of life are associated with a later development of asthma and hay fever, a clear example of the atopic march. We confirm that cat exposure enhances the effect of a *FLG* mutation on the development of eczema and sensitization.

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The development and the phenotypic expression of allergic diseases depend on a complex interaction between genetic factors and environmental exposures to allergens and nonspecific adjuvant factors (1, 2). The impaired skin barrier function is an important factor in the pathogenesis of eczema. Damage to the skin barrier allows allergens to penetrate into the skin. The concept that eczema precedes the development of asthma and hay fever has been denoted as 'the atopic march' (3).

Abbreviations: BHR, bronchial hyper-responsiveness; CI, confidence interval; *FLG*, filaggrin gene; GEE, generalized estimating equations; Ig, immunoglobulin; OR, odds ratio; PIAMA, Prevention and Incidence of Asthma and Mite Allergy.

One of the proteins that contribute to the skin barrier is filaggrin (4). Filaggrin is expressed in the vestibulum of the nose, but not in the mucosa of the lung and the nasal mucosa (5–7). In 2006, Palmer et al. (8) demonstrated that two prevalent loss-of-function *FLG* variants (R501X and 2282del4) were very strong predisposing factors for eczema and concomitant asthma. Three less common mutations (R2447X, S3247X, 3702delG) were significantly associated with eczema in an Irish population (9). The current study investigated *FLG* mutations R501X, 2282del4 and R2447X in a large Dutch birth cohort to assess whether *FLG* mutations contributed to the development of eczema and subsequent development of sensitization, asthma combined with bronchial

hyper-responsiveness (BHR) and hay fever up to the age of 8 years. In addition, we also evaluated whether the recently found gene–environment interaction with cat exposure could be corroborated (10).

Methods

Study population

The Prevention and Incidence of Asthma and Mite Allergy birth cohort (PIAMA) is described elsewhere (11). The medical ethics committee approved the study and all participants provided written informed (parental) consent. Recruitment took place during the first trimester of pregnancy with a validated short screening questionnaire (12) and was conducted at 52 midwife practices in three different regions in the Netherlands (11). Women reporting any of the following symptoms were defined as allergic: a history of asthma, current hay fever, current allergy to house dust mite or pets and their children were defined as ‘high-risk’. A total of 10 232 pregnant women completed the questionnaire and 2949 (29%) of them were ‘allergic’. At baseline, the PIAMA study population consisted of 4146 children. All 1327 children from allergic mothers and a random sample of 663 children from nonallergic mothers were selected for medical examinations at the age of 4 and 8 years. Of these 1990 children, 1808 children were eligible for a medical examination at age 4 years of whom 1288 children participated. DNA was collected from 1046 children at age 4 years. Complete genotypes on *FLG* mutations were obtained from 1012 children. Seventy-eight children were excluded from the analyses because their mother was not Dutch, leaving a study population of 934 children. The 923 children aged 4 years participating in the study were medically examined for atopic eczema and immunoglobulin E (IgE) was obtained from 609 of these children. At the age of 8 years, BHR and IgE were measured in 636 and 678 children respectively.

Clinical evaluation

Eczema. Eczema was defined according to the UK Working Party's Diagnostic Criteria for Atopic Dermatitis at 4 years of age (13). For others, a diagnosis of eczema was based on the ISAAC questionnaire (14) and defined as a history of an itchy rash which was coming and going in the last 12 months on the flexural sites (the folds of the elbows or behind the knees), around the ears or the eyes or on the front of the ankles.

Sensitization. Sensitization was determined by specific IgE levels at 4 and 8 years of age for food (egg, milk) and inhalant allergens [house dust mite (*Dermatophagoides pteronyssinus*), mould (*Alternaria alternata*), cat (*Fel d1*), dog (*Can f1*), birch (*Betula verrucosa*) and grass (*Dactylis glomerata*)], as measured by radioallergen sorbent test. Sensitization was defined as the presence of specific IgE ≥ 0.70 kU/l against at least one tested allergen.

Atopic and nonatopic eczema. Atopic eczema was defined as eczema with sensitization at 4 years of age; nonatopic eczema as eczema without sensitization.

Asthma. Asthma was defined to be present if a parent reported a diagnosis of asthma by a physician and the presence of asthma symptoms in the preceding 12 months at the age 0–8 years. As diagnosis of asthma is difficult to establish in the first years of life, we measured BHR at the age of 8 years as an additional objective

measure indicative of asthma. Bronchial hyper-responsiveness to methacholine was measured according to the protocol of the European Community Respiratory Health Survey (15). A KoKo or Rosenthal-French dosimeter (Koko; Pulmonary Data Services, Doylestown, PA, USA) and DeVilbiss 646 nebulizers (DeVilbiss 646; Sunrise Medical HHG, Somerset, PA, USA) were used to nebulize methacholinebromide with an air pressure of 30 psi (2 bars) up to a cumulative dose of 2.46 mg. Bronchial hyper-responsiveness was present if a decrease of 20% in forced expiratory volume in 1 s occurred at a cumulative dose of ≤ 0.61 mg methacholinebromide.

Hay fever. Hay fever was defined as a positive answer to the question ‘Has your child ever had hay fever’ at the age of 3–8 years.

Genotyping

Children who participated in the medical examination at the age of 4 years in the PIAMA cohort and provided blood or buccal swabs for DNA were genotyped (1037 children) for *FLG* R501X, 2282del4 and R2447X as previously described (8, 9).

Statistical analysis

As a quality check of the genotyping, genotype data were analysed for deviations from Hardy–Weinberg equilibrium using chi-squared statistics. In the PIAMA study, children without Dutch ethnicity (5.0%) were excluded from analysis. The phenotypes eczema, sensitization, asthma combined with BHR and hay fever were tested for association with R501X, 2282del4 and R2447X. Genotypes were compared using chi-squared tests. The effect of *FLG* mutations on the annual incidence (first occurrence) of eczema and hay fever was studied by event history analysis using a discrete time hazard model (16). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated from logistic regression analysis. Generalized estimating equations (GEE) were used to assess the associations between *FLG* mutations and the presence of eczema and asthma in the first 8 years of age simultaneously. Generalized estimating equation analyses on hay fever were not performed, given the lack of data on its annual presence. Generalized estimating equation is a longitudinal statistical technique taking into account correlations between repeated measurements in the same individual to derive correct standard errors and *P*-values of the estimates. A Toepitz7 working correlation structure was used to correct for this within subject correlation. Differences in associations between groups were tested for significance by including an interaction term into a regression model. Calculations were performed using SPSS 14.0 or SAS 9.1 statistical software and considered significant if $P < 0.05$ (two-sided).

Results

A total of 934 children with complete genotypes and Dutch ethnicity were evaluated. Characteristics are presented in Table 1.

Genotyping

All single nucleotide polymorphisms were in Hardy–Weinberg equilibrium. Minor allele frequencies of the mutations R501X, 2282del4 and R2447X were 1.4% (95% CI: 0.9–2.0), 2.7% (95% CI: 1.9–3.4) and 0.4%

Table 1. Characteristics of the participating children

Total number (n)	934
Male, %	52.6 (491)
Phenotypes age 1	
Eczema*	175 (19.2)
Phenotypes age 4	
Eczema†	110 (11.9)
Sensitization‡	166 (27.3)
Phenotypes age 8	
Eczema*	159 (18.0)
Sensitization‡	222 (32.7)
Asthma plus BHR§ at age 8	27 (4.4)
Asthma without BHR§	6 (1.0)
Hay fever ever	54 (6.3)
Atopy mother	610 (65.3)
Atopy father	292 (31.3)
Cat at home at 3 months	275 (29.5)

Results presented as number (%), unless stated otherwise.

*Questionnaire eczema.

†Observed eczema according to criteria UK working party.

‡IgE ≥0.70 kU/l inhalant or food allergens.

§Bronchial hyper-responsiveness: PD₂₀ methacholine ≤ 0.61 mg.

Table 2. Genotypes of 934 participating children

	R501X	2282del4	R2447X	All combined
Wild type% (n)	97.1 (907)	94.6 (884)	99.3 (927)	91.0 (850)
Heterozygous% (n)	2.9 (27)	5.4 (50)	0.7 (7)	9.0 (84)
Homozygous% (n)	0 (0)	0 (0)	0 (0)	0 (0)

(95% CI: 0.1–0.7), respectively, resulting in a combined allele frequency of 4.5% and a combined carrier frequency of 9.0% (Table 2). Children from atopic mothers were more frequently heterozygous for the 2282del4 mutation than children from nonatopic mothers (6.9% vs 2.5%; $P < 0.01$).

The prevalence of atopy was 29% in 10 232 unselected, recruited pregnant women from the general population who completed a screening questionnaire in the PIAMA cohort. Children from atopic mothers had higher allele frequencies; therefore, overall allele frequencies for the general Dutch population were standardized to a population with 29% atopic mothers.

In the general Dutch population, the overall estimated allele frequencies of R501X, 2282del4 and R2447X were 1.4% (95% CI: 0.7–2.1), 1.9% (95% CI: 1.2–2.5) and 0.3% (95% CI: 0.1–0.5), respectively, resulting in a combined allele frequency of 3.6% and a combined carrier frequency of 7.0%.

Association analyses

Association of FLG variants with eczema. The carrier frequency of the combined genotype was significantly higher in children with eczema than those without eczema at the age of 4 years: 20.9% vs 7.5% (OR = 3.3; 95% CI: 1.9–5.5; $P = 0.000004$).

Incidence of eczema: The combined genotype was significantly associated with the incidence of eczema in the first year of life only (OR = 2.6; 95% CI: 1.6–4.2; $P < 0.001$), but not after that (OR = 1.0; 95% CI: 0.6–1.6; $P = 0.98$), the difference between the first year and the subsequent years being significant ($P = 0.007$). The combined genotype was significantly associated with the incidence of eczema in the first year of life in children with a cat at home at 3 months (OR = 3.2; 95% CI: 1.2–8.3; $P = 0.02$) and in children without a cat at home (OR = 2.4; 95% CI: 1.3–4.2; $P = 0.003$), $P = 0.63$ for the difference between children with and without early-life cat exposure.

Stratified analysis on having a cat at home showed a stronger effect of 2282del4 on the development of eczema in the first year in children with early-life cat exposure than those without early-life cat exposure, OR (95% CI) being 8.2 (2.6–25.9) and 3.2 (1.6–6.4), respectively, interaction term being borderline significant ($P = 0.17$).

Prevalence of eczema: Overall, the combined genotype was significantly associated with eczema at the age of 0–8 years (OR = 2.0; 95% CI: 1.4–2.8; $P = 0.0003$) and GEE analysis showed that it was similarly associated with eczema at all ages in the first 8 years of life (Fig. 1). The overall association of the combined genotype with the presence of eczema was not stronger in children with a cat at home in the previous year (OR = 1.9; 95% CI: 1.6–2.9; $P = 0.0029$) than in children without a cat (OR = 2.1; 95% CI: 1.1–3.8; $P = 0.024$), P -value of the interaction term being 0.85.

The overall association of 2282del4 with the presence of eczema was significantly stronger in children with a cat at home (OR = 6.0; 95% CI: 3.2–11.3; $P < 0.001$) than in children without a cat (OR = 2.2; 95% CI: 1.4–3.7; $P = 0.001$), P -value of the interaction term being 0.003.

Association of FLG variants with sensitization. Prevalence of sensitization: The prevalence of sensitization at 4 and 8 years by FLG genotypes is presented in Table 3. The



Figure 1. The combined genotype was similarly associated with eczema at all ages in the first 8 years of life.

Table 3. Prevalence of sensitization to any allergen at the age of 4 ($n = 609$) and 8 years ($n = 678$) and prevalence of atopic and nonatopic eczema at the age of 4 years per *FLG* genotypes

	4 years	8 years	Atopic eczema	Nonatopic eczema	No eczema
	166	222	31	43	531
R501X					
Wild type% (n)	27.4 (162)	32.7 (215)	93.5 (29)	93.0 (40)	97.6 (518)
Heterozygous% (n)	22.4 (4)	33.3 (7)	6.5 (2)	7.0 (3)	2.4 (13)
2282del4					
Wild type% (n)	26.7 (153)	32.3 (208)	83.9 (26)	86.0 (37)	95.3 (506)
Heterozygous% (n)	36.1 (13)	41.2 (14)	16.1 (5)	14.0 (6)	4.7 (25)
R2447X					
Wild type% (n)	27.4 (166)	32.9 (221)	100 (31)	97.7 (42)	99.4 (528)
Heterozygous% (n)	0 (0)	16.7 (1)	0 (0)	2.3 (1)	0.6 (3)
All combined					
Wild type% (n)	27.0 (149)	32.4 (200)	77.4 (24)	76.7 (33)	92.3 (490)
Heterozygous% (n)	29.3 (17)	36.1 (22)	22.6 (7)	23.3 (10)	7.7 (41)

combined genotype was not significantly associated with sensitization to food or inhalant allergens neither at the age of 8 years (OR = 1.2; 95% CI: 0.7–2.0; $P = 0.56$) or in children with or without eczema in the first year (OR = 1.7; 95% CI: 0.6–4.6; $P = 0.30$ and OR = 0.67; 95% CI: 0.30–1.5; $P = 0.34$ respectively). Cat exposure did not significantly modify the association between the combined genotype and sensitization (interaction term $P = 0.30$).

The 2282del4 mutation was significantly more strongly associated with sensitization in children with a cat at home (OR = 5.4; 95% CI: 1.2–23.6; $P = 0.02$) than in children without a cat (OR = 0.9; 95% CI: 0.4–2.2; $P = 0.89$), P -value of the interaction term being 0.04. The prevalence of sensitization to the individual allergens at the age of 8 years is presented in Table 4. The combined genotype was only significantly associated with specific IgE to birch allergen (OR = 2.5; 95% CI: 1.1–5.7; $P = 0.03$), (Table 4).

Association of FLG variants with atopic and nonatopic eczema. In Table 3, *FLG* genotypes according to the presence of atopic and nonatopic eczema at the age of 4 years, an age when 42% (31/74) of children with eczema

had a positive specific IgE are shown. The distribution of *FLG* variants was not significantly different between atopic and nonatopic eczema and both were significantly associated with the combined genotype.

Association of FLG variants with asthma. *Prevalence of asthma:* Overall, the combined genotype was significantly associated with asthma at the age of 0–8 years (OR = 3.7; 95% CI: 1.8–7.5; $P = 0.0003$) (Fig. 2). This overall association was significant in children with eczema in the first year (OR = 3.2; 95% CI: 1.2–8.5; $P = 0.02$), but not in children without eczema in the first year (OR = 1.8; 95% CI: 0.5–6.1; $P = 0.37$), P -value of the interaction term being 0.67. The presence of a cat at home did not significantly modify the overall association between the combined genotype and asthma both in the whole group (interaction term $P = 0.84$) and in the subgroup of children with eczema in the first year (interaction term $P = 0.94$).

The 2282del4 mutation was significantly associated with asthma at the age of 0–8 years (OR = 5.2; 95% CI: 2.4–11.5; $P < 0.0001$, whereas the presence of a cat at home did not significantly modify this association (interaction term $P = 0.92$).

Table 4. Prevalence of sensitization to individual allergens at the age of 8 years ($n = 682$) for the total group and the sub-groups of children with and without the combined genotype

Sensitization	All children % (n)	Children with combined genotype % (n)	Children without combined genotype % (n)	OR	95% CI	P -value
Milk	7.4 (50)	4.8 (3)	7.6 (47)	0.6	0.2–2.0	0.43
Egg	1.3 (9)	3.3 (2)	1.1 (7)	3.0	0.6–14.6	0.18
House dust mite (<i>Der p1</i>)	21.0 (143)	24.2 (15)	20.6 (128)	1.2	0.7–2.3	0.51
Mould (<i>Alternaria alternata</i>)	2.2 (15)	4.8 (3)	1.9 (12)	2.6	0.7–9.4	0.15
Cat (<i>Fel d1</i>)	8.2 (56)	12.7 (8)	7.8 (48)	1.7	0.8–3.8	0.18
Dog (<i>Can f1</i>)	3.1 (21)	4.8 (3)	2.9 (18)	1.7	0.5–5.8	0.42
Birch (<i>Betula verrucosa</i>)	6.2 (42)	12.7 (8)	5.5 (34)	2.5	1.1–5.7	0.03
Grass (<i>Dactylis glomerata</i>)	13.7 (93)	12.7 (8)	13.8 (85)	0.9	0.4–2.0	0.81

Odds ratios for the association of the combined genotype with sensitization to different allergens.

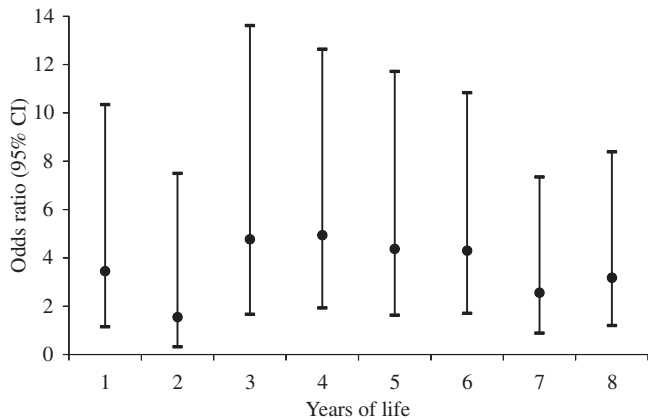


Figure 2. Overall, the combined genotype was significantly associated with asthma at the age of 0–8 years.

Association of FLG variants with hay fever. Incidence of hay fever: The combined genotype was not significantly associated with hay fever (OR = 2.3; 95% CI: 0.9–6.0, $P = 0.10$). The first occurrence of hay fever was significantly associated with 2282del4 at the age of 6–8 years only (OR = 3.9; 95% CI: 1.5–10.5; $P = 0.006$), and not at the age of 3–5 years (OR = 0.5; 95% CI: 0.1–3.7; $P = 0.49$). The association of 2282del4 with hay fever at the age of 6–8 years was significant in children with eczema in the first year (OR = 4.0; 95% CI: 1.2–13.6; $P = 0.03$), but not in children without eczema in the first year (OR = 1.7; 95% CI: 0.2–13.3; $P = 0.60$), P -value of the interaction term being 0.52. Cat exposure did not significantly modify the association between 2282del4 and hay fever (interaction term $P = 0.42$).

Potential confounders. Adjustment for potential confounders, i.e. maternal or paternal atopy, sex or breastfeeding did not relevantly change any of the above associations.

Discussion

Our results show that *FLG* mutations are associated with eczema at the age of 0–8 years when occurring in the first year of life. The higher risk of asthma and hay fever in children with *FLG* variants can be explained by the high-risk of early eczema in these children. We confirmed the findings by Bisgaard et al. (10) that cat exposure enhances the effect of a *FLG* mutation on eczema and we extended this observation by showing that the effect is also relevant for subsequent sensitization.

Previous studies in European populations have reported that variants in the *FLG* gene are associated with eczema and concomitant asthma (8, 17–21) or eczema alone (22). Our data confirm a significant association of the combined genotype R501X, 2282del4,

R2447X and the 2282del4 mutation with eczema, but we did not find a significant association with the R501X and R2447X variants separately. The latter may be explained by the low number of cases with eczema in the PIAMA birth cohort, whereas earlier published studies were based on 100% eczema populations. The PIAMA study was underpowered to make separate conclusions for the minor alleles R501X and R2447X with a low frequency.

Results on *FLG* were reported in four other population-based longitudinal birth cohorts, the MAS, ALSPAC, COPSAC and MAAS birth cohort (10, 20, 23). We have provided an overview of results of the PIAMA, MAS and ALSPAC cohorts in Table 5, because these cohorts demonstrated *FLG* associations with different diseases of the atopic march. The combined *FLG* carrier frequency of 20.9% in eczema cases in the PIAMA cohort is a little higher than in the MAS (16.7%) and ALSPAC cohorts (14.8%). This may be because of the fact that both the PIAMA and MAS cohorts were selected for atopic mothers, whereas the ALSPAC cohort was a nonselected population cohort which could have resulted in a lower carrier frequency. The combined carrier frequencies of R501X and 2282del4 were reported in the ALSPAC and MAS cohorts, whereas we report the combined carrier frequency of R501X, 2282del4 and R2447X. However, the carrier frequency of R2447X is low (0.7%) and hence cannot explain the difference in the combined carrier frequencies. Another explanation for the high carrier frequency in PIAMA birth cohort is the stringent phenotype definition of eczema (13). The distribution of the *FLG* combined genotype was not significantly different between atopic and nonatopic eczema at the age of 4 years in our study, which is comparable with those reported in MAS and ALSPAC cohorts. In contrast, *FLG* mutations were only associated with atopic eczema and not with nonatopic eczema in adults in eczema cohorts (17, 19, 20, 24).

We showed that early-life cat exposure enhances the effect of the 2282del4 mutation on the development of eczema in the first year. Furthermore, our longitudinal analysis demonstrated that the association between 2282del4 and eczema (ages 0–8 years) was stronger in children with a cat at home. Although Bisgaard et al. (10) did not report an association specifically for this mutation, they found a significant effect of the combined R501X and 2282del4 genotypes on the development of eczema in the first year of life in children with early-life cat exposure. The prevalence of the R501X mutation in our population was lower than that reported by others (25). Therefore, it is plausible that we could only find an association with the somewhat higher prevalent genotype 2282del4. The mechanism by which cat exposure drives the development of eczema in *FLG* carriers remains unclear. Bisgaard et al. (10) reported this to be unlikely because of cat-specific IgE. We showed that both atopic and nonatopic eczema were significantly associated with the *FLG* mutations, which indicated that the development

Table 5. Longitudinal studies on the association of *FLG* variants with the atopic phenotypes

MAS cohort	*Marenholz et al. 2006, Germany; <i>n</i> = 871, 0–10 years		
Eczema	PD or parental report of eczema or observed eczema		
Atopic eczema	Eczema and specific IgE to at least one allergen ≥ 0.70 kU/l		
Asthma	At least one wheezing episode during previous 12 months at age 7 or 10		
Allergic rhinitis, hay fever	PD of hay fever		
Sensitization	Specific IgE to at least one allergen ≥ 0.70 kU/l		
Phenotype examined	<i>P</i> -value	OR (95% CI)	Null alleles %
Eczema (<i>n</i> = 186)	3.5×10^{-5}	3.7 (2.0–7.0)	16.7
Atopic eczema (<i>n</i> = 117)	0.00006	3.8 (1.9–7.7)	17.1
Nonatopic eczema (<i>n</i> = 63)	0.00065	3.9 (1.7–8.9)	17.5
Asthma and eczema (<i>n</i> = 40)	5.4×10^{-8}	6.2 (2.6–14.8)	25.0
Asthma, no eczema (<i>n</i> = 77)	0.11	2.5 (1.1–5.8)	11.7
Rhinitis and eczema (<i>n</i> = 44)	1.5×10^{-5}	4.8 (2.0–11.6)	20.5
Rhinitis, no eczema (<i>n</i> = 76)	1.00	1.1 (0.3–3.2)	5.3
Sensitization, no eczema (<i>n</i> = 55)	0.62	1.4 (0.7–2.8)	7.0
ALSPAC cohort	†Henderson et al. 2008, UK; <i>n</i> = 6971, 0–11 years		
Eczema	6, 18, 30, 42, 75, 81 months: reported rash; 7, 9, 11 years: observed flexural dermatitis		
Atopic eczema	Eczema and at least one SPT ≥ 1 mm		
Asthma	91 months: PD asthma ever and wheeze and whistling past 12 months		
Allergic rhinitis, hay fever	54 months: reported allergy to pollen; 81 months and 11 years: reported sneezing, runny or blocked nose in past 12 months exclusively May–September		
Sensitization	7–8 years: SPT positive (≥ 3 mm wheal) to mixed grasses or HDM or cat		
Phenotype examined	<i>P</i> -value	OR (95% CI)	Null alleles %
Eczema (<i>n</i> = 1445)	1.19×10^{-22}	2.5 (2.1–3.1)	14.8
Atopic eczema (<i>n</i> = 193)	3.96×10^{-20}	3.1 (2.1–4.3)	20.7
Nonatopic eczema (<i>n</i> = 863)	1.10×10^{-6}	1.8 (1.5–2.3)	10.7
Asthma (<i>n</i> = 447)	0.00019	1.8 (1.4–2.4)	13.4
Asthma, no eczema (<i>n</i> = 192)	0.664	0.8 (0.4–1.4)	6.8
BHR at 8 years (<i>n</i> = 3106)	0.009	n.a.	n.a.
Multiple sensitization (<i>n</i> = 59)	5.42×10^{-27}	2.8 (1.5–5.3)	20.1
Hay fever at 54 months (<i>n</i> = 142)	0.01	1.7 (1.1–2.8)	14.1
Hay fever at 81 months (<i>n</i> = 275)	0.01	1.3 (1.0–1.8)	21.8
PIAMA cohort	Schuttelaar et al. 2009, The Netherlands; <i>n</i> = 934, 0–8 years		
Eczema	UK diagnostic criteria‡		
Atopic eczema	Eczema and specific IgE to at least one allergen ≥ 0.70 kU/l at 4 years		
Asthma	PD asthma combined with BHR at 8 years of age		
Hay fever	Report of hay fever ever		
Sensitization	Specific IgE to at least one allergen ≥ 0.70 kU/l		
Phenotype examined	<i>P</i> -value	OR (95% CI)	Null alleles %
Eczema at 4 years (<i>n</i> = 110)	0.000004	3.3 (1.9–5.5)	20.9
Atopic eczema at 4 years (<i>n</i> = 31)	0.004	3.5 (1.4–8.7)	22.6
Nonatopic eczema at 4 years (<i>n</i> = 3)	0.001	3.6 (1.6–7.9)	23.3
Asthma (0–8 years)§	0.0003	3.7 (1.8–7.5)	19.5
Asthma (0–8 years)§, no eczema in first year	0.37	1.8 (0.5–6.1)	11.1
Multiple sensitization (<i>n</i> = 62)	0.39	1.4 (0.6–3.3)	12.3
Hay fever (3–5 years)¶ (<i>n</i> = 36)	0.39	1.4 (0.6–3.3)	12.3
Hay fever (6–8 years)¶ (<i>n</i> = 30)	0.10	2.3 (0.9–6.0)	16.7

*Marenholz et al. *J Allergy Clin Immunol* 2006;**118**:866–871.

†Henderson et al. *J Allergy Clin Immunol* 2008;**121**:872–877. MAS cohort and ALSPAC cohort: combined genotype R501X, 2282del4; PIAMA cohort: combined genotype R501X, 2282del4, R2447X; PD, physician's diagnosis; BHR, bronchial hyper-responsiveness (ALSPAC: continuous dose-response slope; PIAMA: PD20 methacholine ≤ 0.61 mg).

‡Williams HC et al. *Br J Dermatol* 1994;**131**:406–416.

§GEE analysis.

¶Event history analysis; n.a., not available.

of eczema in *FLG* carriers did not depend on sensitization. Interestingly, we noted a significant association between 2282del4 and sensitization only in the sub-group

of early-life cat exposure. These findings may indicate that *FLG* carriers should avoid exposure to cats to reduce the risk of developing eczema and sensitization.

The exact relationship between early eczema and subsequent development of sensitization, asthma and hay fever is not clear (26, 27). A disturbed barrier function of the skin because of *FLG* gene mutations may enable allergens to penetrate into the skin, which may contribute to the development of systemic allergic conditions such as sensitization, asthma and hay fever. It is well known that environmental factors may interact with genes and produce a disease phenotype. Here, we confirm that *FLG* mutations are associated with eczema in the first year of life and cat exposure is such an environmental factor in that it enhances the risk of developing eczema and sensitization. In *FLG* carriers, early-life cat exposure seems to stimulate the progress of an atopic march.

To evaluate whether the association between *FLG* mutations and other atopic diseases could be explained by the effect of *FLG* mutations on eczema on one hand and the subsequent higher risk of developing other atopic diseases in patients with eczema on the other hand, we performed all analyses stratified by the presence of eczema in the first year. We did not observe any significant effects of *FLG* mutations on other atopic diseases in children without eczema in the first year of life. This indicates that eczema is a part of the causal pathway between the presence of *FLG* mutations and the development of asthma, allergic rhinitis and sensitization.

In conclusion, our analyses showed that *FLG* mutations are associated with both atopic and nonatopic eczema starting in the first year of life. Furthermore, our longitudinal results showed that there was a higher risk of developing asthma and hay fever at a later stage

during childhood in children with eczema in the first year who had the combined *FLG* mutations. We confirmed that cat exposure enhanced the effect of a *FLG* mutation on the development of eczema and sensitization. The hypothesis of the atopic march in children reflects the clinical observation that once atopic eczema has developed, this progresses to hay fever and asthma. The causal relationships between the atopic phenotypes are not well-established. In our study, both the genetic *FLG* association and cat exposure constitute the mechanisms that contribute to eczema, asthma and hay fever development. Our longitudinal results on atopic phenotypes may furthermore support the concept of an atopic march that starts in the skin, possibly stimulated by cat exposure. Moreover, there is a sub-group of children that has a *FLG* mutation and early onset eczema; yet their atopic march does not appear to proceed towards acquisition of allergic disease. The observation that cat exposure is particularly important in the development of eczema and the subsequent development of hay fever and asthma may have important bearings for preventive strategies.

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