An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma

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Background: Asthma prediction in early infancy is essential for the development of new preventive strategies. Loss-of-function mutations in the filaggrin gene *(FLG)* were identified as risk factors for eczema and associated asthma.
Objective: We evaluated the utility of the *FLG* mutations for the prediction of asthma.
Methods: Eight hundred seventy-one individuals of the prospective German Multicenter Allergy Study cohort were genotyped for 3 *FLG* mutations. Information on asthma, eczema, and food sensitization was available from birth to 13 years of age. Pulmonary function was measured from 7 to 13 years of age. The predictive value of the *FLG* mutations and of atopic phenotypes in infancy was assessed for asthma.
Results: In infants with eczema and sensitization to food allergens, the *FLG* mutations predicted childhood asthma with a positive predictive value of 100% (95% CI, 65.5% to 100%). This subgroup was characterized by a significant decrease in pulmonary function until puberty and represented 8.1% of all asthmatic children and 19.1% of patients with asthma after infantile eczema. We found a strong synergistic interaction between the *FLG*-null alleles and early food sensitization in the disease transition from eczema to asthma (relative excess risk due to interaction, 2.64; 95% CI, 1.70-3.98; $P = .00040$).
Conclusion: *FLG* mutations and food sensitization represent 2 distinct mechanisms interacting in the pathogenesis of asthma. In infants with eczema and food sensitization, genotyping of the *FLG* mutations allows the prediction of asthma before the onset of symptoms. Our findings might facilitate the development of early subgroup-specific interventions to prevent the progression from eczema to asthma. (J Allergy Clin Immunol 2009;123:911-6.)

Key words: *Genetic prediction, interaction, asthma, eczema, food sensitization, filaggrin, mutations, subphenotype, prevention, pulmonary function*

Asthma is a chronic inflammatory lung disease featuring intermittent airway obstruction triggered by environmental allergens, exercise, or viral infections. The increasing prevalence of asthma and the lack of curative therapy underscores the need for effective disease prediction and prevention.[1] Epidemiologic studies indicate that early childhood is a vulnerable phase when environmental exposures modify the disease risk in genetically susceptible individuals.[2] In addition, prospective studies revealed that a decrease in pulmonary function occurs during childhood and often persists into adulthood in these patients,[3-6] indicating that airway remodeling is an early and irreversible event. Therefore the availability of prediction markers in infancy is important to prevent or reduce the burden of asthma and its long-term sequelae.

To date, prediction markers for asthma are lacking. Genetic testing, which is now routine for the diagnosis of single-gene disorders,[7] could have enormous potential for predicting common complex diseases, such as asthma. However, although gene discoveries regarding asthma and other allergic conditions are emerging, their application for disease prediction is unexplored. A question often raised about genetic association findings of complex diseases is whether the information is useful for disease prediction because multiple genes and environmental factors contribute to disease development. Loss-of-function mutations in the gene encoding filaggrin *(FLG)*, which is important for skin barrier function, were identified to be strong genetic risk factors for eczema and eczema-associated asthma.[8] Furthermore, *FLG* mutations participate in the transition from infantile eczema to asthma, which is known as the "atopic march."[9] This process refers to the natural history of allergic disease, which often begins with eczema and food allergy in the young infant and continues with the development of respiratory airways disease later in childhood and adulthood.[10]

We evaluated *FLG* mutations as asthma predictors in the German Multicenter Allergy Study (MAS) birth cohort. For the identification of risk factors for asthma, a multifactorial approach has been proposed, combining information on genetic variations, specific phenotypes, and environmental influences.[11] We therefore investigated whether the predictive value of the *FLG* mutations for asthma was related to infantile eczema. Furthermore, we investigated the role of allergic sensitization to food allergens, which represents the earliest serologic marker for atopy[12] and is a recognized risk factor for chronic asthma.[13,14]

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Abbreviations used
FLG: Filaggrin gene
FVC: Forced vital capacity
MAS: Multicenter Allergy Study
RERI: Relative excess risk due to interaction

METHODS

Study population

The German MAS cohort has previously been described in detail.[15,16] The cohort consists of 1,314 children born in 1990. Children were followed at the ages of 1, 3, 6, 12, 18, and 24 months and at yearly intervals thereafter until age 13 years. Clinical assessment included standardized interviews, questionnaires, and physical examinations. Specific IgE antibodies to hen's egg, cow's milk, wheat, and soy were determined at the ages of 1, 2, 3, 5, 7, and 10 years. DNA samples of 871 children were available for genotyping. The institutional review boards of all centers approved the study, and written informed consent was obtained.

Phenotypes

Eczema was defined by the presence of either (1) the reported physician's diagnosis, (2) parental report of eczema symptoms, or (3) visible eczema at the time of follow-up.[17] Asthma was defined as the presence of 1 or more wheezing episodes during the previous 12 months at the ages of 7, 10, and/or 13 years.[18] Lung function was assessed by using body plethysmography (Masterlab; Jaeger, Würzburg, Germany). The initial measurement was performed at age 7 years in 731 individuals. The final measurement was performed at age 13 years in 642 individuals. For 79 children who did not participate in the final follow-up, we used pulmonary function measurements obtained at 10 years. FEV_1 and forced vital capacity (FVC) values were determined, and the FEV_1/FVC ratio was calculated to assess airway obstruction.

Allergic sensitization was defined as the presence of a specific IgE level of 0.70 kU/L or greater (CAP class II) to at least 1 tested allergen. The absence of specific sensitization was declared only if measurements from at least 2 time points were available. IgE levels to food allergens were available for 618 individuals.

FLG genotyping

Genomic DNA was prepared from whole blood by using standard methods. In all individuals the *FLG* mutations R501X, 2282del4, and R2447X were genotyped by using TaqMan allelic discrimination, fluorescence-based semiautomated allele-sizing technology, and restriction enzyme digestion, respectively, as described previously.[9,19] Two additional mutations, 3702delG and S3247X, which have previously been found at low frequency in an Irish population,[19] were determined in 189 individuals with eczema and 30 unaffected control individuals by sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif). Primer pairs used for amplification of genomic DNA by means of PCR were 3702-forward (5′-gtcaggacaccattcgtgc)/3702-reverse (5′-agacaacctctcggagtcg) and 3247-forward (5′-tctagacactcacaggcagt)/3247-reverse (5′-tgcctgattgtctggagcg), and primers for sequencing were 3702-forward and 3247-forward, respectively. Both mutations were absent in the 189 patients with eczema and 30 healthy control subjects and were therefore disregarded.

Statistical analysis

We evaluated selection bias in the study population by comparing study participants with those individuals who did not participate with respect to eczema, asthma, *FLG* carrier status, sex, parental history of allergy, parental smoking, the presence of older siblings, cord blood IgE levels, specific IgE levels, and asthma age of onset. Significance was obtained from the χ^2 test and the Wilcoxon rank sum statistic for qualitative and quantitative traits, respectively. Heterogeneity of odds ratios was evaluated with the Cochran Q test.[20]

The predictive value of risk factors was evaluated by calculating specificity, sensitivity, positive predictive value, and negative predictive value according to the Standards for Reporting of Diagnostic Accuracy.[21] Logistic regression was used to measure the strength of the association (odds ratio) between risk factors and asthma. Sex, parental history of allergy, parental smoking, the presence of older siblings, and cord blood IgE levels were tested as potential cofactors by using the χ^2 test and the Wilcoxon rank sum statistic for qualitative and quantitative traits, respectively. Marginally significant cofactors ($P < .1$) were included in the model. The significance of the logistic model was expressed as the P value of the likelihood ratio test for the full model (with single or multiple risk factors and cofactors included) versus the null model (cofactors only).

Children with eczema were divided into 3 groups, 2 of which carried either risk factor in the absence of the other factor and the third of which jointly carried both factors, to analyze the combined effect of the *FLG* mutations and sensitization to food allergens in eczema-associated asthma. The relative risk and its 95% CI were calculated by comparing each of the risk groups with the reference group, which lacked both factors. Association analysis was performed in a 2×2 contingency table by using the χ^2 statistic. For small cell counts (<5), the Fisher exact test was used instead. A 2-sided P value of less than .05 was considered statistically significant.

The relative excess risk due to interaction (RERI) was calculated as follows to establish whether an interaction between the 2 risk factors A (*FLG* mutations) and B (allergic sensitization to food allergens) existed:

$$RERI = Relative\ risk\ (A\ and\ B) - Relative\ risk\ (A\ without\ B) - Relative\ risk\ (B\ without\ A) + 1.$$

Interaction was defined as departure from the additive model.[22] There is evidence of interaction at a P value of less than .05 if the RERI 95% CI excludes zero. An RERI of greater or less than zero indicates a superadditive or subadditive effect, respectively. Synergism was said to be present if the combined effect of the 2 factors was greater than the sum of their solitary effects. The 95% CI of RERI was calculated by using a bootstrap percentile method, as suggested by Assmann et al.[23] We chose (with replacement) 100,000 bootstrap samples from the original sample, each of which was the same size as the original sample. We then estimated the CI from the sampling distribution of RERI and obtained a significance level.

The significance of the difference in means of the FEV_1/FVC ratio between 2 groups was assessed by using the t test. All statistical analyses were performed with the software R.

RESULTS

Characterization of the study population

To evaluate the utility of *FLG* loss-of-function mutations in the prediction of asthma, we examined 871 of 1,314 individuals of the MAS birth cohort who contributed DNA samples. These 871 study participants were compared with those individuals who did not participate to assess potential selection bias. No significant differences were found with respect to asthma, asthma age of onset, cord blood IgE levels, specific IgE levels, or *FLG* carrier status, as well as sex, parental history of allergy, parental smoking, or the presence of older siblings (Table I). However, children in the study population were more likely to have eczema (27.6% vs 22.8%). Likewise, the determination of specific IgE levels in a subset of the study population did not introduce a bias (data not shown), with the exception of eczema, which was slightly more frequent among the children with data on IgE levels (30.5% vs 27.6%). Differences in the distribution between each of the 2 subgroups were further tested for heterogeneity of the effect on asthma. The Cochran Q test revealed no evidence for significant heterogeneity.

Of the study population, 236 (27.2%) children had eczema before the age of 3 years, and 168 (20.1%) had asthma up to the

TABLE I. Comparison between the study population and the entire MAS cohort

	MAS cohort (n = 1,314)		Study population (n = 871)	
Male sex	52.1%	(684/1,314)	52.2%	(455/871)
Increased cord blood IgE level (≥0.9 kU/L)	18.3%	(241/1,314)	17.6%	(153/871)
Older sibling	41.1%	(540/1,314)	42.3%	(368/871)
Parental smoking	48.5%	(407/839)	47.3%	(315/666)
Parental history of allergy	48.4%	(627/1,295)	50.5%	(435/862)
Increased specific IgE level (≥0.7 kU/L; 3 y)	25.1%	(185/736)	24.9%	(155/622)
Eczema (3 y)	22.8%	(290/1,272)	27.2%*	(236/867)
Asthma (13 y)	19.8%	(192/970)	20.1%	(168/834)
Mean asthma age of onset	7.3 ± 3.1		7.1 ± 3.1	

*Significant difference ($P < .05$) comparing the study participants with those individuals who did not participate.

TABLE II. Single risk factor and multiple risk factor analysis for asthma in the MAS cohort

	Single risk factors				Multiple risk factors			
Risk factor	**n**	**OR***	**95% CI**	***P* value**	**n**	**OR†**	**95% CI**	***P* value**
FLG mutation	826	2.1‡	1.2-3.5	.0068	584	1.5	0.7-2.8	.27
Eczema	822	2.2‡	1.5-3.2	3.2×10^{-5}	584	1.8‡	1.2-2.8	.0087
Food sensitization	585	3.7‡	2.3-6.0	1.4×10^{-7}	584	3.2‡	1.9-5.2	6.7×10^{-6}

OR, Odds ratio.
*Adjusted for family history of allergy.
†Adjusted for family history of allergy and mutually adjusted for the other risk factors analyzed.
‡Statistically significant effect.

age of 13 years. Within the first 3 years of life, sensitization to food allergens was detected in 104 (16.8%) of 618 children. Three loss-of-function mutations in *FLG* (R501X, 2282del4, and R2447X) were identified within the MAS cohort and analyzed. The carrier frequencies were 4.8% for 2282del4, 4.1% for R501X, 0.7% for R2447X, and 9.4% for any of the *FLG* mutations.

Asthma risk attributable to the *FLG* mutations

The presence of an *FLG* mutation significantly increased the odds for asthma in the MAS cohort. In a logistic regression analysis adjusting for the cofactor of parental history of allergy, the effect of the *FLG* mutations on asthma susceptibility was similar to the effect of infantile eczema. However, adjustment for the effects of food sensitization and eczema revealed that the *FLG* mutations alone had a marginal effect on asthma susceptibility, which was no longer significant (Table II). In contrast, the odds ratios for food sensitization and eczema remained significant, even after adjustment for the other risk factors. Because filaggrin, which is not expressed in human lung, is mainly expressed in the skin, where it is involved in epidermal barrier function, we analyzed whether the onset of eczema was a prerequisite for the enhanced asthma risk attributed to the *FLG* mutations. Indeed, stratifying for eczema yielded a significant effect of the *FLG* mutations on asthma susceptibility only in the subgroup of children with eczema (Table III), whereas food sensitization had a substantial effect in both groups.

TABLE III. Asthma risk dependent on eczema status

	Children with eczema				Children without eczema			
Risk factor	**n**	**OR***	**95% CI**	***P* value**	**n**	**OR***	**95% CI**	***P* value**
FLG mutation	226	2.2†	1.0-4.5	.040	596	1.5	0.7-3.3	.31
Food sensitization	177	3.6†	1.8-7.4	2.5×10^{-4}	407	2.9†	1.4-5.9	.0041

OR, Odds ratio.
*Adjusted for family history of allergy.
†Statistically significant effect.

FLG mutations in the prediction of asthma

We used the longitudinal character of the MAS birth cohort to evaluate whether *FLG* mutations can be used as a genetic predictor for the development of childhood asthma. Because our and other studies[8] have consistently demonstrated an association of the *FLG* mutations with asthma only in children with eczema and because eczema typically predates asthma in the atopic march, we additionally analyzed whether the predictive value of the *FLG* mutations was related to infantile eczema. Furthermore, we included allergic sensitization to food allergens in our prediction model because it represents the earliest serologic marker for atopy[12] and is a recognized risk factor for chronic asthma.[13,14] The positive predictive value of the *FLG* mutations for asthma in the MAS cohort was 32.5% (95% CI, 22.7% to 44.0%), indicating that nearly one third of the children who carried an *FLG* mutation had asthma. Similarly, early sensitization to food allergens was a modestly strong predictor for the future development of asthma, with a positive predictive value of 43.0% (95% CI, 33.3% to 53.3%). Interestingly, the combination of the *FLG* mutations and increased IgE levels to food allergens, which was present in 8.9% of all children with asthma, yielded a strong increase of the positive predictive value to 73.3% (95% CI, 44.8% to 91.1%), exceeding all prediction models for asthma reported previously (Table IV).

We then investigated the utility of the *FLG* mutations in the prediction of the atopic march from infantile eczema to asthma. The subgroup of children with infantile eczema accounted for 42.3% (71/168) of all children having asthma. Although the *FLG* mutations or increased IgE levels to food allergens alone yielded positive predictive values of approximately 50% in this subgroup, again the combination of the 2 risk factors resulted in a strong improvement in performance and predicted future asthma, with a specificity of 100% (95% CI, 96.4% to 100%) and a positive predictive value of 100% (95% CI, 65.5% to 100%; Table V). This specific combination of risk factors defined 8.1% of all asthmatic subjects and 17.2% of children with infantile eczema who had asthma later in childhood.

Interaction between *FLG* mutations and sensitization to food allergens

FLG mutations, as well as food sensitization, enhanced the risk for asthma in children with eczema (Table III). Because in this subgroup the combination of the 2 factors yielded a very high positive predictive value for future asthma, we analyzed whether an interaction between the *FLG* mutations and food sensitization was involved in the disease progression from eczema to asthma.

TABLE IV. Early predictors for asthma in the MAS cohort

Predictor	Frequency	TP	FP	TN	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Eczema	27.6%	71	158	504	97	42.3 (34.8-50.1)	76.1 (72.7-79.3)	31.0 (25.2-37.5)	83.9 (80.6-86.7)
FLG mutation	9.6%	26	54	612	142	15.5 (10.5-22.0)	91.9 (89.5-93.8)	32.5 (22.7-44.0)	81.2 (78.2-83.9)
Food sensitization	16.9%	43	57	411	81	34.7 (26.5-43.8)	87.8 (84.4-90.6)	43.0 (33.3-53.3)	83.5 (79.9-86.6)
FLG mutation and food sensitization	2.5%	11	4	464	113	8.9 (4.7-15.7)	99.1 (97.7-99.7)	73.3 (44.8-91.1)	80.4 (76.9-83.5)

TP, True-positive; *FP*, false-positive; *TN*, true-negative; *FN*, false-negative; *PPV*, positive predictive value; *NPV*, negative predictive value.

TABLE V. The prediction of asthma in children with eczema

Predictor	Frequency	TP	FP	TN	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
FLG mutation	16.2%	17	20	138	54	23.9 (14.9-35.8)	87.3 (80.9-91.9)	45.9 (29.8-62.9)	71.9 (64.9-78.0)
Food sensitization	28.3%	28	23	99	30	48.3 (35.1-61.7)	81.1 (72.8-87.4)	54.9 (40.5-68.6)	76.7 (68.3-83.5)
FLG mutation and food sensitization	5.6%	10	0	122	48	17.2 (9.0-29.9)	100 (96.2-100)	100 (65.5-100)	71.8 (64.3-78.3)

TP, True-positive; *FP*, false-positive; *TN*, true-negative; *FN*, false-negative; *PPV*, positive predictive value; *NPV*, negative predictive value.

TABLE VI. Interaction between *FLG* mutations and sensitization to food allergens in eczema-associated asthma

	Eczema				
	Asthma	No asthma	RR*	95% CI	*P* value
FLG mutation (−) Food (−)	27	83	1.00		
FLG mutation (+) Food (−)	3	16	0.64	0.22-1.91	.56
FLG mutation (−) Food (+)	18	23	1.79†	1.11-2.88	.035
FLG mutation (+) Food (+)	10	0	4.07†	2.94-5.65	3.0×10^{-6}

RR, Relative risk.
*The risk ratio indicates the fold risk of asthma compared with that seen in children having eczema only, who have a 1.78-fold risk of asthma compared with the children lacking all risk factors.
†Statistically significant effect.

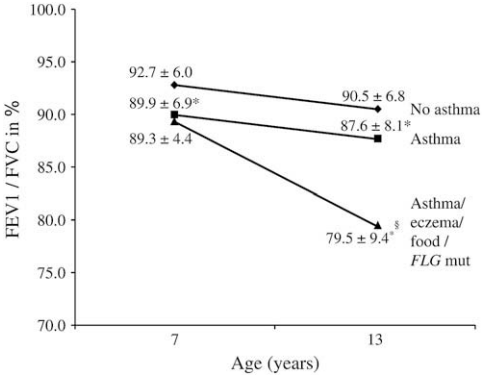

FIG 1. Pulmonary function analysis. Mean FEV_1/FVC values with SDs for children without asthma (n = 583 at age 7 years and n = 567 at age 13 years); for children with asthma, eczema, food sensitization *(food)*, and *FLG* mutations (*FLG mut*; n = 8 and n = 6, respectively); and for all other children with asthma (n = 140 and n = 148, respectively). *Significantly different from the "No asthma" group. §Significantly different from the "asthma" group.

Children with eczema who carried neither risk factor served as the control group. Notably, the control children already carried a significant 1.78-fold risk (95% CI, 1.16-2.71; $P = .00080$) to have asthma compared with children who were not affected by any of the analyzed factors (data not shown). Interestingly, *FLG* mutations provided no additional risk of asthma in children with eczema (Table VI) when sensitization to food allergens was absent. In contrast, early sensitization to food allergens alone yielded a significantly increased asthma risk for children with eczema. The presence of both risk factors, *FLG* mutation and early sensitization to food, strongly enhanced the asthma risk. The RERI of 2.64 (95% CI, 1.70-3.98; $P = 4.0 \times 10^{-4}$) indicated that the risk conferred by the combination of both risk factors was higher than the sum of the independent effects. This finding pointed to a strong synergistic interaction between the skin barrier defect caused by *FLG*-null mutations and early allergic sensitization to food in the cause of asthma. Comparing this high-risk group with the children who carried none of the risk factors analyzed resulted in a 7.23-fold increased risk of asthma (95% CI, 5.55-9.43; $P = 6.5 \times 10^{-9}$).

We next determined whether the *FLG* mutations were associated with increased IgE levels against food allergens within the eczema group. Among the *FLG* mutation carriers, 34.5% (10/29) were sensitized, whereas 28.2% (44/156) of the noncarriers had increased IgE levels to food allergens (relative risk, 1.22; 95% CI, 0.70-2.14; $P = .49$). Thus we found no evidence for an effect of the *FLG* mutations on food sensitization in children with eczema.

Lung function measurement

To further characterize the subgroup of asthmatic subjects that has been predicted by the analyzed risk factors, we compared the pulmonary function of children with asthma belonging to the high-risk subgroup with the lung function parameters of the remaining children with asthma on the one hand and those of the children without asthma on the other hand. Pulmonary function measurements at 7 years showed that the FEV_1/FVC ratio was significantly less in children with asthma compared with that seen in unaffected children (Fig 1). The pulmonary function in the subgroup of children with eczema and the 2 risk factors, *FLG* mutations and early allergic sensitization to foods, was similar to that of the other asthmatic children at that age. However, the prospective re-evaluation of pulmonary function revealed a significant decrease in this subgroup until puberty compared with

the other asthmatic children and the unaffected children whose lung function remained stable at values set at age 7 years (Fig 1).

DISCUSSION

The aim of this study was to investigate the utility of *FLG* mutations in the prediction of asthma. We have analyzed the MAS because it allowed us to assess the predictive power of *FLG* genetic testing in a prospectively evaluated, population-based sample. Furthermore, the MAS cohort enabled us to investigate food sensitization in infancy as a biomarker. Because sensitization to food allergens is usually observed transiently and does not persist until the development of asthma,[12] determination of the sensitization status early in life is required to assess the utility of this factor for disease prediction.

We found that in infants with eczema and sensitization to food allergens within the first 3 years of life, the presence of an *FLG* loss-of-function mutation predicts the future development of asthma with a specificity and a positive predictive value of 100%. The high positive predictive value was attributed to an interaction between the *FLG*-null alleles and sensitization to food allergens, which substantially increased the risk of disease progression from eczema to asthma. These novel findings might facilitate the development of strategies to combat the atopic march. Earlier asthma predictors included a family history of allergic disease, a personal history of eczema, and allergic sensitization. Bergmann et al[24] used eczema and a positive atopic family history to predict allergic airways disease with a positive predictive value of 50.2%. In the Tucson Children's Respiratory Study, Halonen and Stern[25] identified a positive predictive value of 60% for a combination of eczema and sensitization to the fungus *Alternaria* species, which was present in 12% of children with asthma.

In our study the combination of *FLG* mutations and early sensitization to food allergens, which yielded very high positive and negative predictive values, predicted a sizeable proportion (17.2%) of the infants with eczema who made the transition to asthma later in childhood. Although this group comprised only 10 individuals, they accounted for 8.1% of all asthmatic subjects in the MAS cohort. Furthermore, longitudinal pulmonary function measurements demonstrated that this subgroup of asthmatic children identified by the *FLG* mutations had a poor prognosis. In contrast to previous studies on asthma that had reported reduced lung function at school age but no further worsening thereafter,[3,26] the predicted subgroup revealed a steady decrease in pulmonary function until puberty. Hence this subgroup might particularly benefit from early prediction of the disease. Our findings indicate that assessment of the *FLG* carrier status could improve the prediction of eczema-associated asthma considerably. The magnitude of the predictive power surpasses the utility of mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* that account for only 2% to 4% of all breast cancer cases and for 15% to 20% of cases among the high-risk group of women with a strong family history and early onset of the disease[27] in whom targeted genetic testing was recommended.[28] Analogously, in the high-risk group of children with eczema and early food sensitization the genotyping of the *FLG* mutations would identify 35.7% of future asthmatic subjects. The impending risk of asthma is a major concern of parents with children with eczema, and parents of patients with other chronic inflammatory conditions have a positive attitude toward genetic risk assessment.[29] Genetic testing for *FLG* mutations would require prospective genetic counseling and critical risk factor assessment to meet current standards.

For asthma, it has been suggested that multifactorial approaches, which include both genetic and environmental factors, could improve our understanding of the disease.[11] Accordingly, there is growing evidence for gene-environment interactions in asthma and allergic disease.[30-33] An interaction of *FLG* mutations with cat exposure has recently been reported to increase the risk of eczema in infancy.[34] The identification of a synergistic interaction between *FLG* mutations and sensitization to food allergens stresses the importance of such interactions in the development of asthma. It should be noted that statistical interaction does not necessarily imply the biologic mode of action, and therefore further studies are required to resolve the molecular basis underlying this effect. Nevertheless, the strongly increased asthma risk in children with eczema, increased IgE levels to food allergens, and *FLG* mutations might point to key events in disease transition from eczema to asthma. Although the *FLG* mutations cause a molecularly well-characterized skin barrier defect, the mechanisms leading to early sensitization to food allergens are imperfectly understood. Early sensitization to food allergens might indicate an immunologic deviation toward atopy (IgE formation) in general. It might also be due to enhanced penetration of allergens through a defective gastrointestinal barrier, where *FLG* is not expressed. As a consequence, future disease prevention in children at risk for eczema-associated asthma could use a 2-pronged approach focusing on the compensation of the epidermal barrier defect, as well as on the mechanism underlying early sensitization to food allergens.

Asthma is a heterogeneous disease in terms of the age of onset, the severity and persistence of symptoms, and associated allergies. Therefore the identification of asthma subphenotypes has been proposed to improve our understanding of the different mechanisms underlying the disease and to allow for better disease management.[35] A number of preventive treatment modalities have been proposed, with inconsistent results in different patient groups.[36] It is well recognized that a patient's genetic constitution might determine the response to pharmacologic intervention.[37] The response to preventive measures might likewise be affected by genetic variation, and effects might only be detectable in specific genetically defined subgroups.

A few limitations of our study need to be mentioned. Because of incomplete follow-up, our study population comprised only two thirds of the entire MAS cohort and was slightly enriched with children with infantile eczema. This might be attributable to an increased motivation of parents of affected infants to participate in the follow-up period. However, this overrepresentation should not have an effect on the validity of our results because the main outcomes rely on the subgroup of children with eczema. Furthermore, we have shown that the children with eczema among study participants and nonparticipants did not differ significantly with respect to asthma risk. The results presented here were obtained from a large, prospectively evaluated German birth cohort that provided comprehensive data on infantile eczema and childhood asthma (up to 13 years) and repeated measurements of specific serum IgE levels during the first 3 years of life, which allowed a reliable classification of early food sensitization. This was particularly important for prediction purposes because allergic sensitization to food is often a transient phenomenon that does not persist beyond the first few years of life.[12] However, although the effects of the *FLG* mutations on

asthma prediction, as well as in the interaction analysis, were remarkably strong, further studies in different populations need to be initiated to confirm these results.

In this study we demonstrate that the determination of the *FLG* carrier status in infants with eczema and sensitization to food allergens within the first 3 years of life would allow the early prediction of asthma before the onset of symptoms and at a critical time of immune development, when preventive interventions are likely to be effective. We suggest our findings could be of diagnostic and subsequent therapeutic utility.

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Clinical implications: *FLG* mutations can be used for the prediction of childhood asthma and for the definition of a severe asthma subphenotype. This might facilitate the development of early preventive subgroup-specific interventions.

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