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

Ingo Marenholz, PhD,[a,b] Tamara Kerscher, BS,[a,b,*] Anja Bauerfeind, PhD,[a,*] Jorge Esparza-Gordillo, PhD,[a,b] Renate Nickel, MD,[b] Thomas Keil, MD, MPH,[c] Susanne Lau, MD,[b] Klaus Rohde, PhD,[a] Ulrich Wahn, MD,[b] and Young-Ae Lee, MD[a,b] *Berlin, Germany*

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]

From [a]Max-Delbrück-Centrum (MDC) for Molecular Medicine, [b]Pediatric Pneumology and Immunology, Charité, and [c]the Institute for Social Medicine, Epidemiology and Health Economics, Charité.
*These authors contributed equally to this work.
Supported by grants from the German Ministry for Education and Research through the Clinical Research Group for Allergy and the German National Genome Research Network, the European Union through a Marie-Curie fellowship to J.E.-G., and the Global Allergy and Asthma European Network.
Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication June 22, 2008; revised December 1, 2008; accepted for publication January 9, 2009.
Reprint requests: Young-Ae Lee, MD, Pediatric Pneumology and Immunology, Charité, D13353, Berlin, Germany. E-mail: yolee@mdc-berlin.de.
0091-6749/$36.00

doi:10.1016/j.jaci.2009.01.051

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