

Interaction between filaggrin null mutations and tobacco smoking in relation to asthma

Nikolaj Drimer Berg, MSc, PhD,^a Lise Lotte N. Husemoen, MSc, PhD,^a Betina Heinsbæk Thuesen, MSc, PhD,^a Lars-Georg Hersoug, MSc, PhD,^a Jesper Elberling, MD, PhD,^b Jacob Pontoppidan Thyssen, MD, PhD,^c Berit Christina Carlsen, MD, PhD,^c Jeanne Duus Johansen, DMSc,^c Torkil Menné, DMSc,^c Klaus Bønnelykke, MD, PhD,^d Steen Stender, DMSc,^e Michael Meldgaard, MSc, PhD,^e Pal Bela Szecsi, DMSc,^e and Allan Linneberg, MD, PhD^a Glostrup and Copenhagen, Denmark

Background: The mechanisms underlying the association between filaggrin (*FLG*) deficiency and asthma are not known. It has been hypothesized that *FLG* deficiency leads to enhanced percutaneous exposure to environmental substances that might trigger immune responses. We hypothesized that interactions between *FLG* deficiency and environmental exposures play a role in asthma development.

Objective: We sought to investigate possible interactions between *FLG* null mutations and tobacco smoking in relation to asthma.

Methods: A total of 3471 adults from a general population sample participated in a health examination. Lung function and serum specific IgE levels to inhalant allergens were measured, and information on asthma and smoking was obtained by means of questionnaire. Participants were genotyped for the 2 most common *FLG* null mutations in white subjects: R501X and 2282del4. Another Danish population was used for replication.

Results: The *FLG* null mutation genotype was significantly associated with a higher prevalence of asthma and decreased FEV₁/forced vital capacity ratio. In logistic regression analyses with asthma as the outcome, a significant interaction was found between *FLG* null mutations and smoking status ($P = .02$). This interaction was confirmed, although it was not statistically significant, in another Danish population study. Interactions

between *FLG* genotype and cumulated smoking exposure were found in relation to asthma ($P = .03$) and decreased FEV₁/forced vital capacity ratio ($P = .03$). A 3-way interaction was found among *FLG* genotype, smoking, and asthma, suggesting that the *FLG*-smoking interaction mainly played a role in nonatopic subjects.

Conclusion: *FLG* null mutations modified the effects of smoking on the risk of asthma. This finding might have implications for risk stratification of the population. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Asthma, filaggrin, R501X, 2282del4, tobacco smoking, gene-environment interaction, genotype, population based, health examination, Health2006

The main function of the skin is to form a protective barrier between the organism and its external environment, thereby preventing entrance of pathogens, irritants, and allergens, as well as minimizing transepidermal water loss. In the epidermis terminal differentiation of keratinocytes into corneocytes leads to the formation of the stratum corneum, an impenetrable physical barrier consisting of the flat anucleated corneocytes imbedded in an intercellular matrix rich in nonpolar lipids.¹ The cross-linking of filaggrin (*FLG*) monomers derived from profilaggrin produced in the keratinocytes with keratin filaments causes an aggregation of the keratin filaments into tight bundles, which promotes the collapse of the cell into the flat shape characteristic of the corneocyte.² The *FLG* gene is located on chromosome 1q21 residing in the epidermal differentiation complex.³ A 4-base deletion, 2282del4, and a nonsense mutation, R501X, result in complete loss of *FLG* production and are thus functional null alleles for *FLG*,⁴ causing a dysfunctional skin barrier.⁵⁻⁷ In combination, 2282del4 and R501X account for the majority of null mutations in white populations.⁸

FLG deficiency has been shown to be a causative factor for ichthyosis vulgaris,⁴ as well as a predisposing factor for atopic dermatitis.⁹ In recent meta-analyses *FLG* deficiency has been confirmed as a risk factor for atopic eczema, IgE sensitization, allergic rhinitis with or without atopic eczema, and asthma in patients with atopic eczema but not for asthma in patients without atopic eczema.^{10,11} In children and young adults, however, it has been found to be associated with asthma severity independent of eczema status.¹²

The mechanism by which a dysfunctional skin barrier is associated with the development of these diseases is

From ^athe Research Centre for Prevention and Health, Glostrup University Hospital, the Capital Region of Denmark, and ^bthe Danish Research Centre for Chemical Sensitivities; ^cthe National Allergy Research Centre, Department of Dermato-Allergology; ^dCOPSAC, Danish Paediatric Asthma Centre, Department of Paediatrics; and ^ethe Department of Clinical Biochemistry, Gentofte University Hospital, University of Copenhagen.

Supported by the Health Insurance Foundation and the philanthropic foundation TrygFonden.

Supported by the Danish Board of Health; the Danish Environmental Protection Agency; the Copenhagen County Research Foundation; the Aase and Einar Danielsens Foundation; the Velux Foundation, ALK-Abelló A/S, Denmark; and the Danish Scientific Research Council.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication April 1, 2011; revised August 25, 2011; accepted for publication August 31, 2011.

Corresponding author: Allan Linneberg, MD, PhD, Research Centre for Prevention and Health, Glostrup University Hospital, Nordre Ringvej 57, Building 84/85, DK-2600 Glostrup, Denmark. E-mail: allin01@regionh.dk.

0091-6749/\$36.00

© 2011 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2011.08.045

Abbreviations used

FENO: Fraction of exhaled nitric oxide

FLG: Filaggrin

FVC: Forced vital capacity

OR: Odds ratio

unknown. In murine models of *FLG*-deficient skin, an increased penetration of allergens has been shown to cause a secondary local and systemic inflammatory response.^{5,6} Considering atopic dermatitis, it has been shown in mice that *FLG* deficiency in itself confers a reduced inflammatory threshold for irritants and haptens.⁷ With respect to asthma, dermal exposure to chemical allergens in animal models can result in sensitization of the respiratory tract and airway inflammation on subsequent inhalation of the allergen.^{13,14} Studies in human subjects investigating the effect of dermal exposure to allergens on the development of asthma are limited in number. However, questionnaire-determined exposure to methylene diphenyl diisocyanate has been found to be associated with asthma-like respiratory symptoms in wood product plant workers,¹⁵ and in tannery workers asthma has been found to be associated with not wearing protective gloves.¹⁶

These results could suggest that interactions between *FLG* deficiency and environmental exposures might play a role in the development of atopic diseases. An interaction between *FLG* deficiency and cat ownership has been found for the development of eczema in early life, as well as for sensitization to cat,^{17,18} but not for asthma.¹⁸ To the best of our knowledge, there is no previous report on interactions between *FLG* deficiency and other environmental exposures in relation to asthma. In a recent review smoking was found to be associated with the reporting of asthma, as well as several markers of asthma severity, including mortality and exacerbations, and temporal associations between smoking and asthma suggested that the former is a possible risk factor for the latter.¹⁹ Furthermore, *FLG* expression has been found to be upregulated in oral keratinocytes in current smokers compared with that seen in nonsmokers, and in addition, nicotine increased *FLG* expression in oral keratinocytes from nonsmokers.²⁰ The purpose of the present study was to investigate possible interactions between *FLG* null mutations and smoking in relation to asthma.

METHODS**Study population**

A sample of 7931 Danish citizens 18 to 69 years of age, born in Denmark, and living in one of 11 municipalities of Copenhagen, the capital of Denmark, was randomly drawn from the Danish Civil Registration System, and all were invited to participate in a general health examination, Health2006. A total of 3471 (43.8%) subjects were included in the study and were examined between June 2006 and June 2008. Young age and male sex were associated with nonparticipation ($P < .001$).²¹

Assessment of lung function

Spirometry was performed according to international standards²² with the Spiro USB Spirometer (MicroMedical Limited, Rochester, Kent, United Kingdom), and measures of FEV₁ and forced vital capacity (FVC) were obtained. Throughout the data collection, the spirometers were checked every morning with a 3-L syringe, and every 6 months, they were checked with a

decompression flow simulator.²³ Percent predicted FEV₁ was calculated from sex, age, and measured standing height²⁴ and dichotomized for analyses into 80% or less and greater than 80%. The FEV₁/FVC ratio was calculated from the actual measurements and dichotomized into 70% or less and greater than 70% in some analyses.

Fraction of exhaled nitric oxide measurement

Fraction of exhaled nitric oxide (FENO) is a noninvasive marker of airway inflammation. Measurements of FENO were performed with the NIOX MINO (Aerocrine AB, Stockholm, Sweden). Participants were tested in a standing upright position without a nose clip. Participants inhaled to total lung capacity through a mouthpiece and subsequently exhaled at a constant pressure guided by visual and auditory cues to stabilize flow rate. A dynamic flow restrictor yielded a constant flow rate of 50 mL/s, which is in accordance with recommendations of the American Thoracic Society and European Respiratory Society guidelines for FENO measurement.²⁵ The device measured nitric oxide concentrations between 5 and 300 ppb. Measurements less than the detection limit of 5 ppb were automatically set to 0, and all measurements were dichotomized into 20 ppb or less and greater than 20 ppb.

Measurements of allergen-specific IgE

Blood samples from all participants were analyzed for serum specific IgE against the 4 most clinically important inhalation allergens in Denmark (ie, birch, grass, cat, and the house dust mite *Dermatophagoides pteronyssinus*) by using the ADVIA Centaur Specific IgE assay (Siemens, Deerfield, Ill).²⁶ Atopy was defined as at least 1 positive test result (≥ 0.35 kU/L) for specific IgE.

Definition of variables from the questionnaire

All participants completed a mailed questionnaire before the health examination. Questions on asthma were adopted from the stage 1 questionnaire of the European Community Respiratory Health Study.²⁷ Asthma was defined according to the method of Sunyer et al²⁸ as an affirmative answer to at least 1 of the following 3 questions: (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?

Wheezing without a cold was defined as affirmative answers to both of the following questions: (1) Have you had wheezing or whistling in your chest at any time during the last 12 months? (2) If yes, have you had this wheezing or whistling when you did not have a cold?

Chronic cough was defined as confirmative answers to both of the following questions: (1) Have you had coughing most days or nights? (2) Do you cough this way for a minimum of 3 months a year and for at least 2 successive years?

Atopic dermatitis was defined according to the UK Working Party's criteria²⁹ as a history of an itchy skin condition plus a minimum of 2 of 4 minor criteria: (1) onset of rash before the age of 2 years, (2) a history of flexural involvement, (3) a history of generalized dry skin, and (4) a history of asthma/hay fever.³⁰

Smoking status was reported as current, occasional, former, or never smoking. For current and former smokers, daily or former daily tobacco consumption was calculated in grams, equating 1 cigarette or 1 g of pipe tobacco to 1 g, 1 cheroot to 3 g, and 1 cigar to 5 g, and cumulated smoking exposure was calculated in pack years as daily tobacco consumption in grams times years of smoking divided by 20, as previously described.³¹ For analyses, smoking status was entered as current (dichotomized into ≤ 15 g/d and >15 g/d of tobacco), former, or never smoking, whereas occasional smokers were omitted from all analyses because of their low number ($n = 113$ [3.3%]). In stratified analyses 2 of the smoking categories (former smokers and current smokers, ≤ 15 g) were merged to obtain sufficient subjects in all cells. Cumulated smoking exposure was analyzed as a continuous variable.

Social status was reported as very high, high, average, below average, or low, whereas education beyond primary and secondary school was classified into 6 categories: (1) none, (2) less than 1 year, (3) skilled worker, or higher education of (4) less than 3 years, (5) 3 to 4 years, or (6) more than 4 years. Finally, participants were divided into 5 groups (≤ 30 , 31-40, 41-50, 51-60, and >60 years).

Genotyping of *FLG* null mutations

Regions covering the 2 most common null mutations, R501X and 2282del4, of the *FLG* gene were amplified from genomic DNA from 3366 (97.0%) subjects by means of PCR, and the obtained PCR products were hybridized to microbeads carrying DNA probes for mutations and for the wild-type (Luminex, Austin, Tex). Microbeads were subsequently analyzed on a BioPlex 200 (Bio-Rad Laboratories, Hercules, Calif). The genotyping success rates were 99.4% and 99.5% for the R501X and 2282del4 mutations, respectively, and a total of 3346 subjects (96.4% of the total study population) were genotyped successfully for both mutations. Neither of the observed genotype frequencies of the genotyped polymorphisms deviated significantly ($P > .05$) from the expected frequencies under assumption of Hardy-Weinberg equilibrium. Subjects were dichotomized into homozygote wild-types or carriers of at least 1 *FLG* null mutation.

Replication population

Replication of the main findings was sought in another population that was examined between June 1993 and December 1994: the Monica10 cohort.³² The Monica10 population included 2556 subjects aged 41, 51, 61, and 71 years. Extracted DNA was genotyped for the same 2 *FLG* null mutations by using the same method at the same laboratory as in the Health2006 study, and neither of the genotyped polymorphisms deviated significantly ($P > .4$) from the expected frequencies under assumption of Hardy-Weinberg equilibrium. The questionnaire differed from the one used in the Health2006 study, but the questions on asthma attacks within the last 12 months and smoking status were similar in the 2 studies.

Statistical analysis

Hardy-Weinberg equilibrium was calculated by using exact tests with GENEPOP version 4.0.10.^{33,34} All other statistical analyses were generated with SAS software, version 9.2, of the SAS System for Windows (SAS Institute, Inc, Cary, NC).

Crude associations between *FLG* genotype and asthma phenotypes, atopy, atopic dermatitis, smoking status, cumulated smoking exposure, sex, age, social status, and education were examined by using the χ^2 test. The possible confounding effects of sex and age on the associations between *FLG* genotype and asthma phenotypes, atopy, and atopic dermatitis were examined with multivariate logistic regression analyses. Odds ratios (ORs) with 95% CIs adjusted for possible confounding effects of sex and age were calculated. Interactions between *FLG* null mutations and smoking status (or cumulated smoking exposure) in relation to asthma or an asthma attack within the last 12 months were tested in logistic regression models including an interaction term between smoking status (or cumulated smoking exposure) and *FLG* genotype by using the likelihood ratio test. Furthermore, interactions between *FLG* genotype and smoking status (or cumulated smoking exposure) with the FEV₁/FVC ratio as the outcome were tested in linear regression models including an interaction term between *FLG* genotype and smoking status (or cumulated smoking exposure). The interaction between *FLG* genotype, smoking status, and atopy in relation to asthma was tested in a logistic regression model also including all 3 main effects and all 3 two-way interactions. ORs with 95% CIs for reporting asthma in subjects with *FLG* null mutations compared with subjects with wild-type *FLG* were obtained from logistic regression analysis for each combination of atopy and smoking status. P values of less than .05 were considered significant.

Ethics

The Health2006 study was approved by the Danish Data Protection Agency and the local ethics committee (no. KA-2006-0011).

RESULTS

Of the 3346 subjects genotyped successfully for both *FLG* null mutations, 270 (8.1%) carried at least 1 *FLG* null mutation, and of these, 9 were homozygous or compound heterozygous (ie, carried >1 of the 2 null allele mutations). Having at least 1 *FLG* null mutation was not associated with sex, age, social status, education, smoking status, or cumulated smoking exposure (all $P > 0.3$, data not shown).

The *FLG* null mutation genotype was significantly associated with a higher prevalence of asthma, an asthma attack within the last 12 months, and atopic dermatitis, as well as a decreased FEV₁/FVC ratio (Table I). These associations remained significant after adjustment for sex and age (data not shown). Smoking status and cumulated smoking exposure were significantly associated with a higher prevalence of asthma and wheeze without a cold, as well as decreased FEV₁, FEV₁/FVC ratio, and FENO levels and a lower prevalence of atopy (Table I).

In logistic regression analyses with asthma as the outcome, significant interactions were found between *FLG* genotype and smoking status ($P = .02$, Table II), as well as cumulated smoking exposure ($P = .03$, see Table E1 in this article's Online Repository at www.jacionline.org). Although statistically insignificant, similar interactions were seen in logistic regression models with an asthma attack within the last 12 months as the outcome between *FLG* genotype and smoking status in both the Health2006 and Monica10 populations ($P = .09$ and $.11$, respectively; Table III). However, in both populations the association between *FLG* null mutation and an asthma attack within the last 12 months was strong and highly statistically significant in moderate-to-heavy smokers (Table III). Furthermore, in the Health2006 population a similar but statistically insignificant interaction was seen between *FLG* genotype and cumulated smoking exposure with an asthma attack within the last 12 months as the outcome ($P = .08$, see Table E1).

In a linear regression model a significant interaction was found between *FLG* genotype and cumulated smoking exposure with FEV₁/FVC ratio as the continuous outcome ($P = .03$, see Table E1), whereas the relationship between *FLG* genotype and smoking status in relation to FEV₁/FVC ratio was less clear ($P = .54$, see Table E2 in this article's Online Repository at www.jacionline.org).

No significant interactions were found in relation to the different asthma phenotypes between *FLG* null mutations and either atopic dermatitis or atopy (all $P > .1$).

When investigating the relationship between *FLG* null mutations, smoking, and atopy, we found a significant 3-way interaction among *FLG* genotype, smoking status, and atopy in relation to asthma ($P = .011$) in a logistic regression model also including all 3 main effects and all 3 two-way interactions with asthma as the outcome (Table IV). In subjects without atopy, the OR of having asthma associated with *FLG* null mutations increased from 0.53 (95% CI, 0.16-1.72) in the group of never smokers to 1.65 (95% CI, 0.86-3.20) for former smokers and 2.89 (95% CI, 1.08-7.73) and 5.78 (95% CI, 2.17-15.4) for current smokers smoking 15 or less or greater than 15 g/d tobacco, respectively. (Please note that in Table IV the former smokers and

TABLE I. Relationship between *FLG* genotype, smoking status, cumulated smoking exposure, and phenotypes of asthma, atopy, and atopic dermatitis

	FEV ₁ * ≤80%, % (n/total n)	FEV ₁ /FVC† ≤70%, % (n/total n)	FENO >20 ppb, % (n/total n)	Asthma,‡ % (n/total n)
<i>FLG</i> genotype				
Wild-type	10.0 (305/3061)	10.8 (332/3063)	31.0 (871/2810)	10.6 (322/3027)
1 null mutation#	13.1 (34/259)	16.2 (42/259)	24.1 (56/232)	16.0 (41/257)
>1 null mutation**	0.0 (0/9)	0.0 (0/9)	25.0 (2/8)	33.3 (3/9)
<i>P</i> value††	.16	.018	.088	.004
Smoking status				
Never	5.5 (78/1430)	6.0 (86/1430)	37.6 (492/1309)	9.5 (136/1425)
Former	11.0 (122/1108)	13.1 (145/1110)	35.7 (366/1026)	13.1 (145/1103)
Current, ≤15 g/d	13.4 (59/439)	15.3 (67/439)	14.4 (57/397)	9.4 (41/436)
Current, >15 g/d	26.6 (88/331)	23.3 (77/331)	8.4 (25/299)	15.2 (50/329)
<i>P</i> value††	<.001	<.001	<.001	.002
Cumulated smoking exposure				
Never smokers	5.5 (78/1430)	6.0 (86/1430)	37.6 (492/1309)	9.5 (136/1425)
≤20 pack years	8.0 (75/943)	10.2 (96/944)	27.3 (238/872)	11.4 (107/940)
>20 pack years	23.9 (177/741)	23.3 (173/742)	22.1 (149/673)	14.0 (103/736)
<i>P</i> value††	<.001	<.001	<.001	.008

*Percent predicted FEV₁.†The FEV₁/FVC ratio was calculated from the actual measurements obtained.

‡Asthma was defined according to the European Community Respiratory Health Study as an affirmative answer to at least 1 of the following 3 questions: (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?

§Chronic cough was defined as confirmative answers to both of the following questions: (1) Have you had coughing most days or nights? (2) Do you cough this way for a minimum of 3 months a year and for at least 2 successive years?

||Atopy was defined as at least 1 positive test result (≥0.35 kU/L) for serum specific IgE against birch, grass, cat, or house dust mite (*Dermatophagoides pteronyssinus*).

¶Atopic dermatitis was defined according to the UK Working Party's criteria as a history of an itchy skin condition plus a minimum of 2 of 4 minor criteria: (1) a history of flexural involvement, (2) a history of generalized dry skin, (3) onset of rash before the age of 2 years, and (4) a history of asthma/hay fever.

#Defined as exactly 1 of the following mutations: R501X or 2282del4.

**Defined as 2 or more of the following mutations: R501X or 2282del4 (homozygous or compound heterozygous).

††χ² Test.

current smokers smoking ≤15 g have been merged.) This increase in ORs was not found in subjects with atopy (Table IV).

The association between *FLG* genotype and asthma or the interaction between *FLG* genotype and smoking status in relation to asthma were not confounded by either sex or social status, and no interactions were found between *FLG* genotype and sex or social status in relation to asthma (*P* = .45 and .31, respectively).

DISCUSSION

We found that carriers of *FLG* null mutations had a significantly higher prevalence of asthma and lower FEV₁/FVC ratios. Importantly, carriers of *FLG* null mutations were more susceptible to the effects of smoking on the risk of asthma.

In accordance with our results, most previous studies have found a significant association between *FLG* null mutations and asthma. In recent meta-analyses this association was mainly present in persons with atopic dermatitis.^{10,11} In the present study we did not find any differential effect of *FLG* null mutations on asthma between persons with and without atopic dermatitis.

At present, the biological mechanism underlying the association between *FLG* mutations and asthma is not known, although the hypothesis that the skin barrier disruption might facilitate allergen entry and subsequently promote T_H2-like sensitization and asthma has been proposed.^{12-14,35} To the best of our knowledge, there are no published data on interactions

between *FLG* null mutations and environmental risk factors in relation to asthma risk, apart from one study on cat ownership, in which no interaction was found.¹⁸ Our data raise the hypothesis that smoking increases the risk of asthma in carriers of *FLG* null mutations. The biological mechanism could involve systemic inflammation and immunologic alterations that might be common to both patients with atopic dermatitis and those with asthma but could also be local because *FLG* has been found to be expressed in the trachea,³⁶ as well as in the nasal and oral mucosa, where it is presumably involved in epithelial barrier function.³⁷ Importantly, the observed interaction between *FLG* null mutations and smoking was only seen in relation to nonatopic (without IgE sensitization) asthma. This suggests that smoking exerts its *FLG*-dependent effects through nonatopic pathways. Such nonatopic pathway from *FLG* deficiency to asthma is supported by a previous study in infants showing increased risk of wheezing in the very first years of life before development of atopy.³⁸ It might be relevant to note that smoking has been shown to have suppressive effects on eosinophilic inflammatory conditions, such as respiratory allergy³⁹ and colitis ulcerosa, as well as on FENO levels.⁴⁰ These effects could explain the lack of effect of smoking on asthma in atopic subjects in this study. The biological mechanism might involve a smoking-induced suppression of the eosinophilic immune response, a concept that has been used as in the treatment of colitis ulcerosa with nicotine patches.⁴¹

The identification of gene-environment interactions might provide more insight into pathologic mechanisms. Moreover,

TABLE I. (Continued)

Asthma attack within last 12 mo, % (n/total n)	Chronic Cough, [§] % (n/total n)	Wheeze without a cold, % (n/total n)	Atopy, % (n/total n)	Atopic Dermatitis, [¶] % (n/total n)
3.2 (97/3037)	15.2 (462/3042)	15.1 (458/3036)	23.3 (718/3075)	9.0 (263/2934)
6.2 (16/259)	18.3 (47/257)	15.7 (40/254)	21.5 (56/261)	19.7 (49/249)
11.1 (1/9)	33.3 (3/9)	11.1 (1/9)	44.4 (4/9)	66.7 (6/9)
.019	.14	.91	.25	<.001
3.4 (48/1425)	9.2 (132/1428)	9.7 (138/1422)	26.8 (380/1420)	9.7 (133/1367)
3.5 (39/1108)	11.6 (129/1110)	13.8 (153/1108)	20.1 (224/1112)	10.7 (115/1072)
2.5 (11/439)	24.4 (107/439)	21.2 (93/439)	19.2 (84/437)	10.8 (46/427)
4.2 (14/331)	45.0 (148/329)	36.7 (120/327)	18.7 (62/331)	9.5 (31/327)
.61	<.001	<.001	<.001	.80
3.4 (48/1425)	9.2 (132/1428)	9.7 (138/1422)	26.8 (380/1420)	9.7 (133/1367)
3.4 (32/945)	15.3 (145/946)	15.4 (144/938)	23.3 (219/944)	11.5 (105/912)
3.6 (27/742)	28.5 (211/740)	25.6 (190/743)	14.7 (109/744)	9.2 (67/726)
.94	<.001	<.001	<.001	.25

TABLE II. Relationship between *FLG* null mutations and asthma stratified by smoking status

	Asthma*		OR (95% CI) [†]
	Yes, % (no.)	No, % (no.)	
Smoking status			
Never			
<i>FLG</i> genotype			
Null mutation [‡]	9.8 (10)	90.2 (92)	1.02 (0.52-2.02)
Wild-type	9.5 (120)	90.5 (1149)	1.00 (reference)
Former			
<i>FLG</i> genotype			
Null mutation [‡]	18.2 (16)	81.8 (72)	1.57 (0.88-2.80)
Wild-type	12.4 (121)	87.6 (855)	1.00 (reference)
Current, ≤15 g/d			
<i>FLG</i> genotype			
Null mutation [‡]	16.2 (6)	83.8 (31)	1.96 (0.76-5.08)
Wild-type	8.8 (34)	91.2 (352)	1.00 (reference)
Current, >15 g/d			
<i>FLG</i> genotype			
Null mutation [‡]	35.5 (11)	64.5 (20)	3.70 (1.64-8.36)
Wild-type	12.8 (37)	87.2 (252)	1.00 (reference)

$P = .02$ for the *FLG* genotype–smoking status interaction in the logistic regression model also including both main effects with asthma as the outcome.

*Defined according to the European Community Respiratory Health Study as an affirmative answer to at least 2 of the following 3 questions: (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?

[†]ORs with 95% CIs were obtained from multivariate logistic regression models with asthma as the outcome adjusted for sex and age.

[‡]*FLG* null mutation was defined as at least 1 of the following mutations: R501X or 2282del4.

because of the high frequency of *FLG* null mutations and the fact that they carry a considerably increased risk of atopic dermatitis and asthma, these mutations might also potentially be used for screening of the general population and risk stratification. In this way *FLG* null mutations might identify a subgroup that requires special attention and a personalized strategy for treatment and prevention. Our finding that carriers of *FLG* null mutations appear to be at high risk of asthma might emphasize a particular need for smoking cessation/prevention in this subgroup of the population. In line with these findings, subjects with *FLG* mutations might also be more susceptible to related environmental risk factors, such as air pollution, and this should be addressed in future studies.

Several methodological issues should be considered. First, type 1 errors and results that cannot be replicated have been the curse of genetic association studies.^{42,43} However, we were able to replicate the interaction, although it was not statistically significant, between *FLG* null mutation and smoking in another population (Table III). Nevertheless, more studies in other countries and age groups are needed to further investigate the importance of this gene–environment interaction.

Second, the participation rate was moderate, and this might increase the risk of biased risk estimates. We were able to compare the prevalence of smoking with that obtained from a questionnaire study with a response rate of 71% (4242/6000) conducted on another random sample of subjects drawn at the same time and with the same inclusion criteria from the Danish Civil Registration System.⁴⁴ There were statistically significant differences in the prevalence of smoking status between the 2 populations, with the Health2006 study having a lower prevalence

TABLE III. Relationship between *FLG* null mutations and an asthma attack within the last 12 months stratified by smoking status in 2 population samples

	Asthma attack within last 12 mo					
	Health2006			Monica10		
	Yes, % (no.)	No, % (no.)	OR (95% CI)*	Yes, % (no.)	No, % (no.)	OR (95% CI)*
Smoking status						
Never						
<i>FLG</i> genotype						
Null mutation†	4.9 (5)	95.1 (97)	1.49 (0.57-3.86)	1.8 (1)	98.2 (55)	0.74 (0.10-5.73)
Wild-type	3.3 (42)	96.7 (1228)	1.00 (reference)	2.7 (17)	97.3 (606)	1.00 (reference)
Former/current, ≤15 g/d						
<i>FLG</i> genotype						
Null mutation†	5.6 (7)	94.4 (119)	1.82 (0.79-4.19)	4.9 (4)	95.1 (78)	1.05 (0.37-2.99)
Wild-type	3.0 (41)	97.0 (1328)	1.00 (reference)	4.6 (46)	95.4 (956)	1.00 (reference)
Current, >15 g/d						
<i>FLG</i> genotype						
Null mutation†	15.6 (5)	84.4 (27)	5.71 (1.76-18.49)	9.8 (4)	90.2 (37)	4.30 (1.32-14.03)
Wild-type	3.1 (9)	96.9 (281)	1.00 (reference)	2.7 (15)	97.3 (541)	1.00 (reference)

$P = .09$ for the *FLG* genotype–smoking status interaction in the logistic regression model also including both main effects with an asthma attack within last 12 months as the outcome in Health2006. $P = 0.11$ for the *FLG* genotype–smoking status interaction in the logistic regression model also including both main effects with an asthma attack within the last 12 months as the outcome in Monica10.

*ORs with 95% CIs were obtained from multivariate logistic regression models with asthma as the outcome adjusted for sex and age.

†*FLG* null mutation was defined as at least 1 of the following mutations: R501X or 2282del4.

TABLE IV. Association between *FLG* genotype and asthma stratified for atopy and smoking status

	Atopy	
	No, OR (95% CI)*	Yes, OR (95% CI)*
Smoking status		
Never	0.53 (0.16-1.72)	2.19 (0.87-5.54)
Former/current, ≤15 g/d	1.94 (1.12-3.35)	1.08 (0.35-3.34)
Current, >15 g/d	5.78 (2.17-15.4)	1.10 (0.25-4.87)

$P = .011$ for the *FLG* genotype–atopy–smoking status interaction in the logistic regression model also including all 3 main effects and all 3 two-way interactions with asthma as the outcome.

*ORs with 95% CIs for reporting asthma in subjects with at least 1 of the mutations R501X or 2282del4 in the *FLG* gene compared with subjects with wild-type *FLG*.

of current smokers and a higher prevalence of former and never smokers ($P < .001$). Regarding asthma, no difference in prevalence was found between the 2 studies, 11.3 and 11.9%, respectively ($P = .44$). The prevalences of *FLG* null mutations in the 2 populations described in the present article were also comparable (ie, 8.1% and 7.6%; $P = .51$). Thus the observation that the asthma phenotype and *FLG* genotype apparently had no effect on the participation rate makes the concern for substantial bias less serious.

Third, only the 2 most common *FLG* null mutations were genotyped in this study. However, in combination, 2282del4 and R501X account for approximately 70% of null mutations in white populations,⁸ and it is also imaginable that genotyping of additional *FLG* null mutations would strengthen the associations reported.

In conclusion, we found evidence that *FLG* null mutations modify the effects of smoking on the risk of asthma. If confirmed, this finding might have implications for risk stratification of the population and the development of preventive strategies, such as screening for *FLG* null mutations at an early age and intensified measures to prevent these subjects from taking up smoking.

We thank the staff at the Research Centre for Prevention and Health at Glostrup University Hospital for their work.

Key Messages

- Genetically determined *FLG* deficiency is associated with asthma, but the underlying mechanism is unknown.
- In this study the effect of tobacco smoking on the risk of asthma was modified by *FLG* genotype. Thus *FLG*-deficient subjects might be particularly susceptible to the detrimental effects of tobacco smoking on asthma.
- This was especially true for subjects with nonatopic asthma, suggesting that smoking exerts its *FLG*-dependent effects through nonatopic pathways.

REFERENCES

1. Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. *Exp Dermatol* 2008;17:1063-72.
2. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009;122:1285-94.
3. Mischke D, Korge BP, Marenholz I, Volz A, Ziegler A. Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ("epidermal differentiation complex") on human chromosome 1q21. *J Invest Dermatol* 1996;106:989-92.
4. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337-42.
5. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the mouse *Flg* gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 2009;41:602-8.
6. Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009;124:485-93.
7. Scharschmidt TC, Man MQ, Hatano Y, Crumrine D, Gunathilake R, Sundberg JP, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009;124:496-506.
8. Sandilands A, Terron-Kwiatkowski A, Hull PR, O'Regan GM, Clayton TH, Watson RM, et al. Comprehensive analysis of the gene encoding filaggrin uncovers

- prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007;39:650-4.
9. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
 10. Rodriguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown SJ, Cordell HJ, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 2009;123:1361-70.
 11. van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009;339:b2433.
 12. Palmer CN, Ismail T, Lee SP, Terron-Kwiatkowski A, Zhao Y, Liao H, et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol* 2007;120:64-8.
 13. Kimber I. The role of the skin in the development of chemical respiratory hypersensitivity. *Toxicol Lett* 1996;86:89-92.
 14. Redlich CA. Skin exposure and asthma: is there a connection? *Proc Am Thorac Soc* 2010;7:134-7.
 15. Petsonk EL, Wang ML, Lewis DM, Siegel PD, Husberg BJ. Asthma-like symptoms in wood product plant workers exposed to methylene diphenyl diisocyanate. *Chest* 2000;118:1183-93.
 16. Shahzad K, Akhtar S, Mahmud S. Prevalence and determinants of asthma in adult male leather tannery workers in Karachi, Pakistan: a cross sectional study. *BMC Public Health* 2006;6:292.
 17. Bisgaard H, Simpson A, Palmer CN, Bonnelykke K, McLean I, Mukhopadhyay S, et al. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. *PLoS Med* 2008;5:e131.
 18. Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy* 2009;64:1758-65.
 19. McLeish AC, Zvolensky MJ. Asthma and cigarette smoking: a review of the empirical literature. *J Asthma* 2010;47:345-61.
 20. Reno F, Rocchetti V, Migliario M, Rizzi M, Cannas M. Chronic exposure to cigarette smoke increases matrix metalloproteinases and Filaggrin mRNA expression in oral keratinocytes: role of nicotine stimulation. *Oral Oncol* 2011;47:827-30.
 21. Hersoug LG, Husemoen LL, Sigsgaard T, Madsen F, Linneberg A. Indoor exposure to environmental cigarette smoke, but not other inhaled particulates associates with respiratory symptoms and diminished lung function in adults. *Respirology* 2010;15:993-1000.
 22. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319-38.
 23. Pedersen OF, Naeraa N, Lyager S, Hilberg C, Larsen L. A device for evaluation of flow recording equipment. *Bull Eur Physiopathol Respir* 1983;19:515-20.
 24. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report of the Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;16:5-40.
 25. American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
 26. Petersen AB, Gudmann P, Milvang-Gronager P, Morkeberg R, Bogestrand S, Linneberg A, et al. Performance evaluation of a specific IgE assay developed for the ADVIA centaur immunoassay system. *Clin Biochem* 2004;37:882-92.
 27. Pekkanen J, Sunyer J, Anto JM, Burney P. Operational definitions of asthma in studies on its aetiology. *Eur Respir J* 2005;26:28-35.
 28. Sunyer J, Jarvis D, Pekkanen J, Chinn S, Janson C, Leynaert B, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol* 2004;114:1033-9.
 29. Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994;131:383-96.
 30. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994;131:406-16.
 31. Linneberg A, Nielsen NH, Menne T, Madsen F, Jorgensen T. Smoking might be a risk factor for contact allergy. *J Allergy Clin Immunol* 2003;111:980-4.
 32. Eugen-Olsen J, Andersen O, Linneberg A, Ladelund S, Hansen TW, Langkilde A, Petersen J, Pielak T, Møller LN, Jeppesen J, Lyngbæk S, Fenger M, Olsen MH, Hildebrandt PR, Borch-Johnsen K, Jørgensen T, Haugaard SB. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med* 2010;268:296-308.
 33. Raymond M, Rousset F. Genepop (Version-1.2)—population-genetics software for exact tests and ecumenicism. *J Hered* 1995;86:248-9.
 34. Rousset F. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resources* 2008;8:103-6.
 35. Hudson TJ. Skin barrier function and allergic risk. *Nat Genet* 2006;38:399-400.
 36. Wu Z, Hansmann B, Meyer-Hoffert U, Glaser R, Schroder JM. Molecular identification and expression analysis of filaggrin-2, a member of the S100 fused-type protein family. *PLoS One* 2009;4:e5227.
 37. Presland RB, Dale BA. Epithelial structural proteins of the skin and oral cavity: function in health and disease. *Crit Rev Oral Biol Med* 2000;11:383-408.
 38. Bonnelykke K, Pipper CB, Tavendale R, Palmer CN, Bisgaard H. Filaggrin gene variants and atopic diseases in early childhood assessed longitudinally from birth. *Pediatr Allergy Immunol* 2010;21:954-61.
 39. Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Smoking and the development of allergic sensitization to aeroallergens in adults: a prospective population-based study. The Copenhagen Allergy Study. *Allergy* 2001;56:328-32.
 40. Karrasch S, Ernst K, Behr J, Heinrich J, Huber RM, Nowak D, et al. Exhaled nitric oxide and influencing factors in a random population sample. *Respir Med* 2011;105:713-8.
 41. McGrath J, McDonald JW, Macdonald JK. Transdermal nicotine for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2004;(4):CD004722.
 42. Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, et al. Replicating genotype-phenotype associations. *Nature* 2007;447:655-60.
 43. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002;4:45-61.
 44. Berg ND, Linneberg A, Dirksen A, Elberling J. Prevalence of self-reported symptoms and consequences related to inhalation of airborne chemicals in a Danish general population. *Int Arch Occup Environ Health* 2008;81:881-7.

TABLE E1. Relationship between *FLG* genotype and asthma, an asthma attack within the last 12 months, and FEV₁/FVC ratio stratified by cumulated smoking exposure

	Asthma*			Asthma attack within last 12 mo			FEV ₁ /FVC† ≤70%		
	Yes, % (no.)	No, % (no.)	OR (95% CI)‡	Yes, % (no.)	No, % (no.)	OR (95% CI)‡	Yes, % (no.)	No, % (no.)	OR (95% CI)‡
Cumulated smoking exposure									
Never									
<i>FLG</i> genotype									
Null mutation§	9.8 (10)	90.2 (92)	1.02 (0.52-2.02)	4.9 (5)	95.1 (97)	1.49 (0.57-3.86)	4.9 (5)	95.1 (97)	0.80 (0.32-2.04)
Wild-type	9.5 (120)	90.5 (1149)	1.00 (reference)	3.3 (42)	96.7 (1228)	1.00 (reference)	6.1 (78)	93.9 (1196)	1.00 (reference)
≤20 pack years									
<i>FLG</i> genotype									
Null mutation§	17.0 (15)	83.0 (73)	1.72 (0.95-3.13)	5.6 (5)	94.4 (84)	1.82 (0.67-4.90)	16.9 (15)	83.1 (74)	2.02 (1.10-3.71)
Wild-type	10.7 (88)	89.3 (737)	1.00 (reference)	3.1 (26)	96.9 (803)	1.00 (reference)	9.3 (77)	90.7 (751)	1.00 (reference)
>20 pack years									
<i>FLG</i> genotype									
Null mutation§	28.3 (17)	71.7 (43)	2.70 (1.46-4.98)	11.5 (7)	88.5 (54)	3.95 (1.57-9.91)	37.3 (22)	62.7 (37)	2.22 (1.25-3.95)
Wild-type	12.5 (81)	87.5 (569)	1.00 (reference)	2.9 (19)	97.1 (636)	1.00 (reference)	21.8 (143)	78.2 (514)	1.00 (reference)

FLG genotype–cumulated smoking exposure interaction term in logistic regression models with asthma or an asthma attack within the last 12 months as the outcome: $P = .03$ or .08, respectively. *FLG* genotype–cumulated smoking exposure interaction term in a linear regression model with the FEV₁/FVC ratio as continuous outcome: $P = .03$.

*Defined according to the European Community Respiratory Health Study as an affirmative answer to at least 1 of the following 3 questions: (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols, or tablets) for asthma?

†The FEV₁/FVC ratio was calculated from the actual measurements obtained.

‡ORs with 95% CIs were obtained from multivariate logistic regression models adjusted for sex and age.

§*FLG* null mutation was defined as at least 1 of the following mutations: R501X or 2282del4.

TABLE E2. Relationship between *FLG* null mutations and FEV₁/FVC ratio stratified by smoking status

	FEV ₁ /FVC* ≤70%		OR (95% CI)†
	Yes, % (no.)	No, % (no.)	
Smoking status			
Never			
<i>FLG</i> genotype			
Null mutation‡	4.9 (5)	95.1 (97)	0.80 (0.32-2.04)
Wild-type	6.1 (78)	93.9 (1196)	1.00 (reference)
Former			
<i>FLG</i> genotype			
Null mutation‡	23.9 (21)	76.1 (67)	2.40 (1.41-4.09)
Wild-type	11.9 (117)	88.1 (866)	1.00 (reference)
Current, ≤15 g/d			
<i>FLG</i> genotype			
Null mutation‡	16.2 (6)	83.8 (31)	1.28 (0.50-3.31)
Wild-type	15.2 (59)	84.8 (330)	1.00 (reference)
Current, >15 g/d			
<i>FLG</i> genotype			
Null mutation‡	32.3 (10)	67.7 (21)	1.80 (0.76-4.25)
Wild-type	22.0 (64)	78.0 (227)	1.00 (reference)

P = .54 for the *FLG* genotype–smoking status interaction in a linear regression model with FEV₁/FVC ratio as the continuous outcome.

*The FEV₁/FVC ratio was calculated from the actual measurements obtained.

†ORs with 95% CIs were obtained from multivariate logistic regression models with an FEV₁/FVC ratio of 70% or less as the outcome adjusted for sex and age.

‡*FLG* null mutation was defined as at least 1 of the following mutations: R501X or 2282del4.