Filaggrin null mutations are associated with increased asthma severity in children and young adults

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Background: Filaggrin is a key protein involved in skin barrier function. Filaggrin (*FLG*) null mutations are important genetic predisposing factors for atopic disease.

Objective: To study the role of *FLG* null alleles in the clinical phenotype in children and young adults with asthma.

Methods: *FLG* mutations R501X and 2282del4 were assayed in 874 subjects 3 to 22 years old with asthma from Tayside. Lung function and disease severity were also studied.

Results: The filaggrin mutations were significantly associated with greater disease severity for asthma. Independent of eczema, mean FEV_1/forced vital capacity of *FLG* wild-type individuals differed from those carrying either *FLG* null allele (0.89 vs 0.86; $P = .012$). Individuals bearing *FLG* null alleles were more likely to be prescribed increased medication ($\chi^2 = 10.3$; $P = .001$), with the homozygote null individuals having an odds ratio of 6.68 (95% CI, 1.7-27.0; $P = .008$) for being prescribed long-acting β-agonists in addition to inhaled steroids. *FLG* null alleles were also associated with increased rescue medication use ($P = .004$). Individuals with asthma and with *FLG* null alleles were more likely to have eczema, and individuals with eczema tended to have more severe asthma; however, the association of *FLG* null alleles with all markers of asthma disease severity was similar in children with and without eczema.

Conclusion: *FLG* mutations are associated not only with eczema-associated asthma susceptibility but also with asthma severity independent of eczema status.

Clinical implications: *FLG* status influences controller and reliever medication requirements in children and young adults with asthma. (J Allergy Clin Immunol 2007;120:64-8.)

Key words: *Skin barrier, asthma, atopy, eczema, keratinization, treatment, control, lung function*

The protein filaggrin facilitates the terminal differentiation of the epidermis and the formation of the skin barrier.[1] Two independent mutations in the gene encoding filaggrin (*FLG*; R501X and 2282del4) carried by about 9% of people of European origin result in complete loss of processed functional filaggrin in the epidermis.[2,3] We have recently shown that these genetic mutations, previously proven to impair epithelial barrier formation,[3] are strong predisposing factors for childhood eczema (atopic dermatitis) in Scottish, Irish, Danish,[2] German,[4] and English[5] populations. These associations have been well replicated in other white European populations, where these mutations are prevalent,[6-9] as reviewed recently.[10] Analogous loss-of-function mutations in *FLG* have recently been reported to be significantly associated with atopic dermatitis in Japan, showing that this gene may contribute to atopic disease burden to varying degrees worldwide.[11] Importantly, no negative associations have as yet been reported.

Asthma constitutes an important component of the atopic process, often coexisting with dermatitis.[12] The sequence of events, from epicutaneous sensitization by an allergen progressing to airway hyperreactivity, has been reproduced in a mouse model for asthma,[13] with a dose-response relationship between allergen concentrations and skin[14] or bronchial[15] reactivity in atopic individuals. Thus, if the skin barrier plays a key role in preventing sensitization in asthma, a greater entry of allergens through a poorly formed epidermal barrier may speed up or intensify the activation of the immunologic changes of asthma,[16] resulting in patients with asthma with filaggrin gene defects experiencing asthma of greater severity, with less effective day-to-day control and greater airway obstruction, in comparison with patients with asthma with fully functional filaggrin alleles.

Although the combined genotype was of particular interest in our original study,[2] further work has suggested that the R501X mutation may have greater penetrance in determining higher serum IgE levels in patients with

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Abbreviations used
aa: Homozygous R501X or 2282del4 genotype or compound heterozygous genotype
Aa: Heterozygous genotype for either R501X or 2282del4
AA: Wild-type/wild-type *FLG* genotype for R501X and 2282del4 mutation
BTS: British Thoracic Society
FLG: Filaggrin
FVC: Forced vital capacity
OR: Odds ratio

atopic eczema, in comparison with 2282del4.[4] Because similar penetrance differences may occur in asthma, we were interested in comparing the relative effects of the 2 null mutations and the combined genotype on the outcomes of asthma severity and symptomatic control measured for our study.

METHODS

The BREATHE study of childhood asthma has been extended from the time of publication of our initial results.[2] The current data set includes information on demographic, anthropometric, and clinical details from 874 individuals with physician-diagnosed asthma attending primary and secondary clinics in 18 primary care practices and a secondary care asthma clinic in Tayside, Scotland, from 2004 to 2006 (age 3-22 years). The study was approved by the Tayside Committee on Medical Research Ethics. Informed consent was provided by the patient and the parent/guardian as relevant. The methods have been described in detail.[2,17] Briefly, patients were seen in the asthma clinic setting, where a detailed history was obtained, including information on long-term asthma medication. The asthma prescribing level was determined in accordance with the British Thoracic Society (BTS)[18] guidelines for physician-led management of asthma, as follows: step 0, no use of inhaled albuterol on demand within the past month; step 1, inhaled albuterol on demand; step 2, regular inhaled steroids plus inhaled albuterol on demand; step 3a, regular inhaled long-acting β-agonists (salmeterol or formoterol) plus inhaled steroids with inhaled albuterol on demand; step 4, regular inhaled long-acting β-agonists plus inhaled steroids plus oral montelukast with inhaled albuterol on demand. The use of inhaled short-acting β-agonists (bronchodilators) was categorized as follows: 0, rarely or never required; 1, required few times a week but less than once daily; 2, required daily; 3, multiple doses over a 24-hour period on a regular basis. Eczema status was determined using the question, "Does the child have eczema?" Pulmonary function was measured by spirometry as per standard procedure described previously.[17] The ratio of FEV_1 to forced vital capacity (FVC), an established index of airway obstruction,[19] was used as the primary measure of airway obstruction for our study.

Genotyping for *FLG* R501X and 2282del4 was performed as previously described.[2]

AA refers to wild-type/wild-type *FLG* genotype for R501X and 2282del4 mutations, *Aa* refers to heterozygous genotype for either R501X or 2282del4, and *aa* refers to homozygous R501X or 2282del4 genotype or compound heterozygous genotype.

All statistical analysis was performed by using SPSS for Windows version 13 (SPSS Inc, Chicago, Ill) and Instat for Macintosh version 4 (Graphpad, San Diego, Calif). To calculate odds ratios (ORs) for comparison of risk, asthma severity and control outcomes were grouped according to severity. Thus, the asthma treatment steps were grouped as mild to moderate (current use of no medication, use of inhaled albuterol according to need, use of regular inhaled steroids plus inhaled albuterol according to need, ie, BTS steps 0-2) or severe (additional use of regular inhaled long-acting β-agonists and/or oral leukotriene antagonists, ie, BTS steps 3 and 4, respectively).[18] Frequency of inhaled bronchodilator use was grouped into occasional (including the responses "rarely or never required" and "required a few times a week but less than once daily") or daily (including the response "required daily" and "required as multiple doses over 24 hours on a regular basis"). Binary logistic regression analysis was used for comparison of the 2 groups. The effect of *FLG* gene variation on airway obstruction (FEV_1/FVC) was studied by using general linear modeling. Significance was assessed at $P < .05$.

RESULTS

The population characteristics are fairly typical of young individuals with well controlled asthma derived from both primary and secondary care[20] (Table I). The allele frequencies of the *FLG* mutations R501X and 2282del4 in the children with asthma were increased relative to the Tayside population, and this increase in allele frequency was limited to the children with asthma with a self-reported history of eczema as previously reported.[2] Mean FEV_1 and FVC measurements were 96% to 97% predicted, as observed in other populations of children with well controlled asthma.[20] However, the mean FEV_1/FVC ratio was reduced at 0.88 (Table I).

Airway obstruction (measured as FEV_1/FVC) showed a stepwise increase for patients with the combined *FLG* genotype, together with a similar overall trend for patients with R501X and 2282del4 mutations (Fig 1). The contingency table analysis (Table II) shows that the heterozygous and homozygous genotypes for the R501X mutation, or the combined genotype, were associated with a higher BTS treatment step, indicating the need for more intensive asthma medication. This effect is significant for the R501X mutation ($P = 3.02 \times 10^{-5}$) and the combined genotype ($P = .001$; Table II). Thus, while 23% (187/804) of *FLG* wild-type participants were on the higher BTS treatment steps of 3 and 4 (regular long-acting β-agonists with or without oral montelukast in addition to regular inhaled steroids), a significantly greater proportion (40%; 27/68) of *FLG* null allele carriers with asthma were on the higher BTS treatment steps 3 and 4 (Table II). General linear modeling of the treatment step as a quantitative variable demonstrated an increase in the mean BTS step from 2.09 for *FLG* wild-type individuals, 2.29 for *FLG* null allele heterozygotes, and 3.00 for the *FLG* null homozygotes ($P = .002$ for a codominant model).

Binary logistic regression shows a 1.73-fold risk with the codominant model (95% CI, 1.19-2.52; $P = .004$) and a 6.68-fold risk with the homozygous model (95% CI, 1.65-26.99; $P = .008$) of being in the severe (BTS steps 3 and 4) as opposed to the mild to moderate (BTS steps 0-2) asthma category (Table III). Adjusting the analysis

TABLE I. Characteristics of BREATHE study participants with asthma (n = 874)

With eczema:without eczema (n = 863)	458:405
Age	Range, 3-22 (mean, 10.4; SD, 4.0) y
Sex (males:female)	516 (59%):358 (41%)
R501X AA/Aa/aa (%)	805 (92.1)/65 (7.4)/4 (0.5)
2282del4 AA/Aa/aa	826 (94.5)/46 (5.3)/2 (0.2)
Combined genotype AA/Aa/aa	760 (87.0)/105 (12.0)/9 (1.0)
Mean percent predicted FEV_1 (SD) (n = 684)	97.3 (15.2)
Mean percent predicted FVC (SD) (n = 684)	95.8 (13.8)
Mean FEV_1/FVC (SD) (n = 684)	0.88 (0.12)
BTS asthma treatment step (n = 872)	0, 30; 1, 136; 2, 492; 3, 122; 4, 92
Inhaled bronchodilator use (n = 852)	0, 90; 1, 582; 2, 157; 3, 23

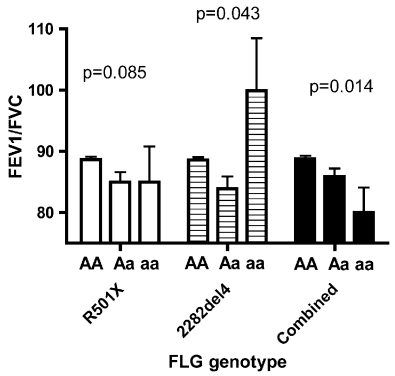

FIG 1. Estimated marginal means with SEs on univariate analysis of variance with the ratio FEV_1/FVC as the dependent variable and filaggrin genotype as the fixed factor with age and sex as covariates (*P* values are for trend per genotypic step). Number of individuals: R501X, 628 (AA), 52 (Aa), 4 (aa); 2282del4, 648 (AA), 34 (Aa), 2 (aa); combined genotype, 595 (AA), 80 (Aa), 9 (aa).

for age, sex, and eczema status did not markedly affect the apparent risk (adjusted allelic *FLG* OR, 1.66; 95% CI, 1.13-2.42; $P = .009$). The association of eczema with asthma severity was very weak (OR, 1.26; 95% CI, 0.92-1.74; $P = .15$). Indeed, the ORs for the association of *FLG* null status and asthma severity were similar in individuals with and without eczema (OR without eczema, 1.93; 95% CI, 0.94-3.95; $P = .074$; OR with eczema, 1.58; 95% CI, 1.014-2.46; $P = .043$). The effect of combined *FLG* gene defects on asthma severity was also reflected in the increased ORs for participants with asthma using inhaled bronchodilators on a daily versus occasional basis (OR, 1.76; 95% CI, 1.18-2.63; $P = .006$) with the codominant model, and OR, 6.91 (95% CI, 1.63-29.25; $P = .009$) with the homozygous model (Table IV). Again, this association was not affected by adjustment by age, sex, and eczema status (adjusted allelic OR, 1.65; 95% CI, 1.11-2.47; $P = .014$) and remained significant when additionally adjusted for the use of long-acting β-agonists (BTS steps 3 and 4; adjusted allelic OR. 1.52; 95% CI, 1.01-2.30; $P = .045$).

TABLE II. Contingency table for filaggrin genotype versus BTS treatment steps 1-4 for asthma*

	Treatment step	0	1	2	3	4	Total	*P* value
R501X	AA	29	132	456	111	76	804	
	Aa	1	4	35	10	14	64	
	Aa			1	1	2	4	
								3.02×10^{-5}
2282del4	AA	28	125	470	115	86	824	
	Aa	2	11	21	7	5	46	
	Aa			1		1	2	
								.963
Combined	AA	27	121	435	106	79	759	
	Aa	3	15	54	13	19	104	
	Aa			3	3	3	9	.001
Total		30	136	492	122	92	872	

*See Methods for details. The *P* values here relate to trend on χ^2 analysis.

DISCUSSION

Both common *FLG* mutations R501X and 2282del4 were overrepresented in the Scottish asthma cohort compared with local population controls, and the *FLG* alleles were overrepresented in a significant fraction of the cohort that had eczema.[2] Since the completion of data collection for our initial study,[2] we have continued recruiting patients with asthma for the Scottish cohort primarily ascertained for asthma to generate the statistical power to investigate further the possible role of filaggrin gene defects on clinical measures of asthma phenotype, including severity, control, and airway obstruction as measured by spirometry. Our data demonstrate that individuals with *FLG* null alleles have a significantly increased disease burden, both in terms of lung function, where we observed greater airway obstruction in the *FLG* null carriers, and in the intensity of medication required for disease control. The individual contribution to the overall signal of the 2284del4 allele was lower than that observed for the R501X mutation, as has been observed in other studies; however, larger studies are required to assess reliably the relative penetrance of the 2 mutations in measures of asthma severity. Interestingly, although the *FLG* mutations are only associated with susceptibility to eczema-associated asthma and not asthma in the absence of eczema, these data suggest that the *FLG* mutations are associated with asthma disease severity even in the absence of a history of eczema. Indeed, in this study, the frequency of the *FLG* null variants in the children with asthma but without eczema remains similar to the local population frequency, as reported in our previous study. Therefore, it would appear that *FLG* mutation carriers that have no symptomatic eczema are not more susceptible to mild asthma, but are at risk of developing

TABLE III. Binary logistic regression analysis of asthma severity as measured by BTS asthma treatment step for filaggrin genotype variation (ORs for BTS step 0-2 (current use of no medication, use of inhaled albuterol according to need, and use of regular inhaled steroids plus inhaled albuterol according to need) versus 3-4 (additional use of inhaled long-acting β-agonists and/or oral leukotriene antagonists)

	OR (codominant)	95% CI	*P* value	OR (heterozygous)	95% CI	*P* value	OR (homozygous)	95% CI	*P* value
R501X	2.16	1.35-3.46	.001	1.96	1.15-3.33	.014	10.45	1.074-101.6	.043
2282del4	1.19	0.65-2.19	.576	1.09	0.56-2.16	.792	3.03	0.19-48.8	.434
Combined	1.73	1.19-2.52	.004	1.47	0.93-2.29	.096	6.68	1.65-26.99	.008

TABLE IV. Binary logistic regression analysis of frequency of inhaled bronchodilator use for filaggrin genotype variation (ORs for daily versus occasional use)

	OR (codominant)	95% CI	*P* value	OR (heterozygous)	95% CI	*P* value	OR (homozygous)	95% CI	*P* value
R501X	1.74	1.05-2.87	.031	1.66	0.93-2.95	.087	4.05	0.57-28.96	.164
2282del4	1.75	0.92-3.33	.088	1.531	0.77-3.07	.765	258.0	$0.00\text{-}2.5X10^{9}$	.498
Combined	1.76	1.18-2.63	.006	1.47	0.91-2.40	.117	6.91	1.63-29.25	.009

quite severe disease. In comparison, significant associations between *FLG* mutations and atopic dermatitis along with weaker associations with disease severity for atopic dermatitis have been reported in another study on families ascertained primarily on the basis of atopic dermatitis.[8]

Although inflamed skin may aggravate allergen entry, there is clear evidence that defective barrier function leading to systemic immune sensitization can occur in the absence of atopic dermatitis. Percutaneous sensitization with allergens through noninflamed, barrier-disrupted skin elicits a T_H2-dominant cytokine response in mice.[21,22] After the induction of T_H2-type immunity in the skin, increased production of IL-4 and IL-13 drives IgE secretion. IgE regulates the expression of its own receptors, the high-affinity FcεRI receptor and the low-affinity IgE receptor (CD23), on mast cells and basophils.[23,24] IgE-dependent upregulation of FcεRI and CD23 receptors subsequently amplifies the immunologic reactions, leading to the greater release of mast cell and basophil mediators at lower concentrations of a specific antigen.[22,24] In addition, the expression of FcεRI on dendritic cells is thought to play a major role in the perpetuation and escalation of allergic disease.[22] Thus, an upregulation of epithelial T_H2-type immunity with preferential activation of the resulting cascade represents a possible mechanism for greater asthma severity in participants with asthma and defective barrier function, even in the absence of symptomatic eczema.

Skin barrier dysfunction is proven in patients with homozygous and heterozygous mutations at both R501X and/or 2282del4. Epithelial barrier function in *FLG* null allele homozygotes and heterozygotes has already been demonstrated, together with evidence that heterozygous carriers have mild features of ichthyosis vulgaris.[3] These individuals had impaired formation of the stratum corneum, which is clinically manifest as scaling of the skin.[3] Genetic linkage studies with ichthyosis vulgaris families[2] subsequently showed that eczema is transmitted as a monogenic disorder with reduced penetrance because of inheritance of either heterozygous or homozygous mutations. Homozygosity was shown to increase penetrance. Thus, the links among heterozygous mutations at both R501X and/or 2282del4, epidermal barrier defects, and eczema have already been well established.

A prospective study from early life of the effects of filaggrin gene defects on IgE levels, FcεRI and CD23 expression on dendritic and Langerhans cells, and the uptake, processing, and presentation of allergens could help elucidate the immunologic pathways that may underlie greater asthma prevalence, greater severity, worse control, and greater reduction in lung function observed in filaggrin-deficient participants. An understanding of the possible relationships between filaggrin gene defects, a T_H2-dominant cytokine response, and asthma could begin to test the exciting hypothesis that primary prevention strategies for asthma and allergy may be more cost-effective for genotype-stratified populations.[25]

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REFERENCES

1. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6:328-40.
2. Palmer CNA, 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.
3. Smith FJD, 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.
4. Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al. Loss-of-function variants within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006;118:214-9.
5. Barker JN, Palmer CNA, Zhao Y, Liao H, Hull PR, Lee SP, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007;127:564-7.
6. Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol 2006;155:1093-4.

7. Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S. Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. J Invest Dermatol 2006;127:722-4.
8. Morar N, Cookson WO, Harper JI, Moffatt MF. Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol 2007; Feb 15 [Epub ahead of print].
9. Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006;118:866-71.
10. Irvine AD. Fleshing out filaggrin phenotypes. J Invest Dermatol 2007;127:504-7.
11. Nomura T, Sandilands A, Akiyama M, Liao H, Evans AT, Sakai K, et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 2007;119:434-40.
12. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003;112(suppl 6):S118-27.
13. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest 1998;101:1614-22.
14. Corbo GM, Foresi A, Morandini S, Valente S, Mattoli S, Ciappi G. Probit analysis applied to the allergen dose-response curve: A method for epidemiologic surveys. J Allergy Clin Immunol 1988;81:41-7.
15. Kopferschmitt-Kubler MC, Bigot H, Pauli G. Allergen bronchial challenge tests: variability and reproducibility of the early response. J Allergy Clin Immunol 1987;80:730-40.
16. Segre JA. Epidermal barrier formation and recovery in skin disorders. J Clin Invest 2006;116:1150-8.
17. Palmer CNA, Doney AS, Lee SP, Murrie I, Ismail T, Macgregor DF, et al. Glutathione-S-transferase M1 and P1 genotype, passive smoking and peak expiratory flow in asthma. Pediatrics 2006;118:710-6.
18. BTS/SIGN. British guidelines on the management of asthma. Thorax 2003;58(suppl 1):1-94.
19. Gilbert R, Auchincloss JHJ. The interpretation of the spirogram: how accurate is it for ''obstruction''? Arch Intern Med 1985;145:1635-9.
20. Timonen KL, Pekkanen J, Korppi M, Vahteristo M, Salonen RO. Prevalence and characteristics of children with chronic respiratory symptoms in eastern Finland. Eur Respir J 1995;8:1155-60.
21. Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. Eur J Immunol 1998;28:769-79.
22. Strid J, Strobel S. Skin barrier dysfunction and systemic sensitization to allergens through the skin. Curr Drug Targets Inflamm Allergy 2005;4:531-41.
23. Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, et al. IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. J Immunol 1999;162:5455-65.
24. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 1997;185:663-72.
25. Arshad SH. Primary prevention of asthma and allergy. J Allergy Clin Immunol 2005;116:3-14.