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Filaggrin Mutations That Confer Risk of Atopic Dermatitis Confer Greater Risk for Eczema Herpeticum

Pei-Song Gao, MD, PhD¹, Nicholas M Rafaels, MS¹, Tracey Hand, MSc¹, Tanda Murray, MHS², Mark Boguniewicz, MD³, Tissa Hata, MD⁴, Lynda Schneider, MD⁵, Jon M Hanifin, MD⁶, Richard L Gallo, MD⁴, Li Gao, MD, PhD¹, Terri H Beaty, PhD², Lisa A Beck, MD⁷, Kathleen C Barnes, PhD^{1,*}, and Donald YM Leung, MD³

¹Johns Hopkins Asthma & Allergy Center, Johns Hopkins University School of Medicine, Baltimore, MD 21224

²Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21287

³Department of Pediatrics, National Jewish Health, Denver, CO 80206

⁴Division of Dermatology, University of California San Diego, San Diego, CA 92093

⁵Division of Immunology, Children's Hospital Boston, Boston, MA 02115

⁶Department of Dermatology, Oregon Health & Science University, Portland, OR 97239

⁷Department of Dermatology, University of Rochester Medical Center, Rochester, NY 14642

Abstract

Background—Loss-of-function null mutations R501X and 2282del4 in the skin barrier gene, filaggrin (*FLG*), represent the most replicated genetic risk factors for atopic dermatitis (AD). Associations have not been reported in African ancestry populations. Eczema herpeticum (ADEH) is a rare but serious complication of AD resulting from disseminated cutaneous HSV infections.

Objective—We aimed to determine whether *FLG* polymorphisms contribute to ADEH susceptibility.

Methods—Two common loss-of-function mutations plus nine *FLG* single nucleotide polymorphisms (SNPs) were genotyped in 278 European American AD patients, of whom 112 had ADEH, and 157 non-atopic controls. Replication was performed on 339 African Americans.

Results—Significant associations were observed for both the R501X and 2282del4 mutations and AD among European Americans ($P=1.46\times 10^{-5}$, 3.87×10^{-5} , respectively), but the frequency of the R501X mutation was three times higher (25.% vs 9%) for ADEH compared to AD without EH (odds ratio [OR]=3.4 (1.7–6.8), $P=0.0002$). Associations with ADEH were stronger with the

*Correspondence to: Kathleen C. Barnes, PhD, Associate Professor of Medicine, Johns Hopkins Asthma & Allergy Center, 5501 Hopkins Bayview Circle, Room 3A.62A, Baltimore, MD 21224, USA; 410-550-2071 (Tel); 410-550-2130 (FAX). kbarnes@jhmi.edu.

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combined null mutations (OR=10.1 (4.7–22.1), $P=1.99\times 10^{-11}$). Associations with the R501X mutation were replicated in the African American population; the null mutation was absent among healthy African Americans, but present among AD (3.2%, $P=0.035$) and common among ADEH (9.4%; $P=0.0049$) patients. However, the 2282del4 mutation was absent among African American ADEH patients and rare (<1%) among healthy individuals.

Conclusion—The R501X mutation in the gene encoding filaggrin, one of the strongest genetic predictors of AD, confers an even greater risk for ADEH in both European and African ancestry populations, suggesting a role for defective skin barrier in this devastating condition.

Clinical Implications—The Filaggrin (*FLG*) R501X Mutation, a major risk factor for atopic dermatitis, confers a greater risk of the severe, HSV-associated complication, eczema herpeticum in diverse ethnic groups.

Capsule Summary—Mutations in the skin barrier function protein, filaggrin, are strong predictors of atopic dermatitis. This report demonstrates an even greater association between one of these mutations (R501X) and eczema herpeticum in ethnically diverse American populations.

Keywords

Atopic dermatitis; Eczema herpeticum; filaggrin; R501X; 2282del4; Single Nucleotide Polymorphisms

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic pruritic, inflammatory skin disease complicated by recurrent bacterial and viral skin infections(1, 2). A rare but serious complication of AD is eczema herpeticum (ADEH), a disseminated infection associated with significant morbidity that had conferred significant mortality prior to introduction of systemic antiviral therapy. Complications include keratoconjunctivitis that can result in blindness, as well as multi-organ involvement with meningoencephalitis. The primary predisposing factor for ADEH is HSV-1 exposure, which is ubiquitous in the general population (*i.e.*, ~20% of children and over 60% of adults are seropositive)(3). However, only ~3% of AD patients develop disseminated cutaneous HSV infections (including HSV-1)(4) despite the high likelihood of HSV-1 exposure, suggesting this complication is not a function of environmental exposure alone, and genetic susceptibility may be at play. Although there is considerable evidence to support a genetic basis for AD(5), virtually nothing is known about the heritability of ADEH.

Two common mutations (R501X and 2282del4) in the gene encoding filaggrin (*FLG*), a critical protein in skin barrier function, have been consistently associated with risk of AD(6) and related traits, including asthma, hay fever, rhinoconjunctivitis, and allergen-specific IgE(7–13). Full sequencing of the *FLG* gene has revealed multiple, additional polymorphisms with varying frequency across ethnic groups(14). With a combined allele frequency among AD patients of 18% and 48% for the R501X and 2282del4 mutations, respectively, these polymorphisms represent the strongest and most compelling genetic risk factors for AD. Little is known regarding the relevance of these mutations in populations of African descent, as nearly all studies to date have been performed in populations of

European or Asian descent. We previously reported genotyping 23 African American individuals for these mutations, and all had wild-type genotypes for both mutations(15), supporting the contention these mutations may be less common in this ethnic group(16). As part of the NIH/NIAID sponsored Atopic Dermatitis and Vaccinia Network (ADVN) to dissect mechanisms increasing susceptibility of AD patients to disseminated viral skin infections, we sought to determine whether the R501X and 2282del4 mutations confer risk of ADEH in a European American group, and to test for association of additional markers across the *FLG* gene. Replication of findings was tested in an independent African American group.

METHODS

Study participants and phenotypes

DNA was isolated from 278 unrelated European American AD patients (112 with ADEH) and 157 healthy controls participating in the ADVN. The same set of markers was genotyped on 187 African American AD patients (32 with ADEH), and 152 healthy controls. Baseline characteristics are presented in Table I.

AD was diagnosed using the US consensus conference criteria(17). ADEH was defined as AD patients with at least one EH episode documented either by an ADVN investigator (or a physician affiliated with the same academic center) or diagnosis by another physician confirmed by HSV PCR, tissue immunofluorescence, Tzanck smear and/or culture. Non-atopic, healthy controls were defined as having no personal history of chronic disease including atopy. AD severity was defined according to the 'eczema area and severity index' (EASI), a standardized grading system(18) and total serum IgE was measured as described in this article's Online Repository at www.jacionline.org. Clinical characteristics are presented in Table I, and further detail of phenotyping is available in this article's Online Repository at www.jacionline.org. The study was approved by the institutional review boards at National Jewish Health in Denver, Johns Hopkins University, Oregon Health and Science University, University of California San Diego, Children's Hospital of Boston, and University of Rochester. All subjects gave written informed consent prior to participation.

An additional group of 177 healthy African American controls was included and genotyped for the two loss-of-function mutations (see details below). Participants were recruited from the BaltimoreWashington, D.C. metropolitan area and self-reported as African American ethnicity. These subjects comprised the consortium for '*Genomic Research on Asthma in the African Diaspora*' (GRAAD) and represent eight separate, NIH-funded studies of asthma in pediatric and adult African American populations, plus one study on healthy African Americans. Informed consent was obtained from each study participant, and the study protocol was approved by the institutional review board at either the Johns Hopkins University or Howard University.

FLG genotyping and quality control

Genotyping was performed as described in this article's Online Repository at www.jacionline.org on the two *FLG* mutations previously reported as associated with AD

(R501X and 2282del4), plus nine additional polymorphisms (rs12730241, rs2065956, rs11582620, rs3126082, rs6587665, rs11204980, rs1933063, rs1933064, rs3126091; details of the polymorphisms and minor allele frequencies (MAF) are presented in Table E1 in this article's Online Repository at www.jacionline.org). To evaluate genetic structure of the two ethnic groups from the three "continental" ancestral populations in the HAPMAP (www.hapmap.org), we genotyped an additional 74 SNPs identified as ancestry informative markers (AIMs) selected for maximal difference between African and European populations and assessed potential confounding due to population substructure using genotype data and the STRUCTURE program (v2.2; <http://pritch.bsd.uchicago.edu/software>) to estimate membership in distinct subpopulations (See Fig E1 in this article's Online Repository at www.jacionline.org). Tests for association were performed using the Cochran–Armitage trend test and as described in this article's Online Repository at www.jacionline.org.

RESULTS

We compared the distribution of observed *P*-values to the expected distribution under the null hypothesis of no genetic association, and concordant with findings reported in other European American populations(19), significant associations were observed for both the R501X and 2282del4 mutations and AD, with allele frequencies of the R501X allele among all AD patients at 7.8% compared to 1.3% among the healthy participants (odds ratio [OR]=7.1 (2.5–20.1), $P=1.46\times10^{-5}$, Table II) and frequencies of the 2282del4 allele at 8.8% compared to 1.6% (OR=5.6 (2.2–14.6), 3.87×10^{-5} ; Table II). Evidence of associations with the R501X mutation and 2282del4 were significantly strengthened when the analyses were limited to the 112 patients with ADEH, where 25.4% of ADEH patients carried the R501X null mutation (OR=13.1 (4.4–38.5), $P=7.25\times10^{-9}$) and 17.8% of ADEH patients carried the 2282del4 null mutation (OR=6.5 (2.4–18.1), $P=3.0\times10^{-5}$). When analyses were restricted to the ADEH patients versus AD patients without EH, a significant, albeit weaker, association was observed for the R501X mutation (OR=3.4 (1.7–6.8), $P=0.0002$), but not for the 2282del4 mutation (OR=1.3 (0.7–2.5), $P=0.465$). Additional analyses were performed for the combined genotype of the R501X and 2282del4 mutations, and the risks for individuals carrying both null mutations for AD (OR=6.4 (3.1–13.2), $P=1.21\times10^{-8}$) and ADEH (OR=10.1 (4.7–22.1), $P=1.99\times10^{-11}$) were remarkably increased compared to the risk for individuals carrying either the R501X mutation or the 2282del4 alone, suggesting that the combination of both R501X and 2282del4 mutations may contribute to an increased risk of both AD and ADEH in this European American group.

In addition to the R501X and 2282del4 mutations, we detected modest associations two SNPs (rs12730241 and rs2065956) in exon 3 localized in the first LD block (see Fig E2 in this article's Online Repository at www.jacionline.org) and a reduced risk of AD (OR=0.61 (0.40–0.91), $P=0.016$, OR=0.62 (0.42–0.91), $P=0.015$), respectively; Table EII in this article's Online Repository at www.jacionline.org) and one SNP (rs1933063) in the promoter region associated with increased risk of ADEH (OR=4.19 (1.22–14.44), $P=0.015$). Association tests for each mutation among European Americans are summarized in Figure I. In sliding-window haplotype analyses using all SNPs, we observed several significant two-, three-, and four-marker haplotypes showing evidence of association for AD ($P=2.43\times10^{-7}$ –0.05, Fig E3 in this article's Online Repository at www.jacionline.org), with the

strongest association for the haplotype carrying SNP 2065956 and two null mutations 2282del4 and R501X ($P=2.43 \times 10^{-7}$). Stronger haplotype associations were also observed for the ADEH phenotype, with the strongest association for haplotypes carrying both R501X and 2282del4 null mutations ($P=0.00067$, Fig E3).

Replication of findings in African Americans

We sought replication of *FLG* associations with both ADEH and AD without EH in African American AD patients and healthy controls. We found the R501X mutation to be completely absent among 152 healthy African Americans, but six AD patients among 188 successfully genotyped individuals (3.2%) were heterozygotes for the mutation. Using Fisher's exact test to account for the absence of the mutation in healthy controls, the R501X mutation was significantly associated with AD among African Americans ($P=0.0351$), as observed in the European Americans. Despite the small number of ADEH patients ($N = 32$), 9.4% of this group carried the mutation and the MAF was considerably higher (4.7%) resulting in a stronger association for ADEH ($P=0.0049$), as observed in the European Americans. When the analysis was restricted to the two case groups (ADEH versus AD without AD), the R501X mutation conferred a greater risk of ADEH ($OR=5.28$ (1.0–27.4), $P=0.0289$). We did not observe an association between the deletion mutation (2282del4) and AD or ADEH among African Americans, likely due to the very low frequency of the deletion among African American healthy controls (<1%) and AD patients (3.2%), and the complete absence of the deletion among the 32 African American ADEH patients. A significant association was observed for one of the same exon 3 markers (rs12730241) associated with a reduced risk of AD in the European Americans; however, among African American AD patients, this SNP conferred a greater risk of disease ($OR=1.95$ (1.15–3.32), $P=0.0127$), and the association was even stronger for ADEH ($OR=4.56$ (1.57–13.21), $P=0.0029$). Flanking marker rs2065956, also associated with a lower risk of AD among the European American group, was associated with a lower risk of ADEH among African Americans ($OR=0.24$ (0.08–0.75), $P=0.0089$). No significant haplotypes were observed among the African American group for any of the markers and risk of AD, and the ADEH group was too small to test for associations independent of AD.

We sought to confirm the absence of the R501X mutation among healthy African Americans by genotyping these markers in an independent reference population of 177 'control' individuals participating in an ongoing asthma genetics study (available in this article's Online Repository at www.jacionline.org). Consistent with our findings in the ADVN healthy African American participants and previous reports of African ancestry populations(15),(16), the R501X mutation was completely absent, with the exception of one individual with a previous history of AD (Table III).

DISCUSSION

This study of *FLG* polymorphisms in two independent and ethnically diverse populations of AD patients stratified by the serious complication, eczema herpeticum (ADEH), demonstrated that the functional R501X mutation confers an added risk of ADEH, with an estimated effect size of nearly 13 among European American patients ($P=7.25 \times 10^{-9}$), and

this association was further replicated among African American patients ($P=0.0049$). Furthermore, we observe a significant association between the 2282del4 mutation and AD and ADEH *per se* among European American patients, but this association was completely absent among the African American ADEH patients. Importantly, the combined R501X and 2282del4 mutations further enhanced the association for AD ($P=1.21 \times 10^{-8}$) and ADEH ($P=1.99 \times 10^{-11}$) among European American patients, suggesting the combination of these two mutations may contribute to an increased risk of AD and ADEH.

Because ADEH manifests as a severe form of AD, it is tempting to attribute the role of the R501X mutation to ADEH susceptibility as it relates to disease severity. Indeed, among both European American and African American ADEH patients, total serum IgE (tIgE) was substantially higher compared to AD patients without EH ($P=6.41 \times 10^{-9}$ and $P=6.16 \times 10^{-8}$, respectively), as was the more direct measure of severity, the 'Eczema Area and Severity Index' (EASI; $P=1.68 \times 10^{-5}$ and $P=7.97 \times 10^{-6}$, respectively; Table I). An association between the R501X mutation and tIgE levels among European American AD patients has been previously reported(19), and in our own study, as shown in Table IV, we observed the significant association between geometric mean tIgE levels and both the R501X ($P=0.023$) and the 2282del4 mutation ($P=2.90 \times 10^{-5}$) among the European American AD group, with the strongest association between tIgE and haplotypes of the combined R501X and 2282del4 (843.7 ± 7.3 vs 197.8 ± 10.2 , $P=5.10 \times 10^{-6}$). Also, we observed significant associations for both mutations and EASI (R501X, $P=0.0075$; 2282del4, $P=0.014$), and this association was further enhanced for the combined R501X and 2282del4 (7.7 ± 3.1 vs 3.9 ± 3.4 , $P=6.79 \times 10^{-5}$). It is possible that the association between these null mutations and ADEH is due to confounding associated with the typically higher levels of tIgE and EASI characteristic of patients with ADEH compared to AD patients without ADEH; unfortunately, it was beyond the scope of the original study design to adequately match ADEH cases and AD, no ADEH controls to address this specifically. However, among all the associations observed, the strongest association was for ADEH, specifically for the combined null mutations ($P=1.99 \times 10^{-11}$). Furthermore, no associations were observed when analyses were restricted to ADEH patients and there were no significant associations at all among African Americans for the outcomes tIgE and EASI. We therefore speculate that the relationship between this null mutation and disease is most likely related to an increased propensity to disseminated viral skin infection resulting from skin barrier dysfunction, which may be independent of serum IgE and skin severity. It also highlights the importance of skin barrier function in anti-viral responses.

The association of *FLG* mutations with ADEH may well be directly causal and have functional consequences relevant for cutaneous viral infectivity. Indeed, it has recently been found that filaggrin-deficient, but not wild-type, mice develop satellite skin lesions when inoculated with vaccinia virus (R. Geha, *personal communication*). These data suggest defects in barrier function may predispose to increased viral penetration and spread in the skin. Because Th2 responses diminish the innate immune response, *FLG* mutations may play a dual role in allowing increased spread and replication of viruses in AD(15, 20, 21). Furthermore, the elevated serum IgE levels and increased allergen sensitization observed in patients with *FLG* mutations may be due to greater allergen penetration through the skin(22). Indeed, a recent study of a newly identified *Flg*-deficient murine model, resulting

from a 1-bp deletion that is analogous to the human null mutations, demonstrated that antigen transfer through a disrupted barrier contributes to an elevated IgE sensitization and initiation of cutaneous inflammation in the context of atopic skin disease(23).

A novel finding from our study is the association between a SNP in the promoter region (rs1933063, -13289A/G) and a reduced risk of ADEH, lower tIgE levels, and a lower EASI among AD patients ($P=0.0032-0.03$). Although this SNP is relatively distant from the transcriptional start site, it is presumably in linkage disequilibrium (LD) with a mutation(s) in the promoter region involved in regulation of gene expression or FLG-mediated signaling. Although nearly 40 mutations in the *FLG* gene have been reported to date(6), none have been found in the *FLG* promoter (I McLean, *personal communication*), suggesting that variants in this region of the *FLG* gene might be uniquely associated with a clinical subset of AD (*i.e.*, disease severity, ADEH). Also, two synonymous SNPs in pairwise LD (rs12730241 and rs2065956, $D'=0.93$, $r^2=0.64$) were associated with a reduced risk of AD among European American subjects ($OR=0.61-0.62$, $P=0.015-0.016$). In contrast, these two SNPs demonstrated an opposite effect in the African American sample, and were significantly associated with an *increased* risk of AD and ADEH ($OR=1.95-4.56$, $P=0.013-0.003$). The opposing associations may be due to variation in allele frequencies according to ethnicity. Interestingly, none of the SNPs were in LD with either R501X or 2282del4 in both European and African American populations (Figure E2). Taken together, these observations suggest there may be a causal variant(s) in LD with these markers (independent of the R501X and 2282del4 mutations) which, together with non-genetic factors (*e.g.*, antigens, herpes simplex virus), may play a role in predicting development of AD and its more serious complication, ADEH.

Ours is the first study to genotype these two purportedly common loss-of-function mutations in a group of African Americans, and concurrent with our preliminary studies(15), we found a complete absence of the R501X mutation and very low frequency (1%) of the 2282del4 mutation among healthy individuals from this ethnic group. As further confirmation for our findings on the R501X mutation in the 152 healthy African Americans participating in the ADVN, we examined an additional reference population (177 African Americans recruited in the Baltimore-Washington, D.C. metropolitan area) and only observed the mutation in a single individual, who upon further examination had reported a history of AD. The low frequency and even absence of this mutation is not novel; elsewhere the prevalence of the R501X mutation among individuals without AD has ranged from 0.8% to 3.0% among European populations, and has been found to be absent in southern European (*i.e.*, Italian(24)) and Asian(16, 25) groups (Table III). In the only summary data available on frequency of this mutation in African populations, it was absent in a cohort of 124 North Africans(16). Although we observed a minor allele frequency of 1.0% among 153 African American AD patients without EH (higher among ADEH patients, at 4.7%), this was considerably less than the observed frequency among European American AD patients (including the findings in this study, 4.8%; Table III). This is lower than their atopic dermatitic, European counterparts, ranging from 5%–16%, and suggests a latitude-dependent distribution with a decreasing north-south gradient of frequency. Although we cannot speculate, at this time, the past selective pressures among Northern Europeans to potentially account for this distribution of *FLG* genotype, we hypothesize that the

considerably lower frequencies of both mutations among the European Americans in our study compared to Northern Europeans in previous reports(16) probably result from the admixture of the African (6%) and Asian (9.5%) ancestry represented in this group (Fig E2). Conversely, the relatively high frequency of the R501X mutation among African Americans with AD, especially those with ADEH, is likely owed to the European (17.7%) and Asian (7.6%) admixture observed in this sample.

Palmer and colleagues(16) have previously noted these differences in mutation frequencies and have suggested that different populations will have different *FLG* mutation profiles. Indeed, this group and others have demonstrated that, in populations where the R501X and 2282del4 mutations are not present, other mutations are prevalent and confer risk of AD, for example, the 3321delA and S2554X mutations among Japanese patients(25). While it is therefore tempting to dismiss the significance of the two mutations common among European ancestry individuals as important risk factors among patients of African descent, the observation that 14% of African Americans with the R501X mutation had ADEH is very intriguing given that the prevalence was also high (25.%) among the European American ADEH group. The significance of this finding – and the importance of the R501X mutation in diseases complicated by AD – is underscored by a recent report (26) where compound heterozygous R501X and 3321delA *FLG* mutations were present in a Japanese family with ichthyosis vulgaris, despite the fact that the R501X mutation failed to be detected in previous studies of both healthy Japanese individuals and Japanese AD patients(16, 25). Eliminating any possibility of European admixture in this Japanese family, Hamada and colleagues concluded that the R501X mutation could therefore occur as a *de novo* mutation in any population regardless of ethnicity. Although the number of African American ADEH patients here was too small to permit full replication of results in European American patients, our findings strongly suggest the R501X mutation, but not 2282del4, confers a substantial risk for ADEH regardless of ethnicity. Moreover, we observe, for the first time, a significant association between the R501X mutation and AD among African Americans, which is particularly relevant given minority groups, including African Americans, suffer from eczema disproportionately(27). We acknowledge that the ADEH samples in this study, especially the African American sample, are small, which is reflective of the rarity of the ADEH phenotype. Although we appreciate the potential for false positive associations, the association between the R501X mutation and ADEH was observed in both (independent) ethnic groups, and was sustained after analyses were repeated using the Fisher's exact tests and permutation tests in the African American patients. Thus, we are encouraged that the observations are likely to be real.

In summary, our findings confirm both R501X mutation and 2282del4 are the strongest predictors of AD, and for the first time this association is reported in a population of African descent. Importantly, the R501X mutation confers a greater risk for ADEH in both European and African ancestry populations. The higher frequency of this mutation among ADEH patients compared to AD patients strongly implicates its role in controlling severity of AD. Novel findings of association with several additional SNPs in *FLG* suggest there may be additional causal variants, independent of the null mutations, contributing to a risk of AD and ADEH. A clearer understanding of genetic risk factors associated with ADEH may

improve the ability of identifying patients at greatest risk for ADEH, which may ultimately lead to early intervention and improved surveillance in this vulnerable population.

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Abbreviations

AD	Atopic Dermatitis
ADEH	Atopic Dermatitis Eczema Herpeticum
HSV	Herpes Simplex Virus
FLG	Filaggrin
SNP	Single Nucleotide Polymorphisms
LD	Linkage Disequilibrium
EASI	Eczema Area and Severity Index
MAF	Minor Allele Frequency
AIM	Ancestry Informative Markers
ADV N	Atopic Dermatitis and Vaccinia Network

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Clinical Implications

The Filaggrin (*FLG*) R501X Mutation, a major risk factor for atopic dermatitis, confers a greater risk of the severe, HSV-associated complication, eczema herpeticum in diverse ethnic groups.

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FIG 1.

Summary of association tests among European Americans showing significance for each *FLG* mutation. The $-\log_{10}$ -transformed P -values on the y axis for all genotyped markers are plotted against their genomic position in NCBI Build 35 in megabase (Mb) along the x axis. Red circles indicate associations for ADEH; green squares indicate associations for AD. The horizontal dotted line indicates nominal significance at $P < 0.05$.

Table 1

Participant characteristics.

Characteristic	European American			African American		
	ADEH+	ADEH-	Healthy	ADEH+	ADEH-	Healthy
Sample size	112	166	157	32	155	152
Males; N (%)	58 (53.7%)	49 (29.7%)	63 (40.1%)	12 (37.5%)	36 (23.2%)	77 (50.7%)
Age; mean (SD)	22.9 (22.1)	38.4 (14.6)	36.5 (13.2)	17.9 (17.8)	36.4 (11.0)	41.1 (10.3)
Geometric mean IgE levels (95% CI) *	2,083 (1399–3100)	366.7 (261–516)	55.5 (44–69)	2,943 (1470–5892)	391.6 (294–522)	142.6 (114–178)
Geometric mean EASI † (95% CI) **	7.4 (6.0–9.1)	3.5 (2.9–4.3)	ND	9.5 (6.3–14.4)	3.1 (2.5–3.9)	ND

The following abbreviations are used: AD, atopic dermatitis; ADEH, atopic dermatitis eczema herpeticum; ADEH+, ADEH positive; ADEH, AD without EH; EASI, Eczema area and severity index; and ND, not done.

† EASI determined by the percentage of eczema area on a 7-point ordinal scale: 0 =<10%; 1=10%–29%; 3=30%–49%; 4=50%–69%; 5=70%–89%; and 6=90%–100%.

* Total serum IgE levels were significantly higher in ADEH+ patients compared to both ADEH- patients and healthy controls.

** EASI was significantly higher among ADEH+ patients compared to ADEH- patients in both ethnic groups.

Table II

Association of *FLG* null mutations with AD and ADEH.

Mutation	Genotype*	No. of subjects (%)		Controls		AD vs. Controls		ADEH vs. Controls		ADEH vs. AD	
		ADEH+	ADEH−		OR** (95%CI)	P-value	OR** (95%CI)	P-value	OR** (95%CI)	P-value	
European American											
R501X	AA	82 (74.6)	151 (91.0)	153 (97.5)	7.1 (2.5–20.1)	1.46 × 10^{−5}	13.1 (4.4–38.5)	7.25 × 10^{−9}	3.4 (1.7–6.8)	0.0002	
	Aa	25 (22.7)	14 (8.4)	4 (2.6)							
	aa	3 (2.7)	1 (0.6)	0							
2282del4	AA	88 (82.2)	142 (85.5)	151 (96.8)	5.6 (2.2–14.6)	3.87 × 10^{−5}	6.5 (2.4–18.1)	3.00 × 10^{−5}	1.3 (0.7–2.5)	0.4651	
	Aa	17 (15.9)	21 (12.7)	5 (3.2)							
	aa	2 (1.9)	3 (1.8)	0							
Combined	AA	66 (61.7)	130 (78.3)	147 (94.2)	6.4 (3.1–13.2)	1.21 × 10^{−8}	10.1 (4.7–22.1)	1.99 × 10^{−11}	2.2 (1.3–3.8)	0.0029	
	Aa	35 (32.7)	33 (19.9)	9 (5.8)							
	aa	6 (5.6)	3 (1.8)	0							
African American											
R501X	AA	29 (90.6)	153 (98.1)	152 (100)		0.0351		0.0049	5.28 (1.0–27.4)	0.0289	
	Aa/aa	3 (9.4)	3 (1.9)	0							
2282del4	AA	32 (100)	148 (96.1)	149 (98.7)	2.5 (0.5–12.5)	0.2542		1.0		0.5919	
	Aa/aa	0	6 (3.9)	2 (1.3)							

The following abbreviations are used: AD, atopic dermatitis; ADEH, atopic dermatitis eczema herpeticum; ADEH+, ADEH positive; ADEH, AD without EH.

* AA, wild type genotype for either R501X or 2282del4; Aa, heterozygous genotype; aa, homozygous mutant genotype.

** OR was calculated by the AA versus combined Aa + aa genotypes.

TABLE III

Minor allele frequencies of the two loss-of-function *FLG* variants in ethnically diverse participants in unrelated population-based studies

Population	R501X MAF		2282del4 MAF	
	With AD (N)	Without AD (N)	With AD (N)	Without AD (N)
<i>American:</i>				
ADVN European American	0.048 (N=166)	0.013 (N=157)	0.082 (N=166)	0.016 (N=156)
European American[Rogers, 2007 #3942]	0.039 (N=179)	0.012 (N=447)	0.050 (N=180)	0.023 (N=449)
ADVN African American	0.010 (N=156)	0.000 (N=151)	0.019 (N=154)	0.007 (N=151)
GRAAD African American	N/A	0.003 (N=177)	N/A	N/A
<i>Other European ancestry:</i>				
Irish[Sandilands, 2007 #3920]	0.136 (N=188)	0.013 (N=736)	0.098 (N=188)	0.013 (N=736)
Scottish [Palmer, 2006 #3636]	N/A	0.030 (N=1,008)*	N/A	0.019 (N=1,008)*
British[Barker, 2007 #4000]	0.156 (N=163)	0.029 (N=1,463)	0.117 (N=163)	0.017 (N=1,463)
French[Hubiche, 2007 #4002]	0.075 (N=99)	0.025 (N=102)*	0.101 (N=99)	0.010 (N=102)*
German[Marenholz, 2006 #3370]	0.051 (N=188)	0.008 (N=319)	0.043 (N=187)	0.017 (N=316)
Italian[Giardina, 2008 #3909]	0.006 (N=178)	0.000 (N=210)	0.008 (N=178)	0.000 (N=210)
<i>Asian ancestry:</i>				
Indian[Palmer, 2006 #3636]	N/A	0.000 (N=83)	N/A	0.014 (N=111)
Japanese[Nomura, 2007 #4001]	0.000 (N=143)	0.000 (N=156)	0.000 (N=143)	0.000 (N=156)
Chinese[Palmer, 2006 #3636]	N/A	0.000 (N=47)	N/A	0.000 (N=49)
<i>African ancestry:</i>				
North African[Palmer, 2006 #3636]	N/A	0.000 (N=124)	N/A	0.000 (N=121)

Abbreviations: MAF, minor allele frequency; AD, atopic dermatitis; ADVN, ; GRAAD, *Genomic Research on Asthma in the African Diaspora*; N, N/A, not available. * Control group selected from the general population and not screened for AD

TABLE IV

Association of null mutations in FLG with total serum levels of geometric mean IgE (KU/L) and EASI in the European American subjects.

Mutation	Genotype*	No. of subjects	Total serum IgE (Mean ± SD, KU/L) [†]	P Value	EASI [†]	P Value
R501X	AA	229	250.1 ± 10.1	0.0234	4.3 ± 3.3	0.0070
	Aa/aa	45	748.8 ± 7.9		8.2 ± 3.4	
2282del4	AA	233	245.5 ± 10.3	2.90×10 ⁻⁵	4.4 ± 3.5	0.0014
	Aa/aa	44	1024.0 ± 6.2		8.0 ± 2.9	
Combined	AA	193	197.8 ± 10.2	5.10×10 ⁻⁶	3.9 ± 3.4	6.79×10 ⁻⁵
	Aa/aa	77	843.7 ± 7.3		7.7 ± 3.1	

* aa: homozygous R501X or 2282del4, Aa: heterozygous genotype for either R501X or 2282del4, and AA: wild type.

[†] Log-transformed adjusted for age and sex.