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Uncommon variants in FLG2 and TCHHL1 are associated with remission of atopic dermatitis in a large longitudinal US cohort

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Abstract

Atopic dermatitis (AD) is a relapsing inflammatory skin disease; filaggrin (FLG) variation has been consistently associated with its pathogenesis. Filaggrin-2 (FLG2) and trichohyalin-like-1 (TCHHL1) are members of the same protein family (S100 fused type proteins), are similar in structure to *FLG*, and may be involved in AD pathogenesis. We sought to evaluate the association between variation in *FLG2*, *TCHHL1* and AD remission. We sequenced *FLG2* and *TCHHL1* in a longitudinal AD cohort using targeted capture based massively parallel sequencing. Association between individual alleles and AD remission was evaluated with generalized estimating equations for binary outcomes. Association between groups of alleles and AD remission was evaluated using a genetic algorithm to group alleles. We identified 2 loss-of-function (LoF) mutations in *FLG2* (Ser2377Ter,Arg2207Ter) and 2 LoF mutations in *TCHHL1* (Gln656Ter,Gln294Ter), none of which were associated with AD remission. Common (MAF>5%) alleles in FLG2 were similarly unassociated with AD. No common alleles in TCHHL1 were associated with AD remission after multiple testing correction. Among self-described whites, a group of 34 uncommon alleles in *FLG2* were associated with increased AD remission (OR 7.64e17; 95%CI 4.41e17–1.32e18; adjusted p<1.0e-16). 12 uncommon alleles in *TCHHL1* were associated with increased AD remission (OR 23.46; 95%CI 7.07–77.89; adjusted p=0.064). Among self-described African

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Code availability: R code for this project is available upon request from the corresponding author.

IRB approval: The PEER study was approved by the Institutional Review Board of the University of Pennsylvania, and written informed consent was obtained from all participants from the participant or his or her caregiver.

Americans, 13 uncommon *FLG2* alleles were associated with increased AD remission (OR 21.01; 95%CI 11.90–37.09; adjusted p<1.0e-16). No *TCHHL1* uncommon allele groups were associated with AD remission among African Americans. Our study supports the role of uncommon alleles in *FLG2* and *TCHHL1* in AD pathogenesis.

Keywords

atopic dermatitis; epidemiology; epidermal biology; epidermal barrier function; filaggrin 2; trichohyalin-like-1; population genetics; S100 fused type proteins

INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disease which typically presents with red itchy patches on the flexural parts of the extremities [1,2,3,4]. AD is common, affecting up to 20% of children and 3% of adults in the industrialized world [5]. As with many common diseases, the pathogenesis of AD likely varies among individuals, and is influenced by both genetic and environmental factors [4,6,7].

Genetic studies of AD have suggested that both barrier dysfunction and immunodysregulation are key in disease pathogenesis [4,8]. Genetic variation in cytokines involved in the TH2 response, such as thymic stromal lymphopoietin (TSLP), and epidermal surface barrier proteins, primarily filaggrin (FLG), have been associated with variation in AD onset and persistence [5,8,15]. Of these, the most consistently associated and widely studied is FLG. Loss-of-function mutations in *FLG* lead decreased protein levels and are thought to produce a barrier defect which predisposes an individual to AD [17].

FLG protein belongs to a family of S100 fused-type proteins (SFTPs), many of which are part of the development of the skin's cornified envelope [5,9,23]. The genes cluster in a region of chromosome 1 called the epidermal differentiation complex (EDC) [5,9,23]. Other proteins in this family include filaggrin-2 and trichohyalin-like 1 protein [5,9,10,11]. Due to these proteins' structural and functional similarity to FLG, it is possible that variation in any of the SFTP genes could be associated with variation in skin barrier function and thus increase AD susceptibility and persistence [5,9,10,11,17,23].

Filaggrin-2 is an SFTP encoded for by the *FLG2* gene. It is located very close to FLG. Previous studies suggest that the FLG2 protein is involved in maintenance of the epidermal barrier and cell-cell adhesion in the cornified layers of skin [11,13]. Decreased expression of *FLG2* has been associated with a thinner epidermis; decreased levels of filaggrin-2 have been observed in the lesional skin of patients with AD and psoriasis [5,10,11,12]. Study of *FLG2* variation in AD has been limited and largely involved studies in African Americans. In 2014, Margolis et al. identified three notable *FLG2* variants in African American individuals, two of which were associated with increased AD persistence [10]. In contrast, in 2015, Pellerin et al. found numerous single nucleotide polymorphisms (SNPs) in *FLG2*, but found no variants associated with the presence of AD [5].

Trichohyalin-like 1 protein is encoded for by the *TCHHL1* gene. Similarly to filaggrin-2, trichohyalin-like 1 protein is an SFTP located near FLG in the EDC. This gene, and its protein's function, is not well-understood [18,19]. A previous investigation, from a small subset of the present cohort, identified loss of function (LoF) mutations in *TCHHL1*, but did not find an association with AD [20]. Another report identified mutations in *TCHHL1* associated with AD, but the sample size was small with no correction for multiple comparisons [21]. Due to *TCHHL1*'s proximity to FLG and limited prior studies, further studies are needed to determine if variation in *TCHHL1* is associated with AD.

Our goal was to examine genetic variation in two filaggrin-like proteins, previously known to have loss-of-function variants, in a longitudinal cohort of individuals with AD, and to better appreciate the relationship between variation in filaggrin-2, trichohyalin-like 1, and AD remission.

METHODS

Genetic data were obtained from a subset of the Pediatric Eczema Elective Registry (PEER) study for which DNA samples were available. Both the overall PEER cohort and the subset with genetic data have been previously described [7,8]. Analyses were conducted on both the overall cohort, and after stratification by ancestry. Determination of ancestry was based on self-report. Self-described ancestry previously has been determined to strongly correlate with genetic markers of ancestry within this cohort [15]. Written informed consent was obtained from all participants. This study was approved by the institutional review board of the University of Pennsylvania.

DNA was collected with Oragene DNA collection kits (DNA Genotek, Ottawa, Canada). Sequencing of *FLG2* and *TCHHL1* was conducted using targeted capture based Massively Parallel Sequencing (MPS) on PEER individuals with sufficient DNA. Sequencing read alignment and variant calling were accomplished as previously described [8,22].

Disease clearance was defined using a self-reported outcome of whether or not a child's skin was symptom-free during the previous six months. As children in PEER could be followed for up to 10 years, PEER participants can have multiple reports of this outcome over time. The association between these outcomes and individual SNPs were evaluated with generalized estimating equations (GEE) for binary outcomes, assuming an exchangeable working correlation structure with empirical standard errors [8]. This GEE model incorporates all individual reports to provide a single point estimate of the likelihood of AD healing over time, which we interpret as a measure of AD remission. An OR>1 indicates an association with greater AD remission, whereas an OR<1 indicates an association with decreased AD remission. GEE models were implemented via the geeglm function from the R package geepack 1.2–1.

Our analysis plan is demonstrated in Figure 1. *FLG2* and *TCHHL1* were evaluated independently, and analyses were conducted in both the pooled cohort and race-stratified cohorts. Association of variants differed depending on whether the SNPs had MAF ≥ 5%. MAF <5% is a commonly used cutoff for distinguishing common from low-frequency and

rare alleles, and has been used by our group in previous work [8,22,26]. We *a priori* decided that SNPs with MAF ≥ 5% would be independently assessed for association with AD remission. SNPs with MAF < 5% were considered to have insufficient data for reliable GEE estimates when evaluated independently, so these variables were grouped according to an algorithm previously developed and validated [22]. Thus, groups of variants with MAF < 5%, rather than individual variants, were evaluated for association with AD remission. All analyses were implemented in R, version 3.6.1.

RESULTS

We obtained genotyping of *FLG2* and *TCHHL1* on 705 PEER individuals; 326 were self-described African Americans and 379 were self-described whites. As of June 2020, over 12,000 follow-up surveys were completed within the PEER study, following participants for a mean of 102 months. Demographic information for the PEER genetic cohort is provided in Table 1.

163 unique variants were identified in *FLG2*, of which three were LoF. 120 unique variants were identified in the African American cohort, and 99 unique variants were identified in the white cohort. Of these, 23 variants in the pooled cohort had MAF ≥ 5%. Supplementary Table S1 presents the unadjusted association of these *FLG2* SNPs with AD persistence. No variants reached significance at a p-threshold of 0.05. Two LoF variants were identified in our cohort; they are presented in Table 2. The previously studied SNP rs12568784 was not associated with AD persistence (OR, 0.98, 95% CI, 0.82–1.16, unadjusted p-value=0.790). Independent GEE estimates for rs377218292 (NC_000001.10:g.152323643G>A) could not be obtained because of insufficient sample size. One stop-loss variant was identified in our sample, NP_001014364.1:p.Ter2392Ser (rs150529054). This variant was only present in three individuals, so it was not studied further.

60 unique variants were identified in *TCHHL1*; there were 41 unique variants in the African American cohort and 26 unique variants in the white cohort. Of these, only three variants had MAF ≥ 5% in the pooled population. Supplementary Table S2 presents the unadjusted association of these TCHHL1 SNPs with AD persistence. None were statistically significantly associated with AD remission after correction for multiple comparisons. Two loss-of-function mutations were identified; they are presented in Table 2. Neither of the LoF variants in *TCHHL1* (rs150014958 and rs61749316) were present at a high enough frequency to assess for association with AD persistence independently.

The rare variant algorithm was run independently on rare variants in *FLG2* and *TCHHL1*, and independently run on white and African American individuals. All p-values were corrected for 250,000 independent tests. Within the white population, thirty-four rare variants were identified in *FLG2*; having any of these was associated with increased AD remission (OR, 7.64e17, 95% CI 4.41e17–1.32e18, adjusted p-value < 1.0e-16). 26 individuals, representing 6.9% of the study population, carried at least one of these variants. These variants are presented in Table 3. Within the self-described African American population, 13 uncommon FLG2 alleles were associated with increased AD remission (OR 21.01; 95%CI 11.90–37.09; adjusted p<1.0e-16). These variants are presented in Table 3.

In *TCHHL1*, in the white population, twelve rare variants were identified, which together trended towards association with increased AD remission (OR, 23.46, 95% CI 7.07–77.89, adjusted p-value 0.064). These variants were identified in 13 individuals, representing 3.4% of the study population. These variants are presented in Table 3. Within the African American population, no TCHHL1 uncommon allele groups were associated with AD remission.

DISCUSSION

FLG LoF variation is an important risk factor for AD and AD persistence. Several genes that code for filaggrin-like proteins (S100FTPs) cluster on chromosome 1 in the EDC region. However, there have been very few studies examining *FLG2* and *TCHHL1*, and prior studies focused on African American individuals. This report is the largest sequencing study of *FLG2* and *TCHHL1* in AD to date, and includes both African American and white individuals. This study is further distinguished by the use of *remission*, rather than presence of AD, as the primary outcome measure.

Taken together, our analyses of both *FLG2* and *TCHHL1* imply that LoF mutations in S100FTPs are not necessarily associated with AD persistence in either whites or African Americans. Since LoF mutations are generally thought to significantly decrease protein production, and since these LoF mutations in *FLG2* and *TCHHL1* are not associated with more persistent AD, it is tempting to speculate that the barrier function defect found in AD is relatively specific—that is, only specific types of skin-barrier alteration, namely those induced by filaggrin loss-of-function mutations, influence AD pathogenesis. Conversely, recent research into filaggrin suggests that filaggrin's contribution to keratohyalin granules, and the skin's barrier function, is dependent upon liquid-liquid phase separation, and that only relatively small amounts of protein are necessary for adequate granule formation and function [24,25]. Extending this hypothesis to *FLG2* and *TCHHL1*, it is possible that the identified loss-of-function mutations within these genes do not sufficiently decrease protein quantity to alter their function.

That said, missense variants have been found to be important in other genes associated with AD remission, so it is reasonable to hypothesize that other variation in *FLG2* and *TCHHL1* may be associated with AD remission [8]. We identified no relatively common variants (MAF ≥ 5%) in *FLG2* and *TCHHL1* associated with AD remission after correction for multiple comparisons. This finding, coupled with the analysis of LoF variants, suggests that *individual, common* variants in FLG2 and TCHHL1 are not significantly associated with AD remission.

Unlike common variants, uncommon alleles, particularly in *FLG2*, were associated with AD remission. We found 34 rare variants in the white population that, when analyzed as a group, are associated with increased AD remission. This represents the first association of *FLG2* variation with AD in white individuals, and the first association of *FLG2* non-LoF variants with AD remission. These variants represent 6.9% of the study population, a considerable number of individuals for a disease with a prevalence of up to 20% of the general population [5]. Similarly, in African Americans, 13 uncommon FLG2 alleles were associated with

increased AD remission. In *TCHHL1*, when analyzed together, 12 rare variants trended towards association with AD remission. These variants were only present in 3.4% of the study population, and the association did not reach statistical significance after adjustment for multiple testing. Future investigations, not subject to such a stringent correction factor, may identify a significant association here.

The primary limitation of our study was that we only studied African American and white individuals, so our results may not apply to AD in patients of differing ancestries. Further studies in diverse populations will be needed to confirm these findings. The incidence of several LoF variants in both genes was very small (<5%), so we were unable to evaluate independent associations between these genes and AD remission.

In this investigation, we report results from the largest sequencing study of *FLG2* and *TCHHL1* in whites with AD. In contrast with previous studies, we observed no common *FLG2* variants associated with AD severity. We also found no common variants in *TCHHL1* associated with AD severity. However, we did find that uncommon allele composites within both *FLG2* and *TCHHL1* were associated with AD remission, implying that these genes may be relevant in certain subsets of AD individuals. This study provides further insight into the genetics of AD, demonstrating that uncommon alleles within non-FLG skin barrier proteins may be involved in AD pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability:

The PEER data (source) is not currently publically available. The PEER study is an ongoing study sponsored by Valeant in response to a post marketing commitment with the FDA. A BED file of the probes used for targeted capture is available upon request from the corresponding author.

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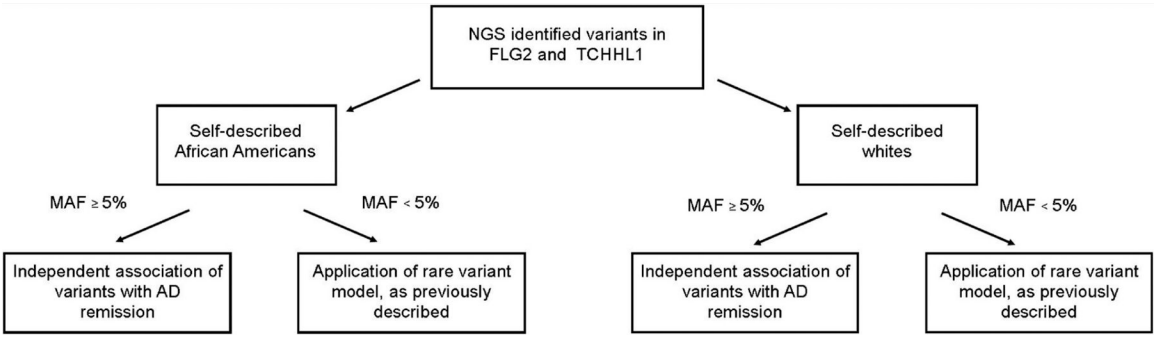

Figure 1:
Schematic of analysis flow.

Table 1:

Participant Demographics

	Whole cohort, n(%)	African American, n(%)	White, n (%)
Number	705	326	379
Age of AD onset, mean (SD)	1.89 (2.65)	2.14 (2.85)	1.84 (2.65)
Sex: male, n (%)	332 (47.1)	136 (42.0)	196 (51.7)
Asthma, n (%)	382 (54.1)	182 (56.2)	200 (52.8)
Seasonal Allergies, n (%)	494 (70.1)	228 (70.4)	266 (70.2)

Table 2:

Loss of function variants in FLG2 and TCHHL1, and their association with AD remission.

			Pooled cohort (n = 705)			African American (n = 379)			White (n = 379)		
Gene	**RSID**	**Location**	**Odds of Clearing (95% CI)**	**raw p value**	**n**	**Odds of Clearing (95% CI)**	**raw p value**	**n**	**Odds of Clearing (95% CI)**	**raw p value**	**n**
FLG2	rs12568784*	NC_000001.10: g.152323132G>T	0.977 (0.822,1.161)	0.790	281	0.944 (0.768,1.16)	0.583	161	1.092 (0.792,1.507)	0.592	120
FLG2	rs377218292	NC_000001.10: g.152323643G>A	***	***	1	***	***	1	***	***	0
TCHHL1	rs150014958	NP_001008536.1: p.Gln656Ter	***	***	12	***	***	4	***	***	8
TCHHL1	rs61749316**	NP_001008536.1: p.Gln294Ter	***	***	16	***	***	6	***	***	10

* Previously identified in Margolis et al. 2014b.

** Previously identified in Margolis et al. 2014c.

*** Could not be estimated, or insufficient sample size for estimation.

Table 3:

Uncommon variants in uncommon variant groupings.

FLG2, whites			FLG2, African Americans			TCHHL1, whites		
Location	**Nucleotide change**	**Amino acid change**	**Location**	**Nucleotide change**	**Amino acid change**	**Location**	**Nucleotide change**	**Amino acid change**
152328471	C>T	p.G597G	152322250	G>A	intronic	152057347	G>A	
152329677	G>A	p.D195D	152322365	T>G	intronic	152059461	G>T	p.Q233K
152331902	G>C		152323269	A>G	p.H2331H	152057769	A>G	p.Y797H
152331264	G>A	p.L33L	152324043	A>G	p.H2073H	152058358	A>G	p.P600P
152330789	G>A		152325368	G>A	p.H1632Y	152058563	C>T	p.G532E
152329534	C>T	p.G243E	152325410	C>T	p.G1618R	152058633	A>T	p.S509T
152329530	C>G	p.L244F	152325444	A>G	p.H1606H	152058648	G>T	p.P504T
152329370	A>G	p.C298R	152325742	C>T	p.S1507N	152059278	G>A	p.Q294X
152328924	T>C	p.V446V	152326154	G>A	p.H1370Y	152059570	T>A	p.I196I
152328475	T>C	p.H596R	152326696	A>G	p.F1189S	152059638	C>T	p.E174K
152328308	A>G	p.S652P	152327295	C>G	p.Q989H	152061454	C>T	
152328190	G>A	p.S691F	152330196	C>T	intronic	152061533	C>T	
152327955	G>A	p.S769S	152330801	C>T	intronic			
152326902	C>T	p.S1120S						
152326715	C>A	p.V1183L						
152326620	T>C	p.Q1214Q						
152325965	G>A	p.H1433Y						
152325911	C>T	p.G1451R						
152325766	G>T	p.S1499Y						
152325522	A>G	p.T1580T						
152325455	C>T	p.G1603R						
152324325	A>G	p.A1979A						
152324316	G>T	p.H1982Q						
152324315	A>G	p.Y1983H						
152324312	G>C	p.P1984A						
152324311	G>C	p.P1984R						

FLG2, whites			FLG2, African Americans			TCHHL1, whites		
Location	**Nucleotide change**	**Amino acid change**	**Location**	**Nucleotide change**	**Amino acid change**	**Location**	**Nucleotide change**	**Amino acid change**
152324146	T>G	p.H2039P						
152324114	C>T	p.A2050T						
152324109	A>G	p.H2051H						
152324106	G?A	p.G2052G						
152323176	C>T	p.G2362G						
152322465	C>T							
152322324	C>T							
152322292	G>A							