

Filaggrin Gene Mutations in African Americans with Both Ichthyosis Vulgaris and Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) and ichthyosis vulgaris (IV) are two common disorders of epidermal homeostasis resulting in dry skin. The profilaggrin gene, located on chromosome 1q22, encodes a keratin filament aggregating protein (filaggrin) that is essential to forming the epidermal barrier and maintaining hydration. Null mutations in filaggrin have been found to underlie IV and are common in patients with AD, but the minority of African Americans with AD or IV show these mutations in filaggrin. We have selectively studied African Americans with both AD and IV to maximize the possibility of finding filaggrin null mutations in this population. DNA was collected using buccal swabs from 18 African American children with both AD and IV and 17 African American controls without either of these diseases. Purified genomic DNA was amplified using polymerase chain reaction from three regions of the filaggrin gene, exon 3, including R501X, 2282del4, E2554X, R2447X, 1249insG, R826X, 2767insT, and E2422X. Of the African American children with both AD and IV, 22.2% were heterozygous for filaggrin null mutations. Out of the control group, one carried a null mutation and was later discovered to have a history of asthma. Null mutations found in this population included R501X ($n = 1$), 2282del4 ($n = 2$), and R826X ($n = 2$, including the control patient). Our data demonstrate a prevalence of filaggrin mutations in the African American population that exceeds previously published data, although the overall prevalence is still lower than in other populations. It is likely that factors other than known *FLG* mutations are involved in African American patients.

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Filaggrin aggregates keratins within the cytoskeleton of keratinocytes during cornification to form the highly insoluble keratin matrix of the stratum corneum and is an important component of the epidermal barrier. Filaggrin is also degraded into hygroscopic amino acids, which promote skin hydration. Profilaggrin (*FLG*) mutations have been unequivocally linked to ichthyosis vulgaris (IV), a common monogenic skin disorder, and atopic dermatitis (AD), the most prevalent inflammatory skin disorder in children. Null mutations in *FLG* have been associated with epidermal barrier abnormalities, including disorganized keratin filaments, abnormal architecture of the lamellar bilayer, and high transepidermal water loss. Two hotspot loss-of-function mutations, R501X and 2282del4, were originally identified in the northern European Caucasian population with a frequency of 7% to 10% (1). Population-based studies have since found other hotspot mutations that are more common in certain ethnic populations, with 49 null mutations previously described (2). In populations with AD, 27.5% of Caucasian children in the United States (3), 31.4% of Han Chinese (4), 20% of Japanese (5), and 0.6% to 0.9% of Italians (6) have shown *FLG* mutations. Two recurrent mutations (R501X and 2282del4) account for 80% of the total risk alleles in Irish people with AD, four different mutations (2554X, 2889X, 3296X, and 3321delA) are found in 22.5% of Japanese people with AD (5), and eight different recurrent mutations contribute 80% of the total risk alleles in Singaporean people with AD (7), demonstrating the population differences in *FLG* null mutations.

Few studies have found *FLG* mutations in individuals of African descent (Table 1). Early studies found no mutations at R501X or 2282del4 in 256 healthy individuals and 12 with moderate AD of African ancestry (1,8). Using African Americans in the Atopic Dermatitis Vaccinia Network registry, Gao et al (9) found R501X and 2282del4 mutations in 3.2% of 187

African American patients with AD and found the 2282del4 mutation in 1.3% of 152 nonatopic African American controls. In a study of Ethiopians, Winge et al (10) found a 632del2 mutation in *FLG* (unknown significance) in one subject with AD but no R501X, 2282del4, S3247X, or R2447X mutations in 103 controls, 103 subjects with AD, and 7 with only IV. Most recently, Margolis et al (3) evaluated the genotype of 370 African Americans with AD, testing for four specific mutations, and found these mutations in only 8.1% of patients (R501X, 3.2%; 2282del4, 0.5%; S3247X, 3%; and R2447X, 1.4%).

MATERIALS AND METHODS

Institutional review board approval was obtained from all institutions and informed consent was obtained from all subjects. We investigated *FLG* mutations in 18 African American children with both AD and dermatologist-confirmed IV. Using the Rajka and Langeland scoring system to determine AD severity, 88.9% of these 18 patients had moderate or severe AD. Because formal criteria for the diagnosis of IV have not been established, investigators created a scoring system to identify persons with likely IV. Potential subjects were examined for the presence of lower extremity ichthyotic scale and were assigned a score from 0 to 3 when scale was absent, mild, moderate, or marked, respectively. They were then given a score from 0 to 2 for palmar markings (normal, increased, or markedly increased). All demonstrated lower extremity scaling and 16 had palmar markings. Mild IV was defined as a combined score of 1 or 2, moderate IV as a score of 3 or 4, and severe IV as a score of 5. All patients fell into the mild ($n = 7$) or moderate ($n = 11$) category. DNA was also evaluated from 17 African American controls without any history or evidence on enrollment examination of IV or AD. We looked for *FLG* null mutations in three regions of the filaggrin coding sequence, all located

TABLE 1. *FLG* Mutations in Africans or Individuals of African Heritage

Study	Population	Mutations in <i>FLG</i>
Palmer et al, 2006 (1)	North Africans	No R501X ($n = 124$) or 2282del4 ($n = 121$) in general population
Howell et al, 2007 (8)	African Americans, United States	None in 12 with moderate AD and 11 healthy controls
Gao et al, 2009 (9)	African Americans, United States	Of 187 with AD, R501X (3.2%) and 2282del4 (3.2%); of 152 healthy controls, 2282del4 (1.3%)
Winge et al, 2011 (10)	Ethiopians	Of 103 with AD, 632del2 (1%); none in 103 controls
Margolis et al, 2012 (3)	African American children, United States	Of 370 with AD, R501X (3.2%), 2282del4 (0.5%), S3247X (3%), and R2447X (1.4%)
Polcari et al, 2014 (this study)	African Americans with IV and AD, United States	Of 18 with AD and IV, R501X (5.6%), 2282del4 (11.1%), and R826X (5.6%); of 17 controls without AD or clinical evidence of IV, R826X (5.9%)

AD, atopic dermatitis; IV, ichthyosis vulgaris.

within exon 3. These regions included *FLG* mutations that are prevalent in AD (2): R501X, 2282del4, and E2554X mutations detected in European and Asian patients; the R2447X mutation detected in Europeans; and the 1249insG, R826X, and 2767insT, and E2422X mutations found in Asian patients. The sequenced portions consisted of the following nucleotide regions, using reference sequence NM_002016 (adjusted so that position 1 equals the A nucleotide in the ATG start codon): 1242-1613, 2225-2763, and 7258-7706.

Briefly, patient DNA was purified from buccal swabs (IsoHelix Swabs, BocaScientific, Boca Raton, FL) and quantified using ultraviolet spectrophotometry. Purified genomic DNA and controls were amplified using polymerase chain reaction (PCR) from three different regions of *FLG* exon 3 using three primer sets. PCR products were analyzed using electrophoresis, purified (Qiaquick, Qiagen, Valencia, CA), and subjected to duplicate cycle sequencing reactions using ABI BigDye v3.1 reagents (Applied Biosystems, Carlsbad, CA). Labeled sequencing products were purified for capillary electrophoresis (ABI3730 or ABI3130 sequencer with POP7 polymer) and sequence results were examined using ABI SeqScape software (Applied Biosystems, Grand Island, NY). All nucleotide changes were noted, including 30 single-nucleotide polymorphisms (SNPs) seen in the samples tested, the most common of which were coding changes at T454A (SNP rs2011331), H2507Q (rs3126074), and G2545R (rs3126072) and a silent change at nucleotide t2508c (rs3120653) (data not shown (11)).

RESULTS AND DISCUSSION

All subjects met Rajka-Langeland criteria for AD and had clinical evidence of mild to moderate ichthyosis. Four subjects with AD and IV (22.2%) and one control (5.9%) were found to be heterozygous for *FLG* null mutations, consistent with the mild to moderate severity of their phenotypes. The control patient who carried an *FLG* null mutation later indicated a history of asthma but no known dermatitis. The subjects with AD with mutations had a severity score of moderate ($n = 2$) or severe ($n = 2$) disease. Detected mutations included R501X ($n = 1$), 2282del4 ($n = 2$), and R826X ($n = 2$, one of whom was the control subject). The R826X mutation has recently been described in Chinese Han people with AD (4), but never before in an African American patient. Neither individual with the R826X mutation possessed any of the four common SNPs in the regions sequenced, but both had two rare SNPs

(rs76955400 and rs13376095) that are more common in the Yoruba population from Ibadan, Nigeria, than the Exome Sequencing Project (ESP) cohort populations (a range of American samples) or the CSAgent cohort (a population of European descent) (11).

Although the number of patients in our study was limited, the much higher percentage of African Americans with *FLG* mutations in our cohort (22.2%), despite investigation of only 11% of the entire coding sequence, could have reflected our inclusion of individuals with both IV and AD rather than with either alone. Nevertheless, the inflammatory cytokines of AD can suppress filaggrin expression, potentially leading to the phenotype of IV. A recent investigation found the percentage of *FLG* mutations to be higher in Chinese patients with only IV (74%) than in those with AD-associated IV (43%) (12).

The low prevalence of *FLG* mutations in African American patients suggests that factors other than known *FLG* mutations are involved. Although the area sequenced would have detected at least 83% of the *FLG* mutations in Irish subjects, it would have detected only 13% of them in subjects from Singapore (7). Given that the entire coding sequence of exon 3 was not evaluated, it is possible that pathogenic mutations reside elsewhere in the *FLG* gene, although it is more likely that African Americans with IV harbor mutations in a gene that encodes another epidermal barrier protein that shares function or interacts with filaggrin. IV has been linked to chromosome 10q22.3-q24.2 in two Chinese families (13), although no candidate gene has been identified. A more likely possibility is *FLG2*, which is expressed in the stratum granulosum and encodes a protein that similarly contributes to epidermal barrier function (14). *FLG2* maps to chromosome 1q21.3 (15), a locus near the *FLG* locus at chromosome 1q22 (1). The recent demonstration using whole-exome sequencing that *FLG2* mutations increase the persistence of AD in African Americans (16) further suggests a pathogenic role for *FLG2* mutations and supports the sequencing of *FLG2* in African Americans with IV with or without AD.

CONFLICT OF INTEREST

M.S. Smith is an employee of Viracor-IBT Laboratories, which performed the filaggrin gene assays.

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