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Exome sequencing of Filaggrin and related genes in African-American children with atopic dermatitis

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Letter

Atopic dermatitis (AD) is a common chronic relapsing disease. There is a considerable body of evidence supporting a genetic basis for AD (Bussman *et al.*, 2011;Ellinghaus *et al.*, 2013). Mutations in the Filaggrin (*FLG*) gene have been consistently found to be associated with AD in people of European and Asian ancestry (Brown and McLean, 2012). More than 40 *FLG* loss-of-function mutations have been described in Europeans and Asians, (Brown and McLean, 2012).However, *FLG* loss-of-function mutations have not commonly been found in Africans or African-Americans (Margolis *et al.*, 2012;Brown and McLean, 2012;Winge *et al.*, 2011a). Loss-of-function mutations in exon 3 of *FLG* result in diminished or absent filaggrin protein, most often due to a premature stop codon or a frameshift mutation resulting in a stop codon further downstream. Interestingly, the absence of profilaggrin protein (precursor of filaggrin) has also been noted in keratohyalin granules in the majority of those with ichthyosis vulgaris (IV) of European and Asian ancestry (Perusquia-Ortiz.A.M. *et al.*, 2013;Thyssen *et al.*, 2013;Fleckman and Brumbaugh, 2002).

FLG is located on chromosome 1q21 in a region called the epidermal differentiation complex (EDC). It is part of a family of genes that code for S100-fused like proteins (SFTP). The SFTPs include the proteins profilaggrin (coded by *FLG)*, hornerin (*HRNR*),

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Conflict of Interest

The authors report no conflicts of interest

filaggrin-2 (*FLG2*), repetin (*RPTN*), cornulin (*CRNN*), trichohyalin (*TCHH*), and trichohyalin-like 1(*TCHHL1*)(Henry *et al.*, 2012). These genes are very similar to one another with respect to structure and function, and lie in close proximity to each other in the EDC (Marenholz *et al.*, 2011;Henry *et al.*, 2012;Pellerin *et al.*, 2013). Based on prior experience with *FLG*, it has been hypothesized that a stop-gain (null) mutation in exon 3 of any of the SFTP genes will result in decreased or absent protein production (Henry *et al.*, 2012;Marenholz *et al.*, 2011;Margolis *et al.*, 2014).

The goal of this study was to identify stop-gain variants in *FLG* and closely related genes in African-Americans with AD from the Pediatric Eczema Elective Registry (PEER)(Margolis *et al.*, 2012). From this cohort we randomly selected 60 subjects for whole exome sequencing to ensure sufficient power to detect variants with a minor allelic frequency (MAF) of greater than 3%. Sequencing was performed by Ambry Genetics (Aliso Viejo, California) using whole exome targeted enrichment by Agilent SureSelectXT Human All Exon 50Mb kit. Quality assessment revealed that most samples were above 50% on target and mean coverage per gene was excellent. The libraries were indexed using 100 base paired ends and processed using Illumina HiSeq2000 at 100x coverage per exon. Data was assessed utilizing a pipeline generated at the University of Pennsylvania based on the best practices protocol from the Broad Institute (Cambridge, MA). This report focused on stop-gain mutations of exon 3 (i.e., loss-of-function mutations) in the SFTP genes because of their likely functional relevance (Brown and McLean, 2012;Henry *et al.*, 2012;Marenholz *et al.*, 2011). Taqman allelic discrimination assays were created for any newly identified *FLG* loss-of-function mutations, which were then used to genotype an additional random sample of 100 African-American PEER children.

Sequencing of the SFTP genes in 60 self-reported (ancestry previously confirmed with ancestral informative markers(Margolis *et al.*, 2012)) African-American children with AD revealed a total of 289 variants in *FLG*, 107 variants in *FLG2*, 339 variants in *HRNR*, 4 variants in *RPTN*, 37 variants in *CRNN*, 88 variants in *TCHH* and 14 variants in *TCHHL1*. However, very few variants resulted in a premature stop codon in exon 3 (Table 1). Each of the three newly identified *FLG* stop-gain mutations, Q570X, R3409X and S3707X, were observed only once. S2392X and S2377X in *FLG2* were noted 1 and 16 times respectively. In *TCHHL1*, the variant Q294X was noted twice. All subjects were heterozygous for the mutations. The MAF for variants noted once, twice and 16 times were 0.008, 0.017 and 0.133, respectively. Next, we used Taqman based allelic discrimination assays to evaluate the three *FLG* mutations, Q570X, R3409X, and S3707X, in an additional 100 African-American PEER children. However, none of these variants could be detected in other members of our cohort

This report is from the largest whole exome sequencing study of African-Americans with AD performed to date. Here we have reported results specific to the SFTP genes. We identified a few new null mutations, albeit the ones in *FLG* had low MAFs. The MAFs noted for S2377X (*FLG2*) and Q294X (*TCHHL1*) vary from the healthy subjects in the 1000 Genomes database (The 1000 Genomes Project Consortium, 2012) (Table 1). S2377X was seen about half as frequently in our cohort as compared to the healthy 1000 Genomes African population; while Q294X, though more common in our cohort, still had a low MAF

(i.e., MAF=0.017) suggesting that these variants may not be clinically important with respect to incident AD.

Our findings are in agreement with that of Winge et al., who also failed to detect common *FLG* loss-of-function mutations in people of African ancestry with AD (Winge *et al.*, 2011a). Our study does have limitations in that we focused only on exon 3 stop-gain mutations in genes. We did not assess copy number variations. We also did not assay protein function. Another point to be noted is that since most African-Americans have their origins in West Africa; our findings may not generalize to everyone with African ancestry. However, based on the experience of others as well as our study, which is the largest whole exome study of African-Americans with AD, it seems unlikely that *FLG* stop-gain mutations have a prominent role with respect to **incident** AD in African-American children(Thaswer-Esmail *et al.*, 2014;Winge *et al.*, 2011b).

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Table 1

Null mutations identified in genes belonging to S100-fused like protein family.

Gene	Variant	Reference Allele	Alternate allele	Location of amino acid change	MAF Current study	MAF African ancestry (Source: 1000 Genomes)	dbSNP Designation
FLG	Stop-gain	G	A	FLG:NM_002016:exon3:c.1708C>T:p.Q570X	0.008	--	--
FLG	Stop-gain	G	A	FLG:NM_002016:exon3:c.10225C>T:p.R3409X	0.008	--	--
FLG	Stop-gain	G	T	FLG:NM_002016:exon3:11120C>A:p.S3707X	0.008	--	--
FLG2	Stop-gain	G	T	FLG2:NM_001014342:exon3:c.7130C>A:p.S2377X	0.133	0.29	rs12568784
FLG2	Stop-loss	C	G	FLG2:NM_001014342:exon3:c.7175G>C:p.S2392X	0.008	0.01	rs150529054
TCHHL1	Stop-gain	G	A	TCHHL1:NM_001008536:exon3:c.880C>T:p.Q294X	0.017	0.01	rs61749316

MAF= Minor allele frequency