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Research article

Genetic polymorphism (rs6587666) in *FLG* protects from eczema in admixed Brazilian children population with high African ancestry

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ABSTRACT

Genetic variants in filaggrin (*FLG*) are key in eczema and are less common in Africans than in Europeans and Asians. Here we examined the association between *FLG* Single Nucleotide Polymorphisms (SNPs) and eczema in a population of admixed Brazilian children and whether African ancestry modifies this association. We included 1010 controls and 137 cases and ran logistic regressions between SNPs in *FLG* and eczema in the studied population and also stratified the analyses according to the degree of African ancestry. In addition, we tested the replication of the findings on an independent set of individuals, as well as, we verified the impact on *FLG* expression according to each SNP genotype. The T allele of SNP rs6587666 was negatively associated with eczema in additive model (*OR*: 0.66, 95% CI: 0.47–0.93, *P*: 0.017). Moreover, African ancestry modifies the association between rs6587666 and eczema. The effect of the T allele was higher among individuals with higher African ancestry and the association with eczema was lost in individuals with lower African ancestry. In our analyses the expression of *FLG* in skin was slightly downregulated by the presence of the T allele of rs6587666. In our population, the T allele of rs6587666 in *FLG* was associated with protection to eczema and the degree of African ancestry was able to modify the observed association.

1. Introduction

Eczema is the most common chronic inflammatory skin disease worldwide, affecting up to 20% of children and 3% of adults [1] involving several targets, including interactions between skin barrier defects and immunological factors [2,3]. Moreover,

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approximately one-third of patients with eczema develop asthma and two-thirds develop allergic rhinitis [4].

The primary function of the skin is to act as a protective barrier between the host organism and its external environment, minimising water loss whilst at the same time preventing the entry of microorganisms and allergens [3,5]. A great number of genes have been linked to eczema susceptibility [6–8]. Interestingly, in several studies characterizing the filaggrin-deficient skin barrier and its consequences, mutations in the filaggrin gene (*FLG*) have been associated with the risk and severity of eczema [6,9–13].

Filaggrin (filament-aggregating-protein) is a key protein in the differentiation of the epidermis. Together with keratin filaments and other proteins, filaggrin forms a cornified cell envelope, which is critical to the skin's barrier function [14]. The role played by filaggrin explains why the lower expression of a single component of the epidermal differentiation complex might have a great influence on the function of the skin barrier.

Several studies have observed ethnic and regional differences in *FLG* variants [13,15]. Palmer and collaborators have shown that 17.5% of European individuals with eczema were carriers of *FLG* null alleles [10]. On the other hand, certain *FLG* variants common in Europeans and Asians are less common in African populations [6,9,12,13,16–18].

The Brazilian population is derived from more than 500 years of genetic admixture between Europeans, Native Americans, and Africans, with the proportions of these ancestral populations varying markedly among Brazilian regions [19]. The Northeast Brazil, mainly Bahia State, received thousands of slaves directly from Africa [20]. Therefore, the influence of African ancestry described in Bahia is different and much higher from other regions of Brazil. In this sense, the African ancestry component is the major contributor of the genetic structure of the population from Salvador, Bahia [21,22].

In this study, we examined the association between Single Nucleotide Polymorphisms (SNPs) in *FLG* and eczema considering whether the association would vary by the degree of African ancestry in a population of Brazilian children from the city of Salvador, Bahia.

2. Materials and methods

2.1. Subjects

Our population-based study design has been described in detail elsewhere [23,24]. The sample comprises 1307 children aged five to twelve years old in the city of Salvador in Northeast Brazil and enrolled in Social Change, Asthma and Allergy in Latin America (SCAALA) Program were included. Written informed consent was obtained from the parents of each child enrolled in the study. The parent/guardian answered the ISAAC phase II core questionnaires for eczema, translated into Portuguese and back-translated into English, and validated [25] as described by Strina and collaborators [23]. This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Institutional Review Board of the Collective Health Institute (register 003–05/CEP-ISC) of the Federal University of Bahia, Brazil.

2.2. Eczema definition

Eczema was defined according to ISAAC studies, as described by Strina and collaborators [23]. In this study, we considered the children as a case when the response was positive for the occurrence of: *i)* itchy rash that came and went for at least 6 months; *ii)* itchy rash at any time in the last 12 months and; *iii*) itchy rash at any time. A rash in the following places of the body was considered for the diagnosis of eczema: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes (ISAAC, 1998). All other children whose answers were not positive to all three questions referred were classified as non-eczema controls.

2.3. Genotyping

DNA was extracted from peripheral blood samples using the Flexigene@ DNA Kit (Qiagen, Hilden, Germany), and genotyping was performed using the Illumina BeadChip HumanOmni 2.5 Kit (www.illumina.com) through EPIGEN-Brazil Consortium (https://epigen.grude.ufmg.br/). One individual was excluded from the analysis due to inconsistency between the sex registered and the genetic sex, based on X chromosome SNPs. Sixty-one individuals were removed from the sample due to the relationship determined by kinship coefficients for each possible pair of individuals. This method was implemented in the REAP software (Relatedness Estimation in Admixed Populations) [26]. We considered a pair of individuals as related if the estimated kinship coefficient between them was $\geq$0.1. This cut-off includes second-degree relatives such as a person's uncle/aunt, nephew/niece, grandparent/grandchild or half-sibling, and any closer pair of relatives. Ninety-eight people had missing values due to the absence of phenotype or covariates. A total of 1147 unrelated children were included in this study.

27 variants in *FLG* were extracted from chromosome 1 positions 152274641 to 152297715 (assembly: GRCh37.p13; location: NC_000001.10). For quality control, we applied the following filters: imbalance of Hardy–Weinberg equilibrium with *P*-value less than 0.05 and for the minor allele frequency – MAF of at least 1%. The genotyping call rate was greater than 99%. After quality control, a total of 18 SNPs were analyzed.

2.4. Stratification of the population according to the African ancestry

For our secondary analyses, subgroup analyses were conducted from the categorization based on different percentiles of the

continuous distribution of individual African ancestry in our population. The calculation of ancestry was performed as described by Kehdy and collaborators [27], using the ADMIXTURE software [28] considering an tri-hybrid model (K = three). Quantitative genetic ancestry was estimated for individuals by including contemporary descendants of the approximate population of origin (pseudoancestors). As external panels samples of African and European individuals from the HapMap project and 93 Native American of the Human Genome Diversity (HGDP) project were used. A total of 370,539 SNPs shared by samples from the HapMap, HGDP and the study population were used for biogeographical ancestry estimate. African ancestry strata were then defined as "high" vs "low" (above and below the median ancestry, respectively), and by tertiles.

2.5. Replication from the Hartford - Puerto Rico (HPR)

Details for the HPR cohort recruitment and procedures have been previously described in detail [29]. Briefly, children 6–14 years old living with four Puerto Rican grandparents were recruited from Hartford, CT, and San Juan, PR. Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards of Connecticut Children's Medical Center (Hartford, CT), the University of Puerto Rico (San Juan, PR), Brigham and Women's Hospital (Boston, MA), and the University of Pittsburgh (Pittsburgh, PA). Genome-wide genotyping was performed using the Illumina HumanOmni2.5 BeadChip platform (Illumina, San Diego, CA). For this analysis, cases with eczema (n = 156) were defined as those reporting an itchy/scaly rash affecting the face, neck, arms, elbows, legs, or knees, and who either still had the rash or had it at least until age 4 years; controls (n = 514) were subjects who never had such rash.

2.6. Statistical analysis

Association analysis were performed using logistic regression model to calculate the *odds ratio* (*OR*) and 95% confidence intervals (CIs). The analyses were adjusted by sex, age, helminth infection, asthma symptoms and principal components (PC1, PC2 and PC3), as previously described [21]. The principal components were estimated from genome-wide genetic variants and used to adjust the effect of population stratification on the different multivariate models tested [30]. We calculated associations using additive, dominant and recessive genetic models. A computationally intensive procedure based on 1000 permutations was used to estimate the statistical significance of multiple correlation tests in the genetic association analysis [31]. Power of each genetic model was estimated. The genetic data and power of the genetic model were analyzed using PLINK 1.9 [32]. The analyses of SNP function were performed using the RegulomeDB Score portal (https://www.regulomedb.org/regulome-search/) [33]. The Expression Quantitative Trait Locus (eQTL) analysis on the skin were performed using Genotype-Tissue Expression (GTEx) Portal (www.gtexportal.org/home/) [34]. The forest plot was performed using GraphPad 6 (GraphPad Software, San Diego, CA, USA). Linkage disequilibrium (LD) was investigated with Haploview [35]. The validation analysis in the HPR cohort used the additive model adjusted for age, sex, study site, and the principal components: PC1, PC2, and PC3. Statistical significance was always defined as $P < 0.05$.

3. Results

Table 1 presents the sample summary information of the 1010 non-eczema and 137 eczema participants of the study. Participants

Table 1
Sample summary information from the Social Change, Asthma and Allergy in Latin America (SCAALA) Brazilian cohort.

	Non-eczema	Eczema	*P*-value
	1010 (88.06%)	137 (11.94%)	
Age			
<6,0	397 (39.31)	53 (38.69)	0.947
6,0 to 7,0	339 (33.56)	45 (32.85)	
>7,0	274 (27.13)	39 (28.47)	
Sex			
Female, No. (%)	467 (46.24)	58 (42.34)	0.390
Male, No. (%)	543 (53.76)	79 (57.66)	
Helminthic infection			
Yes, No. (%)	240 (23.76)	29 (21.17)	0.501
No, No. (%)	770 (76.24)	108 (78.83)	
Asthma symptoms			
Yes, No. (%)	210 (20.79)	47 (34.31)	<0.0001
No, No. (%)	800 (79.21)	90 (65.69)	
Median for African ancestry			
Lower than median, No. (%)	494 (48.91)	73 (52.28)	0.337
Higher than median, No. (%)	516 (51.09)	64 (46.72)	
Tertiles for African ancestry			
1st, No. (%)	330 (32.67)	51 (37.23)	0.503
2nd, No. (%)	344 (34.06)	41 (29.93)	
3rd, No. (%)	336 (33.27)	45 (32.85)	

[a]*P*-value considering the Chi-Square test.

with eczema were more likely to be male than those without eczema. There were no significant differences in age, helminth infection or African ancestry (high vs low or by tertiles) between eczema cases and controls. On the other hand, asthma symptoms were differently significant between eczema and non-eczema participants (*P*-value: <0.0001).

Table 2 showed that the *FLG* SNPs had MAF between 1% and 23% in non-eczema subjects and less than 1% up to 22% in eczema cases. The rs6587666 SNP is located in an intronic region and presented the largest difference in MAF: 16% in individuals with eczema and 23% in subjects from the control group. In the RegulomeDB Score [33], the values for the studied SNPs have ranged from "3" to "7".

The data presented in Table 3 indicate that the T allele of the rs6587666 SNP was negatively associated with eczema in our Brazilian cohort in additive model (*OR*: 0.66, 95% CI: 0.47–0.93, *P*: 0.017). The power analysis performed for this study has demonstrated, approximately, 67%, 55% and 13% of power for additive, dominant and recessive models, respectively (data not shown).

The T allele of *FLG* rs6587666 was more frequent in non-eczema compared to eczema participants (Table 2). No other *FLG* SNP investigated were associated to eczema (data not shown) and also rs6587666 was not in LD with any other SNP evaluated here (see Supplemental Fig. S1).

We then moved to test whether the proportion of individual African ancestry could act as a potential effect modifier of the association between *FLG* variants and eczema in our population. To achieve that, we stratified the cohort according to tertiles of African ancestry. Interestingly, we observed that among participants with the greatest degree of African ancestry (highest tercile, 3rd), the frequency of the T allele of this SNP is higher in non-eczema compared to eczema participants (23% to 10%, respectively). Similarly, for the genotypic frequencies in non-eczema and eczema individuals, we find that the T/C and T/T genotypes are more frequent in controls compared to cases in both 1st and 3rd tertiles. This difference is optimal in the 3rd tercile (Table 4).

In Fig. 1, rs6587666 was negatively associated with eczema, the higher is the African ancestry contribution. Among the sujects in the highest tertile, we found an even stronger negative association between rs6587666 and eczema (*OR*: 0.35; 95% CI: 0.17–0.72; *P*: 0.003) than in the general cohort. Similarly, if we stratified at the median ancestry level, the association was significant in those with high African ancestry (*OR*: 0.53; 95% CI: 0.31–0.89; *P*: 0.016). On the other hand, the association was not significant in those with lower African ancestry, whether defined by tertiles or by the median. Thus, the lower the contribution of African ancestry in the total genome, the lower was the effect observed in both the general cohort analysis and 3rd tertile enriched for African ancestry. This analysis demonstrated a statistical power of 82% in the additive genetic model (data not shown).

In our analyses from GTEX database (www.gtexportal.org/home/) [34], the expression of *FLG* in skin sun exposed was slightly downregulated by the presence of the T allele of rs6587666 (effect size: 0.19) (Table S1). For the CC genotype, the median of the gene expression was ~0.01. For the TC and TT genotypes it was ~0.08 and ~0.11, respectively. Interestingly, for the TT genotype, the median expression was approximately 10 times greater when compared to the CC genotype (Fig. 2). In skin not sun exposed tissue we observed a similar effect.

Other genes close to *FLG* may also have their expression affected by T allele of rs6587666. We observed that its associated with low expression of *HRNR* in skin not sun exposed (effect size: 0.33), as well as with high expression of the *FLG-AS1* in skin sun exposed (effect size: 0.29) and skin not sun exposed (effect size: 0.36) (Table S1).

We were unable to replicate our finding with rs6587666 in Puerto Rican children/adolescents (Table S2).

Table 2
Characterization of the studied *FLG* SNPs in SCAALA Brazilian cohort.

SNP	Position	A1	A2	Non-eczema	Eczema	Function	RegulomeDB Rank
rs115053459	152274909	T	C	0.02	0.01	Utr variant 3 prime	6
rs76330665	152275273	T	G	0.02	0.01	Missense	3b
rs3814299	152275453	A	G	0.01	0.02	Missense	3a
rs3126065	152275559	G	A	0.20	0.22	Missense	5
rs78733053	152275644	A	G	0.02	0.01	Synonymous codon	5
rs62623409	152281745	T	G	0.01	0.01	Missense	4
rs74129458	152281876	C	T	0.02	<0.01	Missense	4
rs12405278	152282267	A	G	0.14	0.11	Missense	5
rs151103850	152282684	A	G	0.01	0.01	Missense	5
rs76586335	152284673	A	G	0.02	<0.01	Missense, upstream variant 2 KB	3a
rs77032592	152285002	A	G	0.02	0.01	Missense, upstream variant 2 KB	5
rs114762657	152285290	T	C	0.04	0.02	Missense, upstream variant 2 KB	5
rs74129464	152285759	G	A	0.02	<0.01	Missense, upstream variant 2 KB	4
rs11588170	152286032	T	C	0.14	0.11	Missense	5
rs76438926	152287078	A	G	0.04	0.03	Intron variant, missense	4
rs115489580	152287213	C	T	0.04	0.03	Missense, nc transcript variant	6
rs76385639	152289573	A	C	0.02	0.01	Intron variant	3a
rs6587666	152296863	T	C	0.23	0.16	Intron variant	7

[a]SNP, single nucleotide polymorphism; [b]Position, location (assembly GRCh37.p13); [c]A1, minor allele; [d]A2, ancestral allele; [e]MAF, minor allele frequency; [f]RegulomeDB rank refers to: 3a: TF binding + any motif + DNase peak; 3b: TF binding + matched TF motif; 4: TF binding + DNase peak; 5: TF binding or DNase peak; 6: Motif hit; 7: Other.

Table 3
Association between rs6587666 and eczema by additive model in SCAALA Brazilian cohort.

Chr	Gene	SNP	Geno	Non-eczema	Eczema	*OR* (95% CI)	*P*-value*
1q21	*FLG*	rs6587666	C/C	609 (60.3%)	96 (70.1%)	0.66 (0.47–0.93)	**0.017**
			T/C	349 (34.5%)	37 (27%)		
			T/T	52 (5.2%)	4 (2.9%)		

[a]Chr, Chromosome; [b]SNP, single nucleotide polymorphism; [c]ADD, additive; [d]Geno, genotype; [e]*OR*, *Odds ratio*; [f]CI, confidence interval. *Adjusted by gender, age, helminthic infection, population structure, and asthma symptoms. **P*-value considering adaptative permutations using the additive model.

Table 4
Genotype frequencies and Minor Allele Frequency (MAF) for T allele on rs6587666 in SCAALA Brazilian cohort, by African ancestry tertile.

African ancestry	Genotype	Non-eczema	Eczema
Tercile, 1st	C/C	190 (57.58%)	34 (66.67%)
	T/C	121 (36.67%)	15 (29.41%)
	T/T	19 (5.76%)	2 (3.92%)
	T allele MAF	0.24	0.19
Tercile, 2nd	C/C	222 (64.53%)	25 (60.98%)
	T/C	105 (30.52%)	15 (36.59%)
	T/T	17 (4.94%)	1 (2.44%)
	T allele MAF	0.21	0.21
Tercile, 3rd	C/C	197 (58.63%)	37 (82.22%)
	T/C	123 (36.61%)	7 (15.56%)
	T/T	16 (4.76%)	1 (2.22%)
	T allele MAF	0.23	0.1

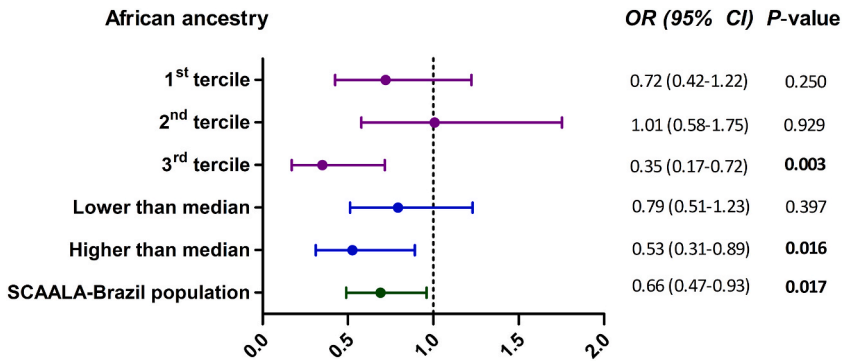

Fig. 1. Associations between rs6587666 and eczema in SCAALA Brazilian cohort. The association analyses were stratified by the degree of African ancestry in each individual using an additive model. Among the sujects in the 3rd tertile was found an stronger negative association between rs6587666 and eczema than in the general cohort. Stratified analyses were not adjusted. *OR*, *Odds ratio*; CI, confidence interval.

4. Discussion

The loss of function of filaggrin may lead to the formation of fragile skin that contributes to an abnormal barrier function. This explains the relationship between mutations in *FLG* and an increased risk of eczema [36]. To the best of our knowledge, this is the first report of an association between the T allele of rs6587666 in the *FLG* gene and a lower risk of eczema in a Latin American population.

Several studies have related genetic variants in *FLG* with eczema in European and Asian populations [10,16]. In contrast, in African populations, *FLG* variants are less common [6,16]. Interestingly, we identified a new polymorphism rs6587666 in *FLG*, associated with protection to eczema in our population. This protection was as high as a ~42%–~62% in participants with higher African contribution in our stratified analyses (*OR*: 0.53 for those with African ancestry above the median; and *OR*: 0.35 for those in the highest tertile of African ancestry).

It is worth mentioning that even with a statistical power below 80% in the general population (data not shown), we found considerable significance in our association analysis. In addition, our study demonstrated a statistical power of 82% in the additive genetic models in the subpopulation of the 3rd tercile (data not shown). We believe that this probably is due to the increased effect of the SNP in this group of greater African ancestry.

The population of Salvador is admixed by Yoruba individuals from Ibadan, Nigeria and northern and western European origin

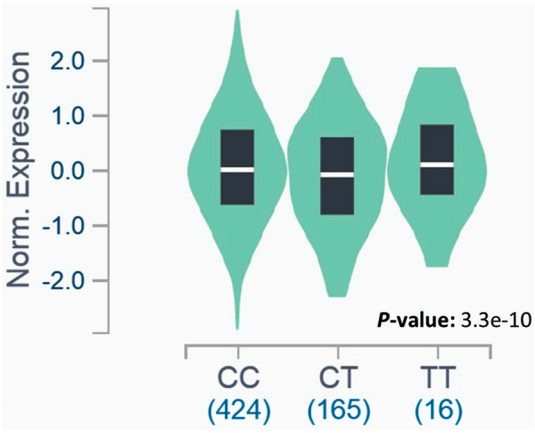

Fig. 2. Impact of the T allele in the rs6587666 on *FLG* expression in skin exposed sun tissue. For the TT genotype, the median expression of *FLG* was approximately 10 times greater when compared to the CC genotype (~0.01 and ~0.11, respectively). The gene expression and the respective figure were obtained from GTEx database (www.gtexportal.org/home/).

populations [21,22]. In descriptive terms, the frequency of the T allele from this ancestral populations is lower than in our admixed population, according with 1000 Genomes Project Phase 3 (see Supplemental Fig. S2), especially when comparing with the controls in our study. Latin American people are the product of an admixture process that has generated chromosomes derived by a complex mixture of ancestry [19]. In relation to the differences in the frequency of alleles, this could explain the peculiar characteristics of our admixed population when compared to African and European ancestral populations.

The rs6587666 was not in LD with any other SNP analyzed herein (see Supplemental Fig. S1). We investigated the LD (r^2) between rs6587666 and other SNPs using available databases. In the Ensembl platform (www.ensembl.org), we found that rs11584340, a missense *FLG* polymorphism, presented high LD (r^2 ~0.92) in the Yoruba in Ibadan (Nigeria) population. According to rSNPBase (rsnp.psych.ac.cn), rs11584340 in *FLG* had a high LD (r^2 ~0.97) in African populations. rs11584340 has been associated with greater eczema severity [37] and to functional parameters such as high levels of free fatty acids and high levels of mean corpuscular haemoglobin concentrations. It is relevant since the disruption of the permeability barrier seem to result in a marked increase in fatty acid synthesis in the skin [38]. However, other earlier studies found no differences or found reduced free fatty acids in atopic skin [39,40]. Kim and collaborators [38] reported that the increase in serum fatty acid levels perceived in their study could be due to other factors such as decreased metabolic utilization or decreased uptake by cells. Our findings indicate that perhaps the rs6587666 may have clinically singular relevant implications on the *FLG* in our population or that may be getting a signal from rs11584340.

The rs6587666 is an intronic polymorphism and as such may modify the gene expression level in the host gene in many different ways. The mechanism whereby it occurs remain unclear [41]. However, specific intron-hosted DNA elements have been identified to regulate transcription initiation affecting the gene expression. In fact, diseases related to genetic variants usually alter gene expression [42,43]. Some studies have observed the reduced expression of *FLG* in eczema as compared to normal subjects [44–46] and loss-of-function mutations in *FLG* associated with severity of atopic eczema [47]. The rs6587666 is intronic and have a functional mechanism other than null mutation. As such, it probably does not cause loss of protein function. In addition, the reduction in gene expression was not drastic from GTEx database in our findings. Therefore, we cannot know how this slight reduction in mRNA expression or whether *FLG-AS1* could affect the expressed protein.

Due to ethical and legal implications we were unable to carry out the gene expression analysis in our own pediatric Brazilian population. The GTEx is a biorepository of samples collected from postmortem adult donors with no evidence of disease from several ancestry (https://biospecimens.cancer.gov/gtexbiobank/donors.asp). Therefore, the characteristics of the participants in GTEx database cannot represent our original population and this is a limitation of our work.

We were unable to replicate the association found to rs6587666 SNP using data from the Puerto Rican children/adolescents. The *OR* was the same direction (*OR*: 0.98). Perhaps, HPR cohort was underpowered for that effect size. Our results reinforce the need to investigate genetic factors to eczema in populations of African origin, which are still underrepresented in genetic association studies.

A limitation of this study is the sample size in the stratified analyses according to the contribution of the African ancestry. However, this was not a sufficient limitation to conceal a likely protective effect of the rs6587666 (T allele) against eczema when the analysis were performed in participants with a great African ancestry contribution. Indeed, to detect the effect size observed in the subgroup with greater individual African ancestry (3rd tercile), the study demonstrated a statistical power of 82% in the additive genetic models (data not shown). In addition, due to ethical and legal implications, we were unable to test gene expression in a pediatric population of Brazilian children similar to our original population.

To our knowledge, this is the first study showing an association between rs6587666 in *FLG* and protection to eczema in an admixed population in which the African ancestry may modify this association herein observed. Functional and selective pressure studies performed later may be able to show how the rs6587666 SNP may have clinically relevant implications on the *FLG* in the human skin, specialy those with high African ancestry contribution in their genome.

Author contribution statement

Raimon Rios: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Thiago Magalhães da Silva: Conceived and designed the experiments; Analyzed and interpreted the data

Agostino Strina: Contributed reagents, materials, analysis tools or data.

Erick Forno, Maurício L. Barreto: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ryan Costa, Juan C. Celedón: Analyzed and interpreted the data.

Camila Alexandrina Figueiredo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13659.

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