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Report

Prevalence of filaggrin loss-of-function variants in Chilean population with and without atopic dermatitis

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Abstract

Background Filaggrin (*FLG*) loss-of-function variants are major genetic risk factors for atopic dermatitis (AD), but these have not been studied in Latin American populations with and without AD.

Methods *FLG* variants R501X and 2282del4 were genotyped in 275 Chilean adults with and without AD from the "Early origins of allergy and asthma" (ARIES) cohort and in 227 patients from an AD cohort based in Santiago, Chile.

Results Among adults in the ARIES cohort, 3.3% were carriers of R501X and 2.9% of 2282del4 variants, all heterozygotes. In this cohort, 6.2% were *FLG* variant carriers: 11.1% of subjects reporting AD were carriers of *FLG* variants vs. 5.2% in those without AD ($P = 0.13$). In this first cohort, *FLG* variants were not significantly associated with asthma, allergic rhinitis, or food allergy. In the AD cohort, the prevalence of *FLG* variants was 7% for R501X, 2.2% for the 2282del4 variant, and 9.3% for the combined genotype. In this cohort, *FLG* variants were present in 15.5% of severe AD vs. 7.1% of mild-to-moderate AD subjects ($P = 0.056$). Evaluation of Chilean population from both cohorts combined (n = 502) revealed that *FLG* variants were not significantly associated with AD (OR = 1.92 [95% CI 0.95–3.9], $P = 0.067$) but were associated with asthma (OR = 2.16 [95% CI 1.02–4.56], $P = 0.039$).

Conclusions This is the first study to evaluate *FLG* loss-of-function variants R501X and 2282del4 in Latin American population, revealing a similar prevalence of these *FLG* variant carriers to that of European populations. Among Chileans, *FLG* variants were significantly associated with asthma but not AD.

Introduction

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease characterized by intense pruritus and recurrent eczematous lesions.[1] It is often associated with a personal or family history of atopy and other allergic diseases as well as increased risk of skin infections, causing a great impact on the quality of life of patients and family members.[2] It is estimated that up to 20% of children and 3% of adults in high-income countries are affected by AD.[3] Genetic and environmental factors contribute to the disease, and both skin barrier dysfunction and immunological derangement appear to play a role in its pathogenesis.[4–6] In addition, over the past decades, increasing evidence has shown that a faulty epidermal barrier is a critical

factor in driving increased transepidermal water loss, allergen permeability and sensitization, and infection susceptibility in patients with AD.[4]

AD susceptibility is genetically related to chromosome 1q at locus 1q21 which contains the epidermal differentiation complex.[7] This complex is a dense set of genes that participate in the terminal differentiation of the epidermis and the formation of the stratum corneum.[8] The filaggrin gene (*FLG*) is located within this complex, and its protein product is key in the formation of the stratum corneum, as it binds keratin within the cytoskeleton, promoting a barrier that seeks to reduce water loss and minimize entry of irritants and allergens.[9] The most significant genetic risk factor for AD is the presence of loss-of-function (LoF) variants of filaggrin.[10,11] Noteworthy, the same *FLG* LoF variants also cause ichthyosis vulgaris, a disease characterized by dry flaky skin that is frequently associated with increased AD susceptibility.[12] *FLG* variants in AD and ichthyosis vulgaris are inherited as a semidominant trait, which is with high penetrance in homozygotes or compound heterozygotes and reduced penetrance in heterozygotes.[11,12] Within the phenotypes of AD, *FLG* LoF variants are often associated with the early-onset persistent disease subgroup and increased prevalence of asthma and allergic sensitization.[13,14]

According to the systematic review by Esparza-Gordillo *et al*, there are 254 known LoF variants in *FLG* to date, many of which have been described to be population specific.[15] Several studies have determined the frequency of *FLG* LoF variants in association with AD incidence and severity in different populations of Europe, North America, Asia, and Africa.[11,16–20] The most frequent LoF variants reported for European populations include p. Arg501Ter (R501X; rs61816761) and c.2282del4 (2282del4; rs138381300) with a prevalence ranging from 7% to 10%.[11,21] By contrast, in other populations such as East Asians, different variants are more commonly found such as the S2554X variant.[17,19]

The specific presence of R501X and 2282del4 *FLG* variants, and their contribution to AD, has not been characterized in any Latin American population. The aim of this study is to describe the frequency of LoF variants in *FLG* and determine associations of these variants with AD in Chileans, an admixed population with ancestral contribution mainly from Europeans and Native Americans. We hypothesized that *FLG* LoF variants associate with AD in Chilean population.

Materials and methods

Study populations

Chilean adults included in this study were recruited from the "Early origins of allergy and asthma" (ARIES) birth cohort (clinicaltrials.gov identifier NCT04186949), an observational prospective longitudinal study based in Santiago, Chile, that is currently ongoing.[22] Children from the ARIES birth cohort were not included because most had not been born or were too young to determine AD prevalence at the time this research was performed. In addition, a cohort of 227 Chilean patients with AD diagnosed by Hanifin and Rajka criteria and confirmed by expert dermatologist or pediatric immunologist was genotyped.[23] In this cohort, AD severity was assessed by trained evaluators using the objective scoring AD (obSCORAD) index. Self-report of AD and other allergic diseases (asthma, allergic rhinitis [AR], and food allergy [FA]) was surveyed in all the individuals from the ARIES cohort, and self-report of asthma, AR, and FA was surveyed in all individuals from the AD cohort. The geographic location and ethnicity of subjects in both cohorts was similar.

This study was approved by the Institutional Review Board of the School of Medicine at Pontificia Universidad Católica de Chile (IRB numbers 170925013, 16-039, and 11-140). A written informed consent was obtained from each participant, and assent was additionally obtained in children aged 7-17 years.

Sampling and DNA extraction

A whole blood sample or buccal swab sample was taken from each participant. Whole blood samples were obtained using an EDTA tube and were stored at −20°C until DNA isolation. The buccal swab (FLOQSwabs, Copan) was rubbed on the inside of the cheeks and stored at −20°C until genomic DNA isolation as well. Genomic DNA was extracted using the Genomic DNA Extraction Kit NucleoSpin 96 Blood (QuickPure) for whole blood and the BuccalAmp DNA Extraction Kit (Epicentre) for buccal swab, according to the manufacturer's instructions.

Filaggrin genotyping

Genotyping for the R501X and 2282del4 variants of *FLG* gene was performed using a TaqMan allelic discrimination assay (Applied Biosystems), using customized TaqMan probes and TaqMan Genotyping Master Mix. The polymerase chain reaction was carried out in a total volume of 10 μL, and the amplification was carried out as follows: 95°C for 10 minutes, then 45 cycles at 95°C for 15 seconds, 60°C for 1 minute, and 60°C for 30 seconds. The allelic discrimination was performed using the QuantStudio Design and Analysis Software (Applied Biosystems).

Whole-exome sequencing studies

Whole-exome sequencing studies from a genomic database of Chilean patients with congenital malformations or primary immunodeficiency, but with unknown AD phenotype, were reviewed in order to identify additional *FLG* LoF variants in Chilean population.

Statistical analysis

All statistical analyses in the association studies were performed using SPSS version 26.0 (Armonk, NY: IBM Corp.). Hardy–Weinberg Equilibrium analysis was performed for each variant. Pearson chi-square test was also used to compare

allele frequencies of the three *FLG* genotypes (AA, Aa, aa) between affected and non-affected subjects. Odds ratios and their 95% confidence intervals for dominant models (AA versus aX) were determined using Fisher exact test.

Results

Given that no previous studies of *FLG* variants had been previously performed in Latin American population, we first evaluated the presence of the LoF variants R501X and 2282del4 in the Latino population included in the Genome Aggregation Database (gnomAD) version 2.1.1.[24] The allelic frequency of R501X and 2282del4 in this database was found to be 0.40% and 0.16% in Latino population, in comparison to 1.63% and 2.16% in the European (non-Finnish) population. We also assessed all other *FLG* LoF variants (e.g. p.R2447X, p.S3247X, or p.L4022X) in gnomAD among Latinos. The allelic frequency of all other LOF variants was <0.001%. Considering these findings, we decided to genotype *FLG* LOF variants R501X and 2282del4 in our study.

We then analyzed 275 Chilean adults from the ARIES study. Demographic and clinical characteristics of this cohort are given in Table 1. A total of 50.2% of the subjects self-reported any allergic disease, with 32.6% of the cohort reporting AD. The *FLG* LoF variant R501X was found in heterozygous form in eight subjects (3.3%) with an allelic frequency of 0.0145. In turn, the 2282del4 was found in heterozygous form in nine subjects (2.9%), with an allelic frequency of 0.016. No individuals in this cohort were homozygous or compound heterozygous for these *FLG* variants. Both variants studied in the ARIES cohort were in Hardy–Weinberg Equilibrium ($\chi^2 = 0.076$, $P = 0.78$, and $\chi^2 = 0.060$, $P = 0.81$, respectively).

The genotyping results of R501X and 2282del4 were then analyzed according to the self-report of AD (Table 2). Among the 45 subjects who reported AD, 11.1% had an *FLG* LoF variant vs. 5.2% among those who did not report AD, although allelic frequency was not significantly different ($P = 0.13$). The odds ratio (OR) of reporting AD in Chilean adult carriers of *FLG* LoF variants resulted in an OR of 2.27 (95% CI 0.76-6.80).

Given that an association of *FLG* LoF variants with asthma has been observed in several populations, we also sought this association in this cohort of Chilean adults. Thus, the R501X and 2282del4 variants were analyzed according to the self-report of asthma in adults from the ARIES cohort. Although the *FLG* variants were more frequent in subjects who reported a history of asthma compared with those that did not (11.4% vs. 5.4%), results were not significant ($P = 0.17$) with an OR of 2.25 (95% CI 0.69-7.35) for the combined genotype. Additionally, we evaluated these *FLG* variants in association with self-report of AR and FA. The *FLG* variants were not significantly associated with AR (OR = 0.98 [95% CI 0.35-2.75], $P = 0.98$) or FA (OR = 2.0 [95% CI 0.54 – 7.43], $P = 0.29$).

We subsequently proceeded to evaluate the prevalence of the *FLG* LoF variants in a cohort of 227 Chilean patients with confirmed AD. Demographic and clinical characteristics of this cohort are given in Table 1. Mean objective Scoring AD (obSCORAD) index in these individuals at the time of DNA sampling was 30 ± 16, with 19% having mild, 55% moderate, and 26% severe AD. In this cohort, 23% of AD patients reported a history of asthma, 45% of allergic rhinitis, and 25% of FA. The prevalence of the *FLG* variants in this AD cohort was 7% for the R501X variant, 2.2% for the 2282del4 variant, and 9.3% for the combined genotype (Table 3). Only one individual (0.4%) had a homozygous *FLG* null variant, specifically of the 2282del4 variant. The allelic frequency for the minor allele in this AD cohort was 0.035 for the R501X variant and 0.013 for the 2282del4 variant. We then assessed the prevalence of *FLG* variants according to AD severity evaluated using obSCORAD in this cohort. The *FLG* LoF variants were present in 15.5% of severe AD vs. 7.1% of mild-to-moderate AD patients ($P = 0.056$). In this AD cohort, the variants were not significantly associated with self-report of asthma ($P = 0.18$), FA ($P = 0.61$), or AR ($P = 0.33$).

Upon combining data of Chilean population from the ARIES and AD cohorts (n = 502), we analyzed the association of *FLG* variants with AD, asthma, AR, and FA (Fig. 1). In this combined population, the presence of *FLG* LoF variants was not significantly associated with AD (OR = 1.92 [95% CI 0.95-3.9], $P = 0.067$). Allele frequency of *FLG* variants was not significantly different between those with AD vs. without AD (0.050 vs. 0.026; $P = 0.081$). By contrast, the *FLG* variants were significantly associated with asthma in our population (OR = 2.16 [95% CI 1.02-4.56], $P = 0.039$), with a significantly higher allele frequency of *FLG* variants among those with asthma compared to those without asthma (0.069 vs. 0.031, $P = 0.047$). *FLG* variants were not significantly associated with either FA (OR = 1.64 [95% CI 0.75-3.62], $P = 0.22$) or AR (OR = 1.32 [95% CI 0.67-2.58], $P = 0.42$).

Table 1 Clinical and demographic characteristics of participants in the ARIES and atopic dermatitis cohorts

	ARIES cohort (n = 275)	**AD cohort (n = 227)**
Age, years (mean ± standard deviation)	33 ± 6	11 ± 11
Gender, female (%)	51.6%	52%
Atopic dermatitis, n (%)	32.6%	100%
Mild	N/A	19%
Moderate	N/A	55%
Severe	N/A	26%
Asthma, n (%)	25.4%	23%
Food allergy, n (%)	10.2%	25%
Allergic rhinitis, n (%)	35.6%	45%

N/A, data not available

Table 2 Frequency of *FLG* loss-of-function variants in the Chilean ARIES cohort with and without AD

	R501X			2282del4			Combined genotype		
Genotype	**Total**	**AD+**	**AD-**	**Total**	**AD+**	**AD-**	**Total**	**AD+**	**AD-**
AA	266 (96.7%)	42 (93.3%)	224 (97.4%)	267 (97.1%)	43 (95.6%)	224 (97.4%)	258 (93.8%)	40 (88.9%)	218 (94.8%)
Aa	9 (3.3%)	3 (6.7%)	6 (2.6%)	8 (2.9%)	2 (4.4%)	6 (2.6%)	17 (6.2%)	5 (11.1%)	12 (5.2%)
Aa	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	275	45	230	275	45	230	275	45	230

FLG variants R501X and 2282del4 in individual from the ARIES cohort with (AD+) and without (AD-) atopic dermatitis. **AA** refers to wild-type/wild-type genotype; **Aa** refers to heterozygous genotype, and **aa** refers to homozygous genotype. Combined genotype refers to the presence of any variants (R501X and/or 2282del4).

Table 3 Frequency of *FLG* loss-of-function variants in the Chilean atopic dermatitis cohort

Genotype	**R501X**	**2282del4**	**Combined genotype**
AA	211 (93%)	222 (97.8%)	206 (90.8%)
Aa	16 (7%)	4 (1.8%)	20 (8.8%)
Aa	0 (0%)	1 (0.4%)	1 (0.4%)
Total	**227**	**227**	**227**

FLG variants R501X and 2282del4 in individuals from the atopic dermatitis cohort. **AA** refers to wild-type/wild-type genotype; **Aa** refers to heterozygous genotype; and **aa** refers to homozygous variant genotype. Combined genotype refers to the presence of any variants (R501X and/or 2282del4).

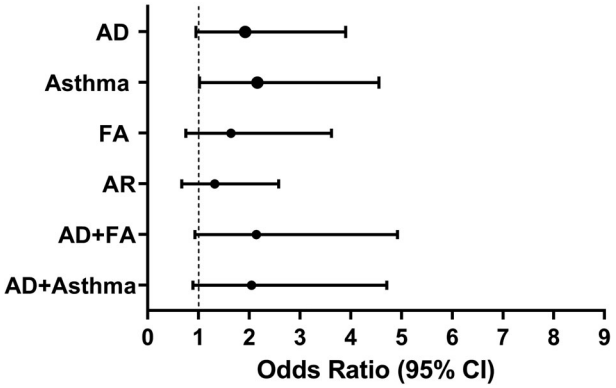

Figure 1 Association of *FLG* loss-of-function variants R501X and 2282del4 with atopic dermatitis (AD), asthma, food allergy (FA), and allergic rhinitis (AR) in Chilean subjects from the combined ARIES and AD cohorts

Comorbid "AD and asthma" was present in 63 subjects (12.5%) and "AD and FA" in 61 subjects (12.2%). We explored the association of *FLG* variants with these combinations. The *FLG* variants were present in 12.7% of those with "AD and asthma" vs. 6.6% in those without this combination (OR = 2.05 [95% CI 0.89-4.71], $P = 0.085$). Similarly, *FLG* variants were demonstrated in 13.1% of subjects with "AD and FA" vs. 6.6% in those without this combination (OR = 2.14 [95% CI 0.93-4.92], $P = 0.068$).

In an attempt to discern the existence of additional *FLG* variants in the Chilean population that may serve as a reference for future studies, we searched for *FLG* LOF variants in 153 available whole-exome sequencing studies from a genomic database of Chilean patients with congenital malformations or primary immunodeficiencies but with unknown AD phenotype. In these, we found two (1.3%) patients with R501X and three with 2282del4 (1.9%). Noteworthy, among these patients five additional *FLG* LOF variants were found: p.R2447X, p.E3616X, p.3625_3625del, p.761_762del, and p.2329_2329del.

Discussion

In this study, we evaluated the frequency of the two common *FLG* LoF variants R501X and 2282del4 in Chilean population with and without AD, finding that these variants are present in our population and, although not significantly associated with AD, we found a significant association with asthma.

The frequency of *FLG* LoF variants has been found to differ among populations. In the seminal research published in 2006 by Palmer et al., variants R501X and 2282del4 were studied in Irish, Scottish, and Danish cohorts (i.e. three different European populations), observing an overall carrier prevalence of ~9%.[11] Thereafter, several different studies have evaluated these and other *FLG* LoF variants reporting similar frequencies in other northern European populations, such as German, Austrian, French, and Swedish[25–28], among which the two most common variants R501X and2282del4 make up ~80% of the variant load.[29] However, lower carrier frequencies have been found in southern European Mediterranean countries such as Spain and Italy.[30,31] By contrast, the carrier frequencies of these two variants in other countries including Japan, China, India, and Ethiopia, as well as in African-American population in the USA, are significantly lower or even absent in some of these studies.[17,19,20,32,33] Given the absence of data from Latin America, for performing this study we first evaluated allelic frequencies of

FLG variants in gnomAD database. Considering that Latino-admixed populations are a small proportion of subjects included in this genomic database, the allele frequencies of *FLG* variants do not necessarily represent the genetic contribution of these variants to AD or other allergic diseases in Chileans. However, it is known that Chileans are admixed with ancestral contribution mainly from Europe and Native America and a minor African component, with imbalanced contribution of European men and Native-American women.[34] Of note, European ancestry representation is highest in the central regions of the country where this study was performed. The carrier frequency in Chilean adults from the ARIES cohort was 6.2%, a rate that closely resembles that observed in Europe.

This is the first study to evaluate the frequency of R501x and 2282del4 variants in AD patients from Latin America (i.e. Latinos). Our results show a carrier frequency of 9.3% of the combined genotype in our AD cohort, a population with expert physician-confirmed AD diagnosis. In the combined cohort analysis, the *FLG* LoF variants were not significantly associated with AD. It is possible these results were influenced by a relatively high prevalence of AD among those with wild-type *FLG* or by the presence of additional *FLG* variants in AD subjects not genotyped in this study. In addition, although *FLG* LoF variants were more than twice as prevalent in severe AD than mild-to-moderate AD (15.5% vs 7.1%), this result was not significant either, probably revealing the study was underpowered to evaluate the association of *FLG* variants with AD severity.

Apart from its role in AD etiology and pathogenesis, the presence of *FLG* variants has been associated with asthma in multiple studies,[11,25] whereas others do not show this association.[18] A meta-analysis showed that *FLG* variants are significantly associated with asthma with an OR of 1.48 (95% CI 1.32-1.66).[35] However, this association was strong in the population with asthma plus AD but not in those with asthma in the absence of AD, suggesting that *FLG* LoF variants increase the risk of asthma through its role in triggering underlying AD first. In our study, carrying *FLG* LoF variants was significantly associated with asthma, although it is important to consider that among those with asthma in our study, 68% also had AD. Given that filaggrin is a major epidermal protein and not present in the airway,[36] as expected and in agreement with other studies,[11,21,25] *FLG* LoF variants were not significantly associated with asthma without AD (data not shown). Moreover, in our study the *FLG* LoF variants also had a relatively high prevalence among those with "AD and FA," a potent surrogate of AD with allergic sensitization. Similarly to asthma, it is likely that the association of *FLG* variants with FA is partly mediated by AD, although heightened epidermal permeability due to filaggrin dysfunction *per se* without AD could increase the risk of FA. This was shown in the study by Brown et al., who found that the association of *FLG* variants with peanut allergy remained significant after controlling for coexistent AD.[37]

Among the limitations of our research is the fact that many of the clinical outcomes which *FLG* variants are associated with were self-reported by the study subjects. However, the prevalence of *FLG* variants in the ARIES cohort self-reported AD subjects and in the physician-confirmed AD cohort subjects was very similar. Another potential weakness of our study is the geographic distribution of participants, since most of the patients came from the central zone of Chile, and thus our results may not accurately represent the prevalence throughout the rest of the country. An additional limitation of our study is that we only genotyped the R501X and 2282del4 variants, although many other less-frequent *FLG* LoF variants have been described in the literature and genomic databases. Although in gnomAD version 2.1.1, other *FLG* variants in Latino population are exceedingly rare, this population is relatively under-represented compared with others, and it does not include Chileans. Thus, to better comprehend the scope of *FLG* variants among Chileans, we searched a genomic database of patients with unknown AD phenotype, finding five additional *FLG* LoF variants. The impact and prevalence of these and other variants in Chilean population with AD remain unknown.

In conclusion, *FLG* LoF variants R501X and 2282del4 are present in Chilean population with a similar prevalence to that observed in European population. Among Chileans, these *FLG* LoF variants were significantly associated with asthma but not AD.

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