Filaggrin Polymorphism Pro478Ser Is Associated With the Severity of Atopic Dermatitis and Colonization by *Staphylococcal aureus*

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Loss-of-function mutations in the filaggrin (*FLG*) gene are associated with increased severity of atopic dermatitis (AD) [1]. Common variants, such as p.Arg501Ter and 2282del4 may be present in up to 50% of Northern European AD patients and absent in Southern European patients [2]. rs11584340 (p.Pro478Ser) is a single-nucleotide polymorphism (SNP) of FLG that is located at codon 478. It is associated with skin barrier disruption, since the 478 serine residue may hinder the action of protease cleavage, thus affecting the rate of aggregation between FLG and keratin filaments [3,4]. Despite having a minor allele frequency of 0.34 worldwide [5], the polymorphism was found to be associated with an increased risk of AD (odds ratio, 1.87) [6,7]. Previous studies have reported that patients with null mutations in FLG have increased transepidermal water loss and increased skin pH, both of which facilitate bacterial growth [8]. However, it remains unknown how p.Pro478Ser affects predisposition to skin colonization by *Staphylococcus aureus* in AD patients.

We performed a cross-sectional analysis to evaluate the association between disease severity/colonization of the skin by *S aureus* and the polymorphism p.Pro478Ser and the null mutations in *FLG* (p.Arg501Terc and 2282del4).

Patients older than 12 years and diagnosed with AD according to the criteria of Hannifin and Rajka provided their written informed consent to participate in the study. In the case of minors, consent was given by the parents, caretakers, or guardians. The Ethics Committee of Porto University, Portugal approved the study. Participants with severe skin disease other than AD, secondary infection (bacteria, fungi, or viruses), or any major systemic disease were excluded. Sample size was calculated based on minimal clinically important differences in the SCORing Atopic Dermatitis (SCORAD) score [9], and post hoc statistical power was set at 95.6% (P=.05) based on the prevalence of FLG mutations in a previous study of Southern European AD patients [2]. We analyzed data from 73 patients (mean age, 30 [13] years; 61% female; 77% atopic) with AD for a mean (SD) of 16 years. Severity was classified based on the SCORAD score as mild (≤15), moderate (16-40), and severe (≥41). Genomic DNA was extracted from peripheral blood samples and analyzed using PCR and direct DNA sequencing for the presence of the 2 null mutations in *FLG* and the p.Pro478Ser polymorphism. The microbiological profile was assessed in the right and left elbow creases, left and right popliteal creases, and neck region (area, 25 cm^2). The number of colony forming units (CFU)/cm^2 of total staphylococci and *S aureus* was determined (Baird-Parker Agar [Lab M] for total staphylococci and Mannitol Salt Agar [Lab M] for *S aureus*). The serum biomarkers assessed were total IgE, eosinophil cationic protein, and specific IgE to a mixture of inhalant allergens (Phadiatop), *S aureus* enterotoxins (A, B, C, and TSST), and *Malassezia* species (ImmunoCap). The Mann-Whitney test or Fisher exact test was used as appropriate (IBM SPSS Statistics for Windows [Version 20.0], IBM Corp).

FLG mutations were present in 15% of patients (9 with p.Arg501Ter and 2 with c.2282del4) and p.Pro478Ser in 38% (3 homozygotes, 25 heterozygotes). p.Pro478Ser was in linkage disequilibrium with the null mutations, and 3 patients with the p.Arg501Ter mutation also had p.Pro478Ser. The presence of p.Pro478Ser was associated with more severe disease, as reflected by the higher SCORAD score and severity class as well as by increased use of oral corticosteroids (Table). Furthermore, significantly more extensive colonization of *S aureus* on 3 of the 5 sampled regions and a higher value of IgE to *S aureus* enterotoxin A were observed. Homozygosity for p.Pro478Ser was not an additional risk factor in this particular group of patients. There were no differences between patients with and without the *FLG* null mutations in terms of AD severity, inflammatory allergic markers, and colonization by *S aureus*.

The novel finding of this study is that, in contrast with the 2 *FLG* null mutations, p.Pro478Ser was significantly associated with more severe disease and greater skin colonization with *S aureus* in AD patients. The 478 serine residue can increase skin permeability, leading to greater skin penetration by bacteria and conferring susceptibility to AD [4]. In addition, the presence of an unrecognized functional mutation at or adjacent to *FLG*, which is in linkage disequilibrium with p.Pro478Ser, could increase the risk for AD [10]. Therefore, our findings indicate that this SNP may have clinically relevant implications with respect to increased bacterial colonization of skin and more severe disease in AD patients.

The limitations of this study are as follows. First, the absence of healthy controls restricts us to speculation on the role of this SNP in patients with AD. Nevertheless, our objective was to study the association between this SNP and bacterial load in patients and not the role of the SNP as a risk factor for AD, in which case it would have been mandatory to include healthy controls. Second, the prevalence of *FLG*

mutations in the Portuguese population as a whole and in AD patients in Portugal is unknown. However, the sample size calculations showed that 42 patients were needed to detect a significant difference in the SCORAD score, and we were able to include more patients to overcome the level of uncertainty regarding the prevalence of genetic mutations.

Importantly, this is the first study to show an association between the presence of p.Pro478Ser and severity of AD and bacterial load in European patients with long-term AD. Only 3 previous studies have investigated this SNP, although these were in Asian patients, suggesting that it confers susceptibility to AD, particularly in patients with high IgE levels [3,6,7]. The low prevalence of *FLG* null mutations in our study is consistent with the wide variation in this gene mutation across the globe and the lower prevalence in Southern European countries. The lack of an association with clinical, microbiological, and allergic parameters reinforces the fact that genetic markers other than *FLG* mutations should be studied.

In conclusion, genetic factors can affect the severity of AD and skin microbiota. Our study shows that the presence of p.Pro478Ser may be related to both increased disease severity and bacterial colonization in patients with long-term AD.

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Table. Characteristics of Patients With Atopic Dermatitis According to Filaggrin Genotype[a]

	FLG Null Mutations Mp.Arg501Ter or C.2282del4			FLG Polymorphism Pro478Ser		
	Yes, n=11	No, n=62	*P* Value	Yes, n=28	No, n=45	*P* Value
Age, y	32 (6.1)	29.6 (1,5)	.91[b]	34.1 (2.7)	27.3 (1.8)	.03[b,d]
Female sex, No. (%)	7 (63.6)	38 (61.5)	.22[c]	16 (57.1)	28 (62.2)	.42[c]
Disease duration, y	15.9 (10.5)	16.3 (10.4)	.23[b]	18.4 (2.3)	14.8(1.3)	.32[b]
SCORAD (0-103)	50.2 (30.9)	41.3 (22.6)	.72[b]	51.8 (4.2)	36.0(3.4)	<.01[b,d]
SCORAD severity, No. (%)						
Mild	2 (18.2)	5 (8.1)	.28[c]	2 (7.1)	5 (11.1)	.40[c]
Moderate	3 (27.3)	26 (41.9)	.81[c]	6 (21.4)	23 (51.1)	.02[c,d]
Severe	6 (54.5)	31 (50.0)	.52[c]	20 (71.4)	17 (37.8)	.01[c]
Oral corticosteroids, No. (%)	3 (27.3)	30 (48.4)	.22[c]	17 (60.7)	16 (35.6)	.03[c,d]
Atopic, No. (%)	6 (54.5)	50 (79)	.53[c]	22 (78.6)	34 (75.6)	.52[c]
Asthmatic, No. (%)	4 (36.4)	36 (58.1)	.64[c]	14 (50.0)	26 (57.8)	.31[c]
Median (IQR) total IgE, IU/mL,	2185 (71.4-5308)	4183 (97.3-3607.8)	.08[b]	6520 (113.6-7935.0)	2240 (88.6-1151.5)	.08[b]
Median Phadiatop, kU_A/L median (P_{25-75})	248.6 (4.5-565.9)	529.9 (0.54-441.0)	.12[b]	763 (9.6-1115.9)	315 (0.4-283.5)	.13[b]
ECP, µg/L	20.7 (14.9)	35.2 (29.1)	.56[b]	37.2 (34.2)	30.5 (21.1)	.52[b]
Specific IgE, kU_A/L						
Enterotoxin A	0.37 (0.2)	2.4 (1.3)	.79[b]	4.5 (13.9)	0.5 (0.9)	.05[b,d]
Enterotoxin B	0.6 (0.3)	1.5 (0.5)	.42[b]	2.4 (5.1)	0.6 (1.3)	.23[b]
Enterotoxin C	1.3 (0.5)	2.2 (0.5)	.38[b]	2.7 (3.5)	1.6 (3.1)	.06[b]
Enterotoxin TSST	0.5 (0.2)	1.4 (0.6)	.52[b]	2.4 (6.7)	0.4 (0.8)	.08[b]
Malassezia species	6.2 (5.8)	4.2 (1.1)	.78[b]	7.2 (13.4)	3.3 (8.7)	.23[b]
Staphylococcus aureus, CFU/cm^2						
Right arm	9471.1	78 152.7	.48[b]	178 083.3	8002.3	.01[b,d]
Left arm	158 909.9	70 271.9	.58[b]	142 859.2	48 310.3	.92[b]
Right leg	23 454.4	39 728.2	.91[b]	89 778.9	8 386.7	.04[b,d]
Left leg	162 754.4	359 865.8	.96[b]	759 552.7	95 528.5	.02[b,d]
Neck	8 994.9	30 732.6	.74[b]	48 538.3	16 244.8	.80[b]

Abbreviation: ECP, eosinophil cationic protein; SCORAD, SCORing Atopic Dermatitis.
[a]Results are presented as mean (SD) unless stated otherwise.
[b]Mann-Whitney test.
[c]Fisher exact test.
[d]Statistically significant.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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