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ARTICLE

Staphylococcus aureus genomic pattern and atopic dermatitis: may factors other than superantigens be involved?

A. Rojo · A. Aguinaga · S. Monecke · J. R. Yuste · G. Gastaminza · A. España

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Abstract The purpose of this investigation was to compare the genotypic profiles of *Staphylococcus aureus* isolated from atopic dermatitis (AD) patients and from control subjects, and to study the relationship between clinical severity, immune response, and genomic pattern of *S. aureus* isolated from AD patients. We selected 32 patients with AD and *S. aureus* skin colonization and 31 atopic controls with no history of AD who where asymptomatic carriers of *S. aureus*. Microarray-based genotyping was performed on *S. aureus* isolates. In AD patients, clinical severity was assessed using the Scoring Atopic Dermatitis index and total IgE levels and staphylococcal superantigen-specific IgE levels (SEA, SEB, SEC, TSST1) were determined. The genes *lukE*, *lukD*, *splA*, *splB*, *ssl8*, and *sasG* were more frequent in isolates from AD patients. CC30 was more common in isolates from atopic controls than in AD patients. There was a correlation between total IgE and clinical severity, but an association between clinical severity, immune response, and the presence of *S. aureus* superantigen genes, including enterotoxin genes, could not be demonstrated. Finally, a correlation was found between AD severity and other *S. aureus* genes, such as *sasG* and *scn*. *S. aureus* factors besides superantigens could be related to the worsening and onset of AD.

A. Rojo (✉) · A. Aguinaga
Department of Clinical Microbiology, Clínica Universidad de Navarra, Avenida. Pio XII nº36, 31008 Pamplona, Spain
e-mail: andrea.rojo.asin@gmail.com

S. Monecke
Institute for Medical Microbiology and Hygiene, Dresden University of Technology, 01307 Dresden, Germany

S. Monecke
Alere Technologies GmbH, 07749 Jena, Germany

J. R. Yuste
Division of Infectious Diseases, Clínica Universidad de Navarra, Avenida. Pio XII nº36, 31008 Pamplona, Spain

G. Gastaminza
Department of Allergology, Clínica Universidad de Navarra, Avenida. Pio XII nº36, 31008 Pamplona, Spain

A. España
Department of Dermatology, Clínica Universidad de Navarra, Avenida. Pio XII nº36, 31008 Pamplona, Spain

Introduction

Atopic dermatitis (AD) is defined by childhood onset, pruritus, chronicity, the presence of other atopic disorders, and a characteristic pattern of skin involvement. It is known that over 90 % of AD patients are colonized with *Staphylococcus aureus*, as compared to 10-40 % in healthy individuals [1, 2]. The contribution of *S. aureus* to the onset and severity of AD is well known. Nonetheless, the mechanisms underlying this process remain unclear.

Possible explanations for the influence of *S. aureus* on AD could be an effect of its multiple factors of virulence or an altered *S. aureus* population structure of the strains colonizing these patients. However, there are only a few studies in the literature on the genetic background of *S. aureus* in AD patients [3–5]. Classically, *S. aureus* exotoxins SEA, SEB, SEC, and TSST-1 have been studied. They can act as superantigens (SAgs) and directly interact with several immunocompetent cell populations and may also act as classic allergens, inducing the production of specific IgE antibodies [6]. These specific IgE have been correlated with the severity of AD [1]. In spite of this, the pathogenic role of SAgs in AD is controversial because only about 50 % of *S. aureus* isolates are SAg-producing and SAg-producing *S. aureus* are also found in healthy carriers [7]. Moreover, the full spectrum of SAgs produced by *S. aureus* isolates from AD patients has not been fully examined [8].

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The aim of our study was, first, to compare the genotypic profiles of *S. aureus* isolated from both AD patients and from atopic control subjects. A second aim was to study the relationship between clinical severity, immune response, and the SAg genes harbored by *S. aureus* in AD patients. Finally, we sought to analyze the relationship between AD severity and a diverse range of *S. aureus* genes related to virulence, adherence, biofilm, and immune evasion.

Patients and methods

This study was performed in Clínica Universidad de Navarra (2009–2011).

Patients

Thirty-two AD patients with *S. aureus* lesional skin colonization were recruited prospectively. Patients were included during phases of an acute worsening of their condition. The severity of AD was assessed using the Score for Atopic Dermatitis (SCORAD). According to the SCORAD result, patients were classified as suffering from mild AD (<25), moderate AD (25–50), or severe AD (>50) [9].

Controls

Thirty-one atopic patients were included as controls if diagnosed with an active allergic disease (rhinitis, asthma, or food allergy), if they had never suffered AD, and if they yielded *S. aureus* cultures from nasal swabs.

Microbiological studies

Four swabs were collected from each AD patient, one from the most affected skin area, and the other three (nasal, inguinal, and perianal) for the detection of carriage of *S. aureus*. Samples were cultured on blood agar and mannitol salt agar plates (bioMérieux, Marcy l'Etoile, France) and incubated at 37 °C for 24 h. The identity of *S. aureus* was confirmed by standard laboratory methods. A single colony of *S. aureus* was selected for subculturing from the lesional area of AD patients and from the nasal swabs of atopic controls. After 24 h of incubation, an enzymatic lysis was performed using the buffers and solutions provided with the StaphyType DNA microarray kit (Alere Technologies, Jena, Germany) and the DNeasy kit (Qiagen, Hilden, Germany). DNA microarrays were performed according to the manufacturer's instructions [10]. This microarray detects species markers and regulatory genes, staphylococcal chromosomal cassette *mec* (SCC*mec*), capsule and *agr* group typing markers, resistance genes, genes encoding virulence factors, genes encoding superantigen-like proteins (*ssl*), biofilm-related genes, genes encoding microbial surface components recognizing adhesive matrix molecules (MSCRAMM), as well as genes encoding several adherence factors. Isolates were assigned to a clonal complex (CC) and strain based on hybridization profiles [11].

Allergological studies

Total IgE and specific IgE against four *S. aureus* SAgs (SEA, SEB, SEC, TSST1) were measured using a commercially available automated system (UniCAP, Thermo Fisher Scientific, Uppsala, Sweden). Results >0.10 kU/L were considered positive [12].

Statistical analysis

Statistical analysis was performed using SPSS 15 for Windows. p-values <0.05 were reported as significant.

Results

Demographic data

The demographic data and atopic background of AD patients and atopic controls are shown in Table 1.

Table 1 Clinical characteristics of atopic dermatitis (AD) patients and atopic controls

	Patients (n=32)	Controls (n =31)
Age, years, mean (SD)	23 (18.8)	31.9 (16.3)
Sex (male), n (%)	21 (65.6)	21 (67.7)
Time evolution of dermatitis, years, mean (SD)	9 (8.11)	–
Atopic diseases		
Dermatitis, n (%)	32 (100)	–
Asthma, n (%)	5 (15.6)	6 (19.3)
Rhinoconjunctivitis, n (%)	11 (34.3)	27 (87.1)
Food allergy, n (%)	3 (9.3)	1 (3.2)
None, n (%)	15 (46.8)	0

S. aureus typing

The most common CC in isolates from AD patients was CC5 (10 isolates, 31.2 %), followed by CC15 (6 isolates, 18.7 %), CC30 (6 isolates, 18.7 %), and CC45 (5 isolates, 15.6 %). Of the remaining isolates, one belonged to CC1 (3.1 %), two to CC8 (6.2 %), one to CC12 (3.1 %), and one to CC188 (3.1 %). Atopic control isolates mainly belonged to CC30 (15 isolates, 48.3 %). CC10, CC5, and CC45 each represented 9.6 % of the atopic controls isolates and seven isolates were assigned to sporadic CCs: one CC8, one CC398, one CC509, one ST942, one CC1, one CC15, and one CC9 (i.e., 3.2 % each). No statistically significant differences were found between the distribution of *S. aureus* CCs in AD patients and atopic control isolates. Isolates from AD patients mostly harbored *agr II* (17 isolates, 53.1 %), while 18 (58 %) isolates from atopic controls were assigned to the *agr* group III.

No methicillin-resistant *S. aureus* was detected in neither strains from AD patients nor from atopic controls.

Analysis of superantigen genes and other virulence factors

SAg gene profiles of the isolates from AD patients and atopic controls are shown in Fig. 1. SAg genes that belong to the *egc* cluster (*selg*+*sei*+*selm*+*seln*+*selo*+*selu*) were the most frequent SAg genes in isolates from both AD patients (21 isolates, 65.6 %) and atopic controls (27 isolates, 87.1 %). The enterotoxin A gene (*sea*) was detected in 14 isolates (43.7 %) from AD patients versus 14 isolates (45.1 %) from atopic controls. The enterotoxin B gene (*seb*) was not detected in isolates from AD patients, and was detected in only 3 (9.6 %) isolates from atopic controls. The enterotoxin C gene (*sec*), which clustered with the enterotoxin-like L gene (*sel*) in all cases, was detected in 7 (21.8 %) isolates from AD patients. Meanwhile, *sec* was detected only in 2 (6.4 %) of the isolates from atopic controls. The gene encoding the toxic shock syndrome toxin (*tst1*) was more frequent in isolates from atopic controls (15 isolates, 48.4 %) than from AD patients (4 isolates, 12.5 %) ($p=0.01$). The enterotoxin E gene (*see*) was not detected in neither isolates from AD patients nor from atopic controls. Twenty-five (78.1 %) of the isolates from AD patients harbored at least one SAg gene, compared to 29 (93.5 %) isolates in atopic controls. The median for the total number of SAg genes detected in isolates from AD patients was 7 (IQR=1–8) and in isolates from atopic controls, it was 8 (IQR=6–8).

The hemolysin gamma cluster genes (*lukS*, *lukF*, and *hlgA*) were detected in all isolates from both groups. Some leukocidin genes such as *lukA*/*B* were also very frequent (31 isolates and 96.8 % in AD patients versus 26 isolates and 83.9 % in atopic controls). In contrast, the leukocidin genes *lukD*/*E*, which form a pathogenicity island with serin protease A gene (*splA*), were more frequent in isolates from AD patients (21 isolates, 65.6 %) than those from controls (9 isolates, 29 %) ($p=0.01$). The gene for serin protease B (*splB*) was also more frequent in isolates from AD patients (21 isolates, 65.6 %) than from atopic controls (8 isolates, 25.8 %) ($p=0.006$) (Fig. 1). No differences were found in the distribution of other genes encoding virulence factors such as aureolysin (*aur*), serin protease E (s*p*/*E*), or staphylococcal complement inhibitor (*scn*). No genes encoding exfoliative toxins (*etA*, *etB*, *etD*) were detected in any of the isolates.

Analysis of *set*/*ssl* genes

As for the major locus of genes encoding staphylococcal superantigen-like proteins (*set*/*ssl* 1–11), most of the genes were ubiquitously present in both groups, except for *ssl8* and *ssl11*. *Ssl8* was detected in 21 isolates (65.6 %) from AD patients versus 6 isolates (19.3 %) from atopic controls ($p=0.006$)

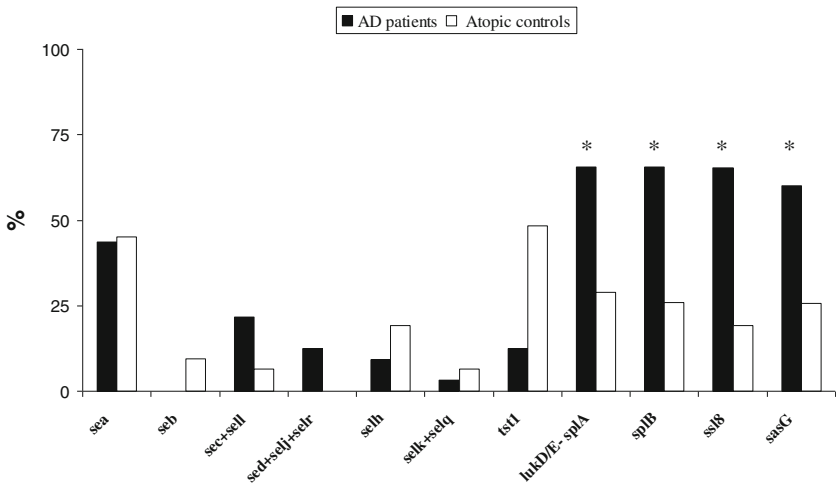

Fig. 1 Prevalence rates of superantigen (SAg) genes and only of the genes statistically more frequent in the isolates from atopic dermatitis (AD) patients than isolates from atopic controls

* Statistically significant $p<0.05$

(Fig. 1). *Ssl11* was detected in 25 isolates (78.1 %) from AD patients versus 19 isolates (61.3 %) from atopic controls.

Analysis of capsule and biofilm genes

Capsule type 8 was the most predominant in both groups. All isolates harboring *agr III* belonged to capsule type 8, with the exception of ST942, which belonged to capsule type 5. Isolates of *agr* groups I and II could belong to either capsule types 5 and 8. Capsule types were uniform within a given CC. Biofilm genes *icaA*/*icaC*/*icaD* were always present. The gene *bap* encoding a surface protein involved in biofilm formation was absent from all isolates.

Analysis of MSCRAMM genes and other adherence factors

MSCRAMM genes were nearly ubiquitously present in the isolates from AD patients and atopic controls, except for the gene for *S. aureus* surface protein (*sasG*) and fibronectin binding protein B (*fnb-B*). *SasG* was detected in 19 isolates (59.3 %) from AD patients versus 8 isolates (25.8 %) from atopic controls ($p=0.01$) (Fig. 1). *Fnb-B* was detected in 25 isolates (78.1 %) from AD patients versus 17 isolates (54.8 %) from atopic controls.

Relationship between clinical, immunological, and microbiological parameters of AD patients

Of the 30 AD patients included in this part of the study, 28 (93.3 %) were nasal carriers of *S. aureus*. Seventeen AD patients (56.6 %) were colonized by *S. aureus* in all samples collected. Seven (23.3 %) patients presented mild AD, 15 (50 %) presented moderate AD, and 8 (26.6 %) presented severe AD. The median for the total IgE levels was 174.5 kU/L (IQR=52.1–1,389.2). The median for the specific IgE levels was: SEA-IgE 0.09 kU/L (IQR=0.01–0.52), SEB-IgE 0.03 kU/L (IQR=0–0.53), SEC-IgE 0.03 kU/L (IQR=0–0.95), and TSST1-IgE 0.15 kU/L (IQR=0.01–0.62). Fourteen patients (46.6 %) showed a positive reaction for SEA-IgE, 8 (26.6 %) for SEB-IgE, 12 (40 %) for SEC-IgE, and 16 (53.3 %) for TSST1-IgE. There was a moderate and significant relationship (cubic model) between SCORAD and the total serum IgE levels ($R^2=37.6$, $p=0.006$). There was no association of specific IgE to SEA, SEB, SEC, TSST-1 with SCORAD.

There was also no statistically significant association between SCORAD and the detection of *sea*, *seb*, *sec*, and *tst1*. Neither was there a statistically significant association between specific serum IgE to SEA, SEB, SEC, TSST-1 and the detection of the respective genes (Table 2).

There was neither a correlation between disease severity scores and *S. aureus* CC or *agr* group in AD patients nor between AD severity and the presence of genes encoding SAgs or the number of SAg genes detected. When other genes included in the microarray were analyzed, two were correlated with AD severity: *scn* and *sasG* (Table 3).

Table 2 Prevalence rates of SAg-specific IgE and SAg genes in AD patients

	Specific IgE+	Specific IgE−
sea+,*n* (%)	8 (57.1)	6 (42.8)
sea−,*n* (%)	7 (43.7)	9 (56.2)
seb+,*n* (%)	0	0
seb−,*n* (%)	8 (26.6)	22 (73.3)
sec+,*n* (%)	2 (33.3)	4 (66.6)
sec−,*n* (%)	10 (41.6)	14 (58.3)
tst1+,*n* (%)	2 (50)	2 (50)
tst1−,*n* (%)	14 (53.8)	12 (46.1)

Discussion

AD pathogenesis is dependent on different factors: immunological dysfunction, skin permeability, and *S. aureus* colonization [13]. In this study, we intended to answer some questions about *S. aureus* and AD. Is the *S. aureus* colonizing AD patients different from that isolated from control subjects? Our study supports the conclusion that there is no characteristic *S. aureus* genotype in AD patients. Is there any gene of *S. aureus* clearly related to the severity of AD? Only a small group of *S. aureus* SAgs has been fully studied in relation to AD, and the wide range of factors present in this microorganism and their relation to this disease remains, as yet, unknown. We found a correlation between the severity of AD and the detection of two *S. aureus* genes: *scn* and *sasG*. *SasG* encodes a protein that belongs to the MSCRAMM, a wide group of surface-bound proteins that are essential for *S. aureus* adherence [14, 15]. The product of *scn* is one of the secreted proteins by *S. aureus* that act as immunomodulators, interfering with activation and regulation of the complement cascade [16].

A clear association of virulence factors such as SAgs with AD severity could suggest addressing treatment with antibiotics, while the detection of adherence factors could mean focusing on the reconditioning of the status of the epidermal barrier. Furthermore, if a clear relation between AD severity and the detection of a particular *S. aureus* gene could be found, the use of specific vaccines or antibiotics that block the expression or the function of that gene might be considered.

Control patients were atopic, meaning that they all had a hyperstimulated immune response. In this way, reliable conclusions regarding the pathogenic role of *S. aureus* in AD could be drawn with no interferences due to altered immunological state of the patient.

Table 3 Prevalence rates of SAgs related to severity and of the genes associated with severity for AD patients

	Total AD (n=30)	Mild AD (n=7)	Moderate AD (n=15)	Severe AD (n=8)
egc cluster, *n* (%)	19 (63.3)	4 (57.1)	9 (60)	5 (62.5)
sea, *n* (%)	14 (46.6)	1 (14.2)	7 (46.6)	6 (75)
sec+*sell*, *n* (%)	6 (20)	3 (42.8)	2 (13.3)	1 (12.5)
tst1, *n* (%)	4 (13.3)	2 (28.5)	2 (13.3)	0
sed+*selj*+*selr*, *n* (%)	4 (13.3)	1 (14.2)	1 (6.6)	2 (25)
selh, *n* (%)	3 (10)	1 (14.2)	1 (6.6)	1 (12.5)
selk+*selq*, *n* (%)	1 (3.3)	0	0	1 (12.5)
*sasG**, *n* (%)	18 (60)	3 (42.8)	8 (53.3)	7 (87.5)
*scn**, *n* (%)	27 (90)	5 (71.4)	15(100)	7 (87.5)

* Statistically significant, $p<0.05$

The most frequent CCs of *S. aureus* found in the isolates from our patients were CC5, CC10, CC15, CC30, and CC45. These data reflect the usual population structure of methicillin-sensitive *S. aureus* of the general population, as also shown in other Spanish and European studies [17, 18]. CC30 is usually prominent in both carriage and invasive disease [4]. Meanwhile, in our study, this CC was under-represented in AD patients (12.5 %). To our knowledge, the only two previous studies on *S. aureus* CC in AD revealed the same low prevalence of CC30 in AD patients [3, 4].

The possible implication of SAgs in the exacerbation of cutaneous inflammation has long been questioned. About 50 % of *S. aureus* isolated from AD patients secrete SAgs [19, 20]. However, SAg-producing *S. aureus* are also found in healthy carriers [7, 21], and some studies have suggested that the expression of an SAg alone does not play an important role in the increasing skin inflammation in AD [4, 5, 22]. Several publications have compared the classical SAg production (SEA, SEB, SEC, TSST1) in isolates from AD patients and controls [7, 8, 20, 23, 24]. No SAg was clearly related with isolates from AD patients or controls. In our study, *sea* was the most frequent in isolates from AD patients. *Sea* and *tst1* were the most frequent SAg genes in isolates of atopic controls. This could be explained by a previously confirmed relationship between CC30 and the carriage of *tst1* [17].

As for the rest of the SAg genes, the *egc* cluster was the most common in isolates from atopic controls and AD patients. This high prevalence has been described in asymptomatic carriers and the relationship of the *egc* cluster with CC30, CC45, CC5, and CC22 has also been described elsewhere [17, 25]. To our knowledge, only one previous report [8] has studied the prevalence of the *egc* cluster in *S. aureus* colonizing AD patients, and they found similar results.

Besides SAgs, other *S. aureus* factors could be involved in the pathogenesis of AD. Although some studies have not found specific genes in AD patients [24, 26], in our study, we found a higher remarkable prevalence of *lukE*, *lukD*, *splA*, *splB*, *ssl8*, and *sasG* in isolates of AD patients. The detection of some of these genes might merely reflect CC affiliations. Several studies have noted that the *lukE*, *lukD*, *splA*, *splB*, and *sasG* genes are not found in CC30 isolates [11, 25]. Thus, it is not clear whether these genes really contribute to the pathogenesis of *S. aureus* in AD or are just a reflection of clonality. To our knowledge, *ssl8* has not been linked to any particular CC. A superantigen-like protein (that could promote a local inflammatory reaction) may play a role in the pathogenesis of a skin disease such as AD. However, it should be noted that the difficulty of assessing the contribution of an individual virulence determinant to *S. aureus* pathogenicity has been highlighted in several reports [17, 27, 28].

In brief, although we found slight differences between the genomic profile of *S. aureus* in AD patients and atopic controls, we conclude that *S. aureus* has no characteristic genotype in AD patients and that the *S. aureus* population is very heterogeneous in these patients. Similar results were obtained in previous studies [3, 29].

AD patients showed increased values of total serum IgE and this value was correlated with the severity of the disease. Previous studies have also demonstrated these facts [30, 31]. IgE inhibits neutrophil adhesion, phagocytosis, respiratory burst, and increases the production of cytokines [32, 33], leading to a higher susceptibility to infection by *S. aureus*. For this reason, total IgE has been proposed as a biological marker of the severity and clinical evolution of AD.

S. aureus toxins can act not only as SAgs, but also as classic allergens inducing the production of specific IgE antibodies [6]. Many reports have been published on IgE- and SAg-specific (SEA, SEB, SEC, TSST1) IgE production in AD patients [34, 35], and this production seems to be quite specific [34, 36]. Our patients showed lower values of specific IgE values compared with previous reports [31, 37]. However, similar results of global positive reaction for each specific IgE were found (25–50 % of our patients and 30–70 % in other studies) [6, 31, 34, 37]. Most of these authors describe a statistical correlation between specific IgE and severity of AD [6, 19, 31, 37]. We found no relationship between these two parameters. We observed that there was not always a relationship between specific IgE production in the AD patient

and the carriage of each particular SAg gene by the isolated *S. aureus* strain. Firstly, specific IgE was detected in some patients, and, surprisingly, there was no detection of the SAg gene. A possible explanation for this discrepancy could be polyclonal colonization by *S. aureus*. As a limitation of our study, we only selected a single colony of *S. aureus* from the culture of the skin swab, and some studies have revealed the presence of various lineages of *S. aureus* in lesions of patients with AD [5]. Secondly, some isolates of *S. aureus* harbored a specific SAg gene but no production of specific IgE against that toxin was found. The detection of genetic material without protein expression could justify the lack of response to that SAg.

There was no correlation between disease severity scores and *S. aureus* CCs. This observation was also found in two previous AD studies [4, 5]. Additionally, no SAg gene had a significant correlation with the severity of AD in our study. Some authors found no relationship between SCORAD and *S. aureus* SAgs [22, 38, 39], while others found a correlation [7, 20, 23, 31]. In our opinion, the wide difference in the number of SAgs that each technique can detect could explain such differences. Some authors detected the production of the classical enterotoxins and others, using molecular tools, detected the presence of a wide range of SAgs genes.

In our study, *sasG* and *scn* were significantly correlated with the severity of AD. *sasG* belongs to the MSCRAMM genes. Its product has been shown to promote the formation of biofilm and intercellular autoaggregation [15], and to participate in the adherence to nasal epithelium cells [40, 41]. Most MSCRAMM genes are usually present in all CCs of *S. aureus*. However, *sasG* is detected usually only in some CCs. The skin of AD exhibits some barrier defects, increasing the adherence of *S. aureus* [1]. This fact could explain the importance of adherence factors in the worsening of AD. *scn* is one of the several *S. aureus* complement-evasion molecules [42]. It blocks the central C3 convertase enzymes and also blocks the conversion of C3 by alternative pathway C3 convertases [43]. *Scn* seems to be randomly distributed across diverse *S. aureus* CCs [44]. AD patients show an altered immunological status, including increased TH2-type cytokine expression in acute lesions, increased numbers of T cells, and deficiency in host defense molecules [13]. For this reason, the carriage of *S. aureus* harboring immunomodulator genes could be involved in the clinical severity of AD.

In conclusion, in our study, we found a higher prevalence of *lukD*, *lukE*, *splA*, *splB*, *sasG*, and *ssl8* in *S. aureus* colonizing AD patients than in atopic controls. We also detected a correlation between the detection of an adherence factor of *S. aureus* (*sasG*) and an immune evasion gene (*scn*) and the severity of AD. However, these results are quite preliminary because of the alleged link of *lukD*, *lukE*, *splA*, *splB*, and *sasG* to certain CCs and the difficulty of determining the real implication in pathogenesis at a molecular level of an individual virulence factor [17, 27, 28]. Further studies should be performed to analyze the association with severity biased by the underlying clonal population structure [17]. Meanwhile, it should be noted in the first place that *sasG*, which produces an adherence factor, was the only gene correlated with AD severity and also statistically more frequent in isolates from AD patients. Secondly, it should also be highlighted that no correlation between SAg genes and SCORAD was found. These two facts reflect the need to expand the studies on *S. aureus* and AD. In our opinion, it is essential that future studies with a greater number of AD patients are performed, including extensive analysis of the genetic profile of *S. aureus*. These screening experiments will single out genes potentially implicated in the pathogenesis of AD that will subsequently be studied individually.

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