

The Skin and Nose Microbiome and Its Association with Filaggrin Gene Mutations in Pediatric Atopic Dermatitis

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Keywords

Eczema · Filaggrin · Microbiome · Skin barrier · Staphylococcus aureus

Abstract

Background: Interactions between the skin barrier, immune system, and microbiome underlie the development of atopic dermatitis (AD). **Objective:** To investigate the skin and nasal microbiome in relation to filaggrin gene (*FLG*) mutations. **Methods:** A cross-sectional study including 77 children with difficult-to-treat AD. The entire encoding region of *FLG* was screened for mutations using single molecule molecular inversion probes and next-generation sequencing. Bacterial swabs from the anterior nares, lesional and nonlesional skin were analyzed using 16S rRNA sequencing. For skin samples, additional qPCR was performed for *Staphylococcus aureus* and *Staphylococcus epidermidis*. **Results:** The prevalence of patients with a mutation in *FLG* was 40%, including 10 different mutations. Analyzing bacterial swabs from all three niches showed a significant effect for both niche and *FLG* mutation status on the overall microbiome composition. Using a subset analysis to test the effect of *FLG* mutation status per

niche separately did not show a significant association to the microbiome. Shannon diversity and *S. aureus* abundance were significantly affected by the niche, but not by the presence of an *FLG* mutation. **Conclusions:** Our results suggest only a minor role for *FLG* mutation status on the overall microbiome, which is rather caused by differences in the present genera than by microbe richness and evenness.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder primarily present in children [1]. This condition is characterized by a dry, itchy skin with eczematous skin lesions that show an age-related distribution [2]. The pathogenesis of AD is complex, including alterations in the skin microbiome, epidermal barrier dysfunction, and immunological changes [1, 3].

The skin microbiome in AD is characterized by a low bacterial diversity, with a high abundance of *Staphylococcus*

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aureus and *Staphylococcus epidermidis* and low abundance of the genera *Propionibacterium*, *Streptococcus*, *Acinetobacter*, *Corynebacterium*, and *Prevotella* [4]. The skin microbiome is in constant interaction with the skin barrier and immune system, leading to inflammation characteristic of AD [5–7]. Previous studies showed that skin barrier impairment, characterized by mutations in the filaggrin gene (*FLG*), causes modified fibronectin binding, higher skin pH, and an increased serine protease activity, which can lead to altered colonization patterns [8, 9]. Interestingly, skin colonization with *S. aureus* leads to the secretion of virulence factors, increasing inflammation and further damage of the skin barrier [8, 10]. Furthermore, activated T helper type 2 (Th2) cells in AD can downregulate the filaggrin expression in the stratum corneum (SC) [11, 12].

Mutations in *FLG* are the strongest genetic risk factors for AD susceptibility [11, 13]. In West-European populations, 2282del4, R2447*, R501*, and S3247* are the most common mutations with a prevalence of around 40% in AD patients [11, 14]. However, up to 113 different mutations have been described in the literature [15], which are present depending on ethnic background [16, 17]. Due to the complexity of *FLG*, with the third exon encoding between 10 and 12 nearly identical repeats, screening for mutations in this gene has been challenging. Classical Sanger sequencing is frequently used, but this method is too cumbersome to screen the whole encoding gene for mutations [18]. A recent method, using single molecule molecular inversion probes (smMIPs) and next-generation sequencing (NGS) permits comprehensive sequencing of the entire gene [15, 19]. Instead of screening for population-specific mutations only, this technique enables to analyze the entire gene and detect population-specific mutations as well as specific mutations not previously published.

Previous studies investigating whether *S. aureus* colonization is more common in patients with a mutation in *FLG* compared to wild-type patients, show conflicting results [10, 20–23]. Publications on the relation between mutations in *FLG* and the composition of the entire microbiome in AD are scarce. It was recently shown that the microbiome on nonlesional skin in adult AD patients was associated with *FLG* mutation status [7]. This study included a smaller number of AD patients and only screened for the most common mutations in *FLG*, which might have led to an underrepresentation of *FLG* mutation carriers. In addition, no other studies are available to validate these results.

The aim of this study was to profile the entire mutational background of *FLG* using a new smMIP sequencing technique in children with difficult-to-treat AD. Second, we describe the microbiome in this population and

investigate differences in the microbiome from nose, lesional and nonlesional skin in AD children with and without a mutation in *FLG*.

Material and Methods

Study Design

This study was performed as part of the DAVOS-trial, a pragmatic randomized controlled trial (RCT) comparing the long-term effectiveness of treatment in alpine climate to treatment in moderate maritime climate in children with difficult-to-treat AD (ISRCTN88136485). The detailed study protocol and primary outcomes have been published previously [24, 25]. Study procedures were reviewed and approved by the Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands (reference 09-192/K). All patients and, if needed, their parents/guardians provided written informed consent. This present study involves secondary outcomes of this trial and included data before the start of the intervention period.

Participants

This study included Dutch children between 8 and 18 years old, with difficult-to-treat AD. Difficult-to-treat AD was determined before the start of the study and defined as use of at least a class 3 topical corticosteroid and not being able to step down, current use of systemic immunosuppressive treatment, repeated treatment with potent topical corticosteroids or systemic immunosuppressive treatment, a history of use of systemic treatment, a significant impact of AD on the child's or the family's quality of life, or seemingly unresponsive to conventional therapy according to current guidelines [25]. Demographic data was extracted from questionnaires and the electronic patient files. Due to the severity of the disease in this patient group, concurrent medication was continued. Disease severity was determined using the Self-Administered Eczema Area and Severity Index (SA-EASI, range 0–96), and microbiome as well as blood samples were collected. Study assessments were performed in the Netherlands.

Microbial Samples

Microbial samples were collected from the anterior nares, lesional and nonlesional skin. Samples taken from the lesional skin were preferably taken from the antecubital fold or the popliteal fold. Nonlesional skin samples were taken from the volar arm if possible (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000520978) [26]. All swabs were collected using sterile cotton swabs soaked in sterile NaCl 0.9% and stored at –80°C until further processing.

DNA Isolation, qPCR, and Sequencing

Extensive description of the procedures have been published previously [26]. Microbiome analysis was performed on bacterial swabs from the nasal cavity, lesional and nonlesional skin with sequencing of the 16S rRNA gene using V4 hypervariable region on the Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). A genus table with raw read counts was generated for downstream analysis. Quantitative (q)PCR was performed on samples from the lesional and nonlesional skin to identify *S. aureus* and *S. epidermidis*. The bacterial DNA concentration was reported as log₁₀-transformed femtogram per microliter (fg/μL) in this paper.

Table 1. Patient characteristics

	Wild-type (n = 46)	FLG mutation carriers ^a (n = 31)	p value ^b
Sex (female), n (%)	20 (43.5)	19 (61.3)	0.13
Age, median (IQR)	13.0 (11.0–15.0)	12.0 (11.0–15.0)	0.64
Age of AD onset <6 months, n (%)	34 (80.4)	28 (90.3)	0.34
Asthma ^c , n (%)	39 (84.8)	26 (83.9)	0.91
Rhinitis ^d , n (%)	38 (82.6)	29 (93.5)	0.30
Food allergy ^{e,f} , n (%)	25 (54.3)	26 (83.9)	<0.01
SA-EASI ^g , median (IQR)	37.2 (17.6–52.6)	41.4 (26.4–60.6) ^h	0.29
Topical corticosteroids previous 7 days ⁱ , n (%)			0.89
None	1 (2.2)	1 (3.2)	
Moderate	2 (4.3)	2 (6.5)	
Potent	40 (87.0)	27 (87.1)	
Very potent	3 (6.5)	1 (3.2)	
Systemic medication, n (%)			1.00
Prednisone	2 (4.3)	1 (3.2)	
Cyclosporine	4 (8.7)	3 (9.7)	
Oral antibiotics	–	1 (3.2)	

AD, atopic dermatitis; FLG, filaggrin gene; SA-EASI, Self-Administered Eczema Area and Severity Index; SD, standard deviation. ^aIncluding 23 patients who were heterozygous, 4 patients homozygous, and 4 patients with two different mutations. ^bp values were analyzed using χ^2 , Fisher's exact test, or Mann-Whitney U when appropriate. ^cAsthma was diagnosed based on spirometry reversibility testing and Methacholine Challenge Test. ^dRhinitis was diagnosed based on assessment by a pediatrician. ^eFood allergy was defined as a positive double-blind placebo-controlled food challenge (DBPCFC) or convincing clinical history (a reported type I allergic reaction with acute symptoms within 2 h after ingestion of the food) in combination with sensitization to the specific food allergen. ^fMissing n = 2 (both groups n = 1). ^gRange 0–96 (<17 mild; 18–46 moderate; >47 severe)¹. ^hMissing n = 1. ⁱUK potency system used.

FLG Mutation Profiling

FLG mutations were determined on DNA isolated from blood samples. We used smMIP-NGS strategy to screen the entire FLG and to identify all mutations resulting in premature protein termination. Detailed information about the sample preparation and smMIP-NGS technique have been described previously [15, 19]. Previous use of this technique showed that more than one mutation may be present on the same allele, especially when this includes rare or new mutations. Therefore, patients in which we found two different mutations in FLG were not classified as compound heterozygous but as patients with more than one mutation. “FLG mutation status” was used throughout this paper to describe the dichotomous outcome as used in our statistical models: FLG wild-type patients (FLG⁺) versus FLG mutation carriers (FLG⁻, patients with one or more mutations in FLG).

Statistical Analysis

To determine differences in demographic data and disease severity between patients with and without a mutation in FLG, χ^2 , Fisher's exact test, or Mann-Whitney U tests were used when appropriate. Descriptive statistics were used to present the FLG mutations in our study population. For analysis of the microbiome, we used non-subsampled genus tables after filtering (Shannon diversity index was calculated on unfiltered data). Filtering was applied by selecting the genera representing 97.5% of all counts. Prior to ordination analysis, the filtered genus tables were square-root transformed with subsequent application of Wisconsin double

standardization. To visualize the microbial composition for all 3 niches sampled (nose, lesional and nonlesional skin) and FLG status (FLG⁺ and FLG⁻), Bray-Curtis distance-based multidimensional scaling (MDS) was used. Permutational multivariable analysis of variance (PERMANOVA) correcting for repeated measurements was used to determine significant differences in microbial composition while accounting for FLG mutation status. We included the covariates “niche” and “mutation status” as interaction terms in this model. When appropriate, PERMANOVA coefficients were obtained to determine the genera that contributed to the significant differences [27]. Second, we performed a subset analysis for the skin samples and the niches individually (nose, lesional and nonlesional skin) to investigate the effect of FLG mutation status on the microbial composition within each niche.

In-depth analysis was performed to assess the effect of FLG mutation status on *S. aureus* and *S. epidermidis* colonization rates using a χ^2 test. Furthermore, the effect on *S. aureus* and *S. epidermidis* abundance in patients with positive bacterial swabs was analyzed with a linear mixed-effect model. This statistical model was also used in analyzing the effect of FLG status on the Shannon diversity index.

Statistical analyses were performed in SPSS (version 21) and R software (version 3.5.1). We used the packages “phyloseq” and “vegan” for microbiome analyses and accounted for repeated measurements using the “strata” argument [28]. R package “ggplot2” was used for visualization [29]. Linear mixed-effect models were performed using “lme” and “lme4” package [30]. A p value of ≤ 0.05 was considered statistically significant.

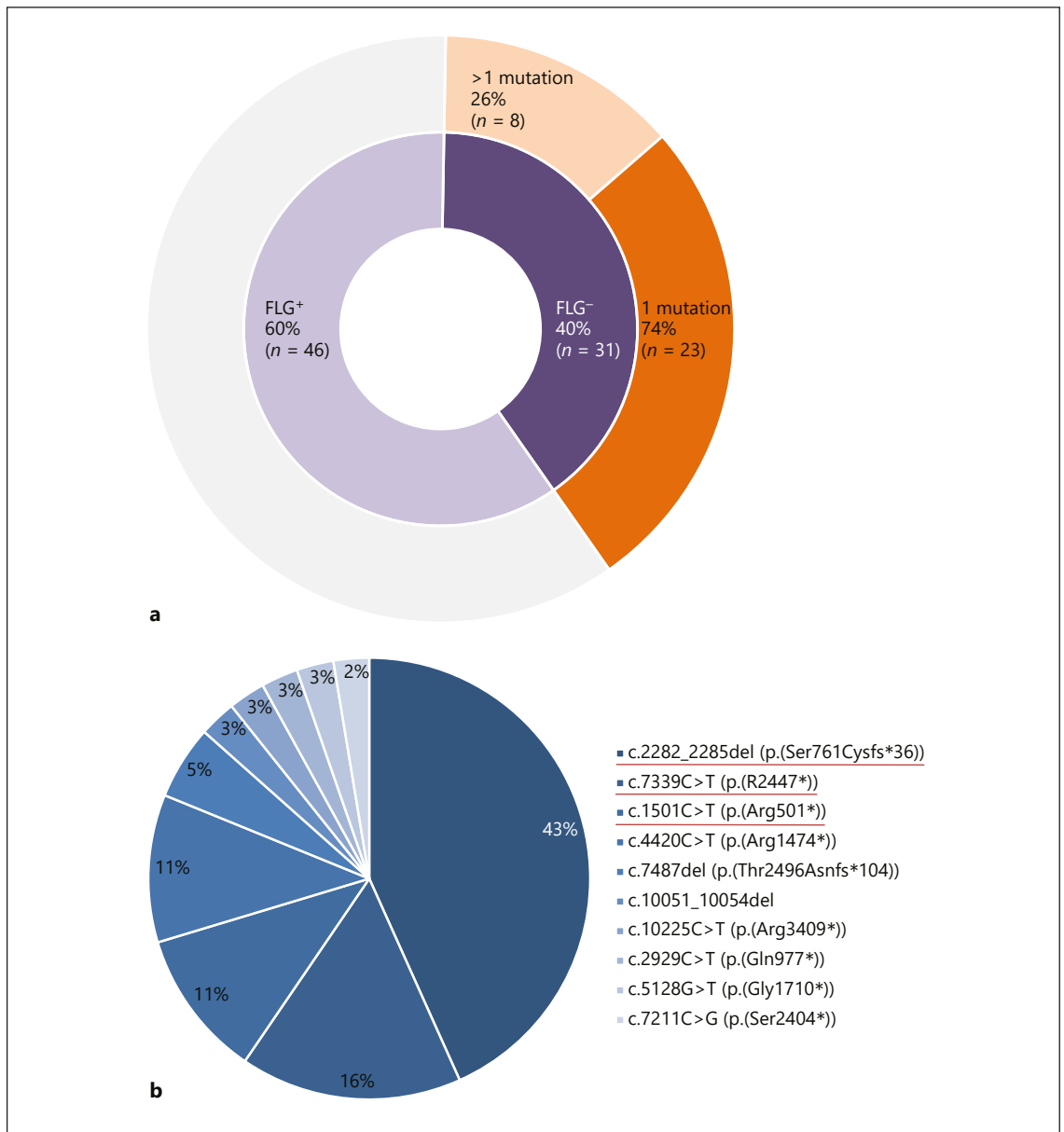


Fig. 1. Prevalence of *FLG* mutations using smMIP sequencing. **a** Prevalence of patients carrying *FLG* mutations. 40% ($n = 31$) of the patients had a mutation in *FLG*. Within this group of mutation carriers, more than one mutation was detected in 8 (26%) patients. **b** Prevalence of the 10 different *FLG* mutations in our study population. Underlined mutations represent mutations which are most commonly detected in West-European populations.

Results

Study Subjects and Sample Characteristics

A total of 77 patients were included in the study. Lesional and nonlesional skin swabs were available for 76 patients (99%) and analyzed using qPCR techniques. Sufficient amount of DNA to perform 16S rRNA sequencing

was available in 63 (82%) patients for lesional skin, 55 (71%) patients for nonlesional skin, and 73 (95%) for the nose (online suppl. Fig. 1). Patient characteristics of missing data did not differ from the study group. Demographic data, comorbidities, medication use, and disease severity are noted in Table 1. The median severity score was 37.2 (17.6–52.6) in the *FLG*⁺ group, compared to 41.4 (26.4–60.6) in the *FLG*⁻ group ($p = 0.29$).

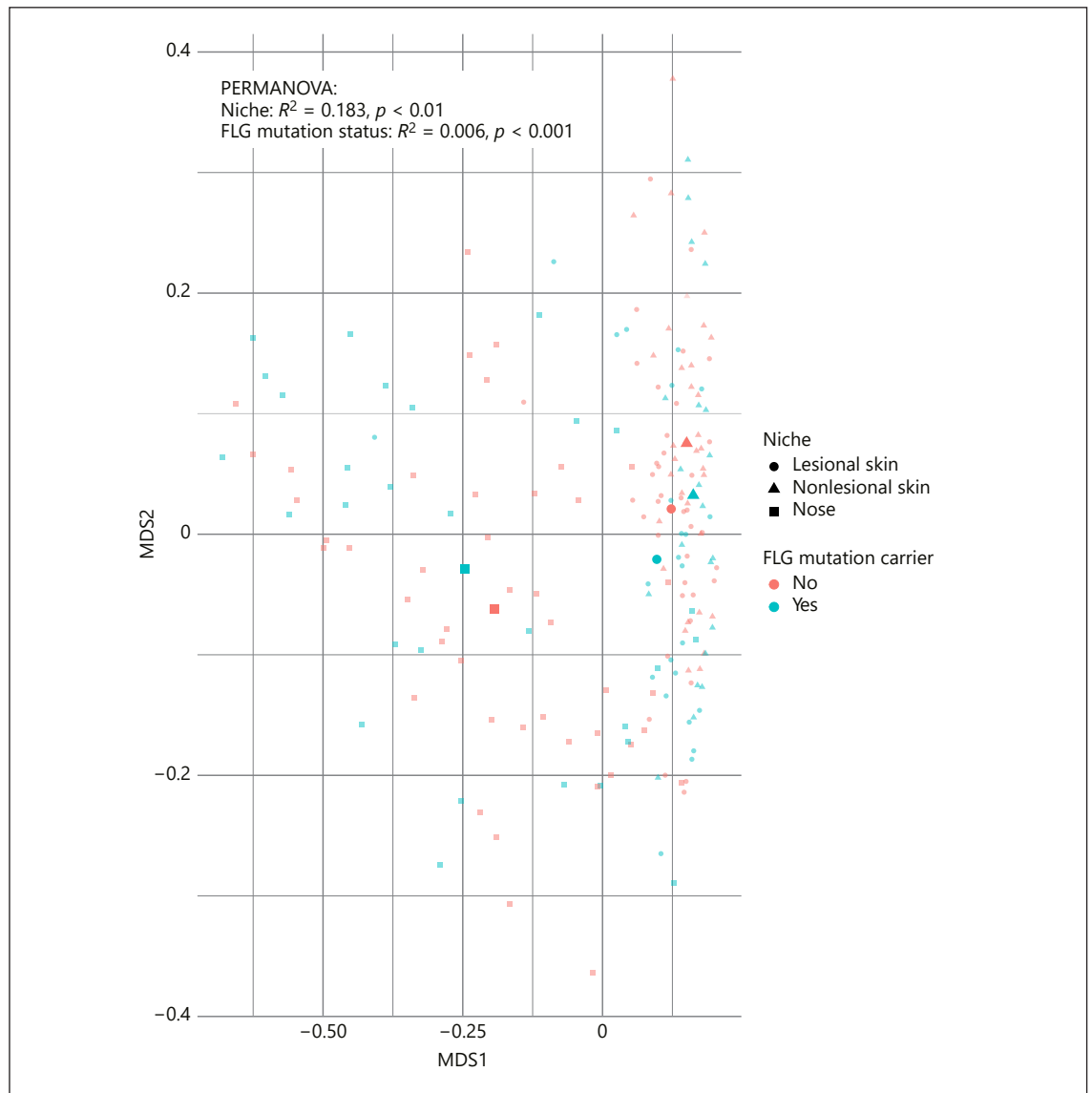


Fig. 2. Bray-Curtis distance-based multidimensional scaling plot showing the microbiota for nose and skin in relation to *FLG* mutation status. For ordination analysis, genus tables were standardized using square-root transformation with subsequent application of

Wisconsin double standardization. *FLG* status was significantly associated with the overall microbial composition using PERMANOVA analysis (niche: $R^2 = 0.183$, $p < 0.01$; *FLG* status: $R^2 = 0.006$, $p < 0.01$; interaction: $R^2 = 0.008$, $p = 0.47$).

Screening the Entire Filaggrin Gene Improves the Sensitivity of Mutation Detection

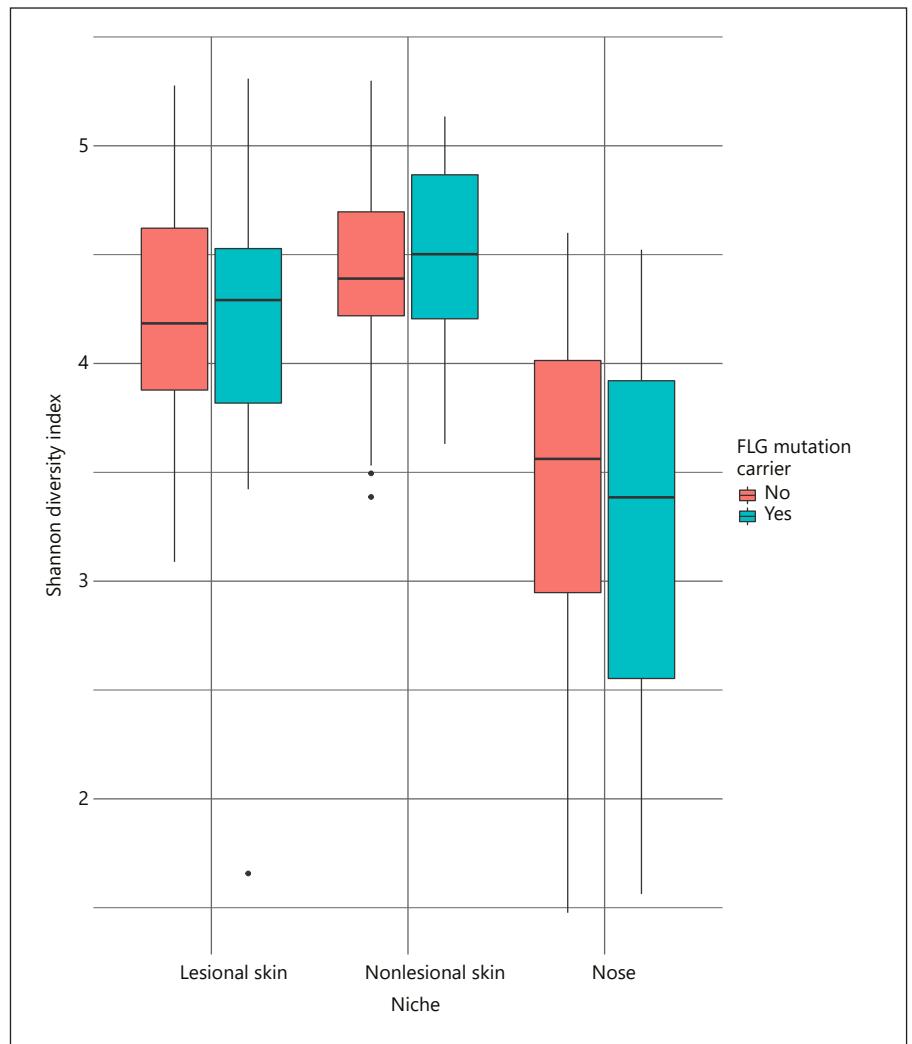
A total of 10 different mutations leading to a premature protein termination were found, of which c.5128G>T (p.(Gly1710*)) in repeat 4 and c.7211C>G (p.(Ser2404*)) in repeat 6 have not been described before (Fig. 1, online suppl. Table 2). Compared to the most common mutations in West-European populations (2282del4, R2447*, R501*, and S3247*), the prevalence of patients with an *FLG* mutation increased from

33% (25 of 77) to 40% (31 of 77) screening the entire encoding gene. This included 8 (26%) patients with more than one mutation.

The Microbial Composition in Children with AD

All sequences belonged to 844 genera, of which 81 genera remained after filtering. The genus *Staphylococcus* was predominant in both lesional and nonlesional skin samples, with a median relative abundance (RA) of 78% and 44%, respectively. This was followed by *Streptococcus*

Fig. 3. Shannon diversity index in relation to *FLG* mutation status. Shannon diversity index represents the number of different genera (richness) and how even they are distributed (evenness). The boxes represent the twenty-fifth percentile, median, and seventy-fifth percentile. *FLG* status did not affect the microbial composition in all three niches as determined using a linear mixed-effect model (niche $p = 0.01$; *FLG* status $p = 0.35$; “niche: *FLG* status” $p = 0.85$).



(median RA 3% and 4%) and *Corynebacterium* genera (median RA 2% and 5%) (online suppl. Fig. 2). Within the nose, the genera *Corynebacterium* (31%), *Staphylococcus* (11%), and *Dolosigranulum* genera (9%) were most prevalent (online suppl. Fig. 2). The differences in microbial composition between the three sampled niches were visualized and tested (online suppl. Fig. 3). All three niches harbored a significantly different microbiome (lesional skin vs. nonlesional skin, nose vs. lesional skin, nose vs. nonlesional skin: PERMANOVA $R^2 = 0.027$, $p < 0.01$; $R^2 = 0.190$, $p < 0.01$; and $R^2 = 0.139$, $p < 0.01$, respectively).

The Overall Microbial Composition Is Affected by FLG Mutation Status

Community composition was visualized considering *FLG* mutation status in Figure 2 (skin samples only in online suppl. Fig. 4). The PERMANOVA analysis showed

that both niche and *FLG* mutation status were significantly associated with the overall microbial composition (niche: $R^2 = 0.183$, $p < 0.01$; *FLG* status: $R^2 = 0.006$, $p < 0.01$; interaction “niche: *FLG* status”: $R^2 = 0.008$, $p = 0.47$). The association between the microbiome and *FLG* mutation status was mainly driven by the *Corynebacterium*, which was less abundant in *FLG* mutation carriers (online suppl. Fig. 5a). Significant effects for niche and *FLG* mutation status were also observed in the subset analysis including only skin samples (niche: $R^2 = 0.027$, $p < 0.01$; *FLG* status: $R^2 = 0.010$, $p < 0.01$; interaction “niche: *FLG* status”: $R^2 = 0.005$, $p = 0.56$; online suppl. Fig. 4). In this model, the *Staphylococcus* (less abundant in *FLG*⁻) and *Acidovorax* genera (more abundant in *FLG*⁻) mainly contributed to the effect for *FLG* mutation status (online suppl. Fig. 5b). Performing subset analysis to test the effect of *FLG* status within each niche separately (nose, lesional

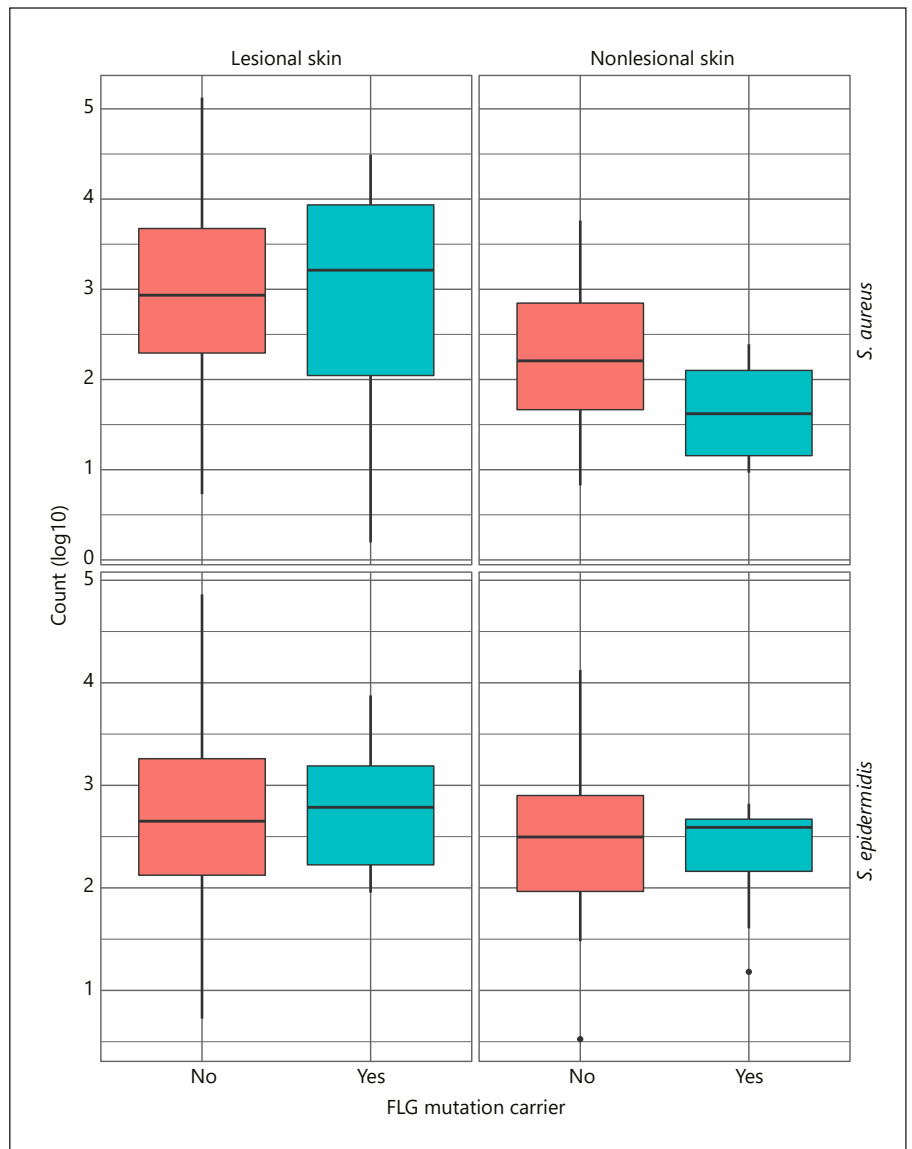


Fig. 4. Quantitative PCR results of the skin samples for *S. aureus* and *S. epidermidis* in relation to *FLG* mutation status. The results for *S. aureus* and *S. epidermidis* load are shown using log₁₀ transformed data. The boxes represent the twenty-fifth percentile, median, and seventy-fifth percentile. *S. aureus* density was significantly associated with the sampled niche, but not with *FLG* status (niche $p < 0.01$; *FLG* status $p = 0.10$).

and nonlesional skin) did not yield any significant associations between *FLG* status and microbiome composition (nose PERMANOVA $R^2 = 0.014$, $p = 0.39$; lesional skin PERMANOVA $R^2 = 0.013$, $p = 0.72$; nonlesional skin PERMANOVA $R^2 = 0.0185$, $p = 0.42$). Alpha diversity, indicated with Shannon diversity index, was significantly affected by niche ($p = 0.01$) but not by *FLG* status ($p = 0.35$, interaction “niche: *FLG* status” $p = 0.85$) (Fig. 3).

Association between Staphylococcus Abundance and *FLG* Status

qPCR was performed to gain more information on the species *S. aureus* and *S. epidermidis*. In the total study population, 56 (72%) patients were positive for *S. aureus*

Table 2. Colonization rates for *S. aureus* and *S. epidermidis*

	Wild-type (n = 46)	<i>FLG</i> mutation carriers (n = 31)	<i>p</i> value ^a
Positive for <i>S. aureus</i>			
Lesional skin	37 (80.4) ^b	19 (61.3) ^c	0.10
Nonlesional skin	26 (56.5) ^c	10 (32.3) ^c	0.04
Positive for <i>S. epidermidis</i>			
Lesional skin	43 (93.5)	28 (90.3) ^b	0.65
Nonlesional skin	43 (93.5)	29 (93.5) ^b	0.54

FLG, filaggrin gene. ^a*p* values were analyzed using χ^2 . Number of missings: ^bn = 1; ^cn = 2.

on lesional skin, with similar proportions in *FLG*⁺ and *FLG*⁻ patients ($p = 0.10$) (Table 2). Interestingly, on non-lesional skin, we found a higher proportion of patients colonized with *S. aureus* in the *FLG*⁺ group than in the *FLG*⁻ group (57% for *FLG*⁺ vs. 32% in *FLG*⁻ group; $p = 0.04$) (Table 2). The mixed model analysis showed that niche (lesional, nonlesional) was significantly associated with *S. aureus* load, but not with *FLG* mutation status (niche $p < 0.01$; *FLG* status $p = 0.10$; interaction “niche: *FLG* status” $p = 0.81$) (Fig. 4). *S. epidermidis* was detected on lesional skin in 70 (91%) participants and in 69 (90%) participants on nonlesional skin (Table 2). The *S. epidermidis* load was not affected by niche ($p = 0.17$), nor by *FLG* mutation status ($p = 0.08$) (Fig. 4).

Discussion

To our knowledge, this is the first study investigating the effect of *FLG* mutation status on the microbial composition in children with AD. In this cohort of children with difficult-to-treat AD, the prevalence of *FLG* mutations was 40% when screening the entire encoding gene, which is consistent with the prevalence of *FLG* mutations in AD groups. We demonstrated that the nose, lesional and nonlesional skin all harbored a significantly different microbiome. In addition, analyzing all samples together (and skin samples only), *FLG* status was of significant influence on the microbial composition, although the impact on the contribution was rather small. *FLG* mutations did not affect the Shannon diversity index or *S. aureus* load. Using subset analysis per niche, we were not able to confirm these results for the effect of *FLG* mutation status on the microbial composition.

The prevalence of *FLG* mutations was increased with 7% using the smMIP-NGS strategy in comparison to including only the four most common mutations in the West-European population. It has been shown previously that only analyzing population-specific mutations gives an underrepresentation of *FLG* mutation prevalence in AD populations [15–17]. Since the C-terminus has a critical role in the processing from profilaggrin to filaggrin and thus the presence of filaggrin in the epidermis [16, 31], all mutations leading to a premature protein termination (and lack of this C-terminal) contribute to the disease burden in AD [16, 32]. This underlines the relevance of screening the entire coding region of the *FLG*, instead of focusing on population-specific mutations. Interestingly, the S3247* mutation, which is often

described as a common European mutation variant, was not detected in our population. Possible explanations for the absence of S3247* is that mutation distribution can vary in every European country. Second, S3247* is described as a less common variant in comparison to R501* and 2282del4 [16].

A significant effect for *FLG* mutation status on the overall skin microbiome was found when analyzing all samples together (and analyzing lesional and nonlesional skin samples together), but not using subset analysis per individual niche. This might indicate that the effect for *FLG* mutation status was only minor (R^2 of 0.006 and 0.010, respectively) and/or that the number of samples per niche in our study was too small, leading to insufficient power to properly detect significant differences. Only one previous publication, including a smaller number of adult AD patients with less severe disease, only screening for four mutations in *FLG*, investigated the association between *FLG* mutations and the microbiome [7]. They found a significant effect of *FLG* mutation status on the microbial composition on nonlesional skin (with *Staphylococcus caprae* being more prevalent in *FLG*⁻), but not on lesional skin or in the nose [7]. Combining these and our results, a role for *FLG* status on the microbial composition is suggested. Interestingly, looking into the Shannon diversity index, our results are in line with two previous publications including AD and ichthyosis vulgaris patients [7, 33], showing that Shannon diversity index was not affected by *FLG* status. The explanation could be that patients with and without a mutation in *FLG* are colonized by different genera and do not differ based on the number of genera (richness) or how even they are distributed (evenness).

We found similar *S. aureus* colonization rates in *FLG*⁺ and *FLG*⁻ patients on lesional skin, but significantly higher colonization rates on nonlesional skin for *FLG*⁺ patients. Literature on this association is conflicting. In vitro studies showed that the absence of filaggrin breakdown products, natural moisturizing factor (NMF), increases *S. aureus* corneocyte adhesion and growth rate [20, 34]. These findings were supported by clinical data in adult AD patients, with higher colonization rates on lesional skin and in the nose of patients with a mutation in *FLG* [21]. In contrast, other clinical studies did not show reduced filaggrin levels in patients colonized with *S. aureus* [23, 35, 36]. The differences in outcomes between clinical studies can be largely affected by disease severity in the studied AD population. In active AD skin, Th2 response is upregulated along with the production of cytokines in-

terleukin (IL)-4 and IL-13, which are known to down-regulate filaggrin expression independent of mutation status [11, 12]. Since our study included patients who were difficult to treat and included 74% with severe or very severe disease (based on SA-EASI score), it is possible that due to an upregulation of the immune system, filaggrin expression was suppressed irrespective of *FLG* mutation status and masked the effect for wild-type patients.

A major strength of this study is the comprehensive sequencing of the entire encoding region of *FLG*, enabling to detect all mutations leading to premature protein termination. This technique enables to identify all patients who are at risk to develop a more severe and persistent disease course and are susceptible for the development of other atopic diseases (i.e., asthma and allergic sensitization). Furthermore, it could be useful to stratify patients in cohort studies. The following limitations need to be mentioned. First, since our cohort included children with difficult-to-treat AD, we did not discontinue concurrent medication. In addition, previous studies have shown that both age (Tanner stage) and disease severity can influence the composition of the microbiome [37, 38]. In this explorative study, we did not correct for these effects in our statistical analysis. However, the use of topical and systemic medication, age, and disease severity did not differ between patients with and without a mutation in *FLG*. Furthermore, excluding patients with systemic treatment from our statistical analysis did not yield different results (data not shown). Larger groups of patients with AD might be helpful in correcting for these effects. Second, heterozygous *FLG* mutations lead to 50% reduction in filaggrin expression as compared to a complete absence of filaggrin with bi-allelic mutations and might lead to different effects [11]. This gene-dosage effect was suggested in a previous study including ichthyosis vulgaris patients, with significant differences in the microbial composition between bi-allelic mutation carriers and wild-type patients, but not between bi-allelic and heterozygous mutation carriers [33]. Although our study included a small group of patients with more than one mutation in *FLG* ($n = 8$), we did not find different results for patients with one and more than one mutation in *FLG* (data not shown). Future studies should include a larger number of patients, investigating the gene-dosage effect on the microbiome in AD. In addition, by measuring *FLG* breakdown products we can determine the effect of the Th2 response in active AD. Lastly, as described previously [26], the impact of the inability to

properly detect the *Propionibacterium* or to classify the *Staphylococci* at species level with the use of the V4 hypervariable region, was only minor due to the sampled body regions and the use of qPCR to detect *S. aureus* and *S. epidermidis* species [39].

In conclusion, sequencing the entire *FLG* for novel, family- and population-specific mutations, contributing to the disease burden in AD, leads to better patient stratification. Our results suggest that the presence of a mutation in *FLG* has only a small effect on the microbial composition. Our results indicate that the difference in the microbiome between *FLG*⁺ and *FLG*⁻ patients is rather caused by the presence of distinct genera than differences in the alpha diversity. More research into the interaction of the microbiome with the skin barrier and immune system in large patient groups will lead to a better understanding of the etiology of AD and will help develop preventive and treatment strategies.

Key Message

Mutations in the filaggrin gene only have a small impact on the skin microbiome in patients with atopic dermatitis.

Acknowledgement

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Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Study procedures were reviewed and approved by the Medical Ethics Committee of the University Medical Center Utrecht, the Netherlands (reference 09-192/K). All patients and, if needed, their parents/guardians provided written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

MMFvM, LMP, KBF, and SGMAP designed the study. SGMAP and KBF designed and supervised the collection of the data. FHJS supervised the DNA isolation, qPCR and sequencing. MvG super-

vised the *FLG* mutation analysis. MMFvM and TJvdB had full access to all of the data and performed the data analyses. MMFvM, LMP, KBF, and SGMAP interpreted the data. MMFvM wrote the manuscript, and all authors critically revised and commented on the manuscript. SGMAP supervised the study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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