Gram-positive anaerobe cocci (GPAC) are underrepresented in the microbiome of filaggrin-deficient human skin

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Letter to the Editor

Gram-positive anaerobe *cocci* are underrepresented in the microbiome of filaggrin-deficient human skin

To the Editor:

Next-generation sequencing technologies and powerful bioinformatics tools have revealed that diversity and composition of the skin microbiota of healthy volunteers strongly depends on the topographical location on the body and has a high degree of interpersonal variation.[1] Nevertheless, the dominant types of bacteria remain relatively stable over time, and specific bacteria are associated with dry, moist, and/or sebaceous microenvironments.[2] Microbial communities, genetic host factors, and the environmental factors at a particular moment constitute a complex relationship that is essential for skin barrier homeostasis.[3] More recent studies have also focused on the microbiota of diseased and injured skin as alterations to microbial communities have been associated with cutaneous disorders.[4,5] In the present study, we selected ichthyosis vulgaris (IV) as a model disease to investigate whether genetic polymorphisms resulting in altered stratum corneum (SC) composition and structure affect the microbiota composition of human skin and the cutaneous host response. Genetic deficiency or haploinsufficiency of the histidine-rich epidermal protein filaggrin (*FLG*) is associated with IV and atopic dermatitis (AD).[6,7] Here, we report 2 novel findings with respect to FLG deficiency and cutaneous microbiota. First, we show that FLG deficiency is associated with a low relative abundance of proteolytic Gram-positive anaerobic

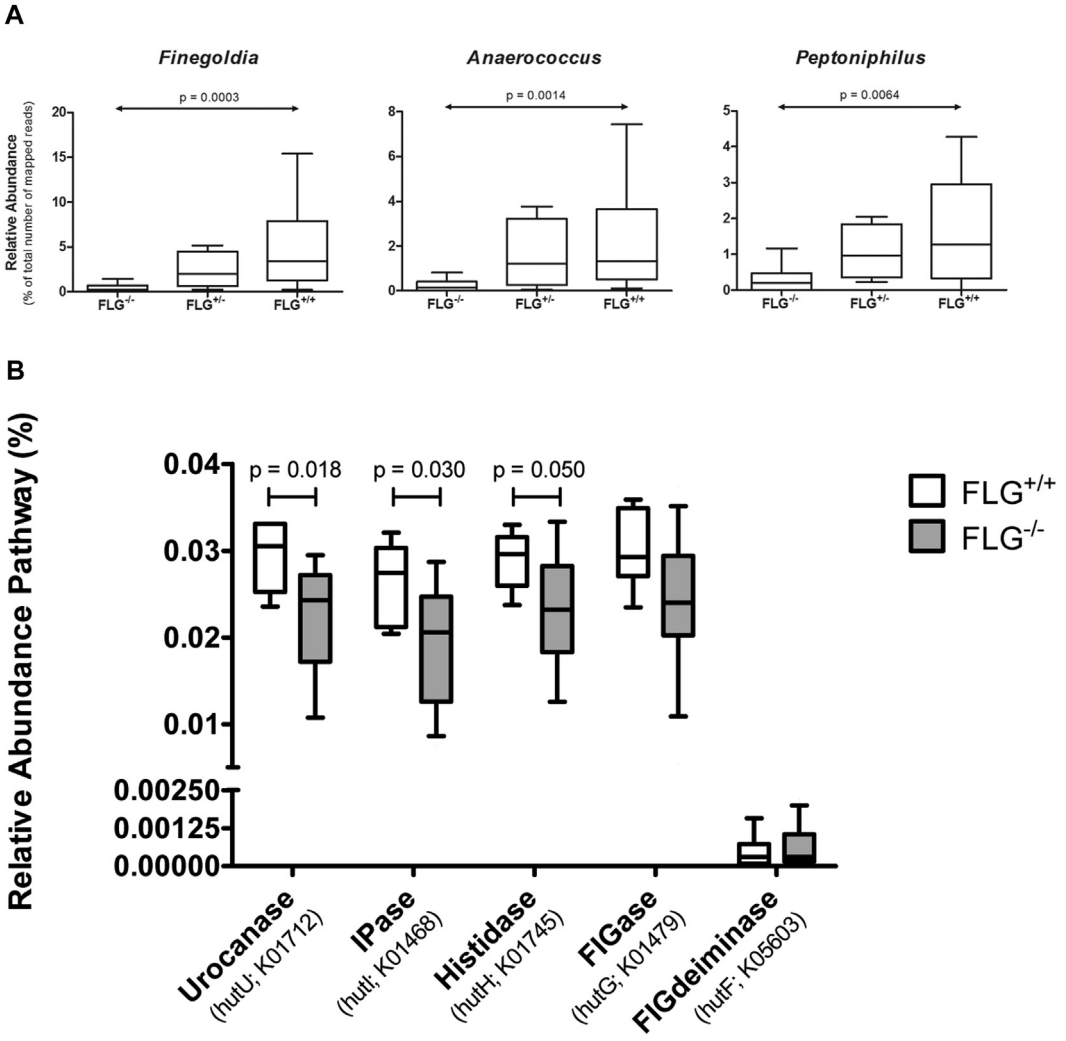

FIG 1. Lower relative abundance of proteolytic GPAC. **A,** Differences in relative abundance between $FLG^{+/+}$ and $FLG^{-/-}$ genotypes for the genera *Finegoldia, Anaerococcus,* and *Peptoniphilus.* Horizontal arrows indicate significant differences (Mann-Whitney *U* test). In $FLG^{+/-}$ individuals, these genera tended to be present at intermediate relative abundance quantities. **B,** Box plots with relative abundances of the candidate *hut* genes for $FLG^{+/+}$ and $FLG^{-/-}$ genotypes.

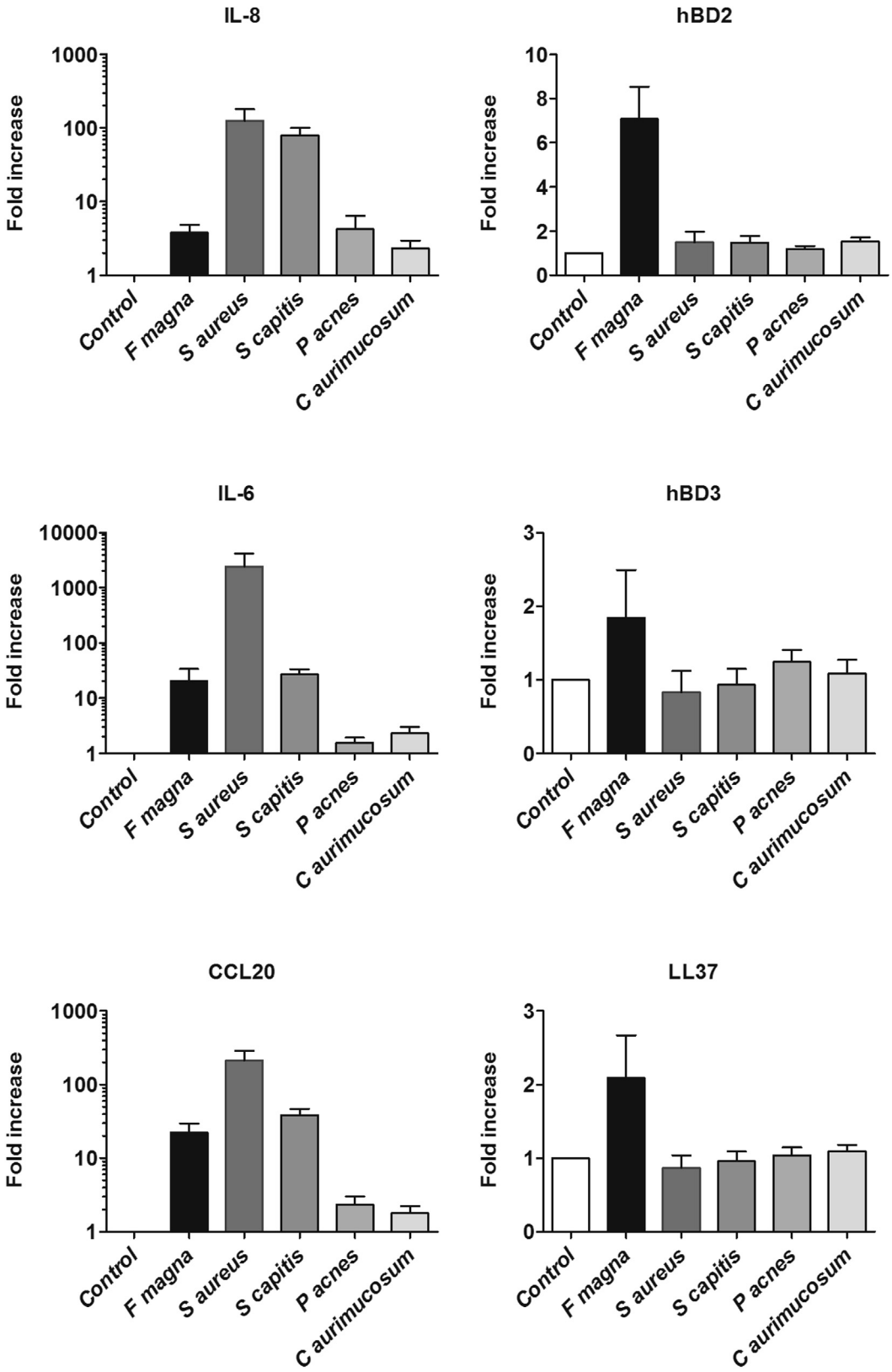

FIG 2. Keratinocyte responses differ between bacterial stimulations. *FLG*$^{+/+}$ keratinocyte cultures (n = 10) were stimulated for 10 hours with bacteria. Expression of 6 host defense genes was measured by quantitative PCR. For each gene, the unstimulated cell culture was set to 1. Data are represented as mean ± SEM. For statistics (repeated-measures ANOVA with Bonferroni *post hoc* test), see Table E5.

cocci (GPAC), and a general underrepresentation of bacterial taxa that are capable of using histidine. Second, we report that exposure of cultured epidermal keratinocytes to *Finegoldia magna* induces the expression of antimicrobial proteins and proinflammatory cytokines, and that this response is distinct from other skin commensals such as *Propionibacterium acnes, Corynebacterium aurimucosum*, and *Staphylococcus capitis*, and the skin pathogen *Staphylococcus aureus*.

All experimental procedures were performed as described in this article's Online Repository at www.jacionline.org. We analyzed the skin microbiota of the lower leg, which is typically a location where the ichthyotic skin alterations (dry and scaly skin) are most prominent. Patients and healthy controls were genotyped for *FLG* mutations (see Table E1 and Fig E1 in this article's Online Repository at www.jacionline.org). None of the patients with IV in our study had eczematous lesions present at the lower leg, thereby excluding (lesional) AD-associated microbiome alterations as a confounding factor. Biophysical measurements showed increased transepidermal water loss and decreased SC hydration in $FLG^{-/-}$ subjects compared with $FLG^{+/+}$ subjects (see Fig E2 in this article's Online Repository at www.jacionline.org). Microbiome samples were subsequently analyzed by barcoded 16S marker gene sequencing (see Tables E2 and E3 in this article's Online Repository at www.jacionline.org). Rarefaction curves show that the phylogenetic diversity does not differ between $FLG^{+/+}$, $FLG^{+/-}$, and $FLG^{-/-}$ samples (see Figs E3 and E4 in this article's Online Repository at www.jacionline.org). Redundancy analysis revealed a significant effect of FLG deficiency on microbiota composition (see Fig E5 in this article's Online Repository at www.jacionline.org). A lower relative abundance of GPAC (average 1.7% of the total genera) was found in $FLG^{-/-}$ skin compared with $FLG^{+/+}$ skin (average 9.3% of the total genera) (see Fig E6 and Excel file E1 in this article's Online Repository at www.jacionline.org). These genera included *Finegoldia*, *Anaerococcus*, and *Peptoniphilus*. Other highly abundant genera did not show significant differences between the genotypes (Excel file E1). We therefore focused on members of the GPAC group for further *in silico* analysis and experimental studies. Most GPAC are asaccharolytic and use the products of protein degradation as substrates for metabolic energy generation.[8] The average relative quantities of these candidate-discriminating genera supported a significant difference between the FLG-deficient and FLG-proficient state, or more specifically, a strong decrease by FLG protein loss (Fig 1, *A*). Indeed, an *in vitro* survival disadvantage of *F magna* on FLG-deficient SC supported the *in vivo* findings (see Figs E7 and E8 in this article's Online Repository at www.jacionline.org). On the basis of bacterial 16S profiles of the skin microbiota, we tested the potential involvement of microbial reactions using natural moisturizing factors such as histidine, urocanic acid, and pyrrolidone carboxylic acid. Histidine is a known carbon source for bacteria that possess the histidine utilization (Hut) pathway (see Fig E9 in this article's Online Repository at www.jacionline.org), which is highly conserved among bacteria and found with high frequency in most phylogenetic groups within the bacterial domain. Contrast analysis of IV versus healthy skin with selected *hut* gene candidates (see Table E4 in this article's Online Repository at www.jacionline.org) revealed that *hutU*, *hutI*, and *hutH* orthologous genes were predicted to be significantly underrepresented in the microbiota of patients with IV (Fig 1, *B*; and Excel file E2 in this article's Online Repository at www.jacionline.org). In conclusion, our study reveals that FLG-deficient skin harbors significantly fewer bacterial taxa capable of using histidine as nutrient (carbon) source, which we postulate to be a consequence of nutrient competition driven by the loss of the histidine-rich protein FLG.

Because microbial factors such as *S aureus* colonization have been implicated in AD,[9] we explored possible interactions of this pathogen and several skin commensals, including *F magna*, with epidermal keratinocytes (Fig 2; and Table E5 in this article's Online Repository at www.jacionline.org). *F magna* induced a significantly higher expression of antimicrobial peptides (AMPs) compared with *S aureus* and the other skin commensals. For *S aureus* and *S capitis*, keratinocyte stimulation caused a significantly higher expression of proinflammatory cytokines than was observed for stimulation with *F magna*. Our observation that keratinocytes appear to exhibit different cytokine/AMP responses toward *S aureus* or *F magna* stimulation is intriguing. Given the known functions and activities of IL-8 (neutrophil chemoattractant), IL-6 (inducer of inflammatory responses), and the chemokine ligand 20 (CCL20, chemoattractant for various white blood cell types, antimicrobial activity), their strong induction by *S aureus* contributes to control of infection. The observed rapid induction of AMPs by *F magna*, however, suggests that this may be an important signaling mechanism to the keratinocytes when the skin barrier is breached and a commensal bacterium comes into close contact with the epidermal keratinocytes. Complete or partial absence of *F magna* could cause an impaired or delayed danger signaling to the keratinocytes in $FLG^{-/-}$ or $FLG^{+/-}$ individuals. This could, speculatively, be a mechanism that favors *S aureus* colonization or infection, but clearly requires further investigation.

In conclusion, we have uncovered new and potentially important biological aspects of a very common genetic polymorphism. Our study has shown this to be the case for the homozygous-deficient FLG state, but our data on heterozygotes suggest a gene dosage effect. This generates new and testable hypotheses to investigate a contribution of microbiome alterations in the pathogenesis of AD.

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