Impaired β-Defensin Expression in Human Skin Links DEFB1 Promoter Polymorphisms With Persistent Staphylococcus aureus Nasal Carriage

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Background. Genetically determined variation in the expression of innate defense molecules may explain differences in the propensity to be colonized with Staphylococcus aureus.

Methods. We determined S. aureus nasal carriage in 603 volunteers; analyzed polymorphisms in the DEFB1 promoter at positions −52 G>A (rs1799946), −44 C>G (rs1800972), and −20 G>A (rs11362); and measured the content of human β-defensin 1 (hBD-1) and hBD-3 messenger RNA (mRNA) in 192 samples of healthy and experimentally wounded human skin.

Results. Compared with GGG at the positions −52/−44/−20, the ACG haplotype was more common among persistent S. aureus nasal carriers (odds ratio, 1.93; 95% confidence interval [CI], 1.2–3.1; P = .006) and was associated with reduced expression of hBD-1 (GGG>ACG>GCA; P < .001) and hBD-3 (GGG>GCA>ACG; P = .04) in skin when measured 72 hours after wounding. Furthermore, a 50% decrease in hBD-1 and hBD-3 mRNA expression in wounded skin increased the odds of persistent carriage by 1.45 (95% CI, .93–2.26; P = .1) and 1.48 (95% CI, 1.01–2.17; P = .04), respectively. Adjustment for known risk factors of persistent S. aureus carriage did not substantially change the associations of both DEFB1 haplotypes and β-defensin expression with S. aureus colonization.

Conclusions. DEFB1 polymorphisms may promote persistent S. aureus colonization by altering β-defensin expression in keratinocytes of human skin.

Keywords. genetic association studies; host-pathogen interactions; nasal colonization; antimicrobial cationic peptides; beta-defensins; single nucleotide polymorphism; innate immunity; Staphylococcus aureus.
this AMP in defense against S. aureus after disruption of the skin’s physical barrier. By contrast, hBD-1 is less active than hBD-3 against S. aureus but is constitutively expressed in human skin without prior stimulation [17]. Interestingly, both induced hBD-3 [14] and constitutive hBD-1 [18, 19] levels show marked interindividual variation, allowing researchers to hypothesize about the role of genetic determinants of hBD expression as determinants of susceptibility to colonization and infection.

In line with this hypothesis, we have reported that patients with more-severe S. aureus infections have lower levels of hBD-3 messenger RNA (mRNA) in lesional skin [20]. By using wounding of extranasal skin as an experimental stimulus to simulate the inflammatory response in the nares of S. aureus carriers [6], we could also demonstrate that impaired hBD-3 induction is associated with persistent S. aureus nasal carriage [15]. A recent study that tried to link S. aureus nasal colonization with DEFB103 copy number, however, failed to demonstrate an association [21], suggesting that other sources of genetic variability are important in defining altered hBD-3 expression.

Polymorphisms in the 5′ untranslated region of DEFB1—a likely nuclear factor κ B (NFκB) binding site [22]—were proposed to regulate β-defensin expression in human epithelia. Kalus et al have described a strong influence of this promoter region on hBD-1 and -3 expression in cultured gingival keratinocytes [23]. This is of interest because single-nucleotide polymorphisms (SNPs) in this region were also reported as a risk factor for Pseudomonas aeruginosa and Candida albicans colonization [24, 25], meningococcal disease [26], and leprosy [22]. Obviously, genes that are transcriptionally regulated by the promoter in the 5′ untranslated region upstream of DEFB1 are coding for components of the human immune system that play a key role in the defense against clinically relevant pathogens.

On the basis of these findings, we hypothesize about a close link between DEFB1 promoter polymorphisms, transcriptional regulation of β-defensin expression in keratinocytes, and the persistent S. aureus nasal colonization phenotype. To our knowledge, an association between DEFB1 polymorphisms and S. aureus infection and colonization has not been reported. Similarly, we are not aware of studies on the influence of genetic variability in the DEFB1 promoter region on human β-defensin expression in human skin in vivo. Most importantly, candidate gene association studies on DEFB1 that simultaneously assess associations of DEFB1 genotype and infectious diseases phenotype with a biological meaningful intermediate have not been published. Such research, however, is needed to strengthen evidence in favor of a biological relationship and render genotype-phenotype associations based on mere genetic linkage less likely.

To this end, we used a population-based, cross-sectional sample of volunteers to study the association of SNPs at positions −52G>A (rs1799946), −44C>G (rs1800972), and −20G>A (rs11362) in the 5′ UTR of DEBB1 with both persistent S. aureus nasal carriage and hBD-1 and hBD-3 expression in healthy and experimentally wounded gluteal skin. Moreover, we newly investigated the association between hBD-1 mRNA expression and persistent carriage and repeated this analysis for hBD-3 to demonstrate consistency with what we reported earlier [15].

**METHODS**

**Study Population**

Between May 2009 and March 2011, volunteers were attracted through public advertising and screened for nasal carriage of S. aureus by taking 4 sequential swabs from the anterior nares. For inclusion, subjects had to reside within 15 km of the study center. Subjects with human immunodeficiency virus infection, diabetes mellitus, immunosuppressive medication, active malignancy, renal insufficiency, or other forms of chronic immunosuppression were not included. At the first visit, information on demographic characteristics, potential confounders, and self-reported race were collected. Four consecutive S. aureus–positive and S. aureus–negative swab specimens defined persistent nasal carriage and noncarriage, respectively. All persistent nasal carriers and a random sample of 2 nasal noncarriers per persistent carrier (without matching) were invited to provide 20 mL of blood. Individuals who were not white and those who were classified as intermittent carriers were not considered for this study. Those consenting to provide blood were also asked to provide 1 sample of healthy and 1 sample of wounded gluteal skin. After taking the specimen of healthy skin (by means of a 4 × 7-mm biopsy punch, Kai Medical), the contralateral gluteal region was wounded using a 1.5 × 7-mm biopsy punch. A mean duration (±SD) of 72 ± 2 hours later, the center of this lesion was excised by a second 4 × 7-mm punch-biopsy specimen. All procedures were performed under aseptic conditions.

**Microbiology Procedures**

The presence of S. aureus in the nares was detected using a selective medium (mannitol salt agar; Oxoid) and confirmed by a latex agglutination test (StaphPlus; DiaMondiAL) or a tube coagulation test (BBL coagulase plasma; Becton Dickinson) as described elsewhere [15].

**DEFB1 Genotyping**

SNPs in the 5′ UTR of DEFB1 were genotyped by fluorescence resonance energy transfer (FRET) and the allelic discrimination principle on a Rotor-Gene 3000 (Qiagen). An asymmetrical quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed in a 20-µL reaction volume using the SensiFAST Probe No-ROX 2× mix, according to the manufacturer’s protocol (Bioline). The cycling conditions were as follows: denaturation at 95°C for 5 minutes, followed by 40 cycles at 95°C for 20 seconds and at 60°C for...
50 seconds. The melting curves were obtained by temperature increments of 1°C every 10 seconds, from 40°C to 85°C. The primers and probes were designed by TibMolbiol, Germany (Supplementary Table 1). In a subset, base calling by FRET was verified through DNA sequencing.

**Human β-Defensin Expression in Healthy and Wounded Skin**

Skin specimens were stored for 48 hours in an RNA-stabilizing reagent (RNAlater, Ambion) and frozen at −80°C until analysis. RNA extraction, complementary DNA synthesis, and real-time PCR were performed as described elsewhere [20], with the modification that only 50 ng of solved RNA were used (primers are specified in Supplementary Table 1). In each run, equal numbers of samples of carriers and noncarriers were amplified simultaneously as triplicates. The cycle-to-cycle fluorescence emissions were analyzed using the threshold cycle (CT) method. The CT value of a specific sample was determined using the comparative quantitation analysis tool, Rotor-Gene 3000 (Qiagen). ΔCT values giving an estimate of the relative expression of the target gene relative to β-actin were calculated by subtracting the β-actin value from the AMP CT value.

**Statistical Analysis**

The associations of *DEFB1* polymorphisms (exposure) with persistent nasal *S. aureus* carriage (outcome) and β-defensin mRNA expression (intermediate) were analyzed using χ² tests, 2-sided t tests, and logistic and linear regression analyses. Differences in AMP expression (intermediate) by *S. aureus* nasal carriage (outcome) were analyzed using the ΔΔCT method and then, for more intuitive presentation, transformed into ratios by calculating 2−ΔΔCT as described elsewhere [15]. Multivariable logistic regression models were fitted to adjust both the association of haplotypes (exposure) and of β-defensin expression (intermediate) with persistent nasal carriage (outcome) for potential confounding by known risk factors of persistent *S. aureus* nasal carriage (current smoking, age, sex, and hormonal contraceptive use). All variables were checked for a normal distribution and were log transformed if required. Haplotypes were reconstructed from population genotype data, using Phase software, version 2.1 [27, 28]. All statistical procedures were done using Stata 11 (StataCorp).

**Ethics**

All participants gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Tübingen Medical School (no. 145/2009BO2).

**RESULTS**

**Study Population**

A total of 603 volunteers were screened for *S. aureus* nasal carriage and provided 325 blood and 192 skin samples (Figure 1). Carriers tended to be younger, and female carriers were more likely to use hormonal contraception (Table 1).

**Association of *DEFB1* Polymorphisms With Persistent *S. aureus* Carriage**

In the total sample of persistent carriers and noncarriers, the genotype frequencies at all 3 positions were in Hardy-Weinberg equilibrium, and there was strong linkage disequilibrium between these 3 loci (Figure 2). The frequency distribution of *DEFB1* genotypes and alleles at positions −52 and −44 differed significantly between persistent carriers and noncarriers (Table 2). At position −52 (G>A), the minor allele was more often found in persistent carriers, and each minor allele more in the genotype increased the odds of persistent nasal carriage by 1.62-fold. Accordingly, subjects who were homozygous for the minor allele at position −52 (A/A) had 2.73-times the odds of persistent carriage as compared to those homozygous for the major allele (G/G) at this position (Table 2). In contrast, the minor allele at position −44 (C>G) was associated with protection from *S. aureus* nasal colonization. There was borderline statistical evidence that each additional minor allele in an individual’s genotype at position −44 decreased the odds of persistent carriage by 0.65. No such associations were present for the genotypes and alleles at position −20. In concordance with findings among German blood donors [29], only 3 haplotypes could be reconstructed. By using GGG at the positions −52/−44/−20 as a reference, the odds of persistent *S. aureus* colonization of the nares were 29% higher for the GCA and 93% higher for the ACG haplotype. Adjustment of the haplotype analysis for known risk factors for *S. aureus* nasal carriage (ie, age, sex, hormonal contraceptive use, and current smoking) somewhat strengthened the association of the ACG haplotype with persistent nasal carriage (adjusted odds ratio, 1.99; 95% confidence interval [CI], 1.23–3.21; P = .005).

**Association of *DEFB1* Polymorphisms With β-Defensin Expression in Human Skin**

As reported before [14, 15], hBD-3 mRNA levels were induced 72 hours after wounding and were about 4.80 times the level found in healthy skin of the same individual (95% CI, 4.00–5.75; P < .001 by the paired t test for H0, the mRNA levels in healthy and wounded skin are the same). By contrast, hBD-1 transcription in wounded skin was on average only 0.47 times the level found in healthy skin (95% CI, 0.42–0.54; P < .0001).

The mean hBD mRNA expression levels in healthy and wounded skin varied substantially when grouped by alleles, genotypes and haplotypes of the *DEFB1* 5′ UTR (Table 2). For hBD-1, the SNPs at positions −52 (G>A) and −44 (C>G) were associated with higher mRNA expression levels in both healthy and wounded skin. By contrast, those subjects with the minor allele at position −20 (G>A) showed lower levels of
hBD-1 mRNA in both types of skin samples. Consequently, individuals with the −52/−44/−20 haplotype GCA, which combines the major alleles at positions −52 and −44 with the minor allele at position −20, had about 50% lower mean hBD-1 mRNA levels in healthy and wounded skin as compared to subjects with GGG. For hBD-3, there was no association between basal expression and DEF1 SNPs. However, in wounded skin, hBD-3 mRNA levels of subjects homozygous for the minor allele at position −52 (G/G) were on average 50% lower than those of subjects homozygous for the major allele (A/A), whereas there was a trend toward a reverse association at position −20. Most interestingly, the ACG haplotype, which was found to be strongly associated with persistent nasal carriage, was at the same time associated with lower hBD-3 and hBD-1 transcription levels in wounded human skin.

### Table 1. Baseline Characteristics of Study Subjects With or Without Nasal Carriage of Staphylococcus aureus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No (n)</th>
<th>Yes (n)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>62 (136/219)</td>
<td>60 (59/106)</td>
<td>.3</td>
</tr>
<tr>
<td>Hormonal contraception use&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51 (69/136)</td>
<td>71 (42/59)</td>
<td>.008</td>
</tr>
<tr>
<td>Current smoking</td>
<td>19 (42/219)</td>
<td>16 (17/106)</td>
<td>.5</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.1 ± 8.6</td>
<td>25.5 ± 6.3</td>
<td>.02</td>
</tr>
<tr>
<td>Time from first to fourth swab, d</td>
<td>75.6 ± 40.4</td>
<td>74.6 ± 45.9</td>
<td>.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are % (proportion) of individuals or mean ± SD.  
<sup>a</sup> By the χ² test or 2-sided t test.  
<sup>b</sup> Data are for women only.  
<sup>c</sup> By the 2-sided t test, after logarithmic transformation.

### Association of β-Defensin Expression and Persistent S. aureus Nasal Carriage

The association of lower hBD-3 mRNA levels in the skin with persistent carriage remained reproducible and consistent with our previous findings, even when 72 additional skin biopsy specimens were included (Table 3) [15]. As a new result, we found an on average 24% lower hBD-1 transcription in wounded skin of persistent S. aureus nasal carriers as compared to wounded skin of noncarriers (Table 3)—a trend that became statistically significant after adjustment for known risk factors of S. aureus nasal carriage (Table 3).
translational activity may biologically link DEFB1 polymorphisms to the persistent carriage phenotype. Indeed, this consecutive work demonstrates substantial variation in AMP expression in healthy and wounded skin, depending on DEFB1 alleles, genotypes, and haplotypes. Moreover, we could confirm our initial observation of hBD-3 expression being associated with persistent nasal carriage in a study population that was substantially enlarged as compared to the population in the initial study [15]. Most importantly, we show that the association of hBD-3 and hBD-1 levels in wounded skin and the odds of persistent carriage are inversely associated with genetic polymorphisms at position −52 (G>A) and −44 (C>G) and with the −52/−44/−20 ACG haplotype. Taken together, these observations lend support to a concept of DEFB1 promoter polymorphisms being the genetic basis of altered hBD expression and provide evidence in favor of impaired hBD expression being the biological link between DEFB1 SNPs and persistent S. aureus colonization.

The findings reported here are consistent with previous reports that linked DEFB1 SNPs with an increased risk of colonization with pathogens other than S. aureus [24, 25]. Similar to what we report, the −52 AA genotype [24] and the −44 CC genotype [25], as well as the ACG haplotype [24], were found to increase the risk of colonization with P. aeruginosa and C. albicans, respectively. In line with findings of the group studying C. albicans [25], however, we did not observe an effect of polymorphisms at position −20 of the 5′ UTR of DEFB1 on colonization reported in the study on P. aeruginosa [24].

In contrast to the presented findings, one earlier study that investigated S. aureus nasal carriage and DEFB1 polymorphisms at position −44 failed to demonstrate an association with this single SNP [30]. That study, however, used a much smaller sample and did not study position −52, which we observed to contribute strongest to both hBD-3 and S. aureus carriage phenotype. Hence, difference in statistical power and choice of SNP are likely to explain the apparent discrepancy between that study and our report.

Human β-defensins are expressed in keratinocytes that line the anterior human nares—the proposed site of S. aureus nasal colonization [1]. In particular, hBD-3 shows marked potency in killing S. aureus within the micromolar range [12, 31] and is further inducible in keratinocytes and skin upon various stimuli [12, 14, 32]. Moreover, hBD-3 was found to be essential for the constitutive capacity of keratinocytes to kill S. aureus in vitro [33]. In line with these observations, we describe persistent nasal colonization being most strongly associated with those DEFB1 alleles, genotypes, and haplotypes that were linked with substantially lower hBD-3 mRNA expression.

In a murine model, the expression of hBD-1 peaked 6 hours after superficial wounding but tended to return to baseline when measured again at 16 hours after the intervention [34]. We found that, irrespective of S. aureus carriage, 72
hours after wounding, human skin contained actually lower relative hBD-1 mRNA levels, compared with levels at baseline. Taken together, this early peak in hBD-1 expression, followed by a progressive downregulation during the later phase of wound healing is, as proposed earlier [35], consistent with constitutively expressed AMPs being of particular importance for the skin’s early response against pathogens.

Interestingly, the lower levels of hBD-1 mRNA in wounded skin 72 hours after wounding differed not only by DEFB1 polymorphism but were also significantly lower in wounded skin of persistent S. aureus nasal carriers as compared to non-carriers (Table 3). However, hBD-1 transcriptional activity in healthy skin, although strongly associated with DEFB1 promoter polymorphisms (Table 2) and, most notably, higher

<table>
<thead>
<tr>
<th>SNP</th>
<th>Nasal Carriage</th>
<th>hBD-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>hBD-3&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (n = 112)</td>
<td>Wounded (n = 80)</td>
<td>Healthy (n = 106)</td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>P</td>
<td>Ratio</td>
</tr>
<tr>
<td>−52 G&gt;A</td>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>0.49 (107)</td>
<td>0.35 (37)</td>
<td>1.00</td>
</tr>
<tr>
<td>G/A</td>
<td>0.43 (94)</td>
<td>0.49 (52)</td>
<td>1.60</td>
</tr>
<tr>
<td>A/A</td>
<td>0.08 (18)</td>
<td>0.16 (17)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.70 (308)</td>
<td>0.59 (126)</td>
<td>1.00</td>
</tr>
<tr>
<td>A</td>
<td>0.30 (130)</td>
<td>0.41 (86)</td>
<td>0.006</td>
</tr>
<tr>
<td>−44 C&gt;G</td>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>0.58 (127)</td>
<td>0.69 (73)</td>
<td>1.00</td>
</tr>
<tr>
<td>C/G</td>
<td>0.36 (79)</td>
<td>0.28 (30)</td>
<td>0.66</td>
</tr>
<tr>
<td>G/G</td>
<td>0.06 (13)</td>
<td>0.03 (3)</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.76 (333)</td>
<td>0.83 (176)</td>
<td>1.00</td>
</tr>
<tr>
<td>G</td>
<td>0.24 (105)</td>
<td>0.17 (36)</td>
<td>0.04</td>
</tr>
<tr>
<td>−20 G&gt;A</td>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>0.28 (60)</td>
<td>0.33 (35)</td>
<td>1.00</td>
</tr>
<tr>
<td>G/A</td>
<td>0.52 (115)</td>
<td>0.49 (52)</td>
<td>0.78</td>
</tr>
<tr>
<td>A/A</td>
<td>0.20 (44)</td>
<td>0.18 (19)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.54 (235)</td>
<td>0.58 (122)</td>
<td>1.00</td>
</tr>
<tr>
<td>A</td>
<td>0.46 (203)</td>
<td>0.42 (90)</td>
<td>0.4</td>
</tr>
<tr>
<td>−52/−44/−20</td>
<td><strong>Haplotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGG</td>
<td>0.24 (105)</td>
<td>0.17 (36)</td>
<td>1.00</td>
</tr>
<tr>
<td>GCA</td>
<td>0.46 (203)</td>
<td>0.42 (90)</td>
<td>1.29</td>
</tr>
<tr>
<td>ACG</td>
<td>0.30 (130)</td>
<td>0.41 (86)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; mRNA, messenger RNA; OR, odds ratio; SNP, single-nucleotide polymorphism.

<sup>a</sup> By the χ<sup>2</sup> test.

<sup>b</sup> From univariable logistic regression analysis comparing the odds of persistent S. aureus carriage for individuals with a given genotype/allele/haplotype with the odds among subjects with the baseline genotype/allele/haplotype.

<sup>c</sup> From univariable logistic regression analysis.

<sup>d</sup> For linear trend over genotypes/haplotypes.

<sup>e</sup> Ratios denote comparisons of the mean mRNA (mRNA) content in skin of individuals with a given genotype/allele/haplotype vs the mRNA content in skin of subjects with the baseline genotype/allele/haplotype. For alleles, P values are from 2-sided t tests for an H0 of no difference in the mean mRNA expression; for genotypes and haplotypes, P values are from linear regression of mRNA expression on genotype/haplotype testing for an H0 of β = 0.

<sup>f</sup> Assuming hBD-1 expression follows GGG>ACG>GCA.

<sup>g</sup> Assuming hBD-3 expression follows GGG>GCA>ACG.

Table 2. DEFB1 Polymorphisms, Persistent Staphylococcus aureus Nasal Carriage, and Human β-Defensin (hBD) Expression in Skin

DEFB1 SNPs and S. aureus Colonization • JID 2013:207 (15 February) • 671
overall than that in wounded skin, was not associated with persistent nasal *S. aureus* carriage (Table 3). Therefore, and in contrast to our findings on hBD-3, our observations do not support that hBD-1 is of biological importance in linking DEFB1 promoter polymorphisms with *S. aureus* colonization of the nares—an interpretation that is consistent with existing knowledge on hBD-1 that shows only low activity against *S. aureus* at physiologic concentrations [17].

Nevertheless, the variation of both hBD-1 and hBD-3 transcription in wounded skin by 5′ UTR haplotype of DEFB1 suggests a role of this region in the transcriptional regulation of both β-defensin genes—a hypothesis put forward earlier in a study on hBD expression in cultured keratinocytes [23]. In fact, Prado-Montes de Oca et al proposed the 5′ UTR region of DEFB1 as putative NF-κB binding site [22]. This is of relevance because Menzies et al observed a marked influence of NF-kB blocking on hBD-3 induction in keratinocytes stimulated with *S. aureus* [32]. The apparent lack of NF-kB binding sites in the direct vicinity of the hBD-3 promoter, however, gave rise to speculations about NF-kB binding motifs nearby that may control hBD-3 gene expression [32]. Our findings lend support to this earlier notion and suggest that, because of its location directly upstream of the defensin gene cluster on chromosome 8p23.1, variation in NF-kB binding in the polymorphic 5′ UTR region of DEFB1 has also an influence on the transcription of the nearby DEFB103 gene coding for hBD-3.

This study has some limitations. First, hBD expression has not been determined at the protein level, and the mRNA expression may not necessarily reflect the protein level in tissues [34]. For hBD-3, however, concordance of mRNA and protein expression 72 hours after wounding was convincingly demonstrated [14], thus strengthening confidence that our findings on mRNA correspond to equivalent hBD-3 peptide concentrations in human skin. Second, it would have been desirable to use living *S. aureus* to stimulate β-defensin induction in vivo. Such an approach, however, is difficult to standardize and, most importantly, raises serious ethical questions. As pointed out earlier by our group [15], wounding offers an attractive alternative for studying genetic determinants of hBD-3 inducibility in vivo. Third, our finding of an association between persistent nasal carriage and DEFB1 polymorphisms may be the mere reflection of linkage disequilibrium between polymorphisms in the 5′ UTR of DEFB1 and a functional locus nearby. However, because these promoter polymorphism also had an effect on the expression of hBD-3—an antimicrobial peptide that was found to be essential for the constitutive capacity of human keratinocytes to kill *S. aureus* in vitro [33]—our observations support a functional role of DEFB1 genotypes in determining the *S. aureus* carriage phenotype. Nevertheless, replication of our findings in other populations and ethnicities will be necessary to further strengthen this interpretation. Finally, it should be noted that persistent *S. aureus* carriage is a complex trait. Thus, the relative importance of DEFB1 polymorphisms, compared with the importance of other known [36–38] and as yet unknown genes involved in defining an individual’s *S. aureus* nasal carriage phenotype, remains to be determined.

In summary, we present evidence for associations between DEFB1 5′ UTR polymorphisms, β-defensin expression, and persistent *S. aureus* nasal carriage. Genetic polymorphisms associated with lower hBD-3 expression were more often found in subjects that were persistently colonized with *S. aureus*.

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**Table 3. Human β-Defensin (hBD) Expression and Persistent *Staphylococcus aureus* Nasal Carriage**

<table>
<thead>
<tr>
<th>hBD Type, Specimen</th>
<th>mRNA Expression, ΔCt</th>
<th>S. aureus Nasal Carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall Carriers Noncarriers Ratio P Crude OR (95% CI)</td>
<td>P Adjusted OR (95% CI)</td>
</tr>
<tr>
<td>hBD-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy skin (n = 112)</td>
<td>3.87 3.85 3.89 1.03 .8</td>
<td>0.95 (.62–1.45) .8</td>
</tr>
<tr>
<td>Wounded skin (n = 80)</td>
<td>5.02 5.25 4.81 0.76 .1</td>
<td>1.45 (1.93–2.26) .1</td>
</tr>
<tr>
<td>hBD-3β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy skin (n = 106)</td>
<td>8.72 8.86 8.59 0.83 .2</td>
<td>1.30 (.89–1.92) .2</td>
</tr>
<tr>
<td>Wounded skin (n = 80)</td>
<td>6.44 6.75 6.15 0.66 .04</td>
<td>1.48 (1.01–2.17) .04</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; Ct, threshold cycle; mRNA, messenger RNA.

* Data are mean values and calculated as the Ct of the target gene minus the Ct of β-actin, where the Ct was determined by quantitative real-time reverse transcription polymerase chain reaction. Thus, a higher ΔCt indicates lower expression of target gene relative to expression of β-actin.

b Denotes a comparison of mRNA expression in nasal carriers vs that in noncarriers, using 2^−ΔΔCt (Ct for carriers – mean ΔCt for noncarriers).

c By the 2-sided test.

d By univariable logistic regression analysis, expressing the increase in the odds of *S. aureus* nasal carriage per 50% decrease in mRNA expression.

* By multivariable logistic regression analysis, with adjustment for age, sex, hormonal contraceptive use, and current smoking.

f Some of these data have been published elsewhere [15].
Colonized individuals, in turn, had lower hBD levels in their skin as compared to those who never had *S. aureus* in their nares. On the basis of these observations and existing knowledge of a marked activity of hBD-3 in killing *S. aureus*, we propose a causal relationship between DEFB1 promoter polymorphisms and the persistent *S. aureus* colonization phenotype, with reduced hBD-3 expression being the likely biological link.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts.

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**References**