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Staphylococcus aureus and the ecology of the nasal microbiome

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The human microbiome can play a key role in host susceptibility to pathogens, including in the nasal cavity, a site favored by *Staphylococcus aureus*. However, what determines our resident nasal microbiota—the host or the environment—and can interactions among nasal bacteria determine *S. aureus* colonization? Our study of 46 monozygotic and 43 dizygotic twin pairs revealed that nasal microbiota is an environmentally derived trait, but the host's sex and genetics significantly influence nasal bacterial density. Although specific taxa, including lactic acid bacteria, can determine *S. aureus* colonization, their negative interactions depend on thresholds of absolute abundance. These findings demonstrate that nasal microbiota is not fixed by host genetics and opens the possibility that nasal microbiota may be manipulated to prevent or eliminate *S. aureus* colonization.

REPORT

Our familiarity with the exterior of the nose belies the intriguing puzzle within. Individuals can have distinctive susceptibilities to nasal colonization by *Staphylococcus aureus*, a major pathogen (*1*); yet, it also appears that host genetics is not a significant determinant of *S. aureus* nasal colonization (*2*). How can this be? One potential explanation is that an individual's susceptibility to *S. aureus* nasal colonization is driven by an environmentally determined phenotype. To satisfy this explanation, the phenotype should have limited association with host genetics, but it should predict *S. aureus* nasal colonization. Because the human microbiome is increasingly considered a host phenotype (*3*–*5*), we examined the potential role of nasal microbiota in *S. aureus* nasal colonization. Testing our hypothesis provided useful insight into the malleability of nasal microbiota and explanations for previous contradictory findings regarding *S. aureus'* negative association with nasal bacteria such as *Propionibacterium* and *Staphylococcus epidermidis* (*6*–*10*). To test our hypothesis, we enrolled and analyzed the nasal microbiome of 46 monozygotic and 43 dizygotic twin pairs from The Danish Twin Registry.

Nasal bacterial density (that is, the total amount of nasal bacteria present) and microbiota composition (that is, the types and proportions of bacteria present in the nasal microbiota) were highly diverse among 178 healthy, community-dwelling middle-aged adults (Table 1). The median nasal bacterial density was 4.4×10^6 16*S* ribosomal RNA (rRNA) gene copies per swab, and it spanned nearly four orders of magnitude, from 6.7×10^5 to 2.1×10^9 16*S* rRNA gene copies per swab [interquartile range (IQR): 1.6×10^6 to 1.7×10^7]. Many bacteria were found in large proportions of subjects, such as *Corynebacterium* (n = 157/178, 88.2%), *Propionibacterium acnes* (n = 149/178, 83.7%), and *S. epidermidis* (n = 161/178, 90.4%), but proportional abundance varied substantially across individuals, contributing to distinctive microbiota compositions. We identified seven major nasal community state types (CST1 to CST17) among our participants (Fig. 1, A to C). Each CST had a uniquely high prevalence and proportional abundance of specific nasal bacteria, as identified by indicator analysis: *S. aureus* defined CST1, Enterobacteriaceae—including *Escherichia* spp., *Proteus* spp., and *Klebsiella* spp.—defined CST2, *S. epidermidis* defined CST3, *Propionibacterium* spp. defined CST4, *Corynebacterium* spp. defined CST5, *Moraxella* spp. defined CST6, and *Dolosigranulum* spp. defined CST7 (table S1). The most prevalent nasal CST was CST4 (n = 51/178, 28.7%), followed by CST3 (n = 40/178, 22.5%) and CST1 (n = 22/178, 12.4%). CST6 was the least common, with only 5.6% prevalence (n = 10/178) (table S2). Thus, our study revealed distinctive nasal CSTs and greater nasal microbiota heterogeneity than previously reported (*6*–*10*), particularly among Enterobacteriaceae, of which *Proteus* and *Serratia* were not previously known to dominate the nasal microbiota.

Was nasal microbiota significantly associated with host genetics?

Host genetics played no significant role in nasal microbiota composition. Among monozygotic twin pairs, only 26.1% had the same nasal CSTs (n =12/46) (Fig. 1C), which was comparable to the 25.6% among dizygotic twin pairs (n = 11/43) (fig. S1, A to C). We confirmed the limited similarity in nasal microbiota composition of monozygotic twin pairs by ecological distance–based analysis, where we found that nasal microbiota of monozygotic twins were not more similar than all or same-sex dizygotic twins, or than unrelated same-sex pairs (table S3).

In contrast, host genetics and nasal bacterial density were significantly linked. Nasal bacterial densities of monozygotic twin pairs were significantly more correlated than those of dizygotic twin pairs [sex- and age-adjusted intraclass correlation coefficient (ICC) in monozygotic twins: 0.42, 95% confidence interval (CI): 0.12 to 0.65, and in dizygotic twins: −0.06, 95% CI: −0.35 to 0.23]. The variations in nasal bacterial density were best explained by a model that comprised additive genetic and nonshared environmental effects (table S4). About 30% of the variation in nasal bacterial density was heritable (95%

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Table 1. Participant demographics and characteristics.

	Monozygotic (n = 46 pairs)	Dizygotic	
		Same sex (n = 23 pairs)	Opposite sex (n = 20 pairs)
	Number of individuals or twin pairs (%)		
Age (years)			
50–54	12 (26.1)	0 (0.0)	0 (0.0)
55–59	13 (28.3)	0 (0.0)	0 (0.0)
60–64	7 (15.2)	4 (17.4)	1 (5.0)
65–69	9 (19.6)	4 (17.4)	11 (55.0)
70–74	3 (6.5)	12 (52.2)	7 (35.0)
75–79	2 (4.4)	3 (13.0)	1 (5.0)
Sex			
Female	25 (54.4)	16 (69.6)	20 (100.0)
Male	21 (45.6)	7 (30.4)	
Smoking			
Smoker	14 (15.2)	9 (19.6)	10 (25.0)
Concordance	40 (87.0)	16 (69.6)	12 (60.0)
History of atopic disease*			
Yes	27 (29.3)	14 (30.4)	13 (32.5)
Concordance	29 (63.0)	15 (65.2)	11 (55.0)
History of psoriasis			
Yes	8 (8.7)	2 (4.3)	5 (12.5)
Unknown	2 (2.2)	1 (2.2)	2 (5.0)
Concordance	38 (82.6)	20 (87.0)	13 (65.0)
Farm exposure			
Yes	1 (1.1)	2 (4.3)	1 (2.5)
Unknown	0 (0.0)	2 (4.3)	0 (0.0)
Concordance	45 (97.8)	21 (91.3)	19 (95.0)

*Atopic diseases include asthma, atopic dermatitis, and allergy.

CI: 6 to 54%) with a large nonshared environmental effect of 70% (95% CI: 46 to 94%).

The sex of the host also significantly influenced nasal bacterial density. On average, nasal bacterial density of women was about half that of men (women median: 2.97×10^6 16S rRNA gene copies per swab, IQR: 1.33×10^6 to 9.11×10^6; men median: 7.94×10^6, IQR: 2.20×10^6 to 4.30×10^7) (Wilcoxon rank sum, $P < 0.001$) (Fig. 2A and table S5). Smoking and the history of atopic diseases or psoriasis had no significant effect on nasal bacterial density (smoking $P = 0.61$, psoriasis $P = 0.22$) (table S5).

The types of nasal bacteria present were also associated with nasal bacterial density, as indicated by the significantly different densities across CSTs (ANOVA, $P < 0.001$). Bacterial density was highest in the two least prevalent CSTs: Enterobacteriaceae-dominated CST2 and *Moraxella*-dominated CST6; in contrast, bacterial densities were lowest in the two most prevalent CSTs: CST3 and CST4 (table S2). The distinctive densities across nasal CSTs indicate that density may be a unique feature of the individual nasal CSTs.

The sex difference in nasal bacterial densities was not due to men's propensity for high-density nasal CSTs. We found no significant sex difference in nasal CST distribution ($\chi^2 = 7.8$, $df = 6$, $P = 0.25$) (table S2). Overall, men had higher nasal bacterial density than women, irrespective of nasal CSTs ($P < 0.001$) (table S6).

Can the nasal microbiota predict *S. aureus* nasal colonization?

The rates and absolute abundance of *S. aureus* differed among nasal CSTs (fig. S2, A and B). Some taxa predict the presence or absence of *S. aureus*, whereas others predict *S. aureus* absolute abundance in a threshold-dependent fashion (Fig. 3A). *Dolosigranulum* spp. was the most informative predictor of the presence or absence of *S. aureus*. Specifically, the rate of *S. aureus* nasal colonization among individuals at or above the *Dolosigranulum* threshold was 16.0% (n = 4/25), as compared with 56.0% among the simulated population (n = 56/100). Likewise, we observed threshold effects for nasal taxa such as *Propionibacterium granulosum* and *S. epidermidis*; however, *P. granulosum* was negatively correlated with the presence of *S. aureus*, but *S. epidermidis* was positively correlated (*P. granulosum* node n = 4/34, 11.8%; *S. epidermidis* node n = 13/14, 92.9%) (Fig. 3A).

The *S. aureus* absolute abundance model indicated that having low *Corynebacterium* abundance predicts high *S. aureus* absolute abundance, that is, category 5, which comprised 10^6 to 10^7 *S. aureus* 16S rRNA gene copies per swab (14/28, 50.0%) (fig. S3A), as compared to the lower category 5 prevalence in the simulated population (n = 16/100, 16.0%) (fig. S3B). Results from validation tests recapitulated and supported the threshold-dependent relationships between *S. aureus* and other nasal taxa from both models (Fig. 3B and fig. S3C). Thus, our findings indicate that nasal taxa determine *S. aureus* nasal colonization through two types of interactions: by exclusion and by limiting *S. aureus* abundance. Ecologically, these relationships may manifest as a result of competition or common sorting along an abiotic axis.

Culture-negative *S. aureus* nasal colonization

In the current study, men and women did not differ in *S. aureus* nasal colonization rates by DNA sequencing (women 52.9%; men 52.6%). This contradicted previous culture-based studies that have shown that men are more likely to be colonized by *S. aureus* (*2*, *11*–*13*). However, this discrepancy could be explained by the higher absolute abundance of *S. aureus* in men and its influence on culture outcomes. Specifically, except in CST1, women frequently had 10- to 100-fold lower *S. aureus* absolute abundance than men (Fig. 2B). At the same time, *S. aureus* absolute abundance had a strong positive link to culture outcome. Each 10-fold increase in *S. aureus* increased the probability of a positive culture by 30.0% ($r^2 = 0.33$, $P < 0.001$) (Fig. 2C). After adjusting for sex and other host factors, *S. aureus* absolute abundance was the key determinant of culture-positive *S. aureus* nasal colonization ($r^2 = 0.33$, $P < 0.05$). This suggests that culture-based methods fail to identify a substantial proportion of *S. aureus* carriers,

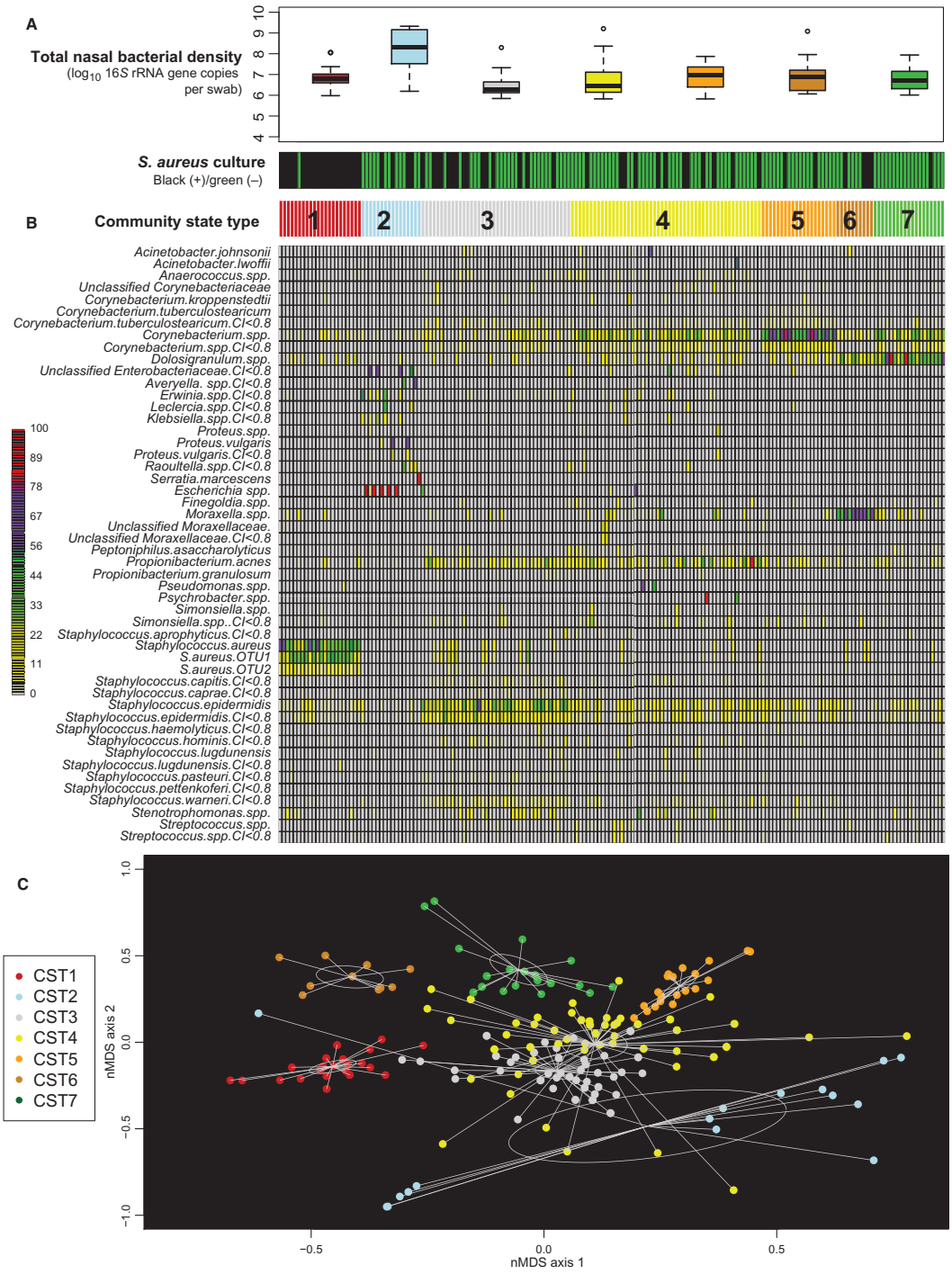

Fig. 1. The seven nasal CSTs and their respective bacterial densities shown in boxplots and composition shown in heatmap visualization and non-metric multidimensional scaling (nMDS) ordination plot. (**A**) In the boxplots, the box of each boxplot denotes the IQR (Q2-Q3) and the corresponding median, whereas the whiskers signify the upper and lower 1.5 × IQR, and the open circles denote outliers beyond the whiskers. The difference in bacterial density was significantly greater across than within CSTs [analysis of variance (ANOVA), $P < 0.001$]. In particular, CST3 had significantly lower bacterial density than all other CSTs except CST4, and CST2 had significantly higher bacterial density than all other CSTs except CST6 (two-tailed Wilcoxon rank sum, $P < 0.05$) (A). (**B**) In the heatmap visualization, each participant's nasal microbiota is represented in a single column, and proportional abundance of each nasal bacterial taxon is shown by row according to the color key to the left. The nasal microbiota is grouped by CSTs, as indicated by the CST color bar above. The *S. aureus* culture result of each participant is noted by the green/black color bar above. (**C**) In the nMDS ordination plot, each participant's nasal microbiota (in proportional abundance) is represented by a single data point, and data points that are closer have a more similar composition than those that are farther apart. The centroids and 95% confidence ellipse for each CST are shown.

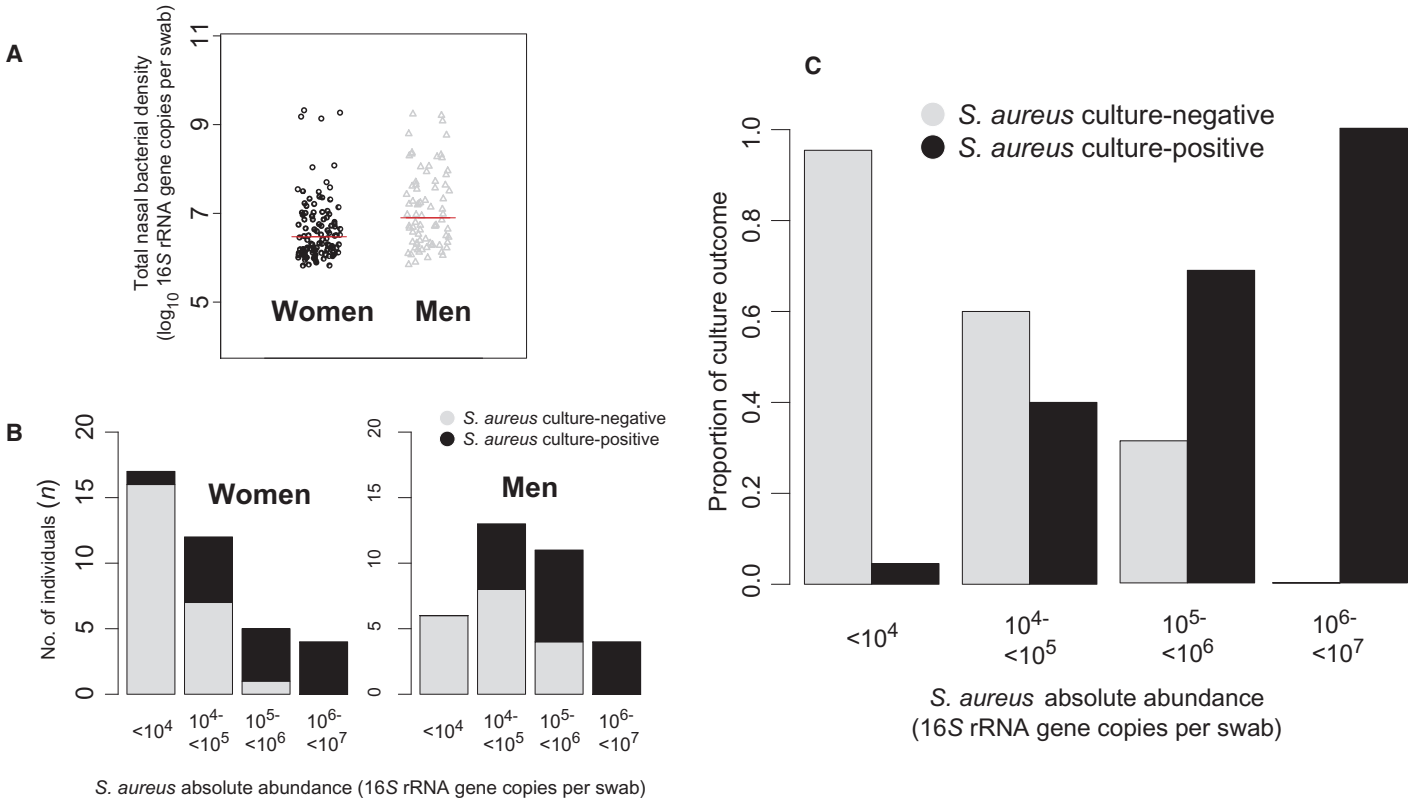

Fig. 2. Nasal bacterial density and *S. aureus* absolute abundance by sex and the relationship between *S. aureus* absolute abundance and *S. aureus* culture. (**A**) The scatterplot shows the higher nasal bacterial density in men than in women. Individuals (non-CST1) with detectable *S. aureus* nasal colonization could be divided on the basis of *S. aureus* absolute abundance into four categories. (**B**) Women were more likely to have the two lowest categories of *S. aureus* absolute abundance (that is, $<10^4$ and 10^4-10^5), whereas men are more likely to have the middle two categories (that is, 10^4-10^5 and 10^5-10^6). (**C**) Culture outcome was strongly linked to *S. aureus* absolute abundance, and each 10-fold increase in *S. aureus* absolute abundance increases the probability of positive *S. aureus* culture by 30%, which suggests that the sex difference in *S. aureus* absolute abundance might explain the lower *S. aureus* culture rates in women than in men.

particularly among women, which could serve as unrecognized reservoirs of *S. aureus* (*11*).

In summary, nasal microbiota is an environmentally derived host phenotype, and nasal taxa determine *S. aureus* nasal colonization by influencing the presence or absence and the absolute abundance of *S. aureus*. Nasal microbiota composition is not fixed by host genetics and is therefore susceptible to environmental modification. Our findings open the possibility for probiotic strategies to eliminate *S. aureus* nasal colonization. One caveat here is the significant influence of sex and host genetics on nasal bacterial density. In addition, although early environment had no significant influence in our cohort, which was middle age or older, it could play a role in a younger cohort. In this study, absolute abundance emerged as a critical factor in nasal bacterial interactions and culture-based detection. In particular, the negative interactions between nasal taxa and *S. aureus* depended on absolute abundance thresholds, consistent with the ecological notion that absolute abundances, not relative abundances, reveal the importance of ecological interactions such as competition (*14*, *15*). Thus, the utility of nasal probiotics will rely on whether nasal microbiota composition trumps nasal bacterial density in determining *S. aureus* nasal colonization. On the basis of the limited influence of host genetics on *S. aureus* nasal colonization (*2*), we predict that the answer will be "yes."

MATERIALS AND METHODS

This study was approved by the Science Ethics Committee for Southern Denmark (project number S- VF-19980072, addendum nos. 8 and 9) with appropriate informed consent following the guidelines of the approved protocol. At the Translational Genomics Research Institute, this study was approved as a study using coded specimens based on the Department of Health and Human Services Office of Human Research Protections Guidance on Coded Private Information of Biological Specimens.

We included 46 monozygotic and 43 dizygotic twin pairs (23 same-sex and 20 opposite-sex twin pairs) from an earlier study of The Danish Twin Registry cohort (*2*). Briefly, each participant's anterior nares were sampled using Copan E-Swabs (Copan Diagnostics Inc.), which was placed immediately in 1 ml of Amies transport medium and stored at 4°C until *S. aureus* testing by standard nonselective medium, as previously described (*2*). The remaining swab eluent was frozen at −80°C until processing. Total DNA was prepared from 100 µl of eluent as previously described (*16*). Briefly, each aliquot was chemically lysed with RLT lysis buffer (Qiagen), mechanically lysed using the Barocycler (Pressure BioScience Inc.), and purified using Qiagen AllPrep DNA/RNA 96-well kit following the manufacturer's instructions, with a final elution volume of 100 µl.

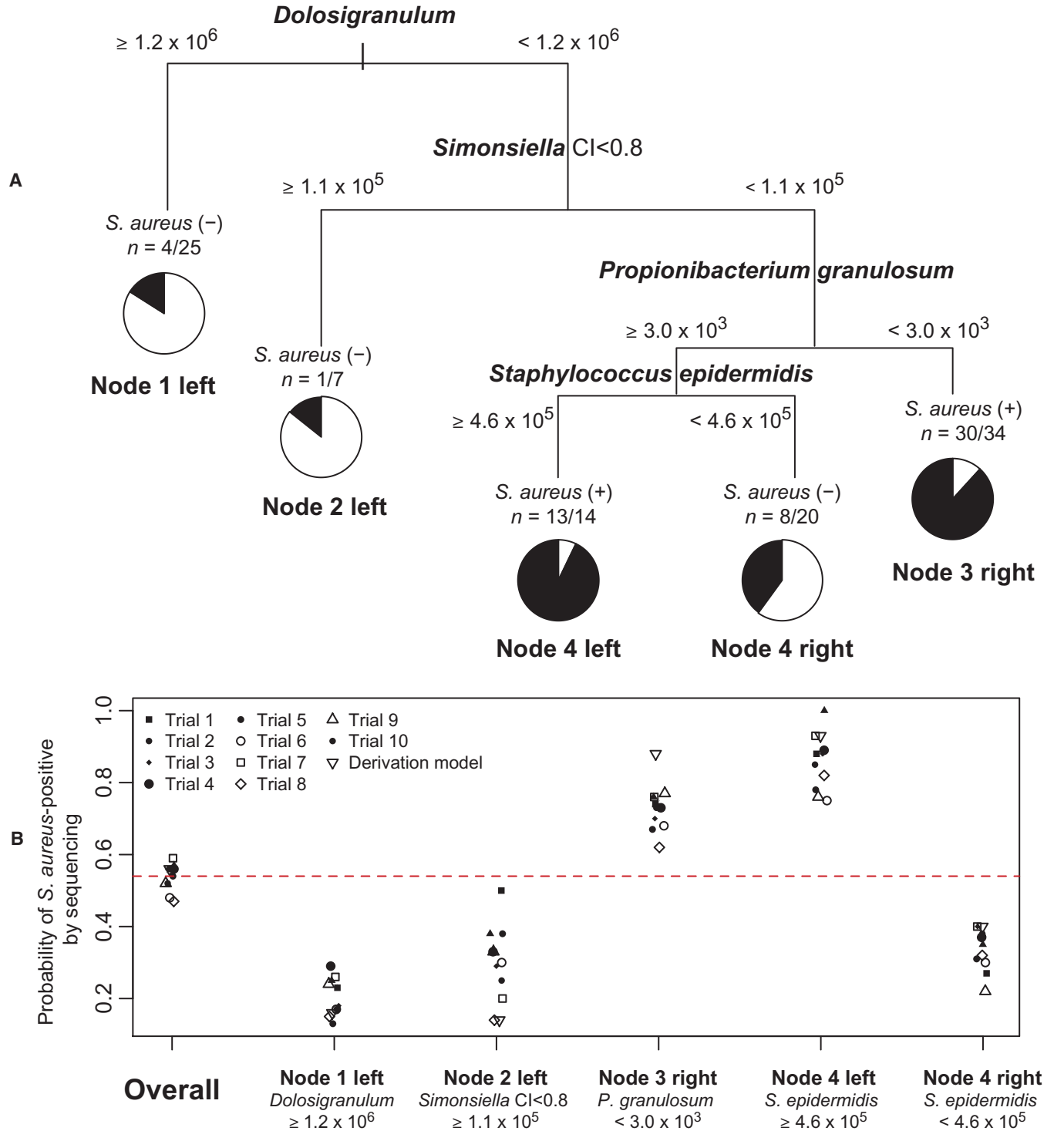

Fig. 3. Results from decision tree model derivation and validation showing threshold-dependent relationships between the absolute abundances of nasal commensals and *S. aureus* presence/absence. (**A**) Model predicting *S. aureus* presence/absence was derived using a randomly drawn group of 100; it showed that the most informative split was a threshold of 1.2×10^6 *Dolosigranulum* 16*S* rRNA gene copies per swab. Having above-threshold *Dolosigranulum* predicts absence of *S. aureus* (n = 4/25, 16.0%), as compared to *S. aureus* nasal colonization rate in the overall derivation group (n = 56/100, 56%). *Simonsiella* had a similarly negative relationship to *S. aureus*, where, among individuals who had below-threshold abundance of *Dolosigranulum*, having $\geq 1.1 \times 10^5$ *Simonsiella* predicts the absence of *S. aureus* (n = 1/7, 14.3%). (**B**) Validation testing using 10 randomly drawn groups of 100 supported the threshold-based relationships between *Dolosigranulum*, *Simonsiella*, *P. granulosum*, and *S. epidermidis* and *S. aureus* presence/absence.

We measured nasal bacterial density using a broad-coverage quantitative polymerase chain reaction (*17*) and characterized nasal microbiota composition by 16*S* rRNA gene-based sequencing and taxonomic classification, as previously described (*18*), with some modifications including a custom classifier for classification of *Staphylococcus* species. Using the taxonomically classified sequence data, we calculated the proportional abundance for each nasal bacterial taxon as: (number of sequences assigned to the taxon from the sample)/(total number of sequences from the sample), which we combined with nasal bacterial density to calculate taxon-specific 16*S* rRNA gene absolute abundance: (proportional abundance of the taxon from the sample) × (nasal bacterial density of the sample) (*18*). Additional methodological details, including development and validation of the custom *Staphylococcus* classifier, can be found in the Supplementary Materials.

All ecological and statistical analyses were performed in R version 3.0.1 (*19*). CSTs were identified using a proportional abundance-based matrix in Euclidean distance by hierarchal clustering with Ward linkage, as previously described (*20*). Through an iterative process, we determined a parsimonious number of distinct CSTs and the CST of each participant. We visualized the nasal microbiota in each twin type and in individuals with each nasal CST by heatmap and non-metric multidimensional scaling using the vegan package (R package version 2.1-10) (*21*) and examined the prevalence of each nasal CST in our study population.

We identified the indicator bacteria for each nasal CST using indicator analysis in the *labdsv* package (R package version 1.6-1) at $\alpha = 0.05$, adjusted for false discovery (*22*). To assess the contribution of host genetics to nasal microbiome composition and bacterial density, we first assessed nasal CST concordance in twin pairs and difference in pairwise ecological distance between twin types. Next, we compared within-pair nasal bacterial density in each twin type by ICCs and its 95% CI based on variance components of a one-way ANOVA and the exact confidence limit using the *ICC* package (*23*). Last, we estimated the relative contribution of genetic and environmental factors to used nasal bacterial density using bacterial density data ($\log_{10}$) in a standard biometrical heritability analysis.

To assess the contribution of nonhereditary traits to nasal bacterial density and nasal microbiome composition, we assessed the influence of nasal CST, sex, history of atopic disease and psoriasis, and smoking status. We compared the nasal bacterial density across CSTs by ANOVA. We evaluated host sex, history of atopic disease and psoriasis, and smoking status with nasal bacterial density using a quasi-Poisson model. To determine nasal microbiome constituents that predict *S. aureus* colonization or 16*S* rRNA gene absolute abundance, we applied decision tree analysis with recursive partitioning and splitting by information criteria in the rpart package (*24*) in the derivation and validation stages. We further assessed the correlation between *S. aureus* 16*S* rRNA gene absolute abundance and culture outcome using a multivariate linear regression model.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/1/5/e1400216/DC1

Fig. S1. Correlation of nasal microbiota composition among monozygotic and among same-sex and opposite-sex dizygotic twin pairs in non-metric multidimensional scaling ordination plots.

Fig. S2. Rates of *S. aureus* nasal colonization by sequencing and by culture and *S. aureus* absolute abundance for the seven nasal CSTs.

Fig. S3. Results from decision tree model derivation and validation showing threshold-dependent relationships between the absolute abundances of nasal commensals and *S. aureus*.

Table S1. The indicator genera for each nasal CST identified on the basis of proportional abundance, where CI < 0.80 denotes bacteria taxa assigned with <80% bootstrap confidence level.

Table S2. Nasal bacterial density (median and IQR) of each nasal CST and the prevalence of each CST by sex.

Table S3. Comparison of within-twin nasal microbiota composition correlation between monozygotic and dizygotic twins based on pairwise ecological distance (Jaccard, Bray-Curtis, and Euclidean distances).

Table S4. Results from standard biometrical heritability analysis using a polygenic model to determine the contribution of additive genetic effects (A), genetics effects due to dominance (D), shared environmental effects (C), and nonshared environmental effects (E) to nasal bacterial density.

Table S5. The associations between nasal bacterial density and host factors, including sex, assessed by Wilcoxon rank sum and Kolmogorov-Smirnov tests.

Table S6. Comparison of nasal bacterial density by sex, adjusted for nasal CST in a quasi-Poisson model.

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