Characterization of conjunctival microbiome dysbiosis associated with allergic conjunctivitis

To the Editor,

Allergic conjunctivitis (AC) is one of the most common ocular surface diseases that encompasses a spectrum of diseases featured by antigen-specific immunoglobulin E (IgE) and T helper type 2 (Th2) lymphocyte-mediated hypersensitivity responses.[1] Among different types of AC, seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) manifest the classical type I hypersensitivity. In contrast to SAC/PAC, the major mechanism of vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) is type IV hypersensitivity.

Increasing evidence shows that the microbiome of skin, gut, and airways play an important role in allergic diseases like atopic dermatitis, food allergies, and asthma.[2] Similar to other mucosal surfaces, conjunctiva is colonized with a variety of microorganisms. We thus hypothesized that the dysbiosis of conjunctival microbiome is associated with AC. To test this hypothesis, we surveyed the conjunctival microbiome of 39 individuals with AC and 48 healthy subjects using metagenomic shotgun sequencing (Table S1). The group of AC comprised 14 patients with PAC, 7 with SAC, and 18 with VKC (Table S2).

We first assessed whether the conjunctival microbiome is generally different between healthy and AC individuals. Overall, bacteria accounted for the majority of the conjunctival microbiota of all individuals (Figure S1). *Malassezia* Fungi (*M. furfur* in particular) were abundant in a fraction of patients with SAC/PAC (Figure 1A). *Malassezia* produces immunogenic proteins that elicit IgE and thus induce pro-inflammatory cytokines and auto-reactive T cells, which contributes to atopic dermatitis pathogenesis.[3] Notably, SAC/PAC is dominated by IgE-mediated reactions in contrast to VKC. The alpha diversity showed no significant difference (Figure 1B), whereas the inter-individual variation within AC groups were slightly lower than healthy groups (Figure 1C). The species composition showed a clear delineation

between healthy and AC participants (Figure 1D), suggesting that dysbiosis of the conjunctival microbiome is associated with AC.

We then used multivariable linear models adjusting for covariates to identify the species that account for the dysbiosis. Numerous associated species were identified (Figure 1E). Interestingly, we detected the enrichment of *Moraxella catarrhalis* in AC (P = 0.0077). *Moraxella catarrhalis* in the upper airways is linked with the development or exacerbation of allergic airway inflammation.[4] This implies that common microbial mechanisms may underlie both ocular allergy and allergic diseases at other sites. Functional profiles of the conjunctival microbiome also showed differences between healthy and AC individuals (Figure S2). In particular, we observed that antibiotic resistance genes are more prevalent in AC patients than healthy individuals.

We next examined whether the conjunctival microbiota is different between SAC/PAC and VKC. The conjunctival microbiota of the patients with SAC/PAC was less diverse than healthy subjects (Figure 2A; P = 0.043). We did not find significant difference in inter-individual variation within samples of SAC/PAC and VKC (Figure 2B; P = 0.31). As expected, the species composition of conjunctival microbiome distinguished between SAC/PAC and VKC (Figure 2C, D; Figure S3). The species associated with SAC/PAC included *Brevibacterium aurantiacum* (P = 0.002) and *Staphylococcus sciuri* (P = 0.021). We observed the enrichment of *Streptococcus* species in VKC (Figure 2E). Colonization of *S. pneumoniae* in airways are the risk factors of asthma.[5] Interesting, alpha-hemolytic *Streptococcus* (which includes *S. pneumoniae* and *S. sanguinis*) were observed on the ocular surface of the patients with ocular graft-versus-host disease.[6] These results indicate that the composition of conjunctival microbiota is associated with specific types of AC.

In summary, the dysbiosis of conjunctival microbiome is associated with AC. Moreover, SAC/PAC and VKC harbor distinct microbial signatures. The microbiome signatures identified here represent potential targets for follow-up studies on the microbial mechanisms that underlie AC and other non-infectious ocular surface

inflammations.

REFERENCES

1. La Rosa M, Lionetti E, Reibaldi M, et al. Allergic conjunctivitis: a comprehensive review of the literature. *Ital J Pediatr*. 2013;39(1):18.
2. Kemter AM, Nagler CR. Influences on allergic mechanisms through gut, lung, and skin microbiome exposures. *J Clin Invest*. 2019;129(4):1483-1492.
3. Glatz M, Bosshard PP, Hoetzenecker W, Schmid-Grendelmeier P. The Role of Malassezia spp. in Atopic Dermatitis. *J Clin Med*. 2015;4(6):1217-1228.
4. Alnahas S, Hagner S, Raifer H, et al. IL-17 and TNF-α Are Key Mediators of Moraxella catarrhalis Triggered Exacerbation of Allergic Airway Inflammation. *Front Immunol*. 2017;8(NOV):1-11.
5. Rahman Fink N, Chawes BL, Thorsen J, et al. Neonates colonized with pathogenic bacteria in the airways have a low-grade systemic inflammation. *Allergy*. 2018;73(11):2150-2159.
6. Shimizu E, Ogawa Y, Saijo Y, et al. Commensal microflora in human conjunctiva; characteristics of microflora in the patients with chronic ocular graft-versus-host disease. *Ocul Surf*. 2019;17(2):265-271.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

L.W. and L.L. conceived the study; Q.L., Yu L, and B.Z. performed data analysis; Jing L., S.Z., Yinglin L., and X.D. collected clinical samples; X.W., S.G., and Juanran L performed the metagenomic sequencing experiments; Q.L. and Jing L. drafted the

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FIGURE LEGENDS

FIGURE 1. Conjunctival microbiome differences between individuals with AC and healthy individuals. A, Genus compositions of healthy subjects (*n* = 48) and AC patients with SAC/PAC (*n* = 21) or VKC (*n* = 18). B, Alpha diversity of AC and healthy samples. C, Beta diversity within AC and healthy samples. D, Principal coordinates analysis of samples from all 87 participants based on species-level Bray-Curtis distance. E, Relative abundance distributions of species accounting for the dysbiosis in AC ($P < 0.05$, fold change > 5). Relative abundances in the heatmap were centered and scaled across all the samples.

FIGURE 2. Conjunctival microbiome differences between individuals with SAC/PAC and VKC. A, Alpha diversity of healthy subjects (*n* = 48) and patients with SAC/PAC (*n* = 21) and VKC (*n* = 18). B, Beta diversity within healthy, SAC/PAC, and VKC samples. C, Principal coordinates analysis of samples from 39 AC participants with SAC/PAC or VKC based on species-level Bray-Curtis distance. D, Model coefficients of species associated with SAC/PAC or VKC ($P < 0.05$, coefficient > 0.2). GLM, general linear model. E, Relative abundance distributions of *Streptococcus* species enriched in VKC.

FIGURE 1

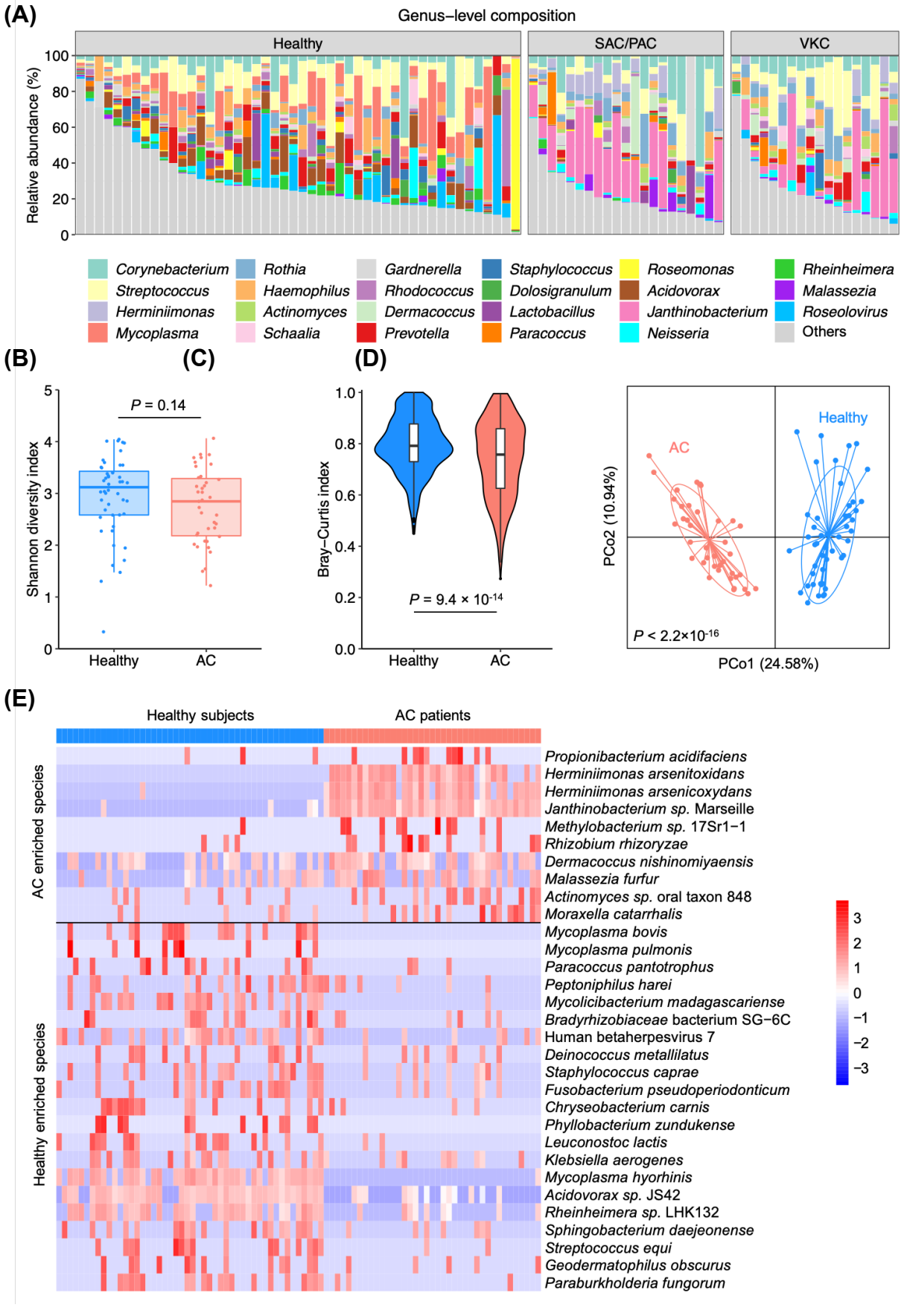
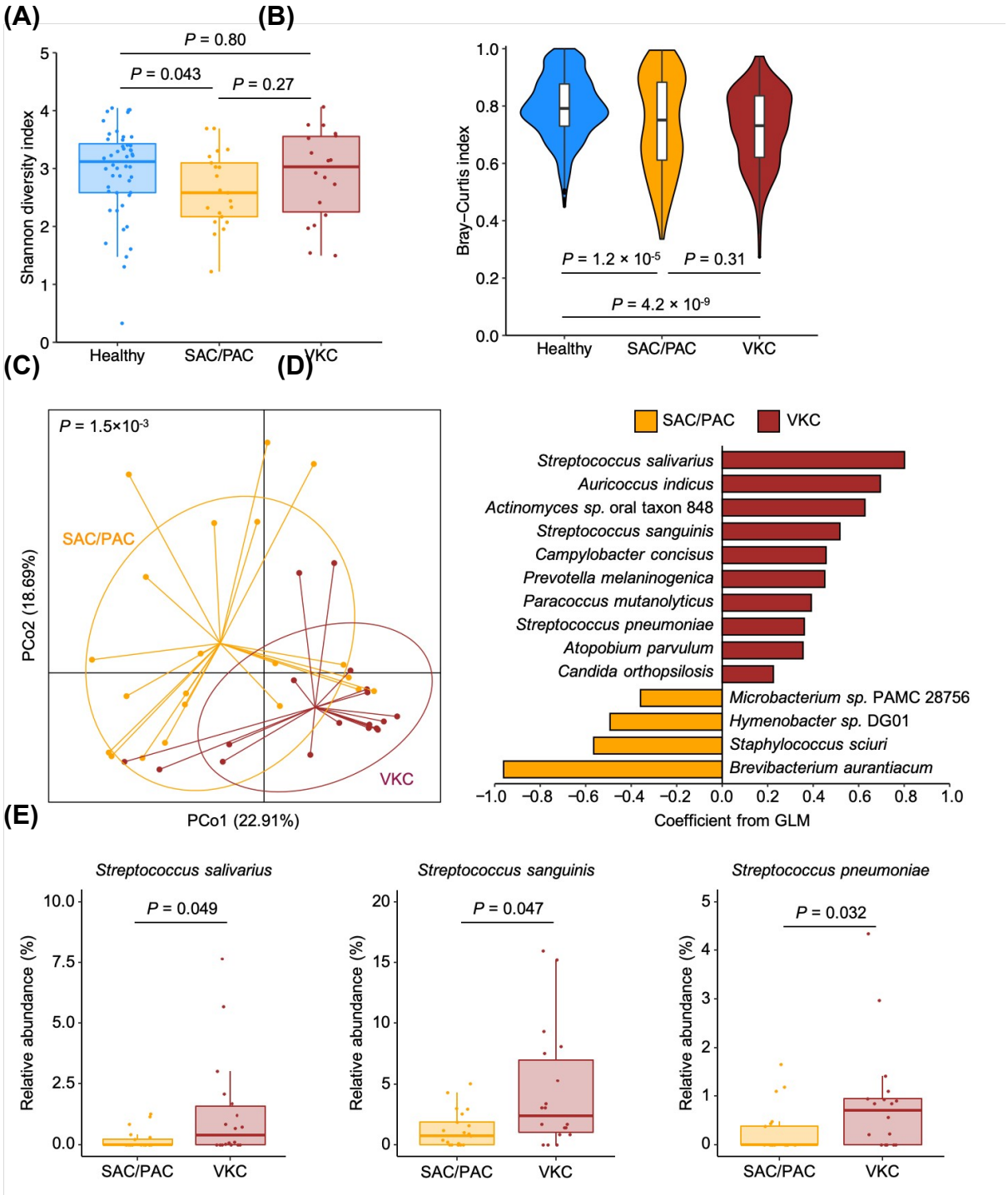

(A)
Genus−level composition
Healthy
SAC/PAC
VKC
Relative abundance (%)
100
80
60
40
20
0
Corynebacterium
Streptococcus
Herminiimonas
Mycoplasma
Rothia
Haemophilus
Actinomyces
Schaalia
Gardnerella
Rhodococcus
Dermacoccus
Prevotella
Staphylococcus
Dolosigranulum
Lactobacillus
Paracoccus
Roseomonas
Acidovorax
Janthinobacterium
Neisseria
Rheinheimera
Malassezia
Roseolovirus
Others
(B)
Shannon diversity index
P = 0.14
Healthy
AC
(C)
Bray−Curtis index
P = 9.4 × 10-14
Healthy
AC
(D)
Healthy
AC
PCo2 (10.94%)
P < 2.2×10-16
PCo1 (24.58%)
(E)
Healthy subjects
AC patients
AC enriched species
Healthy enriched species
Propionibacterium acidifaciens
Herminiimonas arsenitoxidans
Herminiimonas arsenicoxydans
Janthinobacterium sp. Marseille
Methylobacterium sp. 17Sr1−1
Rhizobium rhizoryzae
Dermacoccus nishinomiyaensis
Malassezia furfur
Actinomyces sp. oral taxon 848
Moraxella catarrhalis
Mycoplasma bovis
Mycoplasma pulmonis
Paracoccus pantotrophus
Peptoniphilus harei
Mycolicibacterium madagascariense
Bradyrhizobiaceae bacterium SG−6C
Human betaherpesvirus 7
Deinococcus metallilatus
Staphylococcus caprae
Fusobacterium pseudoperiodonticum
Chryseobacterium carnis
Phyllobacterium zundukense
Leuconostoc lactis
Klebsiella aerogenes
Mycoplasma hyorhinis
Acidovorax sp. JS42
Rheinheimera sp. LHK132
Sphingobacterium daejeonense
Streptococcus equi
Geodermatophilus obscurus
Paraburkholderia fungorum
3
2
1
0
−1
−2
−3

FIGURE 2


(A)
(B)
P = 0.80
P = 0.043
P = 0.27
Shannon diversity index
Healthy
SAC/PAC
VKC
Bray–Curtis index
P = 1.2 × 10-5
P = 0.31
P = 4.2 × 10-9
(C)
(D)
P = 1.5×10-3
SAC/PAC
VKC
PCo2 (18.69%)
PCo1 (22.91%)
Streptococcus salivarius
Auricoccus indicus
Actinomyces sp. oral taxon 848
Streptococcus sanguinis
Campylobacter concisus
Prevotella melaninogenica
Paracoccus mutanolyticus
Streptococcus pneumoniae
Atopobium parvulum
Candida orthopsilosis
Microbacterium sp. PAMC 28756
Hymenobacter sp. DG01
Staphylococcus sciuri
Brevibacterium aurantiacum
Coefficient from GLM
(E)
Streptococcus salivarius
Streptococcus sanguinis
Streptococcus pneumoniae
P = 0.049
P = 0.047
P = 0.032
Relative abundance (%)