Metagenomic analysis of the conjunctival bacterial and fungal microbiome in vernal keratoconjunctivitis

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April 18, 2021

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Short title: Microbiome in VKC

Text Word Count: 597

To the Editor,

there is increasing interest in the role of conjunctival microbiome in the healthy ocular surface (1) and in different ocular diseases. There is also enough evidence that dysregulation of resident microbial communities (dysbiosis) might be associated with allergy risk(2). Vernal keratoconjunctivitis (VKC) is a severe form of ocular allergy affecting mostly male children and young adults with typical seasonal recurrences and potentially visual impairment. We recently found and overexpression of multiple pattern recognition receptors (PRRs) in VKC suggesting the role of host-microbe interaction in VKC pathogenesis(3). To investigate the VKC-associated ocular microbiome, we applied 16S and ITS2 amplicon sequencing (online supplementary MM) in conjunctival swabs obtained from 22 VKC patients and 20 age, sex and ethnicity-matched healthy controls (HC) (Table 1S). Written informed consent was obtained from all parent/subjects enrolled. Type of VKC, allergen sensitization, disease-specific ongoing treatment and results of the Quality of Life in Children with VKC (QUICK) questionnaire were recorded. A 10-items questionnaire investigating the presence of the principal factors related to allergy development was administered to all included subjects.

16S rRNA amplification was successfully carried out in 12/22 VKC and 4/20 HC samples. High-throughput amplicon sequencing produced a total of 734.157 high-quality reads (average of 45.885 reads/sample), which were clustered into 1.241 OTUs (97% sequence identity) and classified according to the Greengenes database. Compared with HC, α-diversity was significantly higher in all VKC (p=0.05), in IgE-negative patients (p=0.03), in tarsal VKC (p=0.03), in formula-fed versus breastfed children (p=0.03) and in children with history of atopic dermatitis (AD) (p=0.01). β-diversity highlighted differences in microbiota composition (Figure 1S) suggesting clusters of subjects with different conjunctival microbiomes. At the phylum level, conjunctival microbiome was dominated by Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes that accounted for >90% of all reads (Figure 1). Of the 132 observed families, Moraxellaceae (W=15) showed a higher abundance in VKC than HC (Figure 2S). At the genus level, Pseudomonas, Staphylococcus, Streptococcus, Acinetobacter, Neisseria, Haemophilus, Prevotella, Corynebacterium, Propionibacterium and Rothia accounted for >70% of sequences (Figure 3S). In VKC, Bacteroidetes and Fusobacteria met the core microbiome’s definition criteria (Table 2S), which includes different species of gram-negative bacteria able to potentially induce an LPS-driven inflammatory response as shown in experimental models(4).
For the fungal microbiome, 10/22 VKC samples produced a detectable ITS2 amplicon. A total of 677,115 high-quality reads (average of 48,365 reads/sample) were clustered into 933 OTUs and taxonomically classified against the UNITE ver. 7.0 database. At the family level, Saccharomycetaceae, Malasseziaceae, Pleosporaceae and Cladosporiaceae accounted for the majority of sequences (Figure 2). OTUs referred to Malasseziaceae were significantly increased in VKC compared with HC (W=42). Malassezia have been associated with AD inducing a mixed Th2/Th17 response(5) interacting with several PRRs and activating multiple signaling pathways. Since multiple PRRs are over-expressed in VKC(3), we suggest that glycan, phospholipid carbohydrate residues of allergens and microbes may engage innate receptors on conjunctival cells priming a type-2 response in VKC.

14/22 VKC children were under topical treatments at the time of swabbing, creating a potential bias (Table 1S). However, only 5/10 patients whose swabs were not sequenced because of absence of amplicons, were under topical medication. In addition, β-diversity didn’t show differences in microbial communities considering the use and type of medications suggesting that factors other than topical eyedrops may alter the conjunctival microbiota. The main limitation was the higher difficulty to obtain amplicon products from HC, which has been already described and attributed to the physiological antimicrobial activity and to the lower microbial load of healthy subjects(6).

In conclusion, the described dysbiosis in VKC highlights the role of the host-microbes interaction in VKC pathogenesis.

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Keywords: ocular surface microbiome, vernal keratoconjunctivitis, 16S rRNA gene amplicon sequencing, ITS2 rRNA gene amplicon sequencing, core microbiome

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Acknowledgments

a. Funding/Support: Supported by MIUR DOR1952345/19 and DOR2092417/20 from Italian Institute of Health.
b. Conflicts of Interest:
Andrea Leonardi: No Conflicts of Interest.
Rocco Luigi Modugno: No Conflicts of Interest
Fabiano Cavarzeran: No Conflicts of Interest
Umberto Rosani: No Conflicts of Interest

c. Contributions to Authors in each of these areas: Andrea Leonardi (AL), Rocco Luigi Modugno (RLM), Fabiano Cavarzeran (FC), Umberto Rosani (UR)

Conception and Design: AL, UR
Analysis and interpretation: AL, RLM, UR
Writing the article: AL, RLM
Critical revision of the article: UR
Final approval of the article: AL, RLM, UR
Data Collection: RLM, UR, FC
Provision of materials, patients, or resources: AL, UR
Statistical expertise: FC
Obtaining funding: AL
Literature search: RLM, AL, UR
Administrative, technical or logistic support: AL, FC
d. Statement about Conformity with Author Information: none
e. Other Acknowledgments: none

Figure legends

Figure 1. Pie charts showing the mean relative abundance of the predominant bacterial phyla in VKC (A) and HC samples (B). Conjunctival microbiomes were dominated by Proteobacteria (VKC 44.8%; HC 67.9%), Firmicutes (VKC 37.0%; HC 22.1%), Actinobacteria (VKC 7.9%; HC 3.7%) and Bacteriodetes (VKC 4.2%; HC 2.7%) that accounted for > 90% of all the reads.

Figure 2. Pie charts showing the mean relative abundance of the predominant fungal families in VKC (A) and healthy control (B) samples. Saccharomycetaceae (VKC 55.8%; HC 62.7%), Malasseziaceae (VKC 15.6%; HC 3.3%), Pleosporaceae (VKC 12.4%; HC 2.6%) and Cladosporiaceae (VKC 4.0%; HC 3.0%) accounted for the vast majority of sequences. “Other” includes genera with <1% mean abundance.

References