

LETTER OPEN ACCESS

Ocular Microbiome in Dupilumab-Induced Ocular Surface Disease

Andrea Leonardi¹  | Riccardo Frizzo² | Fabiano Cavarzeran¹ | Jerome Ozkan³  | Umberto Rosani²

¹Department of Neuroscience, Ophthalmology Unit, University of Padova, Padova, Italy | ²Department of Biology, University of Padova, Padova, Italy | ³School of Optometry and Vision Science, Faculty of Medicine and Health, School of Biological, Earth and Environmental Sciences, Faculty of Science, University of New South Wales, Sydney, New South Wales, Australia

Correspondence: Andrea Leonardi (andrea.leonardi@unipd.it)

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To the Editor,

Patients with moderate–severe atopic dermatitis (AD) receive a great benefit from the use of dupilumab, a human IgG4 monoclonal antibody inhibiting IL-4/IL-13 signaling. However, randomized controlled trials and real-world studies showed, in 30%–50% of cases, the development of dupilumab-induced ocular surface disease (DIOSD), characterized by blepharitis, conjunctivitis, dry eye disease (DED), limbal inflammation, evolving into scarring, ectropion, and keratitis [1]. Higher risk of DIOSD has been associated with AD severity, history of eye disease, high serum IgE levels, and eosinophilia [1]. Metabolic differences between AD patients with and without DIOSD have also been reported [2]. Similarities between DIOSD and DED were suggested because of the decreased number of conjunctival goblet cells and secreted mucins in both [1]. Recently, a shift from a mixed Th2/Th17 toward a Th1/Th17 cytokine profile was shown in AD patients developing DIOSD with a decreased richness of the ocular microbiome [3]. In our real-world cohort of 32 dupilumab-treated AD patients, 17 (53%) had no adverse effects, and 15 (47%) were affected by DIOSD. We conducted a pilot study in 13 dupilumab-treated AD patients, 5 without ocular symptoms (No-DIOSD) and 8 with ocular side effects (DIOSD), and in 6 healthy subjects (CT) applying full-length 16S rRNA gene amplicon sequencing (Oxford Nanopore Technologies, ONT). After recording signs and symptoms (Table S1), each subject was swabbed at the inferior fornix of the right eye, without topical anesthetics, using a single-use, dry, sterile nylon swab (eSwab, Copan Diagnostics Inc., Murrieta, CA, USA), placed in sterile vials and stored at –20°C. The study adhered to the tenets of the Helsinki Declaration and was approved

by the Institutional Review Board. Written informed consent was obtained from all patients/subjects enrolled. DNA was extracted using the NucleoSpin Tissue XS KIT (Machery-Nagel, Düren, Germany) and used as a template to amplify 16S rRNA genes, prepare, and sequence one multiplexed ONT library. Since the quantity of DNA per patient was not sufficient for individual resolution, samples were pooled into 3 groups, CT, No-DIOSD, and DIOSD with total DNA mass of 3.2, 2.1, and 1.6 ng, respectively. We clustered OTUs (similarity threshold 95% over 80% of sequence length) from the 27,173 sequencing reads, and we taxonomically assigned them at the genus level (Table 1, Figure 1). Six phyla were recognized in the DIOSD, 9 in the No-DIOSD, and 15 in the CT group (Table S2). The top 5 genera were *Moraxella*, *Paracoccus*, *Streptococcus*, *Cutibacterium*, and *Staphylococcus* in CT; *Moraxella*, *Paracoccus*, *Massilia*, *Comamonas*, and *Acinetobacter* in No-DIOSD; *Moraxella*, *Streptococcus*, *Paracoccus*, *Acinetobacter*, and *Staphylococcus* in DIOSD (Figure 1; Table S3). Although we can't identify which species prevail over the others between the No-DIOSD and DIOSD patients, we showed some difference in microbiota composition between the 3 groups. Similar to previous studies [4], *Actinomyces* (*Actinobacteria*), *Bacillota* (*Firmicutes*), and *Pseudomonas* (*Proteobacteria*) were the most prevalent phyla in the CT conjunctiva, with evidence of local dysbiosis in AD patients, either with or without DIOSD, both at the phylum and genus levels. A reduced ocular microbial diversity and an increase in the relative abundance of pathogenic bacteria was similarly demonstrated in DED [5], suggesting that higher microbial ocular diversity may help in maintaining ocular surface homeostasis [6]. Alterations in the composition and

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structure of the ocular microbiota in AD patients may disrupt the local microenvironment, triggering inflammatory responses that may be exacerbated by dupilumab treatment. The main limitation in our study was the difficulty in obtaining full-length 16S rRNA gene amplicons from our patients, probably due to the lower microbial load in ocular samples [4]. A longitudinal sampling protocol may have revealed microbiome changes due to therapeutic

strategies for DIOSD. Further investigation is needed to highlight any pathogenic functions related to the specific microbe and the development of DIOSD, as well as to further develop the analysis based on full-length 16S rRNA gene amplicon sequencing starting from ocular samples.

TABLE 1 | Total read counts and number of genera in normal subjects (CT), dupilumab-treated patients without ocular side effects (No-DIOSD), and dupilumab-treated patients with ocular side effects (DIOSD).

	CT	No-DIOSD	DIOSD
Total read counts	10,906	6821	9446
Number of genera	136	59	50
Number of unique phyla	15	9	6

Author Contributions

Conception and Design: A.L., U.R. Analysis and interpretation: A.L., R.F., F.C., J.O., U.R. Writing the article: A.L., U.R., J.O. Critical revision of the article: J.O. Final approval of the article: all authors. Data Collection: A.L., U.R., R.F., F.C. Provision of materials, patients, or resources: A.L., U.R. Statistical expertise: F.C. Obtaining funding: U.R. Literature search: A.L., U.R. Administrative, technical, or logistic support: A.L., R.F., U.R., F.C.

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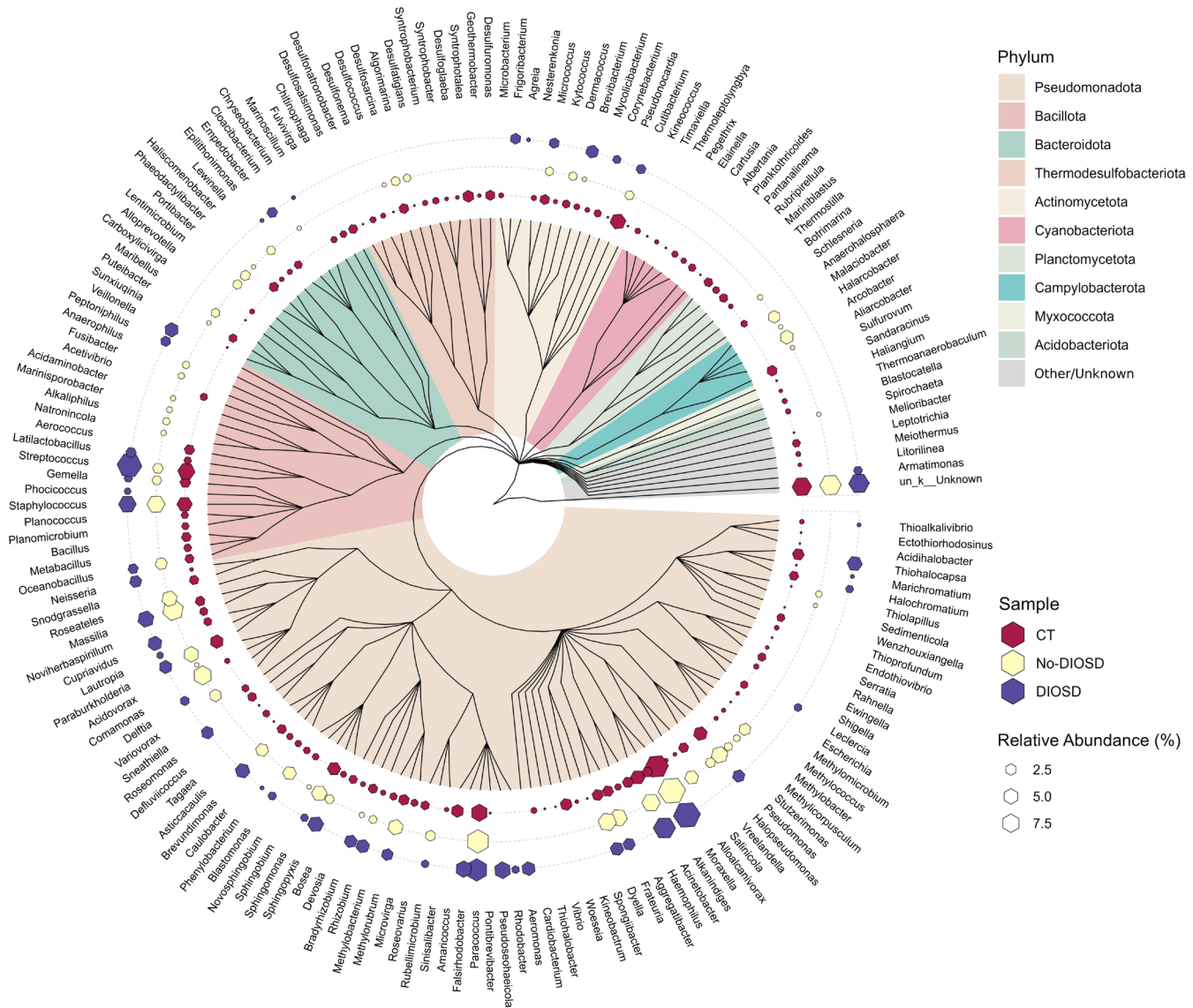


FIGURE 1 | Phylogenetic tree illustrating the relative abundance of the 174 identified genera. From inside outwards: Phylogenetic tree highlighted by phylum; colored hexagons showing the genus-level relative abundance for normal subjects (CT), dupilumab-treated atopic dermatitis patients without ocular side effects (No-DIOSD), and dupilumab-treated patients with ocular side effects (DIOSD); genus classification of identified OTUs.

Conflicts of Interest

Andrea Leonardi: Consultancy for Bausch and Lomb, Dompè, FAES Farma, FIDIA, Santen, Théa, SIFI. The other authors have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** all70104-sup-0001-TablesS1-S3.docx.