Tonsillar microbial diversity, abundance, and interrelations in atopic and non-atopic individuals

To the Editor,

While the prevalence of allergic diseases has increased worldwide, their underlying mechanisms are still not fully understood. Tonsils, consisting of lymphoid tissue, play an important role in local and systemic immune response to bacterial and viral pathogens and allergens. Tonsillar microbiota is influenced by age and health status.

Allergic diseases, as many other noncommunicable diseases, have been associated with imbalance of the gut, respiratory tract, and skin microbiotas. However, to our knowledge, microbial profiling of tonsils comparing atopic and non-atopic subjects has not been performed as of yet. Therefore, as tonsillar microbiota can closely modulate immune responses and serve as an effective in vivo model in investigating their interactions, we wanted to explore whether there are differences in bacterial diversity, abundance, and interrelations in tonsils between atopic and non-atopic elective tonsillectomy patients.

Human tonsil samples were obtained from two consecutive cohorts between 2008 and 2015 from Satakunta Central Hospital, Pori, Turku University Hospital, Turku, and Salo Regional Hospital, Salo, all in Finland. Study patients completed a standard questionnaire to collect information on health, medication, and respiratory symptoms within 30 days before the operation. Tonsillectomy was performed by routine clinical practice, and the tonsil samples were stored in RNAlater. Blood samples were drawn for allergy tests, and nasopharyngeal aspirate was obtained for respiratory virology analysis. Atopy was defined as positive immunoglobulin E antibody (>0.35 kU/L, Phadiatop Combi®, Phadia, Uppsala, Sweden) against any of the common food or aeroallergens. More details in Online Supplementary.

Intratonsillar microbial profiling was performed using RNA sequencing data as previously described. In total, 53 subjects were included consisting of 24 atopics and 29 non-atopics. After filtering out reads mapped to human, the rest were considered microbial reads (~1 M reads on average, 6% of total). These candidate microbial reads were utilized with Kraken2 for investigating microbial composition with default parameters. PhylseqQ was used to measure diversity of microbial communities and EdgeR to assess differential abundance in atopic and non-atopic subjects. Differences were considered significant based on the corrected P value of less than .05. Details in Supplementary methods.

The median age of the study subjects was 11 years (range 2-38) of which 45% were atopic. All the operations were performed during a “cold phase” of chronic tonsil condition. On the operation day, 36 (68%) had no respiratory tract symptoms, 8 (15%) reported mild respiratory symptoms and 9 (17%) had no data. Main indications for tonsillectomy were recurrent tonsillitis (47%) and tonsillar hypertrophy (45%), see details in Table S1.

Alpha diversity (measures of richness and species distribution in a sample) was computed to evaluate the difference in microbial diversity between atopic and non-atopic subjects (Figure 1A). We observed lower alpha diversity in samples from atopic patients compared with non-atopics (P = .02, Figure 1A). After adjusting the analysis to age and self-reported allergy, the difference remained significant (P = .02). Similar finding was observed when those with clinical allergic disease were compared with non-allergic ones (see Online Supplementary and Figure S2). To investigate the difference in microbial diversity at a group level, we used beta diversity (measure of diversity between samples). However, beta-diversity analysis of the tonsillar did not show a significant difference between atopic and non-atopic groups (Supplementary results Figure S3).

The most abundant phylum found in microbial data was Proteobacteria (Figure 1B). No considerable difference (P = .3) in the overall bacterial abundance between atopic and non-atopic individuals was observed. However, there was a difference in the expression of single pathogens between the study groups. The differential abundance of two specific taxa, Clostridium Botulinum and Moraxella Osloensis, was lower (unadjusted P < .05, adjusted P < .05) in atopic compared with non-atopic study subjects (Figure 1C). The heat maps illustrate correlations between the abundances of pathogenic bacteria in the two study groups (Figure 2A,B; network figures in Figure S4). Positive correlations between pathogenic bacteria were stronger in atopic compared with non-atopic individuals (Figure 2C).

Our finding of low microbial diversity in tonsils of the atopic patients is in line with previous studies on other tissues. For example, exacerbation of atopic dermatitis has been shown to associate with decreased microbial diversity of the skin when compared to healthy skin and asthma has been linked to imbalance of detrimental and beneficial microbes of the respiratory and gastrointestinal epithelium when compared to healthy controls. Therefore, as tonsil tissue contains rich microbiome, it is logical that atopy status is also associated with lower bacterial diversity in tonsils. Collectively, these

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findings suggest that systemic dysbiosis, extending to local lymphoid tissue level, is associated with allergic diseases. It should be noted, however, that our study analyzed gene expression by RNA sequencing whereas many earlier studies have analyzed DNA and genetic information.

In our study, the most abundant phylum was Proteobacteria and the most abundant genera were *Salmonella*, *Klebsiella* and *Sodalis*. This is in line with previous studies, in which Proteobacteria have been highly associated with tonsillar hyperplasia and recurrent tonsillitis in children. Also in earlier studies, the increase in *Moraxella* at genus level has been linked to asthma. However, in our study, the differential abundance of taxa *Moraxella Osloensis* was lower in atopic patients as well as taxa *Clostridium Botulinum*. Comparing the interrelations of pathogenic bacteria, we found more significant correlations in the atopic group. The existence of atopy slightly increased the complexity of bacteria-to-bacteria networks. Moreover, intra-individual variation of microbiome has been demonstrated comparing classical secretory otitis media pathogens in adenoid and tonsil tissues.

This study shows four important findings in elective tonsillectomy patients. First, tonsil tissue contains rich bacterial species in direct contact with immune system. Second, this is a first study to demonstrate that atopic individuals have lower tonsillar bacterial diversity than non-atopics. Third, our data indicate that lower overall bacterial abundance and fourth, stronger positive interrelations between pathogenetic bacteria are associated with atopy. Our study provides interesting insights into the microbiome of human tonsils and suggests differences in microbial balance between atopic and non-atopic subjects. Further studies are warranted to investigate interactions between microbes and lymphoid tissue function.

**ACKNOWLEDGEMENTS**

Dr Ivaska reports grants from Turku University Hospital Foundation, grants from Turku University Foundation, during the conduct of the study. MSc. Hanif reports grants from The Ella and George Ehrnrooth Foundation, Finland, grants from Ida Montin Foundation, Finland, grants from Finnish-Norwegian Medical Foundation, during the conduct of the study. MSc. Ahmad has...
nothing to disclose. Dr Tan has nothing to disclose. Dr Altunbulakli has nothing to disclose. Dr Mikola reports grants from The Finnish Cultural Foundation, during the conduct of the study. Dr Silvoniemi has nothing to disclose. Dr Puhakka has nothing to disclose. Dr Akdis reports grants from Allergopharma, Idorsia, Swiss National Science Foundation, Christine Kühne-Center for Allergy Research and Education, European Commission's Horizon's 2020 Framework Programme, Cure, Novartis Research Institutes, Astra Zeneca, Scibase, advisory role in Sanofi/Regeneron, outside the submitted work. Dr Toppila-Salmi reports personal fees from Sanofi Pharma, grants from GSK, personal fees from Roche, personal fees from Novartis, personal fees from ERT, outside the submitted work. Dr Jartti reports grants from the Sigrid Juselius Foundation, Helsinki, Finland, grants from the Allergy Research Foundation, Helsinki, Finland, grants from the Foundation for Pediatric Research, Helsinki, Finland, during the conduct of the study.

CONFLICTS OF INTEREST
The authors have no conflict of interest in connection with this paper.

FUNDING INFORMATION
TJ and his laboratory are supported by the Sigrid Juselius Foundation, Helsinki; Allergy Research Foundation, Helsinki; and the Foundation for Pediatric Research, Helsinki. LEI and TH are supported by the Turku University Hospital Foundation, Turku; and LEI also by the Turku University Foundation, Turku; EM is supported by the Finnish Cultural Foundation and the Allergy Research Foundation Helsinki, all in Finland.

REFERENCES

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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