A dental biofilm microbiome signature associated with pediatric allergies

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To the Editor

It has been proven that a competent immune system is based on the development of a balanced and diverse microbiome throughout childhood. Western life-style conditions are closely linked to dysbiotic colonization patterns and to the occurence of chronic inflammation. While most research concentrates on the skin and gut microbiome , little is known about an association between dental diseases and asthma or other allergic diseases in children . Newer findings gave rise to the question whether oral bacteria, in particular periopathogens, may have a positive impact on or be even a prevention against allergic diseases (5, 6).

To investigate the possible association of saliva and dental biofilm microbiome with pediatric allergy and asthma, a cross-sectional study was conducted in allergic asthmatic children (AA-Chd; n=15; age 10.7±2,9) and atopic/allergic children (AT/AL-Chd; n=16; 11.3±2,9). Allergic asthma was diagnosed by medical history, clinical examination, body plethysmography, positive skin prick test to aeroallergens and/or specific serum IgE. A group of age-matched healthy children (CON-Chd; n=15; age 9.9±2.2) served as control. Age, sex and body mass index (BMI) showed no significant differences. For details, see supplementary table 1.Specific dental indices (see supplement) served as control parameters to confirm comparable dental and gingival conditions and were similar within the three children groups. Oral microbiome from saliva and dental biofilm was assessed by 16S-rRNA gene next-generation sequencing as previously described . Complete material and methods are listed in the supplement.

The β-diversity of the microbiome analysis as visualized in Figure 1 showed significant differences in the structure of the microbiome between saliva and biofilm using PERMANOVA model (r^2= 0.17, p<0.001). When comparing groups the biofilm β-diversity revealed highly similar microbiome structure between the AA-Chd and AT/AL-Chd (r^2=0.02, p=0.796), which was distinct from the CON-Chd (AA-Chd: r^2=0.09, p=0.018; AT/AL-Chd: r^2=0.08, p=0.021).

Concerning α-diversity, the saliva microbiome revealed a slight increase of diversity in the CON-Chd compared to AA-Chd but no significant differences with regard to richness, Pielou´s evenness and dominance

(Suppl. Figure 1A). Consistently, α-diversity in the biofilms microbiome indicated a higher Shannon index in the CON-Chd group, again without statistical significance. Pielou's evenness in CON-Chd compared to AT/AL-Chd (Suppl. Figure 1B) reached the level of statistical significance. A subanalysis considering glucocorticoid intake in the AA-Chd, revealed a significant impact of glucocorticoid on the α-diversity and a higher Shannon index in the biofilm (p=0.04).

All three pediatric groups showed a higher diversity in the biofilm than in the saliva. By using Deseq2 it could be shown that certain taxa were differentially enriched or depleted using between AA-Chd and CON-Chd (Figure 2A, 2B), AT/AL-Chd and CON-Chd (Figure 2C, 2D), and between AA-Chd and AT/AL-Chd (Figure 2E, 2F). Surprisingly, only a limited number of taxa were enriched in AA-Chd versus CON-Chd and in AT/AL-Chd versus CON-Chd (Figure 2G). We observed a major overlap between the differentially abundant taxa of AA-Chd and AT/AL-Chd compared to CON-Chd. In both diseased groups, *Capnocytophaga gingivalis* and the genus *Capnocytophaga, Fusobacterium, Prevotella_6* and*Mannheimia* were enriched but in contrast, *Cardiobacterium hominis, Fusobacterium nucleatum* and *Haemophilus sp.* were depleted. Furthermore, the biofilm microbiome between AA-Chd and AT/AL-Chd differed by only four taxa, *Pasteurellaceae*unclassified and *Kingella* unclassified) were enriched in AA-Chd, and *Capnocytophaga haemolytica* and *Gracilibacteria P22*unclassified were depleted, respectively (Figure 2E, 2F).

These data indicate that allergy and atopy are associated with a strong shift in the biofilm's microbial structure independently of asthma. However, asthma co-morbidity induces a slightly more pronounced shift of the bacterial composition in the biofilm. This important information obtained especially from the analysis of α- and β-diversity in dental biofilm indicates that dental microbiome is informative for future studies especially in discovering biologically plausible mechanisms by which oral pathogens could influence the risk of allergic disease. Longitudinal studies and functional investigations are now required to establish a cause-effect relationship.

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Figure legends

Figure 1: PCoA plot showing the similarity of the microbiota's structure of AA-Chd (green), AT/AL-Chd (red), CON-Chd (blue) for both saliva and biofilm **(A)** , saliva alone **(B)** and biofilm alone **(C)** . The PCoA plots were based on the Morisita-Horn distance. Ellipses represent the 95% confidence ellipse based on a multivariate t-distribution.

Figure 2: Differentially abundant taxa between allergic asthmatic children and age-matched healthy controls. Log2 fold change between AA-Chd and CON-Chd **(A)** , between AT/AL-Chd and CON-Chd**(C)** as well as between AA-Chd and AT/AL-Chd **(E)** . Features were considered significant if their adjusted P -value was $<.05$ and the fold change was higher than 2. Cladogram showing the most differentially abundant taxa enriched in microbiota from AA-Chd (green) and CON-Chd (blue) **(B)** , AT/AL-Chd (red) and CON-Chd (blue) **(D)** as well as AA-Chd (green) and AT/AL-Chd (red) (F). (G) Venn diagram illustrating unique and shared species among child group comparisons.

Figure 1A, B and C

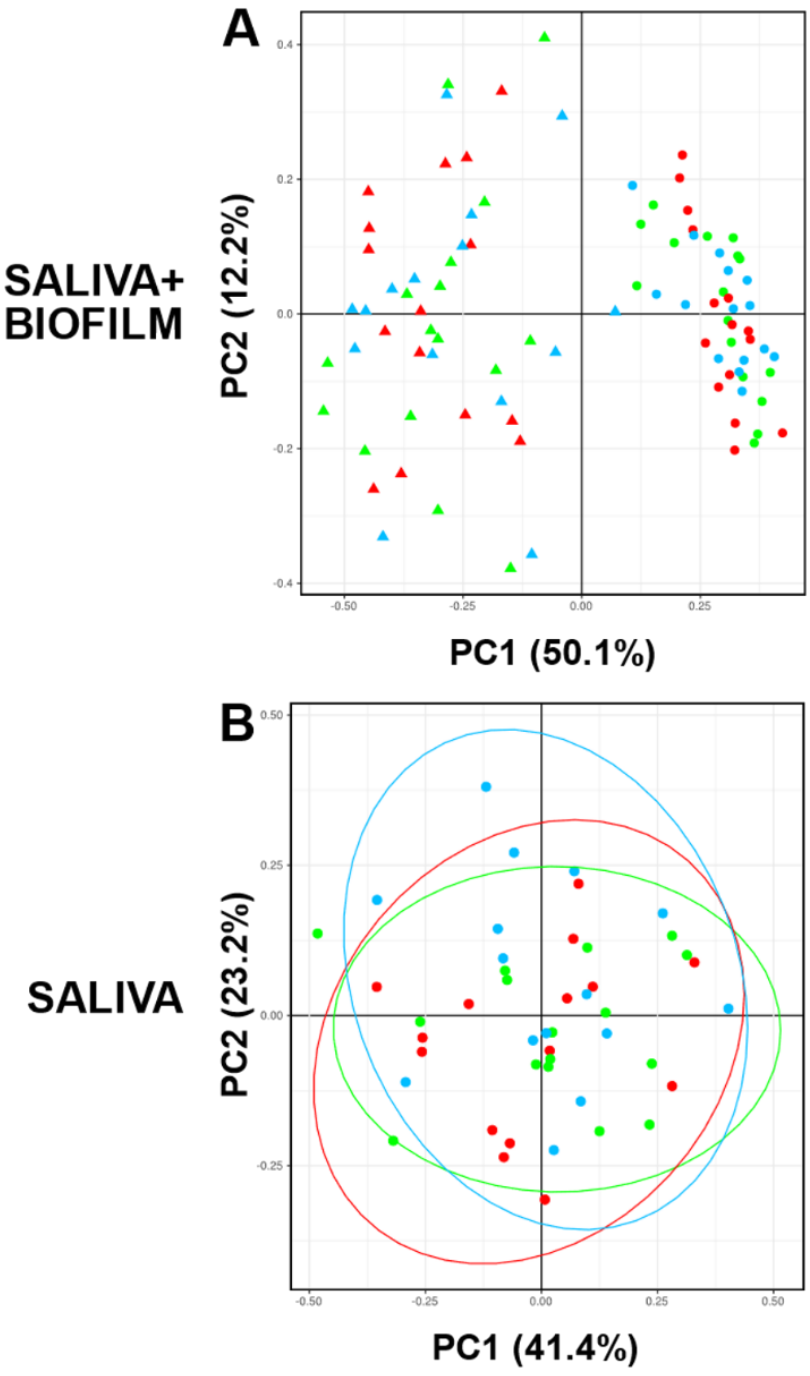

Figure 2A, B, C, D, E, F and G