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Inverse association between the skin and oral microbiota in atopic dermatitis

Short title: Skin, oral and gut microbiota in AD

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Abbreviations: AD, Atopic dermatitis; EOS, Eosinophil; HMP, Human microbiome project;

KEGG, Kyoto encyclopedia of genes and genomes; LDA, Linear discriminant analysis;
LEfSe, The linear discriminant analysis (LDA) effect size; OUT, Operational taxonomic units;
PCoA, Principal coordinates analyses; PERMANOVA, Permutational multivariate analysis of
variance; PICRUST, Phylogenetic investigation of communities by reconstruction of
unobserved states; rRNA, Ribosomal RNA; SCORAD, Scoring of atopic dermatitis

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Abstract

Previous studies have shown independently that the skin and gut microbiota are closely associated with atopic dermatitis (AD); however, the microbiota across different habitats of AD patients as an integrated community has not been characterized. In the present study, we comparatively analysed the structure and function of the microbial communities in the skin, oral cavity and gut of 172 AD patients and 120 healthy controls through 16S rRNA gene amplicon sequencing. The skin and oral cavity, but not the gut, of AD patients demonstrated differential reduction in the microbial diversity, and these were distinctly correlated with disease severity. Different degrees of shifts in the community structure were found among different habitats, and the lineage distance between the skin and oral microbiota of AD patients was closer than that observed in the controls. The different habitats of AD patients exhibited site-specific alterations at the genus level, and many oral-specific microbes of AD showed opposing directions of enrichment in the skin and oral cavity. Most interestingly, an inverse association in the functional pathways was found between the skin and oral microbiota of AD patients. Additionally, the alterations of the microbiota in different body sites of AD patients were differentially affected by age.

Key words: atopic dermatitis, microbiota, oral cavity, skin, gut

INTRODUCTION

Previous studies have independently shown that both the gut and skin microbiota are closely associated with AD. The gut microbiota of infant AD patients exhibits a reduced diversity (Abrahamsson *et al.*, 2012), which is inversely related to the severity of AD (Watanabe *et al.*, 2003; Nylund *et al.*, 2015). The diversity of the skin microbiota in AD lesions is also significantly reduced compared with that of healthy individuals (Chng *et al.*, 2016; Kennedy *et al.*, 2017; Kong *et al.*, 2012; Shi *et al.*, 2016), and the proportion of *Staphylococcus*, particularly *S. aureus*, is increased in AD and correlated with the severity of the disease (Byrd *et al.*, 2017a; Kong *et al.*, 2012). However, the microbiota in different habitats of AD patients as an integrated ecological community has not been characterized, and the role of the microbiota residing in other habitats, *e.g.*, the oral cavity, of AD patients is unclear.

Different body habitats have site-specific microbiota, and these microbiota might be differentially involved in the homeostasis of an individual and in the pathogenesis of various diseases (The HMP, 2012; Lloyd-Price *et al.*, 2017). Under certain disease conditions, the microbial communities in multiple body sites might exhibit alterations simultaneously. For example, dysbiosis in both the gut and oral cavity has been observed in inflammatory bowel disease (IBD) (Lucas *et al.*, 2017), rheumatoid arthritis (RA) (Yeoh *et al.*, 2013; Zhang *et al.*, 2015) and colorectal cancer (Flynn *et al.*, 2016), whereas asthma has been associated with alterations of the microbiota in both the gut and the respiratory tract (Huang *et al.*, 2017). The microbiota in different habitats might contribute synergistically or oppositely to the pathogenesis of a disease, but the exact mechanism has not yet been elucidated. Thus, analyzing the microbiota in a single body habitat might be insufficient to reveal its

relationship with the host.

The oral cavity in human is the second largest bacterial reservoir after the lower gastrointestinal tract (Arweiler and Netuschil, 2016) and is profoundly relevant to host health and disease (Curtis *et al.*, 2011; Dewhirst *et al.*, 2010). The oral microbiota is involved in the aetiology of dental caries and periodontal disease (He *et al.*, 2015), and it is also associated with chronic inflammatory diseases at distant sites, for example, IBD, RA, bacterial endocarditis, atherosclerosis, respiratory diseases and diabetes (He *et al.*, 2015; Yeoh *et al.*, 2013). Most recently, causal relationships between the oral microbiota and colorectal or pancreatic cancer have been confirmed (Fan *et al.*, 2018; Flemer *et al.*, 2017), and ectopic colonization of the oral bacteria in the intestine has been found to drive Th1 cell induction and inflammation (Atarashi *et al.*, 2017). In addition, it has been speculated that oral inflammation is associated with allergic disease (Arbes and Matsui, 2011), but the characteristics of the oral microbiota in AD remain unclear.

In the present study, we aimed to comparatively analyze the microbiota in the skin, oral cavity and gut of AD patients as an integrated community to obtain a more comprehensive understanding of the relationships among the microbiota in different habitats and the host in AD, paying additional attention to the characteristics of the oral microbiota.

RESULTS

Differentially reduced diversity in the skin and oral microbiota in AD patients

We collected 226 skin lesion samples (AD=121, control=105), 161 oral samples (AD=84, control=77) and 171 stool samples (AD=88, control=83) from 172 AD patients and 120 non-AD controls matched in age, gender and body mass index (see Supplementary Table S1 online), and analyzed the 16S ribosomal RNA (rRNA) of bacteria from the samples. Both the operational taxonomic units (OTU) richness and α -diversity of the microbiota from the skin of AD patients were significantly reduced compared with that of healthy controls ($P<0.01$, Wilcoxon rank-sum test, Figure 1a and b). Specifically, the decrease in the Shannon diversity of AD patients remained significant even after the sequence of *S. aureus* was subtracted ($P<0.01$, Wilcoxon rank-sum test, see Supplementary Figure S1 online). The OTU richness and α -diversity of the microbiota of the oral cavity also showed significant reductions in AD patients ($P<0.05$, Wilcoxon rank-sum test, Figure 1a and b). However, no difference in the OTU richness or α -diversity of the gut microbiota was found between AD patients and healthy controls (Figure 1a and b). We then stratified the samples into three age groups: toddlers (1-3 years), children (4-12 years) and teenagers-adults (13-59 years), and obtained similar results for both the skin and gut samples in all age groups; whereas a reduction in α -diversity was observed in the oral cavity of the teenager-adults group (see Supplementary Figure S2 online). Collectively, our data showed that the richness and evenness of the microbiota were differentially reduced in the skin and oral cavity, but not in the gut, under AD conditions.

Closer lineage relationship between the skin and oral microbiota in AD patients

Next we characterized the similarities and differences in the composition of microbial communities between the samples (β -diversity) from AD patients and controls and among the different body sites. The microbial communities in the skin, oral cavity and gut of both AD patients and healthy controls clustered separately (Figure 1c) and showed a pattern similar to that described in a previous report by the Human Microbiome Project (HMP) (The HMP, 2012). However, the microbial community composition of AD patients was significantly different compared with that of healthy controls in all three body sites (permutational multivariate analysis of variance (PERMANOVA), $P < 0.001$, Figure 1c, see Supplementary Table S2 online). The PERMANOVA results based on weighted UniFrac distances among these samples also corroborated these observations. The division of the samples into the three above-mentioned age groups yielded similar results (see Supplementary Table S2, Figure S3 online).

We subsequently investigated the similarities and differences in the microbial communities between two body locations from the subjects with paired sequencing data (see Supplementary Table S1 online). The results showed that the distance between the skin vs oral samples from AD patients was significantly reduced compared with that found for the controls, regardless of whether unweighted or weighted UniFrac distances were used (see Supplementary Figure S4 online, $P=0.0236$ and $P=4.673e-07$, Wilcoxon rank-sum test). The skin vs gut and oral vs gut distances were only significantly reduced only if weighted or unweighted UniFrac distances were used, respectively (see Supplementary Figure S4 online, $P=0.0346$, $P=0.01561$, Wilcoxon rank-sum test). We further performed a SourceTracker

analysis (Knights *et al.*, 2011) and found that the resemblance between the skin and oral microbiota of AD patients was closer than that found for the controls, as demonstrated using oral samples as the ‘source’ and skin samples as the ‘sink’ (see Supplementary Figure S5 online).

Collectively, our data indicated a closer relationship in the microbial lineage between the skin and oral cavity of AD patients than between those of healthy controls.

Distinct abundance of taxa across the three body sites in AD

We found that the skin, oral cavity and gut each harboured niche-specific taxa that were distinctly enriched in AD patients or healthy controls (see Supplementary Table S3-S5 online). For example, at the phylum level, *Proteobacteria* was significantly enriched in the oral samples and significantly decreased in the skin of AD patients, whereas *Firmicutes* was significantly enriched in the skin of AD patients. (see Supplementary Table S3, Figure S6 online). At the genus level, 116, 53 and 39 genera showed significant differences in the skin, oral cavity and gut between AD patients and controls, respectively (adjusted $P < 0.05$, see Supplementary Table S4, Figure S7 online). A stratified analysis of samples from different age groups revealed 98%, 82% and 74% overlap in the genera found in the skin, oral cavity and gut among the different age groups, respectively. Considering the notable similarity in genera from each body site among the three age groups, the subsequent analysis were carried out mainly based on the combined age group. The top 15 genera with the most significant differences in abundance among the three habitats were shown in Figure 2, and 10, 8 and 8 genera were specific to the skin, oral cavity and gut, respectively (Figure 2). The Random

Forest Classifiers that could select the most representative genera that discriminated AD patients from controls also showed that the genera selected from each habitat were distinctive (see Supplementary Figure S8 online). Specifically, 36 out of the 53 oral-specific genera in AD exhibited either the opposite direction of enrichment to that observed in the skin or were not found at a significantly different abundance in the skin (see Supplementary Table S4 online). Thus, at the phylum and genus levels, specific microbes were enriched distinctly in the skin or oral cavity of AD patients.

To further identify the patterns of the differences in the microbial community between AD patients and controls across the three body sites, we established co-abundance associations among the genera from the three body sites and then clustered them into three co-abundance groups (see Supplementary Figure S9a and b online). The most dominant genera in the three groups were *Staphylococcus*, *Streptococcus* and *Bacteroides* (see Supplementary Table S6 online). We then visualized the network of these genera among the three body sites in AD patients and controls according to the Spearman correlation coefficient (Figure 3). The results showed that each habitat had its own dominant cluster of microbes and revealed alterations in both the microbes and the associations among the three habitats in AD patients (Figure 3). Interestingly, 14 of the top 15 genera of the skin microbiota that showed significant differences in abundances, as shown in Figure 2, were classified as belonging to the *Staphylococcus*-dominated group, which was more prevalent in the skin. This feature was also found for the microbes of the oral cavity and gut.

Collectively, AD patients showed site-specific clustering of and alterations in the abundances of microbes across the three major body habitats.

Association between genera and AD-related clinical parameters

The Shannon diversity of the skin microbiota was significantly inversely correlated with the Scoring Atopic Dermatitis (SCORAD) index (Figure 4, $P < 0.001$, Spearman's correlation). Interestingly, the Shannon diversity of the oral microbiota was significantly positively correlated with the SCORAD index, although the correlation was weak (Figure 4, $P = 0.011$, Spearman's correlation). The Shannon diversity of the gut microbiota did not exhibit a significant correlation with the SCORAD index (Figure 4, $P = 0.305$, Spearman's correlation), and no significant correlation between the total IgE level or the eosinophil (EOS) count and the Shannon diversity was found at any of the three body sites (see Supplementary Figure S10 online).

At the genus level, the genera found in the skin, oral cavity and gut were differentially correlated with AD-related clinical parameters, the SCORAD index, total IgE level and EOS count (see Supplementary Figure S11 online). For example, *Staphylococcus* in the skin was positively correlated with the SCORAD index, in contrast to most of the other microbes in the skin; the microbes in the oral cavity were mostly positively correlated with the SCORAD index, and the direction of these correlations was opposite to that of the correlations between most skin microbes and the SCORAD index; many of the microbes in the gut were significantly correlated with the total IgE level and EOS count, and *Phascolarctobacterium* and *Klebsiella* were the most significant genera. Different age group also had its specific correlation between specific genera and clinical parameters (see Supplementary Figure S12 online).

Inverse association in function prediction between the skin and oral microbiota in AD patients

Function profiles of the microbiota obtained for each sample were predicted through a phylogenetic investigation of the communities involving the reconstruction of unobserved states (PICRUSt) based on the closed-reference 16S rRNA gene sequence (Langille *et al.*, 2013). Of the 296 PICRUSt-inferred Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, 108, 71 and 4 pathways showed significant differences between AD patients and controls in the skin, oral cavity and gut, respectively (Figure 5, see Supplementary Table S7 online, linear discriminant analysis (LDA) effect size (LEfSe), LDA score > 2, $P < 0.05$). Interestingly, the enrichment direction of most of the pathways in the skin microbiota was opposite to that found in the oral cavity (Figure 5). For example, the pathways for peptidoglycan biosynthesis, lysine biosynthesis, and galactose metabolism were enriched in the skin of AD patients and decreased in the oral cavity (Figure 5). In contrast, the pathway for tryptophan metabolism was enriched in the oral cavity of AD patients but decreased in the skin (Figure 5). Patterns of enrichment of the significantly different functional pathways of three age groups were similar to that of combined age group (see Supplementary Figure S13 online). We also analysed the correlation between the KEGG pathways and AD-related clinical parameters (see Supplementary Figure S14 online), and significant correlations were mainly found between the pathways of the skin microbiota and the SCORAD index and EOS count (see Supplementary Figure S14 online). The total IgE level showed higher correlations with the pathways in the gut microbiota than with those in the skin and oral cavity. Most of

the enrichment direction of the correlated pathways in the oral microbiota with respect to all three parameters was opposite to that obtained for the skin microbiota (see Supplementary Figure S14 online).

DISCUSSION

In the present study, the microbial structure and function of the skin, oral cavity and gut of AD patients were analysed as an integrated community to our knowledge previously unreported. The results showed that the taxonomic richness of the microbiota was differentially reduced in the skin and oral cavity of AD patients and revealed differential shifts in the composition of the microbial communities, particularly those of the skin and oral cavity, in AD patients; the different habitats in AD patients exhibited site-specific alterations in microbes, and an inverse association in the functional prediction was found between the skin and oral microbiota (Figure 6). The alterations of the microbiota in different body sites of AD patients were also differentially affected by age. Our data revealed the characteristics of the oral microbiota in AD and enriched the understanding of the microbiota in different habitats of AD patients from a global point of view (Figure 6).

Our findings on the microbiota from the skin and gut of AD patients are generally consistent with those obtained in previous studies. The significant reduction in the skin microbial community diversity and the prominent abundance of *S. aureus* species in the lesions of AD patients are highly consistent with prior reports (Kong *et al.*, 2012). The functional predictions based on the KEGG pathways obtained in our study show high concordance with the metagenome data published by Chng *et al.* (Chng *et al.*, 2016). The unchanged α -diversity obtained in our study for the gut microbiota is similar to the results published by Song *et al.* (Song *et al.*, 2016); however, many previous studies have observed a decreased microbial α -diversity in the gut (Abrahamsson *et al.*, 2012; Arrieta *et al.*, 2015; Fujimura *et al.*, 2016). The reason for the discrepancy might be the age range of the enrolled

patients, and both our study and the study conducted by Song *et al.* mainly included AD patients older than one year. Previous study also reported that alteration in specific microbes, such as *Clostridium difficile*, in the gut of infant might lead to eczema (Penders *et al.*, 2007). Thus, our data indirectly confirms that the gut microbiota plays more important roles in the development of atopy during the early stages of life. However, the correlation between the genera in the gut microbiota and the increased IgE level obtained in our study (see Supplementary Figure S11 online) indicates that in adults, the gut microbiota might still be involved in the production of IgE.

The importance of the oral microbiota in diseases at distant sites has attracted increasing attention (Atarashi *et al.*, 2017; Fan *et al.*, 2018; Flemer *et al.*, 2017; Flynn *et al.*, 2016; He *et al.*, 2015; Lucas *et al.*, 2017; Yeoh *et al.*, 2013). In the present study, we revealed a significant reduction in the microbial diversity of the oral cavity in AD patients and an apparent alteration in genera (Figure 1-2). The alteration in the composition of the oral microbiota in AD in our study is different from that observed in oral diseases and chronic inflammatory diseases at distant sites, *e.g.*, IBD. Dental caries show increased abundances of the genera *Prevotella* and *Atopobium*; in periodontal diseases, the abundances of the genera *Prevotella*, *Parvimonas* and *Peptostreptococcus* are increased (He *et al.*, 2015); in the oral cavity of IBD patients, the level of *Prevotella* is increased (Lucas *et al.*, 2017). These genera are hypothesized to produce a pro-inflammatory microenvironment in the oral cavity, which might result in local and systemic inflammation (He *et al.*, 2015; Lucas *et al.*, 2017). However, in the oral cavity of AD patients, the abundances of the above-mentioned genera were significantly decreased compared with those found in healthy individuals, which

suggests that the oral microbiota in AD might play an anti-inflammatory role. Moreover, the direction of most of the KEGG pathways associated with the oral microbiota was opposite to that found for the skin microbiota (Figure 5), and this finding also suggests that the oral microbiota might have an anti-inflammatory function, which contrasts the pro-inflammatory functions of the *S. aureus*-dominant skin microbiota. Specifically, propanoate metabolism, butanoate metabolism and tryptophan metabolism are enriched in the oral microbiota of AD patients (Figure 5). It has been reported that butyrate and propionate play an anti-inflammatory role by induction of Treg via inhibition of histone deacetylase (Arpaia *et al.*, 2014; Sanford *et al.*, 2016), and short chain fatty acids also inhibit the activation of dendritic cells (Nastasi *et al.*, 2015). We just reported that a metabolite of the tryptophan, IAId, attenuated the skin inflammation in a mouse model of MC903-induced AD-like dermatitis by inhibiting the expression of TSLP in keratinocytes in an AhR-dependent manner. (Yu *et al.*, 2018). The increased enrichment of these anti-inflammatory pathways in the oral microbiota of AD patients indicates that the oral microbiota of AD patients may play an anti-inflammatory role. Furthermore, sublingual immunotherapy is effective for the treatment of atopic diseases (Halken *et al.*, 2017), and it has been proposed that the mucosa of the oral cavity is found in a tolerogenic status (Novak *et al.*, 2008). Thus, we speculate that the oral microbiota might create an anti-inflammatory microenvironment and play a regulatory role to counterbalance skin inflammation in AD.

A connection between the oral and gut microbiota has been proposed in gastrointestinal cancer and IBD (Atarashi *et al.*, 2017; Fan *et al.*, 2018; Flemer *et al.*, 2017), and multiple studies have shown that the oral cavity is the source of the microbiota of the nasal cavity

(McLean, 2014) and lower respiratory tract (Segal *et al.*, 2016). However, the relationship between the oral and skin microbiota under physiological and pathological conditions is unclear. Chng *et al.* showed that the majority of species enriched in AD-susceptible subjects are known commensals or opportunistic pathogens of the oral cavity (Chng *et al.*, 2016). Our data demonstrate that the distance between the microbiota from the skin and oral cavity of AD patients is closer than that found in healthy controls (see Supplementary Figure S4 online), and a SourceTracker analysis demonstrated that the resemblance between the skin and oral microbiota of AD patients is higher than that found in healthy individuals (see Supplementary Figure S5 online). The inverse association of the functional pathways between the skin and oral microbiota also suggests a close relationship between the skin and oral microbiota in AD. Thus, we propose that the interplay between the oral and skin microbiota might be involved in the pathogenesis of AD.

Our study has several limitations. We only characterized the microbiota in different body sites at the time of AD flare-up, and an analysis of the microbiota during the remission stage would give more information. The oral samples used in our study consisted of a mixture of the microbiota from the buccal mucosa, keratinized gingiva, supragingival plaque and tongue dorsum, which includes the major sites of the oral cavity and provided abundant bacteria for the analysis; however, the current result might lack site specificity within the oral cavity. Age-associated alterations in the microbial structure and function in AD across the three body sites need further exploration, as the sample size for each age group in the present study is relatively small.

MATERIALS AND METHODS

Study subjects and clinical sample collection

A total of 172 AD patients were recruited, and 120 subjects without AD were selected from the set of volunteers (see Supplementary Table S1 online). None of the subjects had oral or skin diseases other than AD at the time of sampling, and none of the subjects had systemic diseases or received systemic treatment within 6 months before sample collection. Samples of the skin, oral cavity and stool were collected from each patient and control. The subjects were instructed to avoid bathing and the application of topical medications and emollients at the sampling sites for 24 h prior to sampling and to avoid brushing teeth and eating food for 2 h prior to oral sampling. Skin samples were obtained from the lesions on both sides of the antecubital fossae from AD patients or from the same sites of healthy controls using a swab as reported previously (Kong *et al.*, 2012). Oral samples were collected by rubbing the buccal mucosa, keratinized gingiva, supragingival plaque and tongue dorsum with a swab (The HMP, 2012). Stool samples were self-collected, stored on ice and sent to the laboratory within 1 h of defecation. A diagnosis of AD was made based on the UK criteria (Guo *et al.*, 2016), and the severity of AD was assessed using the Scoring Atopic Dermatitis (SCORAD) system. Peripheral blood was obtained for the preparation of serum and the analysis of blood cell counts. The total serum IgE levels were analysed using the ImmunoCap System (Phadia, Uppsala, Sweden). The study was approved by the Ethics Committee of the Institute of Dermatology, Chinese Academy of Medical Science, and written informed consent was obtained from the participants or their parents.

Data availability

Sequence reads for the 558 samples have been deposited in the European Nucleotide Archive under the study number PRJEB23853. The authors declare that all other relevant data supporting the findings of this study are either available in this article and its supplementary files or from the corresponding author upon request.

Detailed information for DNA extraction and 16S rRNA amplicon sequencing, Processing of 16S rRNA sequencing data, SourceTracker analysis, Random-forest classification, Microbial structure network, Prediction of functional potentials from 16S data, and Statistical analysis were provided in Supplementary Material and Methods online.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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References:

- Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012;129 :434-40.
- Arbes SJ, Matsui EC. Can oral pathogens influence allergic disease? *J Allergy Clin Immunol* 2011; 127 :1119-27.
- Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; 504:451-5.
- Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, *et al.* Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med* 2015; 7 :152r-307r.
- Arweiler NB, Netuschil L. The Oral Microbiota. *Adv Exp Med Biol* 2016; 902 :45-60.
- Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, *et al.* Ectopic colonization of oral bacteria in the intestine drives TH1 cell induction and inflammation. *Science* 2017; 358 :359-65.
- Byrd AL, Deming C, Cassidy S, Harrison OJ, Ng WI, Conlan S, *et al.* *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. *Sci Transl Med* 2017; 9: pii: eaal4651
- Chng KR, Tay AS, Li C, Ng AH, Wang J, Suri BK, *et al.* Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nat Microbiol* 2016; 1 :16106.
- Curtis MA, Zenobia C, Darveau RP. The relationship of the oral microbiota to periodontal health and disease. *Cell Host Microbe* 2011; 10 :302-6.
- Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, *et al.* The human oral microbiome. *J Bacteriol* 2010; 192 :5002-5017.
- Fan X, Alekseyenko AV, Wu J, Peters BA, Jacobs EJ, Gapstur SM, *et al.* Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut* 2018; 67 :120-7.
- Flemer B, Warren RD, Barrett MP, Cisek K, Das A, Jeffery IB, *et al.* The oral microbiota in colorectal cancer is distinctive and predictive. *Gut* 2018; 67:1454-63
- Flynn KJ, Baxter NT, Schloss PD. Metabolic and Community Synergy of Oral Bacteria in Colorectal Cancer. *Msphere* 2016; 1. pii: e00102-16.
- Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosch D, *et al.* Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* 2016; 22 :1187-91.

- Guo Y, Li P, Tang J, Han X, Zou X, Xu G, et al. Prevalence of Atopic Dermatitis in Chinese Children aged 1-7 ys. *Sci Rep* 2016; 6 :29751.
- Halken S, Larenas-Linnemann D, Roberts G, Calderon MA, Angier E, Pfaar O, *et al.* EAACI guidelines on allergen immunotherapy: Prevention of allergy. *Pediatr Allergy Immunol* 2017;28 :728-45.
- He J, Li Y, Cao Y, Xue J, Zhou X. The oral microbiome diversity and its relation to human diseases. *Folia Microbiol (Praha)* 2015; 60 :69-80.
- Kennedy EA, Connolly J, Hourihane JO, Fallon PG, McLean WH, Murray D, *et al.* Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. *J Allergy Clin Immunol* 2017;139 :166-172.
- Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, *et al.* Bayesian community-wide culture-independent microbial source tracking. *Nat Methods* 2011; 8 :761-3.
- Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, *et al.* Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; 22 :850-9.
- Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, *et al.* Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 2013; 31 :814-21.
- Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, *et al.* Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* 2017; 550 :61-6.
- Lucas LR, Grande BM, Galvez A, Perez PR The human gastrointestinal tract and oral microbiota in inflammatory bowel disease: a state of the science review. *APMIS* 2017;125 :3-10.
- McLean JS. Advancements toward a systems level understanding of the human oral microbiome. *Front Cell Infect Microbiol* 2014;4 :98.
- Nastasi C, Candela M, Bonefeld CM, Geisler C, Hansen M, Krejsgaard T, *et al.* The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep* 2015; 5:16148.
- Novak N, Haberstok J, Bieber T, Allam JP. The immune privilege of the oral mucosa. *Trends Mol Med* 2008; 14 :191-8.
- Nylund L, Nermes M, Isolauri E, Salminen S, de Vos WM, Satokari R (2015) Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria. *Allergy* 2015; 70 :241-4.
- Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2017; 56:661-7.

Sanford JA, Zhang LJ, Williams MR, Gangoiti JA, Huang CM, Gallo RL. Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks immune tolerance of the epidermis to TLR ligands. *Sci Immunol* 2016; 1. pii: eaah4609.

Segal LN, Clemente JC, Tsay JC, Koralov SB, Keller BC, Wu BG, *et al.* Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol* 2016; 1 :16031.

Shi B, Bangayan NJ, Curd E, Taylor PA, Gallo RL, Leung D, *et al.* The skin microbiome is different in pediatric versus adult atopic dermatitis. *J Allergy Clin Immunol* 2016;138 :1233-6.

Song H, Yoo Y, Hwang J, Na YC, Kim HS. *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *J Allergy Clin Immunol* 2016;137 :852-60.

The HMP C. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 486 :207-14.

Watanabe S, Narisawa Y, Arase S, Okamatsu H, Ikenaga T, Tajiri Y, Kumemura M. Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects. *J Allergy Clin Immunol* 2003;111:587-91.

Yeoh N, Burton JP, Suppiah P, Reid G, Stebbings S. The role of the microbiome in rheumatic diseases. *Curr Rheumatol Rep* 2013; 15 :314.

Yu J, Luo Y, Zhou Y, Zhu Z, Sun L, Gao J, *et al.* A tryptophan metabolite of the skin microbiota attenuates inflammation in atopic dermatitis via the aryl hydrocarbon receptor. *J Allergy Clin Immunol*. 2018 Dec 19. pii: S0091-6749(18)32777-5. doi: 10.1016/j.jaci.2018.11.036. [Epub ahead of print]

Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, *et al.* The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* 2015; 21 :895-905.

FIGURE LEGENDS:

Figure 1. Community-level analysis of the microbiota in the skin, oral cavity and gut. (a and b) Richness (a) and α -diversity (Shannon index, b) of the microbiota in the three body sites at the OTU level. Skin (AD n=111, control n=102); Oral (AD n=83, Control n=77); Gut (AD n=88, Control n=83). * $P<0.05$, ** $P<0.01$. A two-tailed Wilcoxon rank-sum test was used to determine significance. Boxplots represent the medians and interquartile ranges with whiskers, as determined by Tukey's method; black dots represent data points beyond the whiskers. (c) A principal coordinates analyses (PCoA) based on unweighted UniFrac distances is shown along the first two principal coordinate (PC) axes. Each point represents a single sample. Box plots indicate the distribution of samples along the given axis.

Figure 2. Differential abundance of genera in the three body sites. Relative abundances of the top 15 taxonomic genera that showed clear significant differences in abundances between AD patients and controls in the skin, oral cavity and gut. Only the significant genera are coloured. Adjusted $P<0.05$, two-tailed Wilcoxon rank-sum test. * denotes that the genus is ranked within the top 15 in at least two body sites. Skin (AD n=111, control n=102); Oral (AD n=83, Control n=77); Gut (AD n=88, Control n=83). Boxplots represent the medians and interquartile ranges with whiskers, as determined by Tukey's method; black dots represent data points beyond the whiskers.

Figure 3. Co-abundance associations between genera in the three body sites. Network plots indicate the patterns in the variations of the genera between AD patients and controls

across the three body sites. Each network shows a correlation between the genera in the respective dataset (Skin: AD n=111, Control n=102; Oral: AD n=83, Control n=77; Gut: AD n=88, Control n=83). Each node represents a bacterial genus, and its dimension is proportional to the mean relative abundance across the three body sites. The three groups are named after the most abundant genera, and the colour is coded as follows:

Staphylococcus-dominated group (yellow), *Streptococcus*-dominated group (orange), and *Bacteroides*-dominated group (purple). The colours indicate that the genera are significantly different between AD patients and controls in the specific body site, and grey indicates no significant difference between AD patients and controls. Only the top 15 genera shown in Figure 2 are labelled. Blue edges, Spearman correlation coefficient > -0.3 , adjusted $P < 0.05$; red edges, Spearman correlation coefficient < 0.3 , adjusted $P < 0.05$.

Figure 4. Associations of microbial diversity with SCORAD index. Scatter plot of Shannon index against the SCORAD index in the three body sites. The linear regression line and the 95% confidence interval are also visualized. The correlations between the Shannon index and the SCORAD index were calculated by Spearman's correlation analysis (skin AD n=111, oral AD n=83, gut AD n= 88).

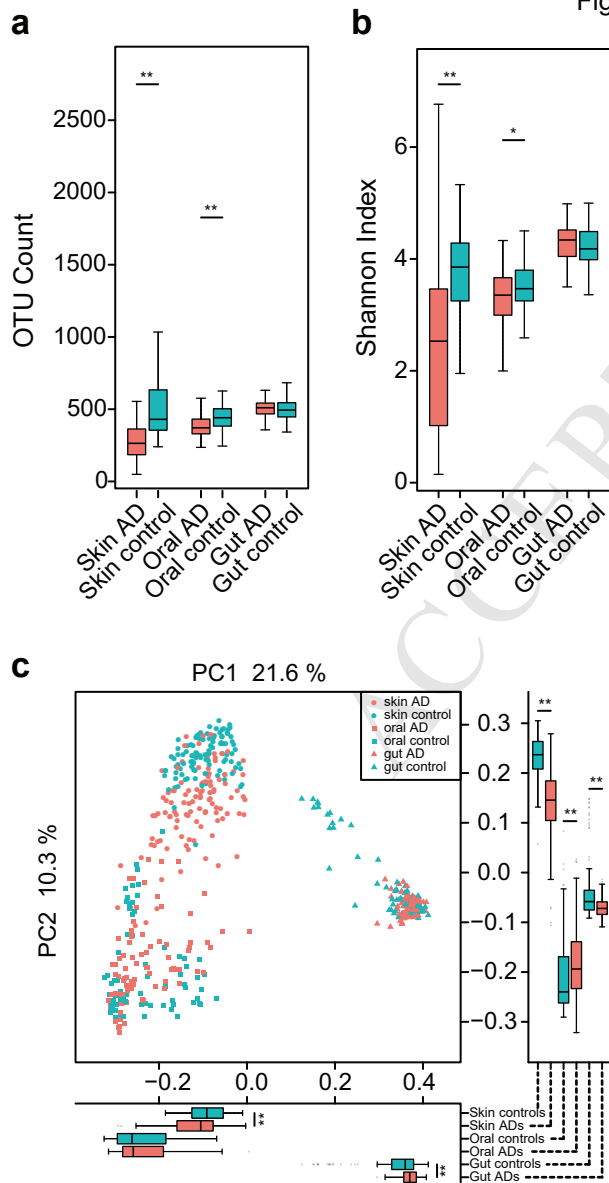
Figure 5. The inferred microbial functions in the three body sites. Heat map of the results of the LEfSe performed on the pathway abundance between AD patients and controls across the three body sites (skin: AD n=111, controls n=102; oral: AD n=83, Control n=77; gut: AD n=88, Control n=83). Blue, pathways enriched in the controls; Red, pathways enriched in AD

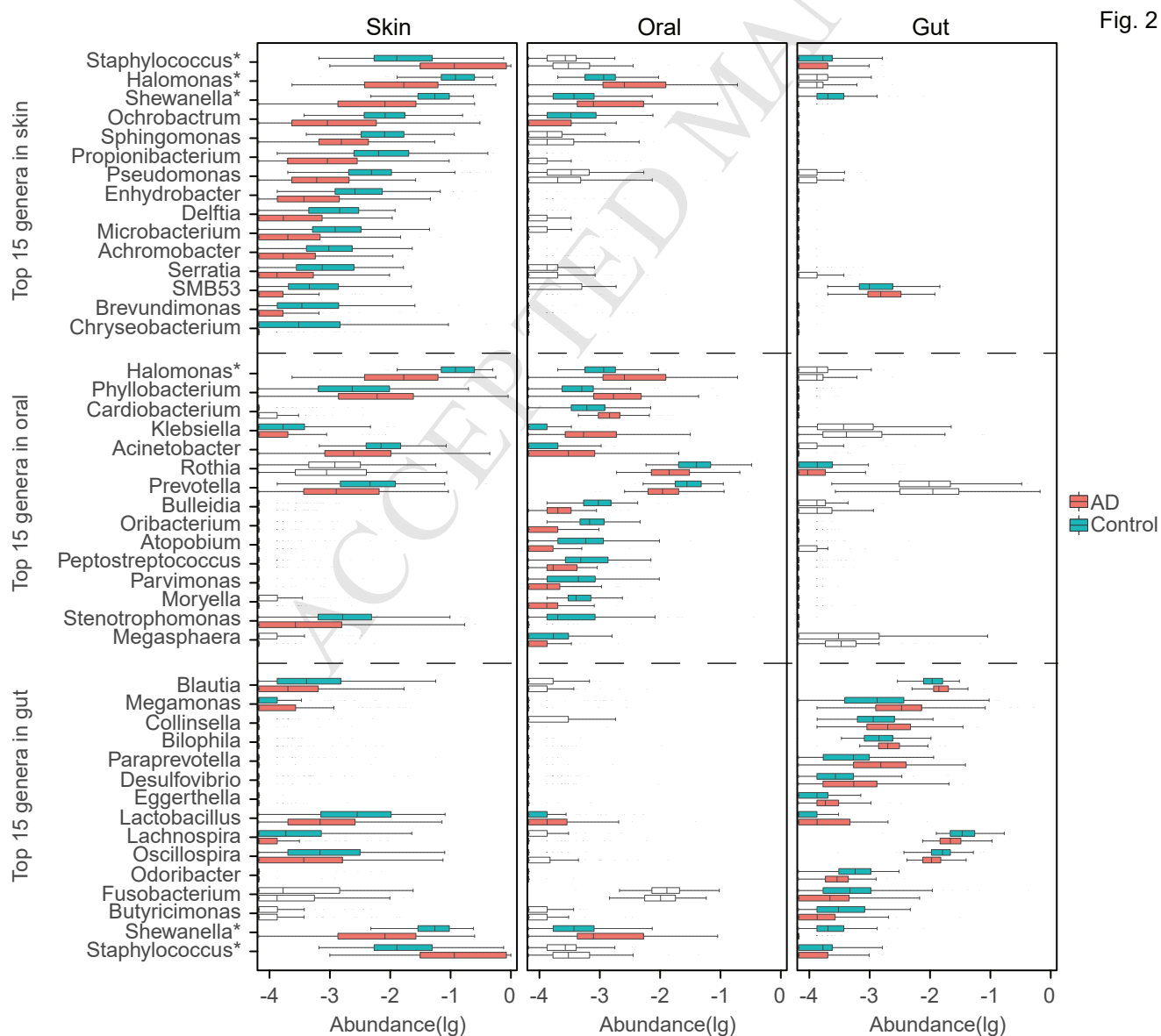
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Figure 6. Summary for the structure and function of the microbiota in different body

sites. There are differential reduction in the microbial diversity of the skin and oral cavity, but not the gut, of AD patients. The lineage distance between the skin and oral microbiota of AD patients is closer than that of the controls. Each habitat of AD patients has site-specific changes in microbes, and many of the microbes show opposing directions of enrichment in the skin and oral cavity. The inferred microbial function enrichment in the skin and oral cavity of AD patients is inversely associated.

Fig. 1

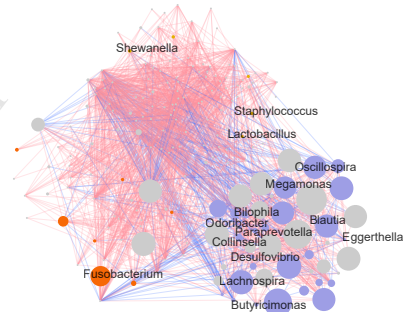
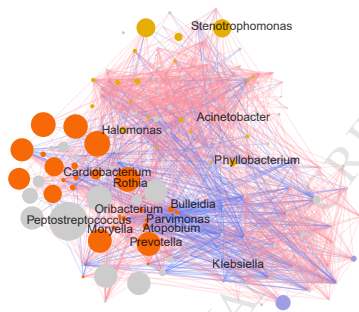
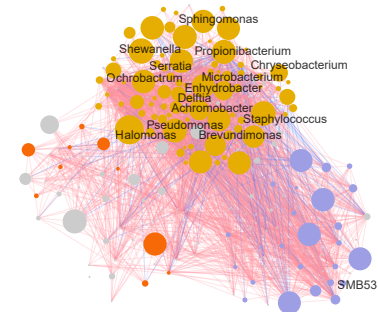




Skin

Oral

Gut



Control

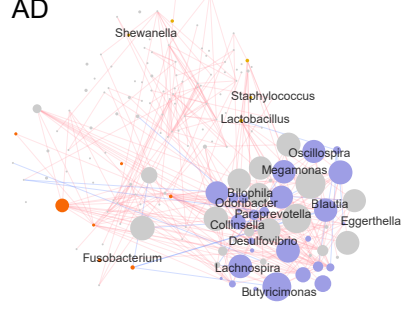
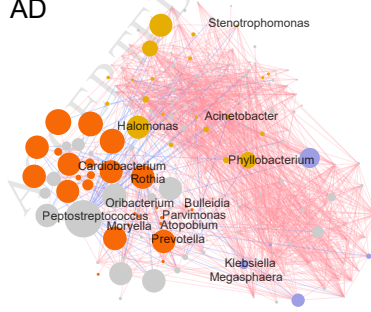
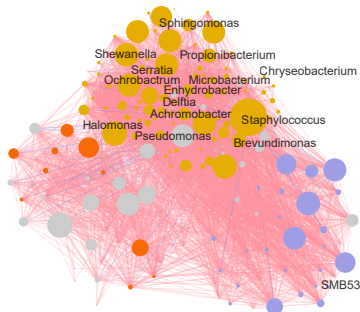
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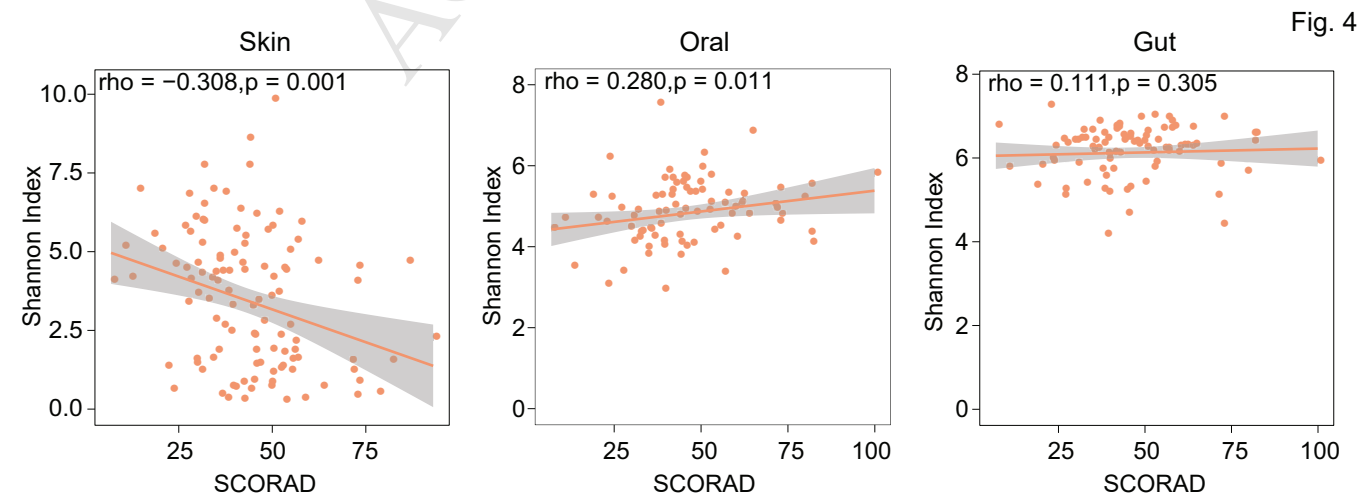
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AD

AD





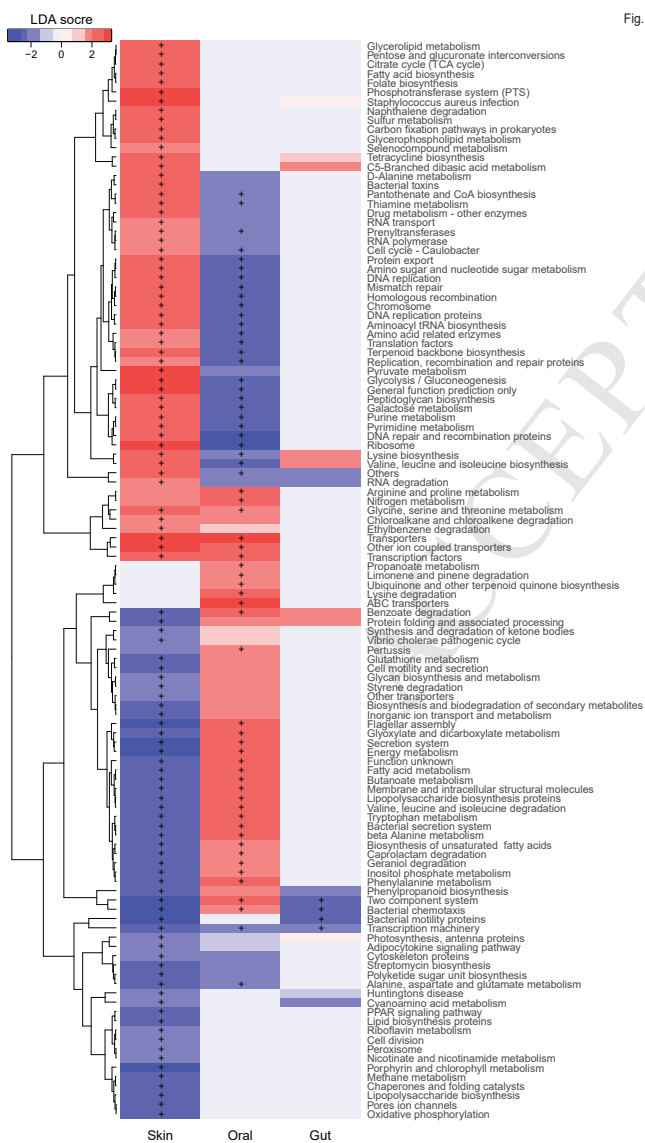


Fig. 6

