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MICROBIAL PATTERNS IN PATIENTS WITH HISTAMINE INTOLERANCE

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Histamine intolerance represents a controversially discussed disorder. Besides an impaired degradation of orally supplied histamine due to diamine oxidase (DAO) deficiency, a deranged gut flora may also contribute to elevated histamine levels. Our aim was to determine the intestinal bacterial composition in patients with proven histamine intolerance in comparison to other food intolerances and healthy controls. A total of 64 participants were included in the study, encompassing 8 patients with histamine intolerance (HIT), 25 with food hypersensitivity (FH), 21 with food allergy and 10 healthy controls (HC). All participants underwent blood testing for total and food-specific immunoglobulin E, plasma histamine and DAO serum activity. Stool samples were used to analyze stool histamine and zonulin levels and bacterial composition by 16s rRNA sequencing. No significant differences in stool histamine levels were observed, but HIT patients showed elevated levels of stool zonulin. Microbiota analysis revealed increased levels of Proteobacteria (5.4%) and a significantly reduced alpha-diversity in the HIT group ($P = 0.019$). On family level, HC showed a significantly higher abundance of Bifidobacteriaceae compared to other study groups ($P = 0.005$), with lowest levels in the HIT group ($P = 0.036$). Also significantly reduced abundances of the genera Butyricimonas ($P = 0.026$) and Hespellia ($P = 0.025$) were observed in the HIT patients, whereas Roseburia were significantly elevated ($P = 0.021$). We concluded that the altered occurrence of Proteobacteria and Bifidobacteriaceae, reduced alpha-diversity as well as elevated stool zonulin levels suggest a dysbiosis and intestinal barrier dysfunction in histamine intolerant patients, which in turn may play an important role in driving disease pathogenesis.

Key words: *dysbiosis, food intolerance, gastrointestinal microbiome, histamine, intestinal barrier, diamine oxidase, lactic acid bacteria*

INTRODUCTION

The prevalence of patients suffering from gastrointestinal and extra-intestinal afflictions after food ingestion is rising. The spectrum of food intolerances reaches from carbohydrate malabsorption (*e.g.* lactose, fructose) to immunological IgE or non-IgE-mediated food allergies (1, 2). In addition, histamine intolerance (HIT) is also often considered to be responsible for gastrointestinal symptoms after food intake. Thereby, histamine intolerance is defined as an adverse reaction of ingested histamine that affects different organ systems and results in various intestinal and extra-intestinal symptoms (3). Ingestion of histamine containing foods and beverages, including fish, cheese or red wine, are supposed to trigger symptoms like flush, pruritus, nausea, vomiting, diarrhea and abdominal pain (3). Other foods like citrus fruits or various drugs further contribute to an elevated histamine concentration through their histamine-liberating effect (4). Although the exact mechanism of the pathogenesis is still unclear, a reduced intestinal diamine oxidase (DAO) activity, which is important for degradation of exogenously supplied histamine, is presumed (4). This leads to an insufficient degradation of food derived histamine, which passes into the blood stream leading to increased plasma histamine concentrations and evoking the described symptoms by affecting various organ systems (*e.g.* cardiovascular system, respiratory tract, skin, nervous system, intestine) (4, 5).

However, also other factors are discussed to affect histamine intolerance, for example an alteration of the intestinal bacteria. Various bacteria, which are able to convert histidine from proteins into histamine, naturally occur in the digestive tract as part of the normal intestinal gut flora (6, 7).

Interestingly, some probiotic strains including several lactic acid bacteria, like *Lactobacillus reuteri*, *Lactobacillus casei* and *Lactobacillus delbrueckii subsp. bulgaricus*, possess the enzyme histidine decarboxylase (HDC) and are therefore able to generate biogenic amine (8, 9). The presence of these bacteria in the human intestine might contribute to increased histamine levels and promote histamine sensitivity in some persons.

It is well known that alterations of the human intestinal microbiota are linked to various diseases. Besides obesity or cardiovascular disease (10, 11), a dysbiosis is discussed in the pathogenesis of different autoimmune diseases including type 1 diabetes, rheumatoid disease, inflammatory bowel disease or celiac disease (12, 13). But even in patients with an allergy, the influence of the microbiota as a triggering factor for asthma and food allergy is discussed (14, 15). Several studies revealed a

correlation between a low microbial exposure in childhood and an increased risk for allergies. Thereby, multiple factors can influence this risk positively or negatively by altering the intestinal microbiota, *e.g.* mode of birth, duration of breastfeeding, treatment with antibiotics, infections, living with older siblings or furred pets (16). Interestingly, the use of probiotics seems to have immunomodulatory effects in allergic disease by suppressing histamine signaling (17). *Via* the induction of regulatory T cells (Tregs) some Clostridia species seem to suppress symptoms in murine models of intestinal allergy (18). All these facts underline the importance of intestinal bacteria in human immunity and health.

To determine the influence of the human gut microbiota in the pathogenesis of histamine intolerance, we analyzed the intestinal bacterial composition by sequencing the bacterial 16S rRNA of stool samples derived from patients with a confirmed diagnosis of histamine intolerance. These data were compared with the microbial patterns of stool samples from healthy individuals, patients with food allergy or food hypersensitivity. The measurement of histamine concentrations in stool samples was done to evaluate the histamine production by intestinal bacteria. To assess the gut permeability, zonulin, a regulator of tight junctions, was measured in serum and stool samples.

MATERIALS AND METHODS

Study participants

Patients with histamine intolerance, food hypersensitivity and allergies were recruited over a 12-month period *via* the outpatient clinic for nutritional medicine of the Medical Department 1 of the University Hospital Erlangen as well as social media platforms. Healthy controls were recruited from the circle of friends or colleagues. A total of 64 patients were included in the study. Exclusion criteria were pregnancy, lactation, being underage and current intake of antibiotics, anti-histamines or anti-inflammatory medication.

All participants were informed in detail by a doctor about the aim and procedure of the study and gave their written informed consent prior study inclusion. The study was approved by the ethics committee of the Friedrich-Alexander-University Erlangen (application number: 231_14B) and in accordance with the declaration of Helsinki.

Group allocation

Blood samples were taken of all study participants to determine total immunoglobulin E (IgE) as well as ten food-specific IgEs (chicken's egg white, milk protein, wheat flour, rye flour, nut mixture, soy bean, tomatoes, salmon, casein and celeriac). Participants with gastrointestinal (diarrhea, nausea, vomiting, abdominal pain) and extra-intestinal symptoms (allergic rhinitis, oral allergy syndrome, headache, fatigue, skin changes, asthmatic symptoms) briefly after food ingestion and positive serological food-specific IgE antibodies and significantly elevated total IgE (361.2 kUA/L; $P > 0.001$) were classified as food allergy patients (FA group). Individuals with symptoms, but negative food-specific IgE antibodies and low total IgE levels (< 100.0 kUA/L) underwent further measurements of plasma histamine levels and serum DAO activity. Patients with impaired histamine degradation, characterized by elevated plasma histamine levels and decreased DAO activity in serum and an alleviation of symptoms during a histamine-free diet, were further validated by repeated blood samples over a period of 24 hours, and allocated to the histamine intolerant group (HIT group). The remaining participants without IgE antibodies and without validated histamine intolerance, but clinical symptoms including abdominal pain, diarrhea, nausea, headache, skin changes or allergic rhinitis, were classified as food hypersensitive patients (FH group). The healthy controls (HC group) showed no clinical symptoms and no signs for food allergy and histamine intolerance.

The whole diagnostic procedure for group allocation was published by Pinzer *et al.* (19).

Nutritional assessment

To determine daily intake of macronutrients the Freiburger Diet Protocol (Nutri-Science GmbH, Freiburg, Germany) was used. Therefore, all study participants were asked to fill in a three-day nutritional dairy at the beginning of the study. Daily intake of energy, carbohydrates, fats, fibers and alcohol was evaluated by PRODI® (version 6.5 expert, Nutri-Science GmbH).

Sample collection and analysis

Venous blood samples were taken from every study participant and plasma histamine, DAO activity, total IgE concentration and food-specific IgE antibodies were quantified in blood serum. Serum zonulin and TNF-α concentrations were measured by ELISA (IDK® Zonulin ELISA Kit and IDK® TNF-α ELISA, Immunodiagnostic AG, Bensheim, Germany) following manufacturer's instructions. Histamine and zonulin from stool samples were determined with the Histamine ELISA and IDK® Zonulin ELISA from Immunodiagnostic AG, Bensheim, Germany. Stool samples were collected once at study beginning from all study participants and were immediately cooled at 4ºC and stored within 4 hours at –20ºC till analysis. Bacterial DNA was isolated with the QIAamp Fast DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) following manufacturer's instructions. DNA concentration was measured by NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, USA).

For 16S-based microbiome analysis, the amplification of the V3-V4 region of bacterial 16S rRNA was realized by NEBNext Q5 Hot Start Hifi PCR Master Mix (New England Biolabs, Ipswich, USA). Amplicons were purified with AMPure XP Beads (Beckman Coulter Genomics, Indianapolis, USA), and the DNA content was measured by fluorometric quantitation using the Qubit® dsDNA-Kit (Thermo Fisher Scientific, Germany). DNA samples were pooled and analyzed by 2 × 300 bp paired-end sequencing on the Illumina MiSeq platform. Quality control, OTU table generation and taxonomic classification against the database of the 'Ribosomal database projects' (RDP, version 16) was performed using Usearch 10 (64 bit version).

Statistical analysis

Statistical analyses were performed using SPSS version 21 (IBM SPSS Statistics, Ehningen, Germany) and GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA, USA). Bioinformatics analysis for bacterial composition were performed with METAGENassist (20) and MicrobiomeAnalyst (21). Characteristic data are described as means ± standard deviation (SD), median and range (min-max) or in number (n) and percent (%). All data for bacterial proportions are described as median with minimum to maximum values (min-max). For statistical evaluation data were checked by Kolmogorov-Smirnov-test for normal distribution. Differences between study groups were determined using Kruskal-Wallis-test for non-parametric data. Due to the exploratory character of this pilot study, no correction

for multiple testing was applied. For categorical variables differences between study groups were analyzed by Pearson's chi-squared test. The alpha-diversity was described using Shannon-Weaver-Index (SWI) and Simpson's reciprocal index (SI). The Permutational Multivariate Analysis of Variance (PERMANOVA) was used for analysis of pairwise inter-sample distances with Bray-Curtis method. Correlation analysis of variables was computed using the non-parametric Spearman rank correlation.

All tests for significance were two sided, and a P-value of $P < 0.05$ was considered as statistically significant.

RESULTS

Characteristics

Overall 64 study participants (38.3 ± 14.2 years, 84.4% female) were enclosed to the study, and classified according to a previous study (19). Briefly, 33 patients (age 38.4 ± 13.4 years, 84.8% female) had suspected histamine intolerance. The 24 h histamine profiling and the measurement of serum DAO activity revealed 8 out of these 33 patients (12.5%) with histamine intolerance by definition (decreased DAO activity) and these patients were allocated to HIT-group. The remaining 25 patients (age 41.4 ± 12.8 years; 80.0% female) with normal DAO activity were considered as food hypersensitive (FH-group). Additionally, 21 patients with proven food allergy (age 41.4 ± 14.9 years, 81% female) and 10 healthy volunteers (age 31.3 ± 13.9 years, 90% female) without gastrointestinal complaints were acquired.

Patients with proven food allergy showed positive IgE antibodies against nut mixture (76.2%), wheat flour (47.6%), celery (42.9%), tomato (23.8%), rye flour (23.8%), soybean (14.3%), and milk protein (4.8%) as well as significantly increased total IgE levels (361.2 ± 911.2 kUA/l; $P < 0.001$). None of the individuals from the other groups showed specific IgE antibodies against foods.

The presence of further comorbidities including asthma, atopic eczema, cardiovascular disease, depression, endometriosis, fibromyalgia, gastrointestinal disease, hypothyreodism was not significantly different between all study groups.

Patients characteristic are shown in *Table 1*.

Serum and stool parameters

Patients in the HIT group revealed elevated levels of TNF-α compared to other study groups ($P = 0.097$) (*Table 1*). However, only one HIT patient showed a serum TNF-α concentration above the reference threshold value of 20 ng/ml (*Fig. 1a*). Zonulin levels in stool and serum were similar in all participants ($P = 0.726$ and $P = 0.595$) (*Fig. 1b* and *1c*), with highest median levels for stool zonulin in patients that belong to the HIT or FH groups (*Table 1*, *Fig. 1b*). Concerning the stool histamine concentrations one patient of the FH and one patient of the FA group showed very high stool histamine levels (> 24.000 ng/ml) (*Fig. 1d*). The

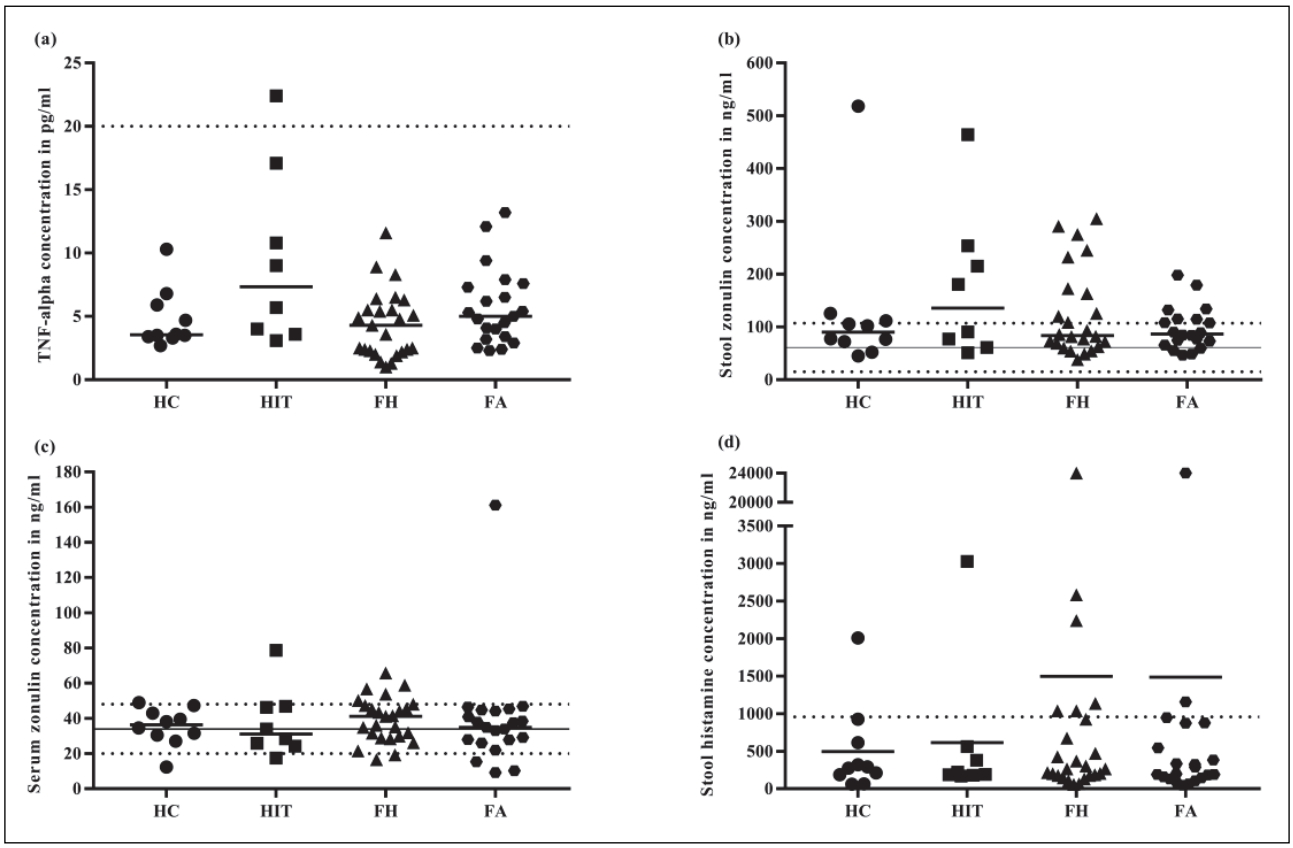

Fig. 1. Blood and stool parameters of study groups. *Fig. 1a* shows individual serum TNF-α concentrations with mean values. The dotted line indicates the reference threshold for normal TNF-α values (< 20 pg/ml). *Fig. 1b* and *1c* show individual zonulin concentrations in stool (*b*) and serum (*c*) with median (horizontal line). The solid lines mark the reference medians and dotted lines indicate the under and upper threshold of normal concentrations. *Fig. 1d* shows individual histamine stool concentrations of study groups with mean values (horizontal line). Dotted line indicates reference threshold for normal histamine values (< 959 ng/ml). Kruskal-Wallis test was used for multiple comparisons of laboratory values between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

Table 1. Patient characteristics of study groups.

Characteristic	HC	HIT	FH	FA	P-value
Demographic					
Amount [n(%)]	10 (15.6)	8 (12.5)	25 (39.1)	21 (32.8)	–
Age [years]	31.3 ± 13.9	28.9 ± 11.2	41.4 ± 12.8	41.4 ± 14.9	**0.026***
Male [n(%)]	1 (10.0)	0 (0.0)	5 (20.0)	4 (19.0)	0.518
Female [n(%)]	9 (90.0)	8 (100.0)	20 (80.0)	17 (81.0)	
Body mass index [kg/m²]	21.2 ± 2.0	24.6 ± 6.5	24.2 ± 4.6	23.4 ± 4.1	0.273
Alcohol consumption [n(%)]	8 (80.0)	6 (75.0)	15 (60.0)	17 (81.0)	0.397
Nicotine abuse [n(%)]	3 (30.0)	1 (12.5)	2 (8.0)	3 (14.3)	0.411
Probiotic use [n(%)]	0 (0.0)	0 (0.0)	4 (16.0)	1 (4.8)	0.248
Comorbidities					
Asthma [n(%)]	0 (0.0)	0 (0.0)	3 (12.0)	3 (14.3)	0.447
Atopic eczema [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	3 (14.3)	0.432
Cardiovascular disease [n(%)]	1 (10.0)	0 (0.0)	2 (8.0)	1 (4.8)	0.805
Depression [n(%)]	0 (0.0)	1 (12.5)	2 (8.0)	0 (0.0)	0.357
Endometriosis [n(%)]	0 (0.0)	1 (12.5)	0 (0.0)	2 (9.5)	0.271
Fibromyalgia [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)	0.359
Gastrointestinal disease [n(%)]	0 (0.0)	1 (12.5)	3 (12.0)	1 (4.8)	0.578
Hypothyreodism [n(%)]	0 (0.0)	2 (25.0)	4 (16.0)	6 (28.6)	0.265
Migraine [n(%)]	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)	0.359
Serum and stool parameters					
Total IgE [kUA/l]	14.3 ± 9.6	38.7 ± 29.9	25.9 ± 22.7	361.2 ± 911.2	**> 0.00**
TNF-α [pg/ml]	3.6 (2.7 – 10.3)	7.4 (3.1 – 22.4)	3.6 (1.0 – 11.6)	5.0 (2.3 – 13.2)	0.097
Zonulin serum [ng/ml]	36.4 (12.3 – 48.9)	31.2 (17.5 – -78.7)	41.2 (16.6 – 65.8)	34.9 (9.2 – 161.2)	0.595
Zonulin stool [ng/ml]	90.0 (45.1 – 518.2)	135.7 (51.2 – 464.2)	85.7 (38.0 – 305.0)	84.6 (46.8-198.3)	0.726
Histamine stool [ng/ml]	283.7 (62.6 – 2008.0)	206.0 (169.8 – 3027.5)	265.2 (57.9 – 24000.0)	196.6 (45.7 – 24000.0)	0.828
Nutritional intake					
Energy intake [kcal/d]	2081.7 ± 818.6	2431.3 ± 362.3	2873.3 ± 1218.1	2862.9 ± 1552.8	**0.025***
Carbohydrates [g/d (%TE)]	232.2 ± 104.2 (44.5)	302.4 ± 69.4 (50.4)	291.4 ± 112.1 (40.9)	258.3 ± 94.0 (42.1)	0.097
Fat [g/d (%TE)]	85.9 ± 34.6 (37.6)	86.1 ± 15.9 (31.8)	126.9 ± 66.3 (38.4)	103.5 ± 34.1 (37.1)	0.059
Protein [g/d (%TE)]	79.6 ± 33.0 (15.3)	87.1 ± 24.9 (14.5)	116.1 ± 51.4 (16.6)	94.1 ± 28.2 (16.1)	**0.026***
Fiber [g/d (%TE)]	19.4 ± 2.5 (1.9)	31.7 ± 0.0 (1.9)	68.3 ± 81.5 (2.2)	22.8 ± 12.6 (1.7)	0.261
Alcohol [g/d (%TE)]	4.3 ± 2.4 (0.7)	5.0 ± 6.2 (1.0)	13.6 ± 14.7 (2.0)	15.3 ± 9.9 (3.2)	**0.028***

Data are presented as number and proportions (%), mean ± standard deviation. Laboratory values (except total IgE) are expressed as median and range (min to max). Comparisons between HC, HIT, FH and FA group are assessed by Pearson's chi-squared test, respectively, for categorical variables and Kruskal-Wallis test for continuous variables. Statistically significant differences are indicated by *P < 0.05; ***P < 0.001 and marked in bold type. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers; %TE, Total energy percent.

analysis of stool histamine concentrations revealed no significant differences between study groups (P = 0.828) (*Table 1*).

Nutritional intake

The nutritional assessment revealed significant differences in the daily protein (P = 0.026) and alcohol (P = 0.028) intake between the study groups (*Table 1*). Our healthy controls ingested lower amounts of protein, reaching significance compared to the FH group (P = 0.005). Both, patients of the HIT group and healthy controls, consumed significantly less alcohol than patients of the FA group (P = 0.013 and P = 0.023). Daily carbohydrate intake was highest in the HIT group.

Correlation analysis

Correlation analysis between measured blood and stool parameters *via* Spearman rank method revealed significant correlations. Thereby the stool concentrations of histamine and zonulin showed a moderate positive correlation (r = 0.454; P < 0.001). Both, parameters for α-diversity, SWI (r = 0.339; P = 0.007) and SI (r = 0.337; P = 0.007), were correlated with TNF-α concentrations.

Microbiome analysis

To compare the microbial composition between different study groups, we converted the bacterial counts into percentages. Bacterial phyla, families and genera with an overall percentage below 0.01% were excluded from analysis.

The microbial patterns showed differences between all study groups. On phylum level, Bacteroides (61.9%), Firmicutes (31.7%) and Proteobacteria (3.7%) were most abundant in all study participants. Significant differences were observed for Verrucomicrobia (P = 0.030) with elevated numbers in patients with FH [0.35% (0.0 – 16.4%)], but minor proportions in HC [0.02% (0.00 – 2.36%)], HIT [0.00% (0.00 – 0.07%)] and FA groups [0.08% (0.00 – 3.05%)], respectively (*Fig. 2a*, *Table 2*). Interestingly, patients from the HIT group showed very low levels of Verrucomicrobia, without any abundance in five patients reaching significance to FH and FA group (P = 0.003 and P = 0.019). In contrast, HIT patients had elevated proportions of Proteobacteria [5.36% (1.34 – 34.59%)] compared to the other study groups, although significance was not reached, because of great variations between the HIT patients (*Fig. 2b*, *Table 2*).

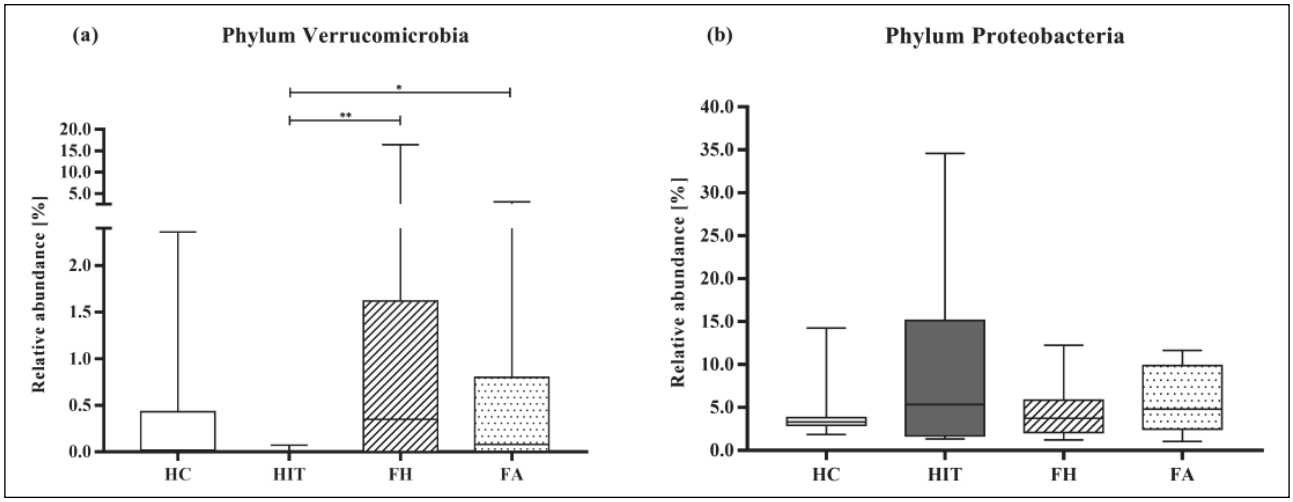

Fig. 2. Differences on phylum level between study groups. *Fig. 2a* shows significant higher relative abundance of the phylum Verrucomicrobia in FH and FA group compared to the HIT group (P = 0.003 and 0.019). *Fig. 2b* showed highest abundance for the phylum Proteobacteria in the HIT group [5.36% (1.34 – 34.59%)]. Kruskal-Wallis test was used for multiple comparisons between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers. Significance: *P < 0.05 and **P < 0.01.

Table 2. Phylum level - relative abundance.

Phylum	HC	HIT	FH	FA
Bacteroidetes	56.94 (37.94 – 74.03)	63.97 (15.80 – 86.00)	63.94 (27.56 – 83.22)	61.19 (27.56-83.22)
Firmicutes	37.00 (22.29 – 54.78)	25.51 (12.43 – 46.50)	28.87 (14.40 – 58.82)	34.07 (10.63-53.84)
Proteobacteria	3.33 (1.85 – 14.25)	5.36 (1.34 – 34.59)	3.75 (1.22 – 12.26)	4.82 (1.04-11.64)
Actinobacteria	0.45 (0.06 – 3.31)	0.12 (0.01 – 7.39)	0.25 (0.03 – 3.97)	0.13 (0.07-2.86)
Verrucomicrobia	0.02 (0.00 – 2.36)	**0.00 (0.00 – 0.07)†,††**	**0.35 (0.00 – 16.44)††**	**0.08 (0.00-3.05)†**
Tenericutes	0.0013 (0.00 – 0.92)	0.00 (0.00 – 0.00)	0.00 (0.00 – 2.32)	0.00 (0.00-4.45)
Synergistetes	0.00 (0.00 – 0.28)	0.00 (0.00 – 0.13)	0.00 (0.00 – 0.72)	0.00 (0.00-0.03)
Lentisphaerae	0.00 (0.00 – 0.20)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.93)	0.00 (0.00-0.35
Acidobacteria	0.00 (0.00 – 0.01)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.19)	0.00 (0.00-2.26)
Elusimicrobia	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 2.04)	0.00 (0.00-0.00)

Data are presented as median and range (min-max). Kruskal-Wallis test was used for multiple comparisons between study groups. Statistically significant differences are indicated by P < 0.05 and marked in bold type. Significance: †P<0.05, ††P<0.01 comparison between HIT, FH or FA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

Table 3. Family level - relative abundance.

Family	HC [%]	HIT [%]	FH [%]	FA [%]
Bacteroidaceae	32.24 (10.61–58.06)	52.17 (3.82–76.56)	42.50 (3.04–75.28)	22.75 (0.03–77.76)
Ruminococcaceae	15.74 (8.83–20.91)	5.78 (1.44–20.52)	9.31 (1.27–26.26)	13.90 (3.79–25.85)
Lachnospiraceae	9.94 (2.79–17.24)	10.03 (3.52–16.04)	5.14 (1.15–17.19)	7.09 (2.07–20.86)
Porphyromonadaceae	9.40.(2.75–16.99)	7.24 (0.05–16.08)	6.22 (2.54–22.57)	8.18 (2.04–30.51)
Rikenellaceae	5.84 (1.36–14.05)	2.77 (0.00–9.51)	4.13 (0.09–14.68)	5.27 (0.02–21.57)
Oscillospiraceae	3.43 (0.56–16.35)	1.10 (0.11–4.29)	1.12 (0.04–8.46)	1.13 (0.03–11.84)
Veillonellaceae	1.88 (0.01–5.51)	2.99 (0.00–7.62)	1.24 (0.01–9.74)	2.14 (0.39–8.93)
Eubacteriaceae	1.45 (0.23–4.39)	1.23 (0.05–12.16)	0.70 (0.04–4.11)	0.90 (0.06–3.90)
Prevotellaceae	1.13 (0.00–47.46)	0.03 (0.01–13.93)	0.08 (0.00–63.35)	3.09 (0.01–69.12)
Sutterellaceae	0.52 (0.00–12.11)	1.02 (0.00–33.65)	0.96 (0.00–7.20)	0.68 (0.00–3.52)
Clostridiaceae	0.48 (0.01–4.79)	0.06 (0.00–0.79)	0.14 (0.01–9.78)	0.22 (0.01–4.99)
Desulfovibrionaceae	0.37 (0.00–2.31)	0.27 (0.03–0.90)	0.31 (0.00–1.68)	0.45 (0.00–3.39)
Hyphomicrobiaceae	0.37 (0.07–1.72)	0.44 (0.07–1.38)	0.41 (0.01–7.13)	0.56 (0.01–8.00)
Acidaminococcaceae	0.32 (0.00–3.87)	0.00 (0.00–5.54)	1.54 (0.00–29.46)	0.002 (0.00–9.92)
Bifidobacteriaceae	**0.30 (0.02–1.58)***,**	**0.02 (0.00–6.65)***	**0.09 (0.00–3.86)***	**0.06 (0.00–2.43)****
Coriobacteriaceae	0.20 (0.04–1.74)	0.09 (0.01–0.72)	0.06 (0.01–1.36)	0.07 (0.00–0.50)
Lactobacillaceae	0.14 (0.00–1.19)	0.17 (0.00–1.36)	0.10 (0.00–5.27)	0.06 (0.00–1.14)
Pasteurellaceae	**0.12 (0.00–1.13)***	0.02 (0.00–0.11)	**0.003 (0.00–1.62)***,†	**0.025 (0.00–0.93)**†
Peptostreptococcaceae	0.11 (0.01–0.28)	0.18 (0.00–9.09)	0.06 (0.00–13.68)	0.20 (0.03–2.34)
Peptococcaceae	0.10 (0.00–0.84)	0.02 (0.00–0.53)	0.05 (0.00–0.92)	0.08 (0.00–0.58)
Erysipelotrichaceae	0.08 (0.02–0.33)	0.05 (0.00–0.81)	**0.06 (0.01–0.45)**††	**0.16 (0.03–3.34)**††
Clostridiales Family XIII, Incertae Sedis	0.06 (0.02–0.42)	0.06 (0.00–0.21)	0.21 (0.00–0.27)	0.06 (0.00–0.400)
Streptococcaceae	0.06 (0.00–1.10)	0.07 (0.00–0.43)	0.03 (0.00–1.55)	0.03 (0.00–0.35)
Enterobacteriaceae	0.05 (0.00–2.18)	0.17 (0.00–6.39)	0.05 (0.00–11.54)	0.11 (0.00–9.05)
Clostridiales Family XII, Incertae Sedis	0.02 (0.00–0.34)	0.05 (0.00–1.69)	0.02 (0.00–12.37)	0.03 (0.00–0.85)
Verrucomicrobiaceae	0.025 (0.00–2.39)	0.00 (0.00–0.07)	0.16 (0.00–16.51)	0.08 (0.00–3.06)
Graciibacteraceae	0.009 (0.00–0.03)	0.00 (0.00–0.55)	0.00 (0.00–0.10)	0.008 (0.00–0.25)
Bdellovibrionaceae	0.00 (0.00–1.37)	0.001 (0.00–0.37)	0.00 (0.00–3.27)	0.00 (0.00–2.83)
Comamonadaceae	0.00 (0.00–1.31)	0.00 (0.00–1.19)	0.002 (0.00–1.98)	0.002 (0.00–3.94)
Sphingobacteriaceae	0.00 (0.00–1.31)	0.00 (0.00–0.01)	0.00 (0.00–0.14)	0.00 (0.00–8.22)
Rhodospirillaceae	0.00 (0.00–0.96)	0.00 (0.00–5.33)	0.004 (0.00–2.41)	0.23 (0.00–3.66)
Spiroplasmataceae	0.00 (0.00–0.92)	0.00 (0.00–0.00)	0.00 (0.00–0.15)	0.00 (0.00–0.63)
Acholeplasmataceae	0.00 (0.00–0.42)	0.00 (0.00–0.00)	0.00 (0.00–1.54)	0.00 (0.00–3.99)
Synergistaceae	0.00 (0.00–0.28)	0.00 (0.00–0.13)	0.00 (0.00–0.72)	0.00 (0.00–0.03)
Anaeroplasmataceae	0.00 (0.00–0.21)	0.00 (0.00–0.00)	0.00 (0.00–0.80)	0.00 (0.00–0.11)
Victivallaceae	0.00 (0.00–0.20)	0.00 (0.00–0.00)	0.00 (0.00–0.94)	0.00 (0.00–0.35)
Oxalobacteraceae	0.00 (0.00–0.14)	0.00 (0.00–0.20)	0.00 (0.00–0.12)	0.00 (0.00–0.11)
Desulfobacteraceae	0.00 (0.00–0.05)	0.00 (0.00–0.00)	0.00 (0.00–0.35)	0.00 (0.00–0.12)
Flavobacteriaceae	0.00 (0.00–0.03)	0.00 (0.00–0.00)	0.00 (0.00–0.98)	0.00 (0.00–0.19)
Elusimicrobiaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.08)	0.00 (0.00–0.00)
Marinilabiaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–1.74)	0.00 (0.00–0.00)
Succinivibrionaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.63)	0.00 (0.00–0.08)
Puniceicoccaceae	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.36)	0.00 (0.00–0.26

Data are presented as median and range (min–max) in %.
Kruskal-Wallis test was used for multiple comparisons between study groups. Statistically significant differences are indicated by $P < 0.05$ and marked in bold type. Significance: $^{*}P < 0.05$; $^{**}P < 0.01$, comparison HC to HIT, FH or FA; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, comparison between FH and FA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

The most abundant bacterial families found in all study groups were Bacteroidaceae (38.7%), Ruminococcaceae (11.2%), Lachnospiraceae (8.1%), Porphyromonadaceae (7.8%), Rikenellaceae (4.6%) and Veillonellaceae (2.1%). Moreover, the detailed analysis of bacterial families revealed significant differences in the proportions of Bifidobacteriaceae (class Actinobacteria; P = 0.050), Erysipelotrichaceae (class Erysipelotrichia; P = 0.018) and Pasteurellaceae (class Gammaproteobacteria; P = 0.031) between study groups.

On family level, the HC group harbored a significant higher proportion of Bifidobacteriaceae [0.30% (0.02 – 1.58%)] compared to the HIT [0.02% (0.00 – 6.65%); P = 0.036], FH [0.09% (0.00 – 3.86%; P = 0.027)] and FA [0.06% (0.00 – 2.43%); P = 0.007] group (*Fig. 3*, *Table 3*).

The percentage of Erysipelotrichaceae was significantly elevated in the FA group in comparison to FH group [0.16% (0.03 – 3.34%) versus 0.06% (0.01 – 0.45%); P = 0.012]. HC and FA group showed highest proportions of Pasteurellaceae [HC

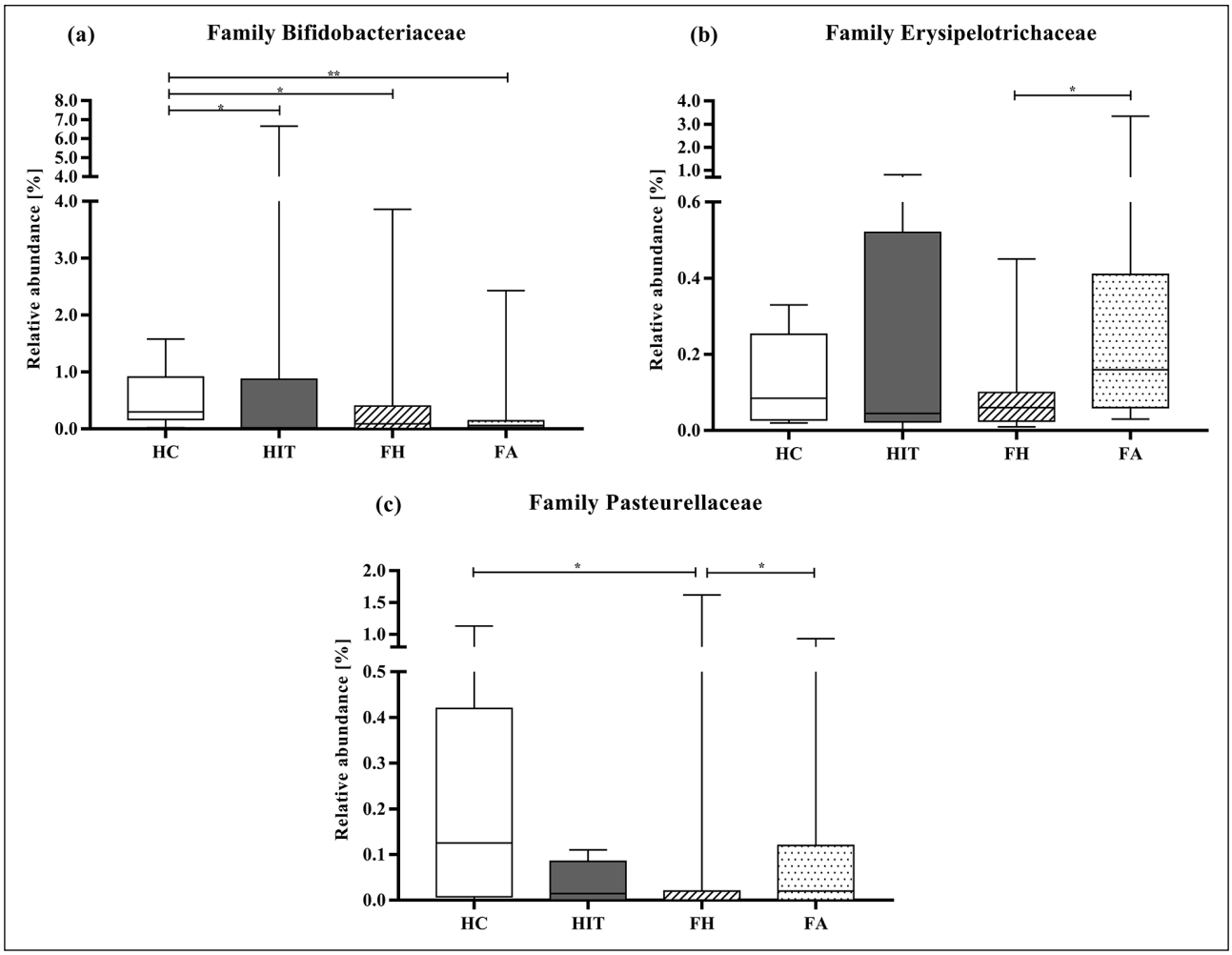

Fig. 3. Differences on family level between study groups. *Fig. 3a* shows significantly highest relative abundance of the family Bifidobacteriaceae in the HC group compared to the HIT (P = 0.036), FH (P = 0.027) and FA group (P = 0.007). *Fig. 3b* shows highest abundance of the family Erysipelotrichaceae in the FA group with significance compared to the FH group (P = 0.012). *Fig. 3c* displays Pasteurellaceae with significant highest levels for the HC and the FA group compared to the FH group (P = 0.014 and P = 0.021). Kruskal-Wallis test was used for multiple comparisons between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers. Significance: *P < 0.05 and **P < 0.01.

0.12% (0.00 – 1.13%) and FA 0.03% (0.00 – 0.93%)], reaching significance between HC and FH group (P = 0.014) and between FA and FH group (P = 0.021).

The analysis on genus level, the most detailed bacterial classification in our analysis, revealed significant differences in six bacterial genera between the study groups (*Fig. 4*). These include Roseburia (family Lachnospiraceae; P = 0.021), Dendrosporobacter (family Veillonellaceae; P = 0.008), Butyricimonas (family Porphyromonadaceae; P = 0.026), Haemophilus (family Pasteurellaceae; P = 0.034), Hespellia (family Clostridiaceae; P = 0.025), and Obesumbacterium (family Enterobacteriaceae; P = 0.001) (*Table 4*).

Thereby patients of the HIT group showed elevated proportions of Roseburia [0.45% (0.07 – 6.72%)] reaching significance compared to the FH group [0.11% (0.00 – 0.82%); P = 0.004)] (*Fig. 4a*). The genus Obesumbacterium [0.00% (0.00 – 1.31%)] was enriched in 2 of 8 HIT patients resulting in significantly higher values in the HIT group compared to all other study groups (HC: P = 0.001; FH: P < 0.001; FA: P = 0.001) (*Fig. 4b*). In addition, the proportion of Veillonella was elevated in the HIT group [0.13% (0.00 – 7.66%)], but did not reach statistical significance.

In contrast, bacteria of the genus Butyricimonas were significant less abundant in the HIT group [0.00% (0.00 – 0.24%)] compared to the HC [0.42% (0.00 – 1.18%); P = 0.002] and FH group [0.04% (0.00 – 2.20%); P = 0.046] (*Fig. 4c*). Additionally, the genus Hespellia was significantly higher in the HC [0.03% (0.00 – 0.20%); P = 0.03] and FA [0.03% (0.00 – 0.12%); P = 0.013] group compared to the HIT group [0.00% (0.00 – 0.06%)] (*Fig. 4d*).

Differences were also observed for genus Haemophilus with elevated percentages for the HC [0.14% (0.00 – 1.33%)] and FA group [0.003% (0.00 – 1.76%)], which were significant compared to the FH group (P = 0.015 and P = 0.025). The FA group [0.37% (0.00 – 2.61%)] showed a significant higher abundance of Dendrosporobacter compared to the HIT [0.00% (0.00 – 1.09%); P = 0.012] and FH group [0.00% (0.00 – 0.77%); P = 0.002].

In accordance with the elevated proportions of Bifidobacteriaceae on family level, the HC group showed also an increased amount of the genus Bifidobacterium [0.35% (0.02 – 1.93%)] compared to the HIT [0.02% (0.00 – 12.91%)], FH [0.09% (0.00 – 6.81%)] and FA group [0.07% (0.00 – 2.95%)], even though the values did not reach significance (P = 0.052).

Bacterial diversity and cluster analysis

The PERMANOVA of the Shannon-Weaver-Index (H) revealed significant differences in bacterial α-diversity between the study groups (P = 0.019) (*Fig. 5a*). Especially patients from the HIT group showed a significant lower diversity compared to all other study groups. Healthy individuals (HC group) displayed the highest α-diversity index with HHC = 3.2 ± 0.7 compared to HHIT = 2.4 ± 1.0 (P = 0.043). The FH (HFH = 2.8 ± 0. 8) and FA group (HFA = 3.0 ± 0.9) showed lower diversities than the HC group, but significantly higher values compared to the HIT group (P = 0.009 and P = 0.002). In accordance, the Simpson's reciprocal index confirmed the significantly lower α-diversity of the HIT group compared to all other groups (*Fig. 5b*).

On genus level, the beta-diversity of HIT patients partly differ from the other study groups using multidimensional scaling by principal coordinates analysis (PCoA) or non-metric multidimensional scaling (NMDS) (*Fig. 6*).

Table 4. Genus level - relative abundance.

Genus	HC	HIT	FH	FA
Bacteroides	37.99 (12.15–60.74)	65.49 (7.42–84.62)	47.55 (3.65–81.65)	34.72 (0.04–81.73)
Alistipes	6.50 (1.65–16.64)	3.14 (0.00–12.63)	4.44 (0.16–16.09)	6.94 (0.03–31.33)
Faecalibacterium	5.56 (2.35–16.09)	3.79 (0.00–6.91)	3.10 (0.00–16.68)	2.38 (0.06–11.48)
Parabacteroides	4.85 (2.38–8.33)	0.24 (0.00–11.07)	4.34 (0.02–10.14)	1.96 (0.00–18.87)
Oscillibacter	4.05 (0.59–19.94)	1.38 (0.12–5.42)	1.33 (0.06–10.28)	1.65 (0.05–13.54)
Barnesiella	2.18 (0.02–12.27)	1.98 (0.04–13.44)	1.01 (0.00–14.35)	2.42 (0.02–30.74)
Ruminococcus	1.87 (0.20–13.95)	1.03 (0.00–3.70)	1.53 (0.00–8.41)	1.46 (0.00–15.51)
Dialister	1.69 (0.00–4.08)	2.40 (0.01–7.41)	1.34 (0.00–9.88)	0.87 (0.00–9.38)
Eubacterium	1.65 (0.25–4.76)	1.50 (0.05–23.48)	0.72 (0.04–6.77)	1.02 (0.07 –4.44)
Odoribacter	0.82 (0.30–2.13)	0.27 (0.00–1.58)	0.66 (0.00–2.93)	0.80 (0.00–2.16)
Sporobacter	0.54 (0.00–4.36)	0.34 (0.00–1.00)	0.10 (0.00–4.58)	0.38 (0.00–2.04)
Gemmiger	0.45 (0.08–1.86)	0.51 (0.07–2.68)	0.43 (0.01–8.05)	0.74 (0.02–8.84)
Butyricimonas	**0.42 (0.00–1.18)****	**0.00 (0.00–0.24)****,†	**0.04 (0.00–2.20)**†	0.003 (0.00–1.46)
Blautia	0.36 (0.07–0.96)	0.24 (0.03–4.08)	0.26 (0.04–1.10)	0.28 (0.13–3.05)
Bifidobacterium	0.35 (0.02–1.93)	0.02 (0.00–12.91)	0.09 (0.00–6.81)	0.07 (0.00–2.95)
Sutterella	0.34 (0.00–2.01)	0.00 (0.00–0.15)	0.006 (0.00–2.65)	0.00 (0.00–4.26)
Bilophila	0.28 (0.00–2.12)	0.005 (0.00–0.96)	0.14 (0.00–1.26)	0.17 (0.00–2.11)
Coprococcus	0.28 (0.01–1.01)	0.13 (0.01–4.35)	0.13 (0.00–3.27)	0.18 (0.01–1.2)
Prevotella	0.25 (0.00–51.46)	0.02 (0.01–16.23)	0.08 (0.00–76.14)	0.85 (0.01–69.41)
Alkaliphilus	0.21 (0.00–0.41)	0.002 (0.00–0.49)	0.007 (0.00–7.59)	0.14 (0.00–3.88)
Lactobacillus	0.16 (0.00–1.43)	0.23 (0.00–1.43)	0.10 (0.00–9.28)	0.07 (0.00–1.50)
Roseburia	0.14 (0.00–1.91)	**0.45 (0.07–6.72)**††	**0.11 (0.00–0.82)**††	0.27 (0.00–1.32)
Haemophilus	**0.14 (0.00–1.33)***	0.02 (0.00–0.14)	**0.003 (0.00–1.76)***,†	**0.03 (0.00–1.01)**†
Dorea	0.14 (0.02–0.47)	0.06 (0.00–2.15)	0.05 (0.00–2.16)	0.09 (0.00–2.39)
Thermotalea	0.14 (0.00–0.17)	0.00 (0.00–0.10)	0.00 (0.00–10.04)	0.003 (0.00–0.14)
Veillonella	0.12 (0.00–0.98)	0.13 (0.00–7.66)	0.04 (0.00–2.11)	0.06 (0.00–2.05)
Desulfotomaculum	0.09 (0.00–0.69)	0.02 (0.00–0.40)	0.04 (0.00–1.16)	0.05 (0.00–0.63)
Collinsella	0.09 (0.02–0.66)	0.003 (0.00–0.74)	0.03 (0.00–1.51)	0.06 (0.00–0.55)
Anaerovorax	0.07 (0.01–0.44)	0.07 (0.00–0.20)	0.02 (0.00–0.29)	0.06 (0.00–0.46)
Pseudoflavonifractor	0.07 (0.01–0.29)	0.09 (0.00–0.39)	0.07 (0.01–0.10)	0.10 (0.00–0.62)
Anaerotruncus	0.07 (0.00–0.19)	0.05 (0.00–0.37)	0.05 (0.00–1.26)	0.06 (0.00–1.48)
Streptococcus	0.06 (0.01–1.32)	0.09 (0.00–0.47)	0.03 (0.00–1.98)	0.02 (0.00–0.37)
Anaerostipes	0.05 (0.01–0.45)	0.10 (0.02–0.67)	0.05 (0.00–0.37)	0.06 (0.00–0.49)
Dendrosporobacter	0.03 (0.00–2.26)	**0.00 (0.00–1.09)**†	**0.00 (0.00–0.77)**††	**0.37 (0.00–2.61)**†,††
Acidaminobacter	0.03 (0.00–0.41)	0.06 (0.00–3.28)	0.02 (0.00–15.09)	0.04 (0.00–0.80
Hespellia	**0.03 (0.00–0.20)***	**0.00 (0.00–0.06)***,†	**0.014 (0.00–0.09)**†	**0.03 (0.00–0.12)**†,†
Clostridium	0.02 (0.00–1.82)	0.01 (0.00–0.10)	0.01 (0.00–1.36)	0.04 (0.00–2.39)
Acetanaerobacterium	0.02 (0.00–1.51)	0.00 (0.00–0.67)	0.01 (0.00–1.56)	0.015 (0.00–2.28)
Papillibacter	0.02 (0.00–0.26)	0.01 (0.00–0.07)	0.006 (0.00–0.85)	0.02 (0.00–1.23)
Peptococcus	0.02 (0.00–0.09)	0.00 (0.00–0.17)	0.00 (0.00–0.20)	0.01 (0.00–0.47)
Desulfovibrio	0.01 (0.00–2.79)	0.07 (0.01–1.09)	0.02 (0.00–1.79)	0.12 (0.00–3.94)
Enterorhabdus	0.01 (0.00–1.05)	0.00 (0.00–0.05)	0.00 (0.00–0.14)	0.00 (0.00–0.30)
Adlercreutzia	0.01 (0.00–0.66)	0.02 (0.00–0.27)	0.007 (0.00–0.17)	0.014 (0.00–0.21)
Turicibacter	0.01 (0.00–0.22)	0.002 (0.00–0.07)	0.00 (0.00–0.34)	0.003 (0.00–0.24)
Anaerofilum	0.01 (0.00–0.09)	0.005 (0.00–0.06)	0.015 (0.00–0.16)	0.01 (0.00–0.10)
Filifactor	0.01 (0.00–0.08)	0.008 (0.00–0.38)	0.003 (0.00–0.27)	0.009 (0.00–0.33)
Lutispora	0.01 (0.00–0.05)	0.002 (0.00–0.07)	0.006 (0.00–2.88)	0.02 (0.00–2.20)
Hydrogenoanaerobacterium	0.01 (0.00–0.04)	0.011 (0.00–0.10)	0.009 (0.00–0.09)	0.015 (0.00–0.17)
Gracilibacter	0.01 (0.00–0.03)	0.00 (0.00–0.73)	0.00 (0.00–0.13)	0.01 (0.00–0.33)
Akkermansia	0.00 (0.00–5.04)	0.00 (0.00–0.13)	0.18 (0.00–17.02)	0.09 (0.00–3.48)

Paraprevotella	0.00 (0.00–5.04)	0.00 (0.00–0.00)	0.00 (0.00–3.73)	0.00 (0.00–13.33)
Phascolarctobacterium	0.00 (0.00–4.65)	0.00 (0.00–7.36)	1.74 (0.00–31.49)	0.003 (0.00–10.29)
Robinsoniella	0.00 (0.00–4.60)	0.00 (0.00–0.00)	0.00 (0.00–4.52)	0.00 (0.00–0.99)
Geosporobacter	0.00 (0.00–3.55)	0.00 (0.00–0.34)	0.00 (0.00–4.06)	0.003 (0.00–2.13)
Acidaminococcus	0.00 (0.00–1.74)	0.00 (0.00–0.00)	0.00 (0.00–0.05)	0.00 (0.00–0.13)
Vampirovibrio	0.00 (0.00–1.64)	0.001 (0.00–0.43)	0.00 (0.00–3.66)	0.00 (0.00–3.24)
Schlegelella	0.00 (0.00–1.57)	0.00 (0.00–1.32)	0.00 (0.00–2.26)	0.00 (0.00–4.60)
Insolitispirillum	0.00 (0.00–1.10)	0.00 (0.00–7.08)	0.00 (0.00–2.69)	0.03 (0.00–4.09)
Spiroplasma	0.00 (0.00–1.06)	0.00 (0.00–0.00)	0.00 (0.00–0.16)	0.00 (0.00–0.72)
Olivibacter	0.00 (0.00–0.80)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–4.32)
Parapedobacter	0.00 (0.00–0.75)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–5.26)
Fastidiosipila	0.00 (0.00–0.49)	0.00 (0.00–0.00)	0.00 (0.00–0.19)	0.00 (0.00–0.27)
Acholeplasma	0.00 (0.00–0.46)	0.00 (0.00–0.00)	0.00 (0.00–2.06)	0.00 (0.00–4.56)
Paraeggerthella	0.00 (0.00–0.42)	0.00 (0.00–0.07)	0.00 (0.00–0.05)	0.00 (0.00–0.01)
Victivallis	0.00 (0.00–0.25)	0.00 (0.00–0.00)	0.00 (0.00–1.26)	0.00 (0.00–0.46)
Cloacibacillus	0.00 (0.00–0.24)	0.00 (0.00–0.15)	0.00 (0.00–0.67)	0.00 (0.00–0.02)
Anaeroplasma	0.00 (0.00–0.24)	0.00 (0.00–0.00)	0.00 (0.00–1.06)	0.00 (0.00–0.00)
Caloramator	0.00 (0.00–0.23)	0.00 (0.00–0.04)	0.00 (0.00–0.17)	0.003 (0.00–0.20)
Pelotomaculum	0.00 (0.00–0.21)	0.00 (0.00–0.00)	0.00 (0.00–0.04)	0.003 (0.00–0.04)
Alkalibaculum	0.00 (0.00–0.18)	0.00 (0.00–0.13)	0.002 (0.00–0.27)	0.02 (0.00–0.28)
Oxalobacter	0.00 (0.00–0.16)	0.00 (0.00–0.24)	0.00 (0.00–0.14)	0.00 (0.00–0.12)
Erysipelothrix	0.00 (0.00–0.16)	0.00 (0.00–0.11)	0.00 (0.00–0.01)	0.00 (0.00–0.10)
Roseateles	0.00 (0.00–0.15)	0.00 (0.00–0.00)	0.00 (0.00–0.58)	0.00 (0.00–0.01)
Ethanoligenens	0.00 (0.00–0.13)	0.00 (0.00–0.12)	0.005 (0.00–0.63)	0.003 (0.00–0.74)
Rhodospirillum	0.00 (0.00–0.12)	0.00 (0.00–0.28)	0.00 (0.00–1.10)	0.00 (0.00–0.95)
Paenibacillus	0.00 (0.00–0.12)	0.00 (0.00–0.00)	0.00 (0.00–0.18)	0.00 (0.00–0.13)
Slackia	0.00 (0.00–0.08)	0.00 (0.00–0.09)	0.00 (0.00–0.17)	0.00 (0.00–0.17)
Desulfonema	0.00 (0.00–0.06)	0.00 (0.00–0.00)	0.00 (0.00–0.39)	0.00 (0.00–0.14)
Mitsuokella	0.00 (0.00–0.05)	0.00 (0.00–0.01)	0.00 (0.00–0.20)	0.003 (0.00–0.71)
Capnocytophaga	0.00 (0.00–0.04)	0.00 (0.00–0.00)	0.00 (0.00–1.19)	0.00 (0.00–0.01)
Tindallia	0.00 (0.00–0.02)	0.00 (0.00–0.20)	0.00 (0.00–1.71)	0.00 (0.00–0.03)
Acetivibrio	0.00 (0.00–0.02)	0.00 (0.00–0.15)	0.00 (0.00–1.27)	0.00 (0.00–0.23)
Obesumbacterium	**0.00 (0.00–0.00)****	**0.00 (0.00–1.31)****,††,†††	**0.00 (0.00–0.00)**†††	**0.00 (0.00–0.00)**††
Elusimicrobium	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.78)	0.00 (0.00–0.00)
Anaerophaga	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.31)	0.00 (0.00–0.00)
Megamonas	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–1.29)	0.00 (0.00–1.29)
Succinivibrio	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.84)	0.00 (0.00–0.00)
Coraliomargarita	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.48)	0.00 (0.00–0.32)
Enterobacter	0.00 (0.00–0.00)	0.00 (0.00–0.07)	0.00 (0.00–0.18)	0.00 (0.00–1.51)
Selenomonas	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.64)

Data presented as median and range (min-max). Kruskal-Wallis test was used for multiple comparisons between study groups. Statistically significant differences are indicated by $P < 0.05$ and marked in bold type. Significance: *P<0.05; **P<0.01, comparison HC to HIT, FH or FA; †P<0.05; ††P<0.01; †††P<0.001 comparison between HIT, FH or FA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers.

Hierarchical clustering using Euclidean distance measurement and Ward's method did not reveal any cluster building of study groups considering bacterial distribution on family level (*Fig. 7*).

DISCUSSION

To our knowledge this study provides the first detailed microbiome analysis of patients with histamine intolerance. It reveals significant differences in the bacterial pattern from patients with histamine intolerances, and patients with other food intolerances or allergies in comparison to healthy controls. It is generally known, that the intestinal microbiome has a huge influence on immunological processes of the host. Thereby, not only the taxonomic diversity of bacteria affects the immune tolerance, but also bacteria derived metabolites have an important effect on human health (22, 23). Besides the well-studied short-chain fatty acids, including butyrate, acetate and propionate (24), also the biogenic amine histamine is produced by intestinal bacteria (23, 25). Several bacterial strains possess the enzyme histidine decarboxylase and are able to produce histamine by decarboxylation of histidine (23). Most of these strains were found in foods, mainly fermented products like cheese, meat, sauerkraut, wine or beer (26, 27).

Pugin *et al.* were able to isolate the histamine-producing strains *Morganella morganii* and *Lactobacillus vaginalis* from human feces (28). The gram-negative *Morganella morganii* belongs to the family of Enterobacteriaceae of the phylum Proteobacteria and was already described in former studies for its histamine-secreting properties (29). Even though our study patients showed no significant differences in the relative abundance of Enterobacteriaceae, they were increased in histamine intolerant patients with 0.17%. Moreover, on phylum level, the Proteobacteria were elevated in the HIT group. This is of special interest, since several studies suggested an increase in

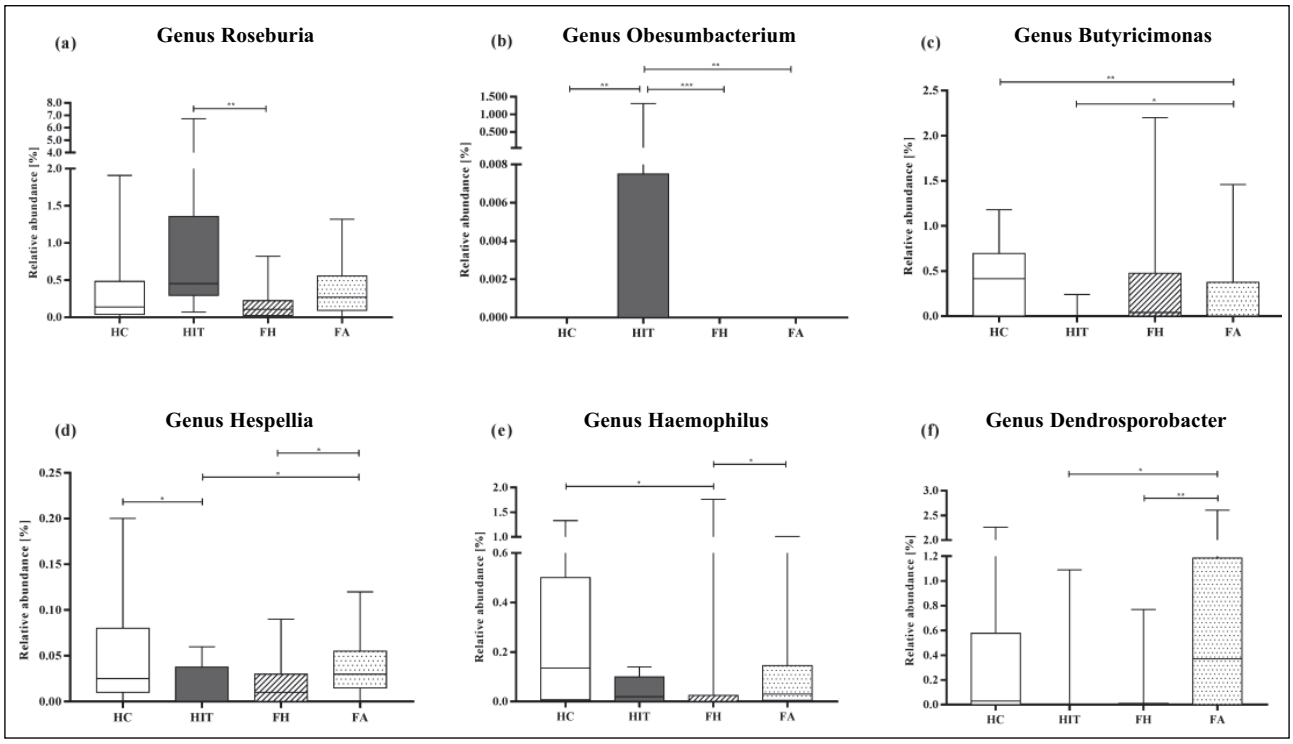

Fig. 4. Differences on genus level between study groups. *Fig. 4a* shows a significantly higher relative abundance of Roseburia in the HIT group compared to the FH group (P = 0.004). *Fig. 4b* shows the significantly highest abundance of Obesumbacterium in the HIT group compared to HC (P = 0.001), FH (P < 0.001) and FA (P = 0.001). *Fig. 4c* shows an elevated abundance of Butyricimonas in the HC and FH group and an absence of this genus in the HIT group. *Fig. 4d* shows significantly higher proportions of Hespellia in the HC compared to the HIT group (P = 0.03) and FA showed significant higher proportions compared to FH and HIT group (P = 0.034 and P = 0.013). *Fig. 4e* shows a significantly elevated abundance of Haemophilus in the HC and FA group compared to the FH group (HC: P = 0.015 and FA: P = 0.025). *Fig. 4f* shows a significantly elevated abundance of Dendrosporobacter in the FA group, reaching significance compared to the HIT (P = 0.012) and the FH group (P = 0.002). Kruskal-Wallis test was used for multiple comparisons between study groups. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers. Significance: *P < 0.05, **P < 0.01 and ***P < 0.001.

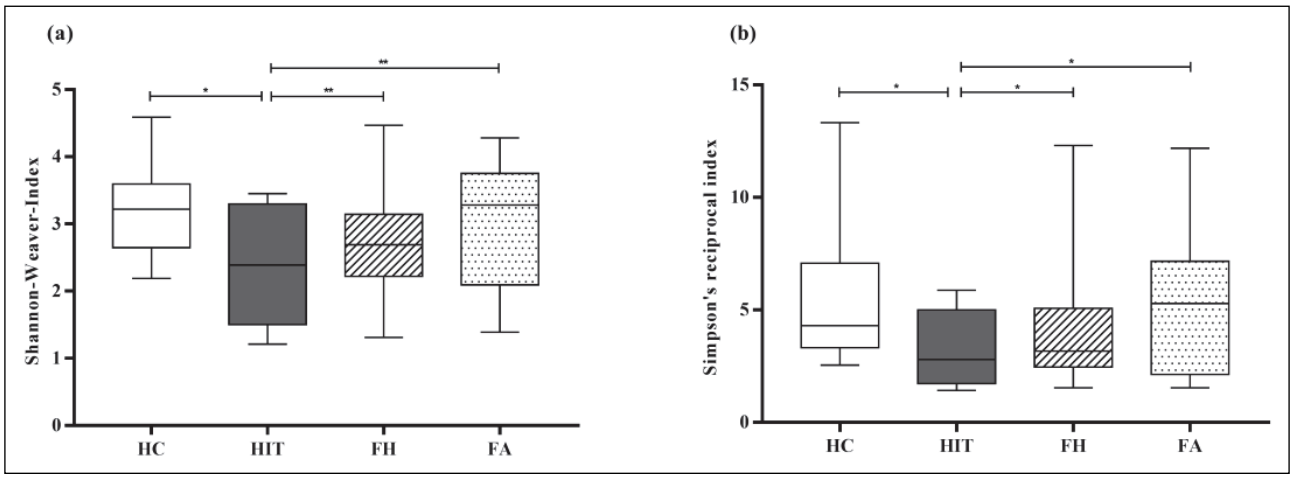

Fig. 5. Alpha-diversity of study groups. *Fig. 5a* shows α-diversity using Shannon-Weaver-Index (SWI). HIT patients showed significantly lower SWI compared to HC (P = 0.017), FH (P = 0.009) and FA (P = 0.002). *Fig. 5b* shows α-diversity using Simpson's reciprocal index (SI). HIT patients showed significantly lower (SI) compared to HC (P = 0.027), FH (P = 0.022) and FA (P = 0.006). Multiple comparisons between study groups were calculated by PERMANOVA. *Abbreviations:* HC, healthy controls; HIT, histamine intolerants; FH, food hypersensitives; FA, food allergy sufferers. Significances: *P < 0.05; **P < 0.01.

Proteobacteria as a sign for dysbioses (30). Interestingly, a dysregulation of the innate immune response was suggested to promote the intestinal overgrowth with Proteobacteria resulting in a low-grade intestinal inflammation (30, 31). An intestinal inflammation may cause an epithelial dysfunction and increase the oxygen levels in the colon. This promotes the growth of facultative anaerobic bacteria, *e.g.* various species of Proteobacteria, that compete against obligate anaerobic bacteria

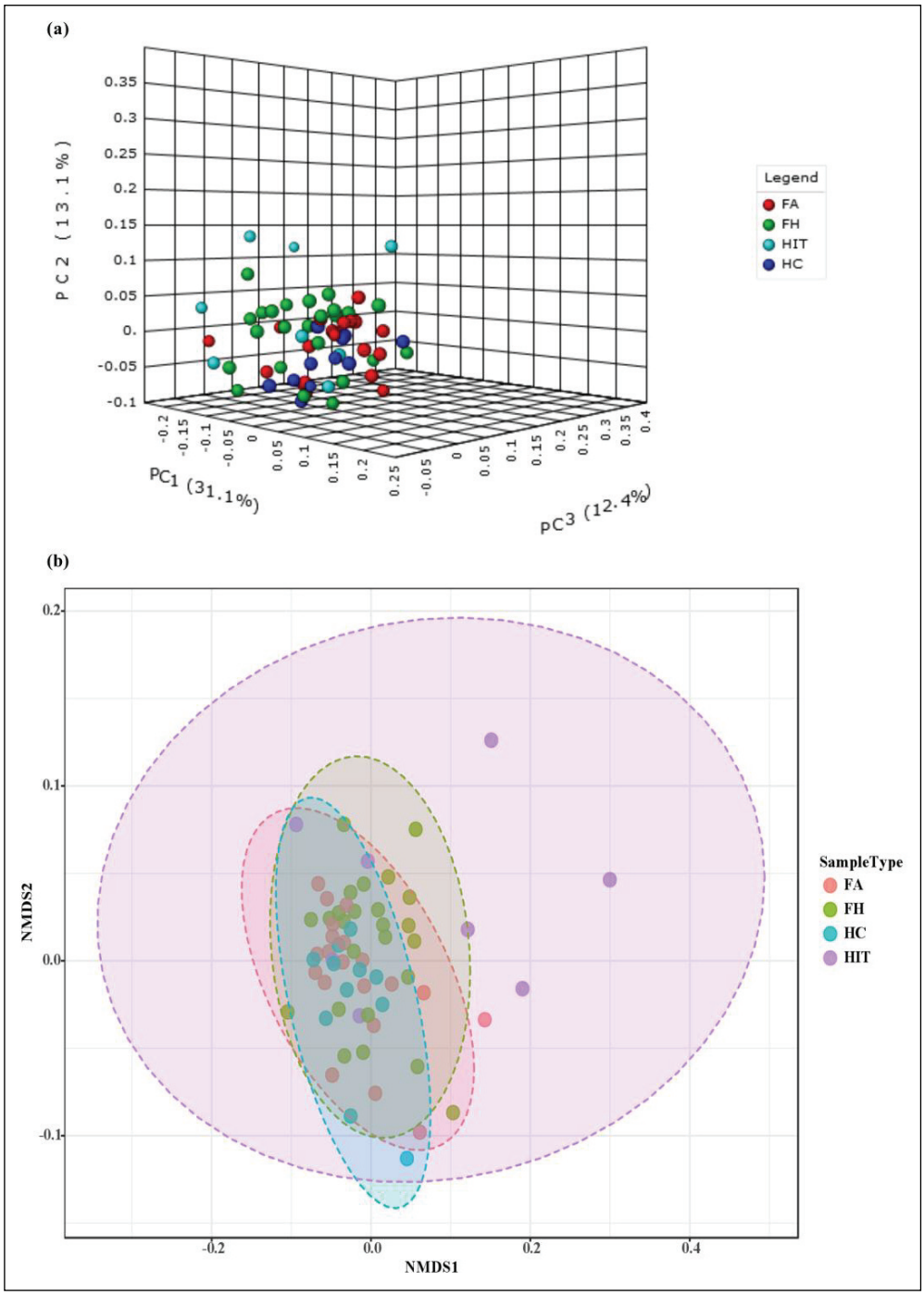

Fig. 6. Beta-diversity of study groups. *Fig. 6a* shows 3D ordination plot based on principal coordinate analysis plot. *Fig. 6b* shows 2D ordination plot based on non-metric multidimensional scaling. For beta-diversity analysis unweighted UniFrac distance was calculated based on genus level. *Abbreviations:* HC, healthy controls; HIT, histamine intolerance; FH, food hypersensitivity; FA, food allergy.

(32) comprising beneficial ones like Bifidobacteria. Therefore, an increase in Proteobacteria is also suggested as a marker for epithelial dysfunction (32). An overgrowth of this phylum was already described in patients with inflammatory bowel disease (33, 34). Also patients with post-infectious or diarrhea-predominant irritable bowel syndrome showed an increase of Proteobacteria in comparison to healthy controls (35, 36). In healthy persons, an intestinal abundance of Proteobacteria is described between 2.5 – 4.6% (30). The increased abundance of Proteobacteria (5.4%, range 1.3 to 34.6%) in our study patients with histamine intolerance compared to healthy controls indicates a dysbiosis and/or altered epithelial function in this patient group.

Within the Lactobacillus family some species display amino acid decarboxylase activity, *e.g. Lactobacillus casei* or *Lactobacillus delbrueckii subsp. bulgaricus*, resulting in histamine formation and secretion (8). Interestingly, data of murine models and patients with inflammatory bowel disease suggest a protective effect of HDC-positive bacteria like *Lactobacillus reuteri* on developing colorectal neoplasia by the suppression of chronic intestinal inflammation (37).

However, none of our study participants showed a significant increase in Lactobacillus. Thus, we could not detect a significantly higher abundance of histamine-producing bacteria in histamine intolerant patients, at least on phylum, family or genus level. A more precise characterization of bacteria on species level, especially of the phylum Proteobacteria, may reveal more detailed results (38) and should be examined in the future. The influence of bacterial derived histamine as cause of an elevated intestinal histamine exposure in histamine intolerant subjects is therefore questionable.

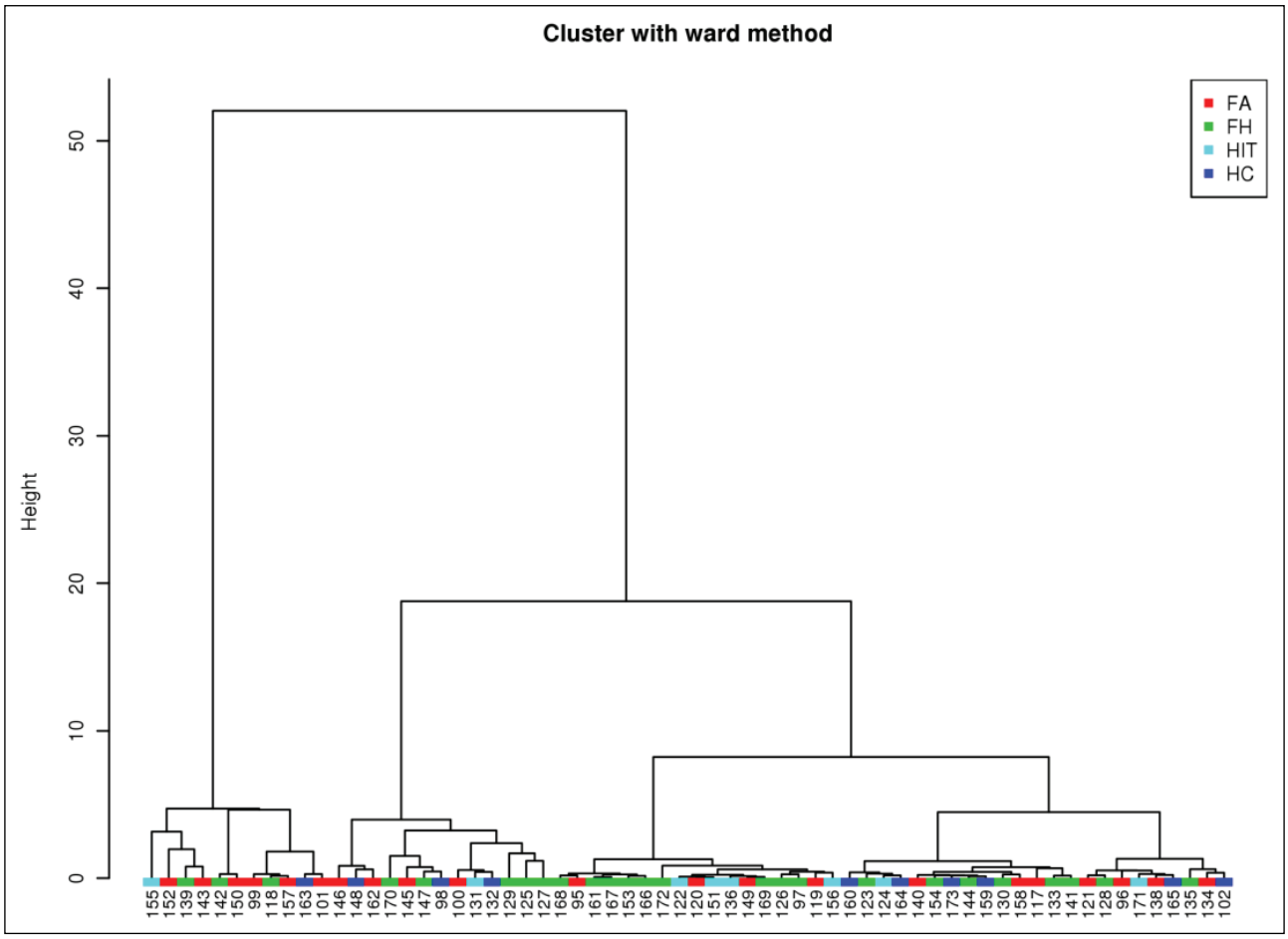

Fig. 7. Cluster analysis. Dendrogram displays cluster analysis with Ward method on family level. Abbreviations: HC, healthy controls (dark blue); HIT, histamine intolerance (light blue); FH, food hypersensitivity (green); FA, food allergy.

Nevertheless, a dysbiosis may promote a mucosal inflammation in the gut. Since the histamine-degrading DAO is synthesised by mature enterocytes and stored in the mucosal epithelial cells (39), a disruption of these cells caused by inflammation may also contribute to a reduced DAO synthesis. This could lead to a reduced degradation of exogenous histamine and results in increased endogenous histamine levels, which cause the typical symptoms of histamine intolerance. Since we did not examine the intestinal inflammation status, future studies should include the analyses of molecular inflammation markers, e.g. of intestinal mucosa samples, to evaluate the association between dysbiosis and intestinal inflammation.

However, we observed a significantly higher abundance of the bacterial genera Roseburia in patients with histamine intolerance compared to healthy controls and the other food intolerant subjects. Interestingly, Roseburia is known to produce butyrate and health-promoting effects were attributed to this bacterial genus (40). In accordance, a reduced abundance of *Roseburia spp*. was found in patients with ulcerative colitis (41) or in patients with chronic kidney disease (42). Different species of Roseburia produce short-chain fatty acids by metabolizing indigestible carbohydrates, for example starch, inulin or xylan, in the human colon (43). The increased proportions of Roseburia in the stool samples of our histamine intolerant patients could be caused by the carbohydrate and fiber-rich diet of these patients, which might exert prebiotic properties and promote the growth of butyrate-producing bacteria (44). However, histamine intolerant patient showed significantly lower levels of the bacterial genus Butyricimonas which also belongs to the butyrate-producers. Decreased levels of colonic butyrate could lead to an impaired barrier function and were already noticed in inflammatory bowel diseases (45-47). Maybe the lower abundance of these bacteria in our histamine intolerant patients may exert some unfavorable effects on patient's health. But, even though the health promoting effects of butyrate and its important role as energy source for enterocytes is well accepted, there are also contrary reports. In this context, increased butyrate levels were described in patients with self-reported food hypersensitivity and elevated levels of butyrate also induced visceral hypersensitivity in rats (48, 49).

In comparison to all other study groups, the healthy persons of our study showed the highest abundance of the family Bifidobacteriaceae with a median of 0.3%. This bacterial family harbors health-beneficial species (44). Bifidobacteriaceae decrease the intestinal pH value through production of acetic and lactic acids and thus inhibit the growth of potential pathogenic bacteria and block their adhesion to the intestinal mucosa (44). Also an alleviation of gastrointestinal symptoms (*e.g.* diarrhea, constipation) and immune stimulating properties were described (50, 51), and reduced numbers of Bifidobacteria were already observed in several disorders including allergies, irritable bowel syndrome and inflammatory bowel disease (50). An *in vitro* study of Hsieh *et al.* demonstrated that the supplementation of the probiotic *Bifidobacterium bifidum* induced an enhanced epithelial function by promoting the epithelial tight junction integrity in the human intestinal epithelial cell line Caco-2 (52). This suggests a protective effect of Bifidobacterium species on gut barrier, and a lack or reduced numbers of Bifidobacteria may

contribute to an impaired gut barrier in different patient groups, *e.g.* in our histamine intolerant patients.

Furthermore, we observed an increased abundance of the phylum Verrucomicrobia, especially in patients with food hypersensitivity, but very low numbers in patients with HIT. A high colonization with Verrucomicrobia was described in patients treated with a broad-spectrum antibiotic therapy (53). However, neither patients with FH nor patients of the other study groups had a recent antibiotic treatment.

Interestingly, the food allergy patients of our study showed elevated levels of the family Erysipelotrichaceae (54), which belongs to the phylum of Firmicutes. A high abundance of this family was described in patients with inflammatory bowel disease and metabolic disease. An association between Erysipelotrichaceae and the lipid metabolism of the host is assumed (54), but species of this family are also supposed to mediate strong immunogenic properties and promote inflammation. In this context, a study observed a positive correlation of the relative abundance of Erysipelotrichi with TNF-α (55), an important mediator of immune and inflammatory responses, which is also involved in the process of allergic reactions (56), but we could not find any correlation in our study (data not shown).

Furthermore elevated levels of the phylum Firmicutes are described in infants with food allergy (14), but we could not confirm these findings in our study. With regard to the whole bacterial composition, individuals that were classified as histamine intolerant patients showed a significant reduced α-diversity compared to all other study groups. Furthermore, the beta-diversity revealed a distinct tendency to a modified microbial pattern at least in some HIT patients.

The α-diversity describes the mean species diversity within a certain habitat, in this case the human gut. A reduced α-diversity was already described in patients with inflammatory bowel disease, irritable bowel syndrome, obesity or diabetes (57-59). A reduction in bacterial diversity in patients with histamine intolerance might represent another hint for a deranged microbial pattern in the patients gut. Nevertheless, we were not able to detect disease-specific microbial clusters for each study group, and every patient rather showed an individual intestinal bacterial composition. Perhaps the analysis of mucosa-associated bacteria rather than bacteria from stool samples will provide deeper insight in disease associated microbial patterns.

Furthermore, we found a moderate positive association between histamine and zonulin concentrations in the stool samples from all our study participants. Although we did not detect any significant differences in stool zonulin concentration between study groups, our histamine intolerant patients showed elevated levels of zonulin. With a mean of 136 ng/ml the stool zonulin concentration was slightly increased compared to the recommended values for healthy persons (122 ng/ml) (60). This finding suggests a mild alteration of gut permeability in patients with a high intestinal histamine exposure. In this context, increased gut permeability is discussed in the pathogenesis of various diseases, *e.g.* inflammatory bowel disease, irritable bowel disease or liver diseases (61, 63). It facilitates the penetration of pathogenic bacteria, bacterial metabolites or fragments as well as other toxins that might trigger inflammation (64) and in turn lead to gastrointestinal or extra-intestinal symptoms.

In summary, our results suggest an alteration of the microbial composition in food intolerances, especially in patients with histamine intolerance. The increased abundance of Proteobacteria, the decreased abundance of Bifidobacteriaceae/Bifidobacterium and the lower bacterial diversity points to a dysbiosis and an impaired intestinal barrier in this patient group. The positive correlation between intestinal histamine and stool zonulin levels indicates a negative effect of histamine on gut permeability. However, we did not find elevated stool histamine concentrations in patients with histamine intolerance nor an enrichment of known histamine-producing bacteria. Nevertheless, a dysbiosis in histamine intolerant patients may contribute to the mucosal inflammation. This in turn could favor the development of a leaky gut and the reduction of intestinal DAO leading to elevated histamine levels and clinical symptoms in sensitive patients.

In addition to the necessity of a larger cohort with histamine intolerant patients to confirm our preliminary findings, the determination of bacterial species as well as the identification of mucosa-associated bacteria might provide an even more detailed insight in the intestinal bacterial pattern in future studies.

Clinical Trials Registration: NCT02293343.

M. Schink and P.C. Konturek contributed equally to this work.

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