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Title page

Upper-airway dysbiosis related to frequent sweets consumption increases the risk of asthma in children with chronic rhinosinusitis.

Short running title: Sweets-related childhood asthma

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Abbreviations

CRS- chronic rhinosinusitis

MALT- mucosa-associated lymphoid tissue

T1R3- taste receptor type 1 member 3- sweet taste receptor

TSLP- thymic stromal lymphopoietin

DAFB2- Beta-defensin 2

IL- interleukin

Abstract

Background: Innate immunity response to local dysbiosis seems to be one of the most important immunological backgrounds of chronic rhinosinusitis (CRS) and concomitant asthma. We aimed to assess clinical determinants of upper-airway dysbiosis and its effect on nasal inflammatory profile and asthma risk in young children with CRS.

Methods: We recruited one hundred thirty-three children, aged 4-8 years with doctor-diagnosed CRS with or without asthma. The following procedures were performed in all participants: face-to-face standardized Sinus and Nasal Quality of Life questionnaire, skin prick test, taste perception testing, nasopharynx swab, and sampling of the nasal mucosa. Upper-airway dysbiosis was defined separately by asthma specific microbiome composition and reduced biodiversity. Multivariate methods were used to define the risk factors of asthma and upper-airway dysbiosis and their specific inflammatory profile of nasal mucosa.

Results: The asthma-specific upper-airway microbiome composition reflected by the decreased ratio of *Patescibacteria/Actinobacteria* independently of atopy increased the risk of asthma (OR:8.32; 95%CI:2.93-23.6). This asthma-specific microbiome composition was associated with ≥ 7 /week sweets consumption (OR:2.64; 95%CI:1.11-6.28), reduced biodiversity (OR:3.83; 95%CI:1.65-8.87), the presence of *Staphylococcus* strains in the nasopharynx (OR:4.25; 95%CI:1.12-16.1), and lower expression of beta-defensin 2, IL-5, IL-13 in the nasal mucosa. The reduced biodiversity was associated with frequent antibiotic use and with a higher nasal expression of IL-17 and T1R3 (sweet taste receptor). In asthmatic children reduced sweet taste perception was observed.

Conclusions: Specific upper-airway dysbiosis related to frequent sweets consumption, frequent antibiotics courses, and altered nasal immune function increases the risk of asthma in young children with CRS.

Key Message

Our data point out a vicious circle between frequent sweets consumption and upper-airway dysbiosis with different immune responses of nasal epithelium and finally increased asthma risk with sweet taste perception impairment.

Introduction

Dysbiosis is an imbalance of the local microbial composition that can be defined by the loss or gain of bacteria that promote health or disease, respectively¹⁻². An emerging hypothesis postulates that dysbiosis increases the risk of chronic inflammatory diseases, including asthma¹⁻⁵. The upper

and lower airway microbiota are two environments that host different types of commensal bacteria and consequently different dysbiotic bacterial composition⁶. Importantly, both lower and upper airway dysbiosis could be found in asthmatics^{1,5}.

Various internal (fibrosis, genetic deficiency of Muc5b) and external (antibiotics, cigarette smoking, or dietary changes) factors lead to airway microbiota dysbiosis^{1,2}. Once established, airway dysbiosis induces a local and systemic immune response, thereby creating a feedback loop where local immune cells and microbiota cross-talk with each other. This cross-talk seems to be the immunological background of associations between gut microbiota and asthma observed in children^{7,8}. Different innate and adaptive immune changes have been found in the airways with dysbiotic microbiomes such as Muc5ac and IL-17 overproduction, the cellular response of TH17, invariant natural killer T cells, and lung-resident $\gamma\delta$ T cells⁹.

Collectively, all the above facts encouraged us to conduct a multi-omic study focused on the interaction between upper-airways dysbiosis and local inflammatory response in young children suffering from chronic rhinosinusitis (CRS) with or without asthma. We aimed to assess clinical determinants of upper-airway dysbiosis and its effect on nasal inflammatory profile and asthma risk in young children with CRS.

Patients and methods

We recruited one hundred thirty-three children, aged 4-8 years with CRS, diagnosed by otorhinolaryngologists according to the EPOS (European Position Paper on Rhinosinusitis and Nasal Polyps) criteria¹⁰. The sample size justification is given in Online Supporting Material. Recruitment for the study took place in our Allergy Outpatient Clinic during two subsequent autumn-winter seasons. Asthma was diagnosed by a pulmonologist according to positive Asthma Predictive Index and confirmed by clinical improvement during anti-inflammatory therapy and/or positive bronchial reversibility test¹¹. The following exclusion criteria were considered: food allergy with symptoms affecting the respiratory tract, contraindication to nasal endoscopy or

contraindication to nasal mucosa biopsy, hypertrophy of the third tonsil exceeding 60% of the nasopharynx (confirmed by endoscopy of the upper-respiratory-tract), confirmed immunodeficiency, exacerbation of asthma requiring systemic administration of glucocorticosteroids, obesity, exposure to tobacco smoke or other chronic illnesses and clinical conditions, which in the opinion of the researcher may influence the evaluation and the course of the study. Receiving intranasal corticosteroids within 4 weeks and/or acute respiratory tract infection within 2 weeks before the visit were temporary exclusion criteria.

During the visit the following procedures were performed in all patients: i) standardized questionnaire - Sinus and Nasal Quality of Life Survey (SN-5), ii) questionnaire which assessed the frequency of sweets consumption and the frequency of antibiotic therapy, iii) skin prick test, iv) taste perception assessment, v) nasopharynx swab, vi) and nasal mucosa sampling. A full description of all methods is given in the Online Supporting Material. Key methods are described briefly below.

Questionnaires

SN-5: Face-to-face validated questionnaire to assess the quality of life in children with CRS (5 symptom-cluster items covering the domains of sinus infection, nasal obstruction, allergy symptoms, emotional distress, and activity limitations) was carried out¹². Additionally, parents were asked to report: i) the average number of times in a week the child had eaten sweets over the past month, and ii) the number of antibiotic courses over the past year. The list of sweets adopted from the Food Craving Inventory included: candy, brownies, cookies, chocolate, donuts, cake, cinnamon rolls, and ice cream¹³.

NGS library preparation and sequencing

DNA was extracted from nasal swabs using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). The nuclease-free water was used as a negative control for the background of 16S rRNA contamination. NGS libraries preparation was performed according to Illumina's "16S Metagenomic Sequencing Library Preparation". Then, the samples were pooled

at an equimolar concentration and sequenced as paired-ends using the Illumina MiSeq platform (Illumina, CA). Biodiversity was assessed by Shannon index.

Nasal mucosa sampling, mRNA isolation, and Q-PCR

Nasal mucosa was sampled using a plastic curette (Rhino-probe™, ASI, Arlington, TX, USA) from the anteromedial aspect of the inferior turbinate (according to the manufacturer's instructions). This procedure was performed by ENT specialists. The nasal epithelium specimen was transferred to 100 µL of TRI Reagent® and stored at -80°C until further processing. Total RNA was isolated by the method of Chomczynski and Mackey¹⁴. Reverse transcription reaction was performed in triplicate by the protocol of Wiame et al.¹⁵ using random primers and Maxima™. The primers used for evaluation of specific gene expression are listed in Supplemental Table 2. The expression of specific genes was compared to 18SrRNA as a reference gene and expressed as $\Delta\Delta Ct$.

Ethics

The protocol has been approved by the Medical University of Lodz Ethics Committee. Written consent from the patients and their parents was obtained.

Statistical methods

First, the Analysis of Composition of Microbiomes (ANCOM) was performed to identify taxa that were differentially abundant across asthmatics vs non-asthmatics (bioinformatics details are given in the Online Supporting Material). A receiver operating characteristic curve (ROC) analysis was used to point out the optimal cut-point value which, allowed to defined subgroups of children with the asthma-specific microbiome composition and reduced biodiversity (reflected by Shannon-index).

Next, logistic regression analysis was used to defined variables independently associated with asthma, asthma-specific microbiome composition, and reduced Shannon-index separately. Logistic regression analysis was performed in the univariate model followed by the multivariate model. The multivariate model was built according to the step-forward selection approach; coefficients associated with the dependent variable in the univariate model with p-level <0.1 were only included.

Finally, nasal expressions of preselected molecules were compared between defined subgroups of patients using the Mann-Whitney test with Benjamini and Hochberg correction for multiple comparisons. Transform variables were included in the multifactorial analysis of variance to check for significant interactions with potential confounders. A p-value of less than 0.05 was assumed as statistically significant. Statistica 13.1 (*TIBCO Software Inc.*) was used to perform all analyses.

Results

One hundred thirty-three children with CRS were included in the analysis. The clinical characteristics of participants are presented in Table 1. Asthma was diagnosed among 82(61.6%) children.

Asthma-specific microbiome composition/reduced Shannon index

Analysis of the composition of microbiomes (ANCOM) showed asthma-specific composition defined by the reduced relative frequency of *Patescibacteria* with concomitant increased frequency of *Actinobacteria* in the nasopharynx of our asthmatic vs non-asthmatic patients (Figure 1A). Both of them have W statistic equal to 1. Both differences were included in the ratio *Patescibacteria/Actinobacteria* calculated for all children and compared between asthmatics and non-asthmatics (Figure 1B). Dychotomized ratio, according to the optimal cut-point value from ROC-curve analysis, allowed to defined the sub-group of children with asthma-specific microbiome composition. Next, a significant difference in the Shannon index (biodiversity indicator) between these sup-groups was found (Figure 1C). Dychotomization of the Shannon index using the optimal cut-point value from the corresponding ROC-curve analysis defined the next sub-group of patients with the Shannon index reduced according to the asthma-specific microbiome composition. Both variables were next included in the logistic regression analysis together with other potential confounders to defined independent asthma risk factors (Table 1).

Asthma risk factors

First, in univariate analysis, a set of variables associated with asthma was defined (Table 1) and included in the multivariate model. The logistic regression analysis in a multivariate approach (Supplemental Table 3) showed that independent asthma risk factors are: asthma-specific microbiome composition (OR: 8.32; 95%CI: 2.93-23.6), atopy (OR: 7.96; 95%CI: 2.88-22.0), and

reduced sweet taste perception (OR: 3.09; 95%CI: 1.18-8.07). Details on the definition of reduced sweet taste perception are given in Supplemental Table 4.

Associations with the asthma-specific microbiome composition and reduced Shannon index

Variables associated with asthma-specific microbiome composition in univariate analysis were defined (Table 2) and included in the multivariate model. A multivariate model of logistic regression (Supplemental Table 5) showed the following variables associated with asthma-specific microbiome composition: *Staphylococcus* strains present in the nasopharynx (OR: 4.25; 1.12-16.08), reduced Shannon index (OR: 3.83; 95%CI: 1.65-8.87) and frequent (≥ 7 /week) sweets consumption (OR: 2.64; 95%CI: 1.11- 6.28). The reduced Shannon index was associated with frequent antibiotics and, higher than the median *Pseudomonas* strains frequency in the nasopharynx (Supplemental Table 6); multivariate analysis showed that only frequent antibiotics independently increased the risk of reduced Shannon index (OR: 2.34; 95%CI: 1.12-4.90).

Nasal inflammation according to the asthma-specific microbiome composition and reduced Shannon index

Nasal expressions of preselected molecules were compared according to the asthma-specific microbiome composition and reduced Shannon index separately since there was no interaction between both variables in the analysis of asthma risk factors.

The nasal inflammation of children with asthma-specific microbiome composition compared to others was characterized by a lower expression of defensin beta 2 ($p=0,003$; after adjustment for multicomparison $p=0,018$), IL-13 ($p=0,017$; after adjustment for multicomparison $p=0,051$) and IL-5 ($p=0,031$; after adjustment for multicomparison $p=0,062$) (Table 3, Figure 2). In children with reduced Shannon index compared to others, the nasal inflammation was characterized by a higher expression of IL-17 ($p=0,048$; after adjustment for multicomparison $p=0,08$) and T1R3 ($p=0,019$; after adjustment for multicomparison $p=0,06$), and lower TSLP expression ($p=0,038$; after adjustment for multicomparison $p=0,08$) (Table 3, Figure 2).

The multifactorial analysis of variance with log-transform mRNA expression did not show any significant interaction with asthma or atopy (Supplemental Tables 7, 8, and Supplemental Figures E1, E2).

Discussion

In the present study, we showed the results of an integrated multi-omics approach to dysbiosis-related asthma in young children observed after preschool initiation.

First, we showed that in our asthmatic patients compared to children without asthma specific upper-airway dysbiosis could be defined. This dysbiosis was characterized by a disproportion between the main bacterial phyla reflected by a decreased ratio of *Patescibacteria/Actinobacteria*.

Dysbiosis in many current models has been associated with chronic inflammation in the upper and lower respiratory tract^{1,7}. This phenomenon can be explained in two ways. First, it is suggested that dysbiosis-related inflammation may result from the lack of protective strains of specific saprophytic bacteria that can tune immunoregulatory mechanisms². In our study, asthma was

independently associated with dysbiosis defined by the low abundance of *Patescibacteria*. In another study in the pediatric population, loss of asthma control was associated with a lower abundance of *Campylobacter* and *Dolosigranulum* in anterior nares⁴. These differences can be explained by microbiome biogeography. In our study, we evaluated the microbiome in the nasopharynx, where the healthy composition of bacterial phyla is significantly different compared to anterior nares⁶. Secondly, inflammation can be a natural and obvious response to pathogenic flora⁵. We have strong evidence to prove that colonization of the upper airways with bacterial pathogens including *Streptococcus*, *Moraxella*, and *Haemophilus* is associated with asthma¹⁶. It has also been shown that pathogenic flora, in particular *Staphylococcus aureus*, was associated with a risk of losing control of asthma symptoms in children⁴. The results of our study seem to confirm this concept. Asthma-specific microbiome composition in our patients was associated with the presence of *Staphylococcus* strains in the nasopharynx. What is more, nasal inflammation in these patients was characterized by a significantly lower beta-defensins expression, which could be an immunological background of ineffective antimicrobial response, leading to the biofilm formation of pathogens like *Staphylococcus aureus*.

Importantly, asthma-specific microbiome composition in our population was related to frequent sweets consumption. Previous epidemiological studies showed that high glucose intake increases the risk of asthma¹⁷. Increased airway glucose concentration was observed in patients with stable COPD and further increased during COPD exacerbations¹⁸. Also, it is well documented, that the overconsumption of sugar alters the gut microbiome, leading to gut dysbiosis¹⁹. On the other hand, glucose levels in the airways rapidly deplete during a bacterial infection due to the bacterial load²⁰. Here we have shown, for the first time, that sweets consumption more than 7-times per week significantly increased the risk of asthma-specific microbiome composition of upper-airways in young children without the current infection. This phenomenon seems to be of high interest to a large audience of clinicians and scientists. Based on previous and also our results we may suspect that high glucose concentration in the microenvironment may negatively interact with the antimicrobial activity of MALT via taste receptors²¹. It has been shown that bitter compounds secreted by microbes bind to and activate bitter taste receptor (T1R3) resulting in rapid secretion of antimicrobial peptides (ex. beta-defensins) via calcium signaling and this pathway could be attenuated by glucose in the airway surface liquid via sweet taste receptors, which suppress the calcium response^{21,22}. Therefore we postulate that the reduced expression of defensins followed by

more frequent *Staphylococcus* strains observed in our study among children with asthma-specific dysbiosis could be a consequence of frequent sweets consumption. What is more, the reduction of sweet taste perception was an independent asthma risk factor in our study. In the other study, a higher intensity of sweet taste was observed in adult patients with CRS compared to healthy control²³. We compared taste perception within-group of children with CRS and we suspect that observed differences were caused probably by changes in airway glucose concentrations and/or by microbiota composition, not by the disease itself. It has been shown that sensitivity to sweet taste negatively correlated with salivary concentrations of glucose²⁴ and sweet taste intensity correlated negatively with the frequency of sweet food intake and sweet beverage liking²⁵. Importantly, some bacterial taxa on the tongue dorsum were associated with gustatory functions and with vegetable-rich (e.g., *Prevotella*) or protein/fat-rich diets (e.g., *Clostridia*)²⁶. Indeed, it has been shown that the microbial composition of the tongue microbiota of people with higher taste responsiveness is different from that of people of reduced taste responsiveness²⁷. Although our case-control model did not allow us to fairly identify what was a cause and what was a consequence, it clearly showed a vicious circle between frequent sweets consumption and asthma-specific microbiome composition with different immune responses and finally asthma-related sweet taste perception impairment (Figure 3).

Asthma-specific microbiome composition was related also to lower biodiversity, reflected in our study by the reduced Shannon index. Among these patients we observed higher nasal production of IL-17 and higher expression of T1R3. After correction for multiple comparisons, there was no evidence of statistical significance. What is more, the reduced Shannon index was related to frequent antibiotic use and associated with more frequent *Pseudomonas* strains in the nasopharynx. This finding stays in agreement with the previously published data^{28,29}. It has been shown recently that oral antibiotic administration suppresses the intestinal metabolites processed by the microbiota and up-regulates inflammation associated with ILC3s and IL17+ cells³⁰. T2R3 has been found as a link between the microbiome and obesity³¹. Based on the above data we may speculate that higher local expression of IL-17 and T1R3 is an immunological signature of reduced biodiversity of the upper-airway microbiome.

Importantly, higher nasal production of IL-17 in children with reduced biodiversity suggests that this phenomenon is related mainly to the third type of inflammation. This finding may have

serious clinical consequences since we cannot expect an optimal response to local corticosteroids in the third type of inflammation.

Another independent risk factor of asthma in our population was atopy. Specific dysbiosis was described in eosinophilic inflammation and atopic subjects³². Therefore, it was suspected that local dysbiosis is not only disease-specific but also depends on disease endotype³³. However, we were not able to define distinct dysbiosis profiles in atopic subjects. These unexpected results could be due to possibly low leucocyte fraction in nasal tissue reflected by a low number of samples with a detectable expression of IL-5 and 13.

Nutrition status was not a primary covariate in the original protocol of our study. Full and adequate assessment of key dietary contributors to the microbiota environment demands, in our opinion, a prospective approach and consequently additional visit, which negatively affects patients' compliance. Nevertheless, in the context of results obtained, lack in the analysis other than sweets dietary determinants, is an important limitation of our study. Another important limitation is the lack of a healthy control group. Although all study procedures are well tolerated, we were not able to recruit a healthy children group due to a lack of parents' consent.

In conclusion, our data point out a link between food habits, upper-airway dysbiosis, and asthma risk in young children. Further clinical and scientific research to find novel therapeutic and prevention strategies of dysbiosis are strongly encouraged.

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MP, PC, and KP designed research; MK, ML, RB, WM, BIA, BA, GM, LJ, SKM, BM, KK, SD, MJ, and CD conducted research; MP analyzed data; MP, MK, CP, and ML wrote the paper; KP had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1 Clinical characteristics of asthmatics vs non-asthmatics. Data are presented by number (%) of observation, otherwise indicated. Odds ratios with 95%confidence intervals from univariate logistic regression analysis are given.

	total group (n=133)		non-asthmatics (n=51)		asthmatics (n=82)		P	OR ^a	95%CI	
	N	%	N	%	N	%				
age (years), median(quartiles Q1-Q3)	6(5-7)		6(5-7)		6(5-7)		0.4279	0.92	0.74	1.14
male gender	81	60.9	30	58.8	51	62.2	0.6985	1.15	0.56	2.35
preterm delivery	27	20.3	9	17.6	18	22.0	0.5492	1.31	0.54	3.20
natural delivery	63	47.4	22	43.1	41	50.0	0.4413	1.32	0.65	2.66
older siblings in family	46	34.6	18	35.3	28	34.1	0.8924	0.95	0.46	1.98
family history of allergy	88	66.2	31	60.8	57	69.5	0.3020	1.47	0.71	3.06
food allergy in infancy	58	43.6	16	31.4	42	51.2	0.0262	2.30	1.10	4.78
atopic dermatitis	9	7.8	3	6.8	6	8.3	0.7676	1.24	0.29	5.24
atopy	54	42.5	10	20.4	44	56.4	0.0001	5.05	2.21	11.53
environmental tobacco smoke (ETS)*	18	13.5	6	11.8	12	14.6	0.6387	1.29	0.45	3.67
BMI (kg/m2), median(quartiles Q1-Q3)	15(14.6-17.3)		15(14.5-16.3)		16(14.7-18.1)		0.2383	1.10	0.94	1.27
SN-5 (points), median(quartiles Q1-Q3)	3.6(2.8-4.2)		3.6(2.8-4.2)		3.6(2.8-4.2)		0.3007	1.19	0.86	1.64
SN5>50 percentile	72	54.1	27	52.9	45	54.9	0.8275	1.08	0.54	2.18
SN5>75 percentile	31	23.3	10	19.6	21	25.6	0.4272	1.41	0.60	3.30
reduced sweet taste perception	95	72.0	31	60.8	64	79.0	0.0250	2.43	1.12	5.28
reduced bitter taste perception	100	75.8	36	70.6	64	79.0	0.2733	1.57	0.70	3.51
total number of antibiotic courses/year, median(quartiles Q1-Q3)	2(1.2-3)		1.6(0.8-2.4)		2.5(1.4-3.6)		0.0052	1.48	1.13	1.95
antibiotic courses/year <25 percentile	35	26.3	20	39.2	15	18.3		ref.		
25-50 percentile	35	26.3	16	31.4	19	23.2	0.3398	1.58	0.62	4.07
50-75 percentile	31	23.3	9	17.6	22	26.8	0.0238	3.26	1.17	9.08
>75 percentile	32	24.1	6	11.8	26	31.7	0.0020	5.78	1.90	17.56
antibiotic courses/year>median	70	52.6	17	33.3	53	64.6	0.0006	3.66	1.75	7.64
sweets consumption (episodes per week), median(quartiles Q1-Q3)	6(4-7)		6(4-7)		6.5(4-7)		0.2568	1.06	0.96	1.17
sweets consumption <25percentil	43	32.3	15	29.4	28	34.1		ref.		
25-50 percentile	30	22.6	17	33.3	13	15.9	0.0674	0.41	0.16	1.07
50-75 percentile	43	32.3	15	29.4	28	34.1	1.0000	1.00	0.41	2.43
>75 percentile	17	12.8	4	7.8	13	15.9	0.3974	1.74	0.48	6.29
sweets consumption>median	60	45.1	19	37.3	41	50.0	0.1524	1.68	0.82	3.44
pathogens, relative frequency>0										
<i>Staphylococcus</i>	26	19.5	8	15.7	18	22.0	0.3777	1.51	0.60	3.79

<i>Haemophilus</i>	131	98.5	50	98.0	81	98.8	0.7351	1.62	0.10	26.48
<i>Moraxella</i>	48	36.1	19	37.3	29	35.4	0.8254	0.92	0.45	1.90
<i>Pseudomonas</i>	26	19.5	11	21.6	15	18.3	0.6435	0.81	0.34	1.95
pathogens, relative frequency>median										
<i>Staphylococcus</i>	13	50.0	5	62.5	8	44.4	0.3993	0.48	0.09	2.65
<i>Haemophilus</i>	65	49.6	27	54.0	38	46.9	0.4311	0.75	0.37	1.53
<i>Moraxella</i>	24	50.0	10	52.6	14	48.3	0.7680	0.84	0.26	2.67
<i>Pseudomonas</i>	14	53.8	6	54.5	8	53.3	0.6917	0.73	0.15	3.47
reduced Shannon index	89	66.9	31	60.8	58	70.7	0.2372	1.56	0.75	3.26
asthma-specific microbiome composition	97	72.9	27	52.9	70	85.4	0.0001	5.19	2.28	11.81
non-asthma-specific composition	36	27.1	24	47.1	12	14.6		ref		
asthma-specific microbiome composition	24	18.0	5	9.8	19	23.2	0.001	7.6	2.28	25.34
asthma-specific microbiome composition with reduced Shannon index	73	54.9	22	43.1	51	62.2	0.0001	4.64	1.97	10.90

^a dependent variable asthmatics vs non-asthmatics

*One or more smoking person at home

ref- reference category of categorized variable

Table 2 Clinical characteristics of the study population by asthma-specific microbiome composition. Data are presented by number (%) of observation, otherwise indicated. Odds ratios with 95%confidence intervals from univariate logistic regression analysis are given.

	others (n=36)		asthma-specific composition (n=97)		P	OR	95%CI	
	N	%	N	%				
age (years), median(quartiles Q1-Q3)	6(5-7)		6(5-7)		0.8793	0.98	0.78	1.24
male gender	24	66.7	57	58.8	0.4076	0.71	0.32	1.59
preterm delivery	8	22.2	19	19.6	0.7373	0.85	0.34	2.17
natural delivery	18	50.0	45	46.4	0.7113	0.87	0.40	1.86
older siblings in family	12	33.3	34	35.1	0.8532	1.08	0.48	2.42
family history of allergy	20	55.6	68	70.1	0.1177	1.88	0.85	4.13
food allergy in infancy	11	30.6	47	48.5	0.0673	2.14	0.95	4.82
atopic dermatitis	1	2.9	8	9.8	0.2394	3.57	0.43	29.69
atopy	16	44.4	38	41.8	0.7826	0.90	0.41	1.95
environmental tobacco smoke (ETS)*	6	16.7	12	12.4	0.5214	0.71	0.24	2.05
BMI (kg/m2), median(quartiles Q1-Q3)	15(14.7-16.4)		16(14.6-17.8)		0.7391	1.03	0.87	1.21
SN-5 (points), median(quartiles Q1-Q3)	3.5(2.7-4.2)		3.6(3-4.2)		0.4832	1.13	0.80	1.61
SN5>50 percentile	18	50.0	54	55.7	0.5602	1.26	0.58	2.70

SNS>75 percentile	7	19.4	24	24.7	0.5218	1.36	0.53	3.51
reduced sweet taste perception	24	66.7	71	74.0	0.4073	1.42	0.62	3.25
reduced bitter taste perception	26	72.2	74	77.1	0.5622	1.29	0.54	3.09
total number of antibiotic courses/year, median(quartiles Q1-Q3)	1.5(0.8-2.7)		2.1(1.4-3.3)		0.0249	1.42	1.04	1.92
antibiotic courses/year <25 percentile	13	36.1	22	22.7		ref.		
25-50 percentile	9	25.0	26	26.8	0.3051	1.71	0.61	4.74
50-75 percentile	10	27.8	21	21.6	0.6779	1.24	0.45	3.44
>75 percentile	4	11.1	28	28.9	0.0262	4.14	1.18	14.47
antibiotic courses/year>median	15	41.7	55	56.7	0.1252	1.83	0.84	3.98
sweets consumption (episodes per week), median(quartiles Q1-Q3)	5(3.5-7)		7(4-7)		0.0505	1.14	1.00	1.31
sweets consumption <25percentil	14	38.9	29	29.9		ref.		
25-50 percentile	11	30.6	19	19.6	0.7160	0.83	0.31	2.22
50-75 percentile	11	30.6	32	33.0	0.4770	1.40	0.55	3.58
>75 percentile	0	0.0	17	17.5				
sweets consumption>median	11	30.6	49	50.5	0.0425	2.32	1.03	5.23
pathogens, relative frequency>0								
<i>Staphylococcus</i>	3	8.3	23	23.7	0.0580	3.42	0.96	12.19
<i>Haemophilus</i>	36	100.0	95	97.9	-	-	-	-
<i>Moraxella</i>	13	36.1	35	36.1	0.9976	1.00	0.45	2.21
<i>Pseudomonas</i>	8	22.2	18	18.6	0.6362	0.80	0.31	2.04
pathogens, relative frequency>median								
<i>Staphylococcus</i>	1	33.3	12	52.2	0.5465	2.18	0.17	27.56
<i>Haemophilus</i>	19	52.8	46	48.4	0.6563	0.84	0.39	1.81
<i>Moraxella</i>	4	30.8	20	57.1	0.1120	3.00	0.77	11.63
<i>Pseudomonas</i>	4	50.0	9	50.0	1.0000	1.00	0.19	5.29
reduced Shannon index	16	44.4	73	75.3	0.0011	3.80	1.70	8.49

ref- reference category of categorized variable

*One or more smoking person at home

Table 3 Nasal expression of various molecules by reduced Shannon index and by asthmatic microbiome composition. Data are presented as relative to 18SrRNA expression $\times 10^5$. The number of observations given for each comparison indicates the number of the sample with a detectable expression included in

the analysis. Sample of cellular material from the nasal mucosa was taken from 121 patients; missing data (n=12) was a result of a lack of patients' compliance during the sampling procedure.

	others, (n=39)	reduced Shannon index, (n=82)	difference*	95% CI of difference	p-level	p-level #
	median, n	median, n				
IL-17	0,017, n=35	0,044, n=71	0.013	0,0002 to 0,155	0.0487	0,080
T1R3	0,072, n=38	0,275, n=79	0.050	0,006 to 0,430	0.0199	0,060
TSLP	0,002, n=11	0,0003, n=31	-0.0009	-0,006 to -2,710e-005	0.0381	0,080
DAFB2	9,501, n=35	5,929, n=71	-1.025	-6,324 to 1,797	0.3879	0,447
IL-13	0,002, n=21	0,001, n=42	-0.0005	-0,002 to 0,0005	0.3067	0,447
IL-5	0,0002, n=7	0,0001, n=15	-0.00007	-0,001 to 0,0007	0.4475	0,447
	others, (n=32)	asthma-specific composition, (n=89)	difference*	95% CI of difference	p-level	p-level #
	median, n	median, n				
DAFB2	22,07, n=29	3,335, n=77	-12.64	-24,86 to -3,444	0.0035	0.018
IL-13	0,004, n=20	0,001, n=43	-0.001	-0,012 to -0,0003	0.0176	0.051
IL-5	0,001, n=5	9,578e-005, n=17	-0.0008	-0,013 to -0,0002	0.031	0.062
IL-17	0,026, n=27	0,024, n=79	0.001	-0,011 to 0,029	0.857	0.857
T1R3	0,096, n=32	0,259, n=85	0.012	-0,034 to 0,276	0.5652	0.847
TSLP	0,0006, n=8	0,0005, n=34	-0.00003	-0,0006 to 0,002	0.8382	0.857

* Hodges-Lehmann

Benjamini and Hochberg correction for multiple comparisons

Figure legends:

Figure 1

Bar plots as a visualization of analysis of the composition of microbiomes (ANCOM) showing the reduced relative frequency of *Patescibacteria* and increased frequency of *Actinobacteria* in the nasopharynx of asthmatic vs non-asthmatic patients with chronic rhinosinusitis, (A). The *Patescibacteria/Actinobacteria* ratio comparison between asthmatics vs non-asthmatics with corresponding ROC curve and estimated optimal cut-point value defining sub-group of children

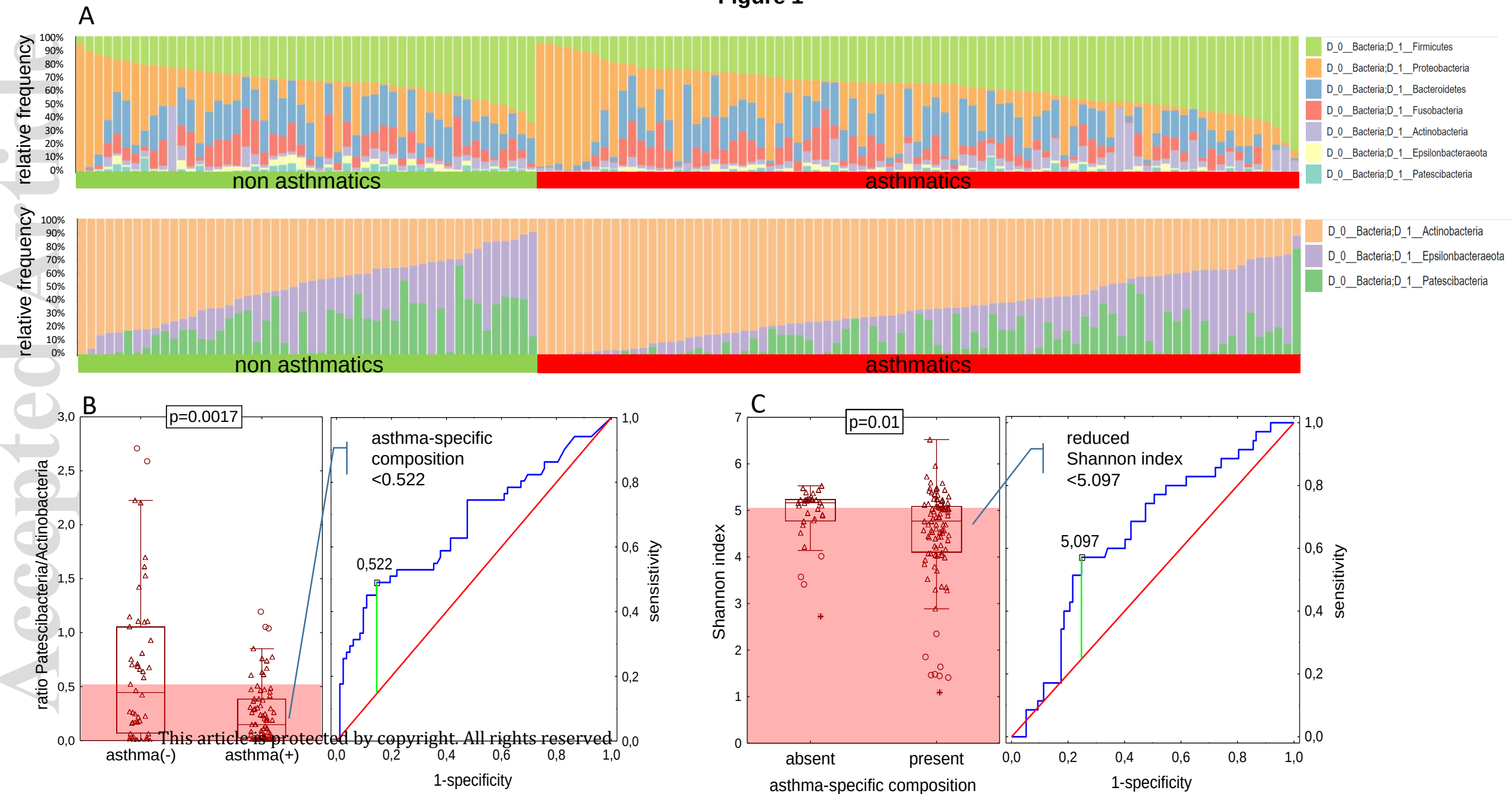
with the asthma-specific composition (red area), (B). Shannon index comparison between children with or without asthma-specific composition with corresponding ROC curve and estimated optimal cut-point value defining sub-group of children with reduced Shannon index (red area), (C).

Figure 2

Relative expression of various molecules in the nasal mucosa of children with chronic rhinosinusitis and with vs without asthma-specific composition (right panel) and with vs without reduced Shannon index (left panel). The visualizations of subgroups analyses are shown. Data are presented as individual values (points), lower-upper quartiles (boxes) and min-max values (whiskers).

Figure 3

A vicious circle of asthma-specific microbiome composition and reduced biodiversity leading to increased asthma risk in young children with chronic rhinosinusitis. Study results visualization.



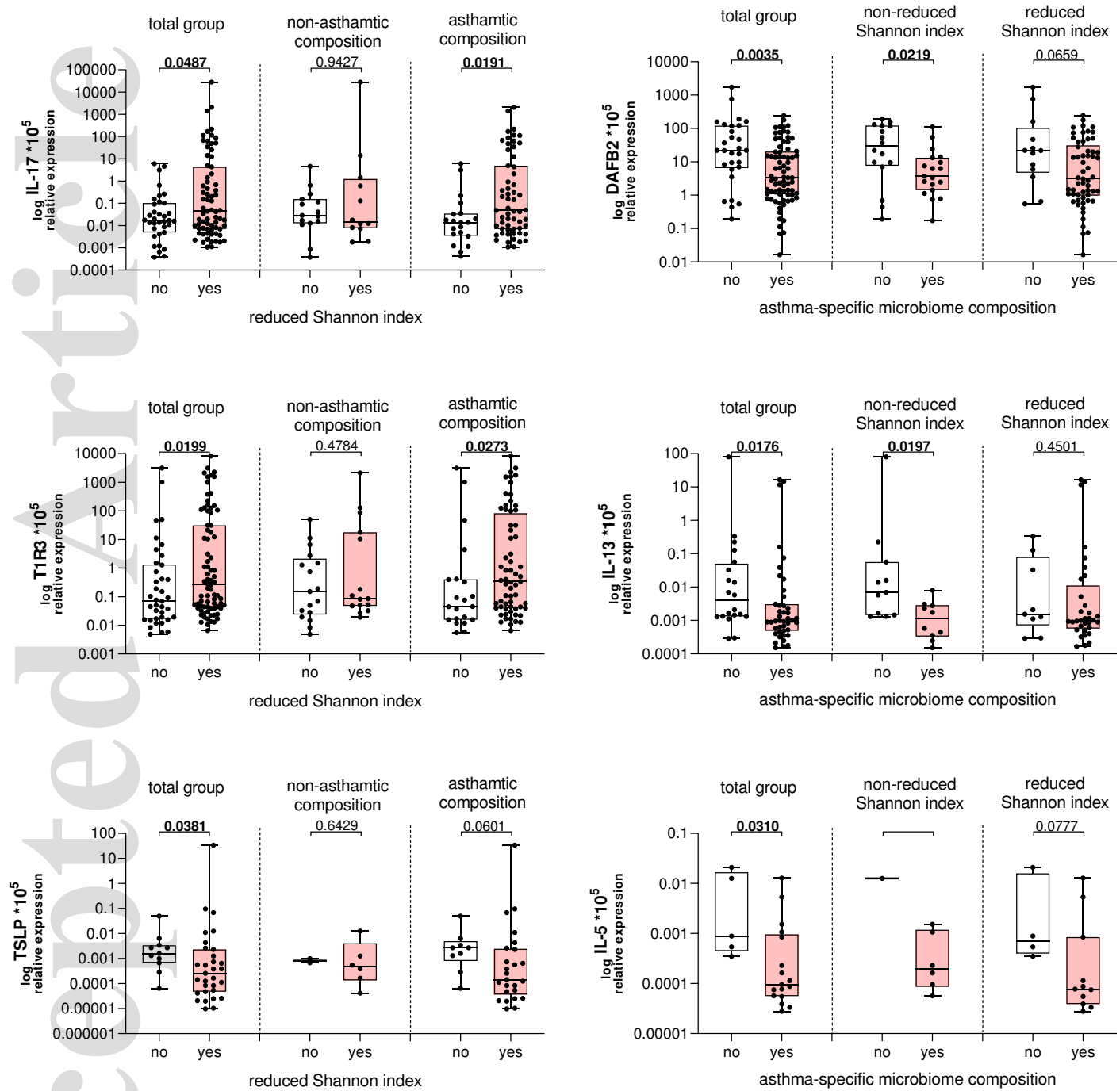


Figure 2

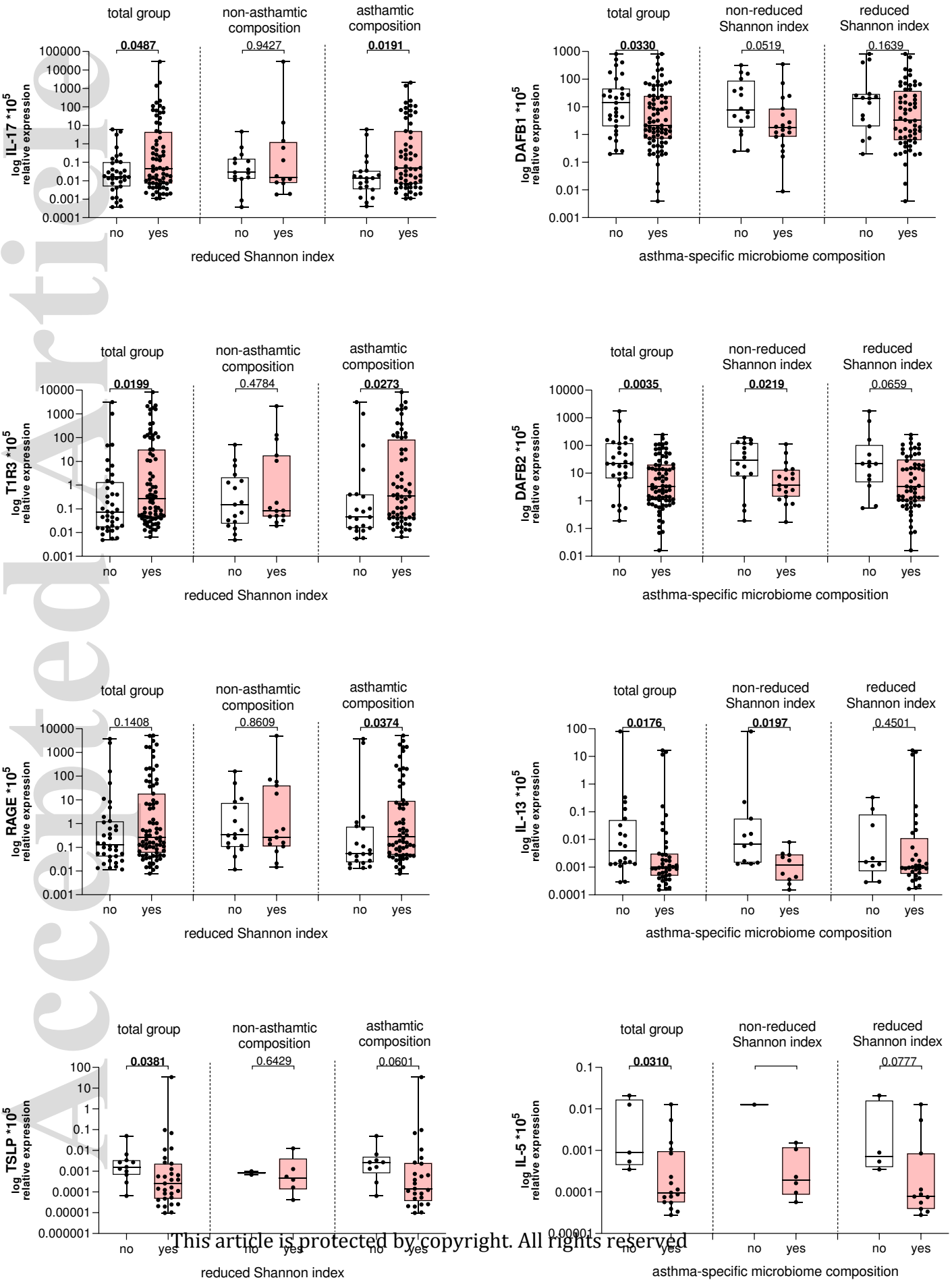


Figure 2

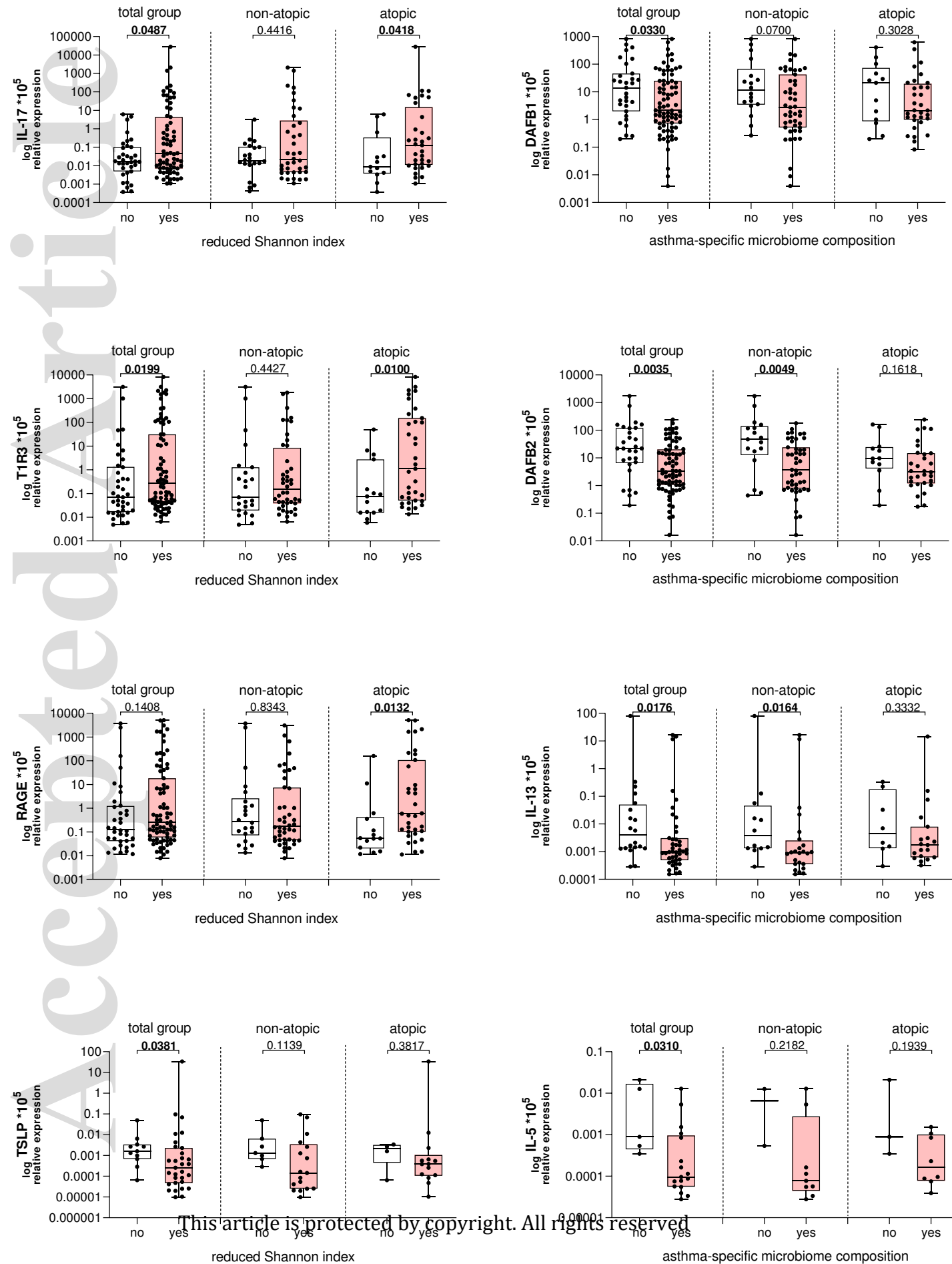
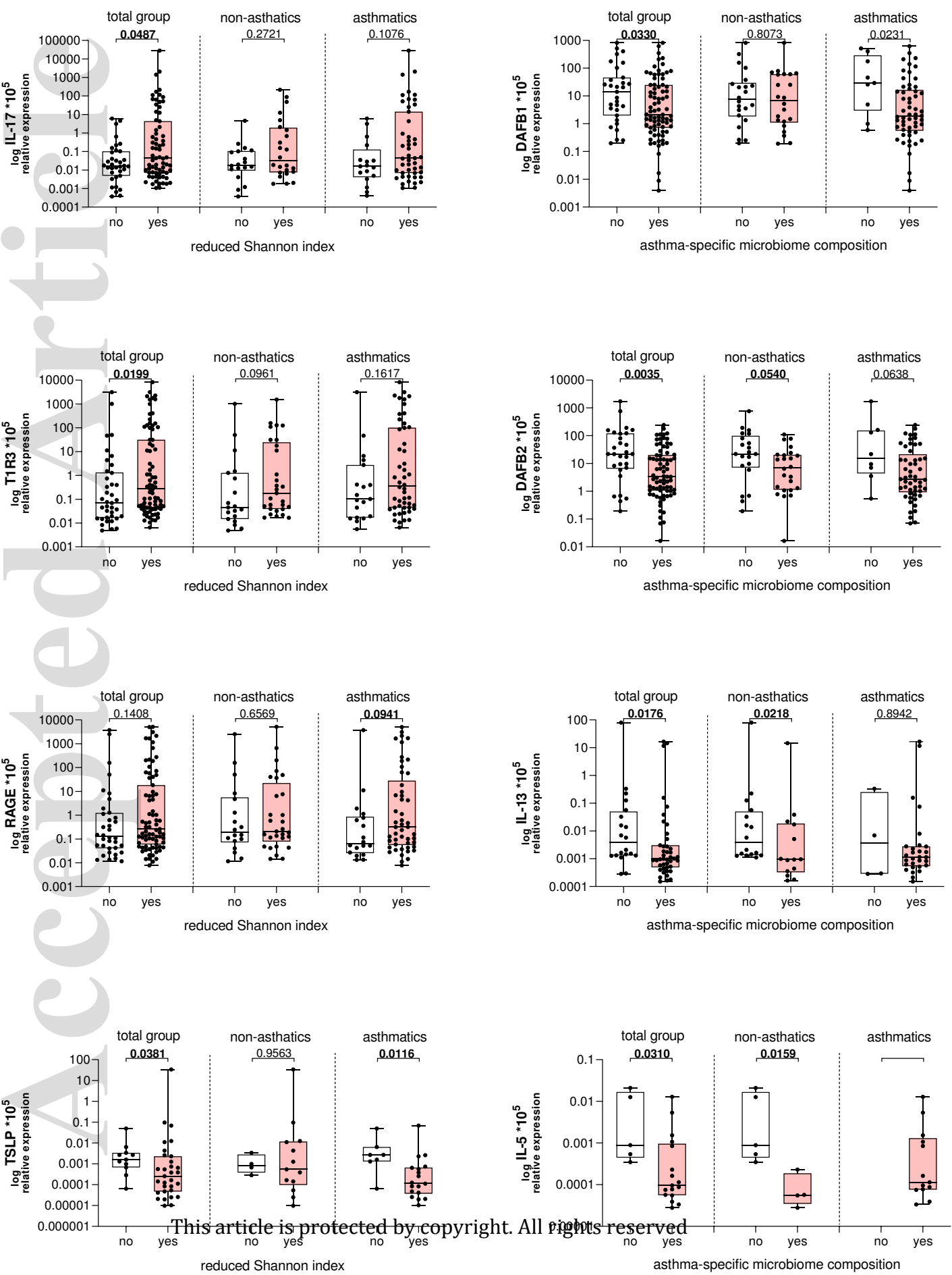


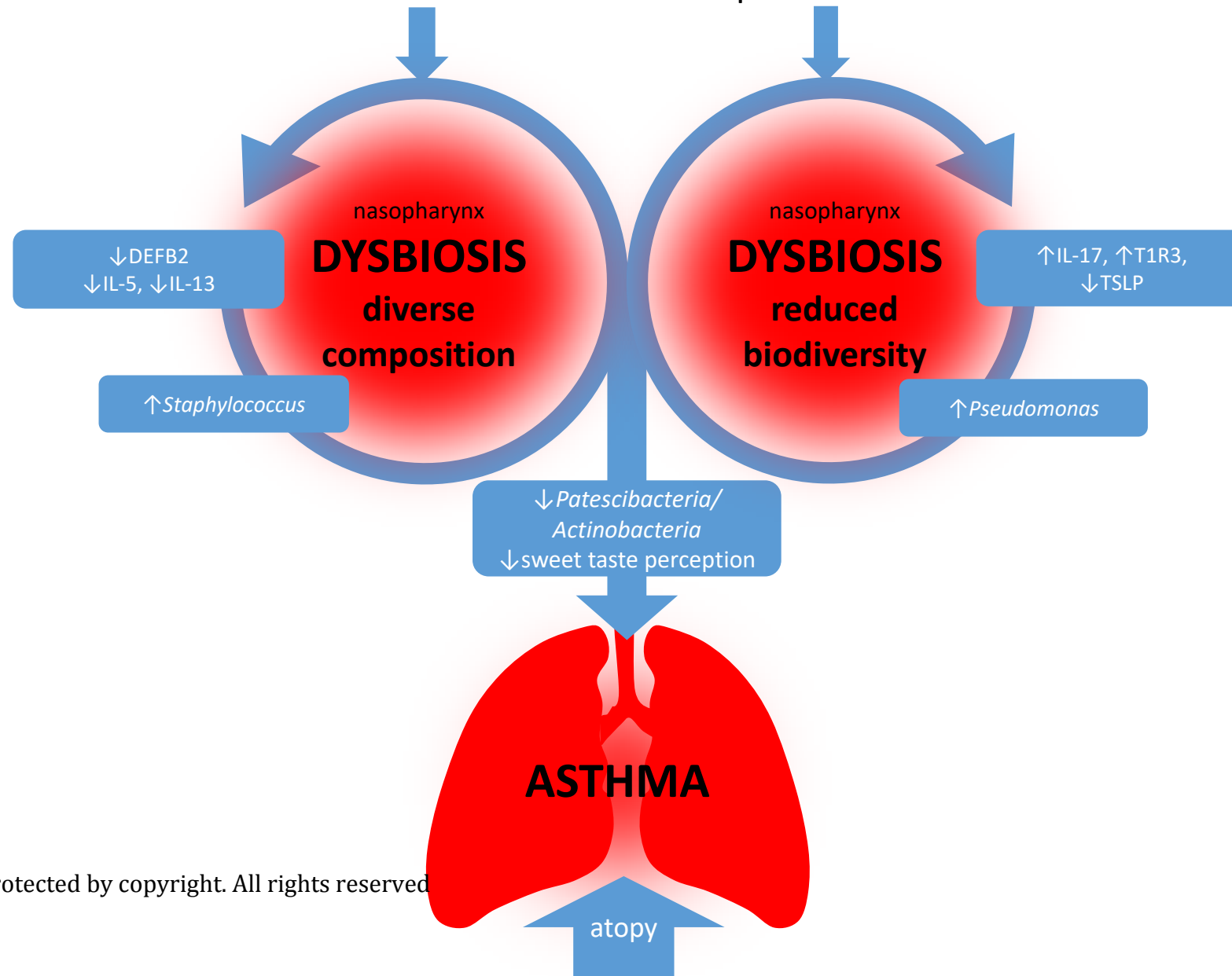
Figure 2



Preschoolers with chronic rhinosinusitis and with:

frequent sweets consumption

frequent antibiotics



Upper-airway dysbiosis related to frequent sweets consumption increases the risk of asthma in children with chronic rhinosinusitis

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Patients

The project RAISe (*Response of the Airway in Sinusitis and Asthma*) was conducted in two phases.

The first phase (case-control study- presented in the current manuscript) included children who presented symptoms of a running nose, cough or recurrent episodes of bronchial obstruction during the autumn-winter season (see flow chart below).

After Allergology and Laryngology consultations, children were included in the following groups:

- CRS+/asthma+: a group of children (planned n=90, achieved n=89) suffering from CRS (fulfilling the EPOS criteria, European Position Paper on Rhinosinusitis and Nasal Polyps) and asthma (fulfilling the API criteria, Asthma Predictive Index),
- CRS+/asthma-: a group of children (planned n=30, achieved n=52) suffering from CRS (fulfilling the EPOS criteria) but without asthma symptoms (negative API criteria),
- CRS-/asthma-: a group of children (planned n=30, achieved n=0) without symptoms of CRS (negative EPOS criteria) and asthma symptoms (negative API criteria).

The second phase (intervention study: intranasal mometasone group vs control group) planned to include children from the CRS+/asthma+ group.

Although all study procedures are well tolerated, we could not recruit a healthy children group due to a lack of parents' consent. We were able to include symptomatic children only. Unexpectedly, all of them fulfilled EPOS' criteria of CRS. Therefore we had no healthy control group and larger CRS+/asthma- group. Due to a lack of compliance with children during ENT procedures we were able to take 133 nasopharynx swab for microbiome analysis and 121 nasal mucosa sample. Therefore in the current analysis 133 patients (asthmatics, n=82) were included.

Planned Samples size

We estimated 30 patients in each group would be sufficient. The following criteria of estimation were included:

- 20% difference between groups in the relative expression of molecules in the nasal mucosa
- nonparametric distribution of variable, SD according to available data
- 20% missing data
- 80% power of conclusion with p level below 0.05

Analysis in subgroups according to atopy status justifies sample size (n=90) for CRS+/asthma+ group during the second phase of the project

The study flow chart in line with the STROBE statement

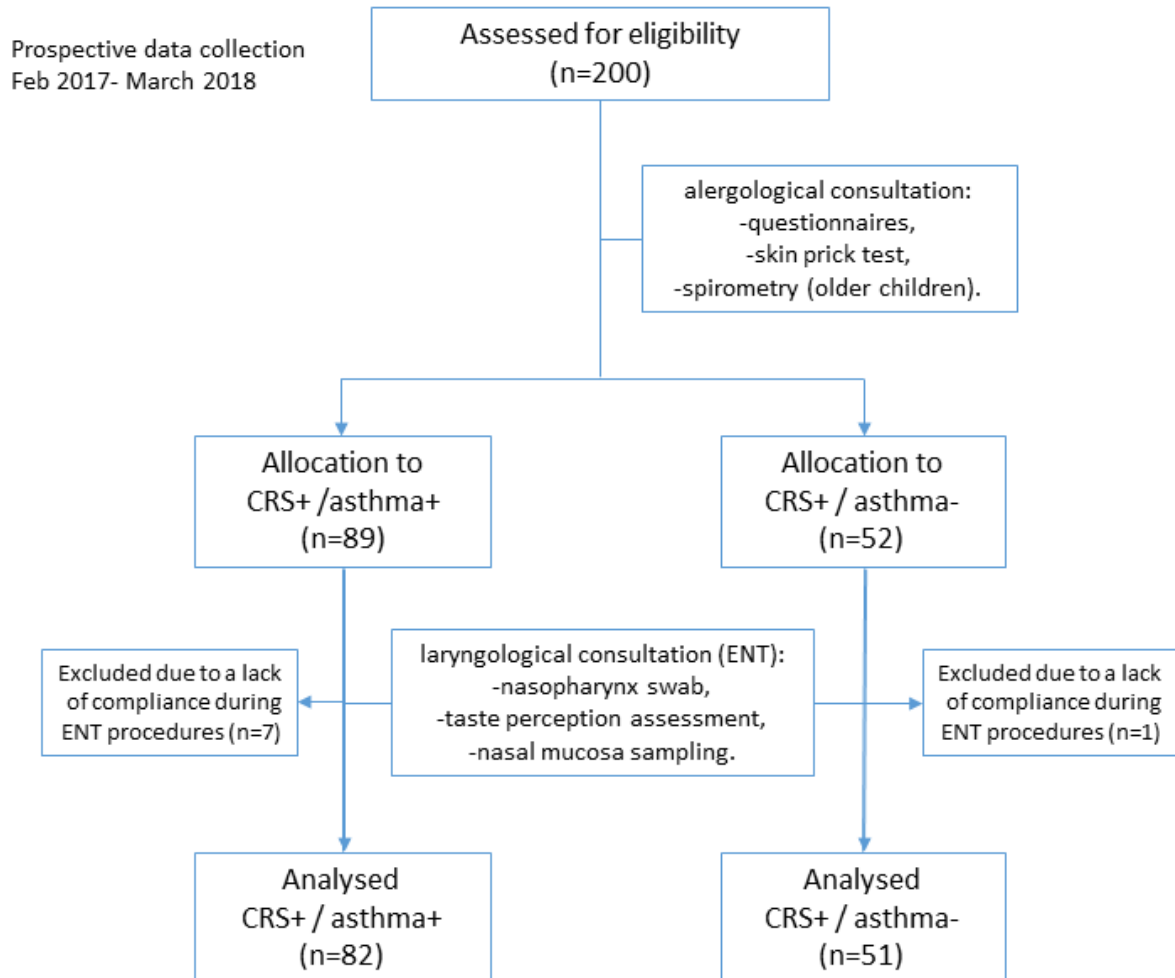


Table 1 Additional clinical characteristics of study cohort.

Variable:	
chronic rhinosinusitis (n=133)	
duration of the disease [months], median(lower-upper quartile)	8 (6-11)
nasal corticosteroids (<2 weeks)	82(61.6)
nasal corticosteroids (from 2weeks to 2 months)	18(13.5)
nasal corticosteroids (>=2 months)	0(0)
asthma (n=82):	
duration of the disease [months], median(lower-upper quartile)	4 (3-7)
episodic* inhaled corticosteroids, n(%)	80(97.5)
daily inhaled corticosteroids, n(%)	0(0)
daily leukotriene receptor antagonist, n(%)	27(32.9)
atopy**(n=54):	
house dust mite, n(%)	27(50)
molds (<i>Alternaria</i>) , n(%)	10(18.5)
cat, n(%)	12(22.2)
dog, n(%)	6(11.1)
tree, n(%)	29(53.7)
birch, n(%)	21(38.9)
pet at home, n(%)	5(3.7)

* inhaled corticosteroids taken whenever SABA taken (up to 2 weeks during infection)

**positive skin prick test and/or presence of specific IgE in serum

Methods

Taste-testing

The taste test was carried out using validated taste strips (taste strips; Burghart, Wedel, Germany). The set consisted of sixteen stripes: four different flavors (sweet, sour, salty, and bitter) each in four concentrations. The following concentrations were used: sweet: 0.4, 0.2, 0.1, and 0.05 g/mL sucrose; sour: 0.3, 0.165, 0.09, 0.05 g/mL citric acid; salty: 0.25, 0.1, 0.04, 0.016 g/mL sodium chloride; and bitter: 0.006, 0.0024, 0.0009, 0.0004 g/mL quinine hydrochloride. Tests were carried out from the lowest to the highest concentrations in a pseudo-random manner. The taste strips were placed in the middle of the tongue, and then the patients were allowed to close their mouths and move the strip [1,2].

Skin prick tests (SPT)

SPTs were performed according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines. All patients underwent skin prick tests performed with common aeroallergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grasses, birch, hazel, alder, mugwort, cat, dog, *Alternaria tenuis*, and *Cladosporium herbarum* (Allergopharma). Histamine 1.7 mg/mL (Allergopharma) and standard glycerol saline solution (Allergopharma) were used as a positive and negative control, respectively. A wheel diameter ≥ 3 mm was considered a positive result.

Endoscopy

This procedure was performed by ENT (ear, nose, and throat) specialists according to GCP (good clinical practice) requirements to excluded children with adenotonsillar hypertrophy from the study.

Processing of nasal epithelium

The curette with a nasal epithelium specimen was placed in a 15-mL-tube containing 2 mL of cold sterile RPMI 1640 (Gibco, Thermo Fisher Scientific Inc., Waltham, MA USA) and transported to the biological laboratory within 3 hours since the collection. The curette was vigorously shaken to dislodge the tissue sample into the medium. Then the curette was removed using sterile forceps and the sample was vortexed for approximately 30 seconds. The medium was then centrifuged ($100 \times g$, 10 minutes, RT) and the cell and tissue pellet further processed as specified below.

mRNA isolation

RPMI 1640 residues were carefully removed by gentle suction and the pellet was suspended in 100 μ L of TRI Reagent® (Merck KGaA, Darmstadt, Germany) by repetitive pipetting. The resulting mixture was transferred to a sterile 1.5 mL-Eppendorf tube and stored at -80°C until further processing.

Total RNA was isolated by the method of Chomczynski and Mackey [3]. The sample was allowed to thaw at room temperature (RT), vortexed and then further incubated for 15 minutes at RT to allow nucleoprotein complexes to dissociate. After the addition of 10 μ L of 1-bromo-3-chloropropane (BCP), the sample was vigorously vortexed for 15 seconds and left for 15 minutes at RT to allow phase separation. After centrifugation ($12,000 \times g$, 15 minutes, 4°C), the uppermost water phase was gently transferred to an RNase-free tube and thoroughly mixed with 50 μ L of isopropanol. The sample was incubated for 10 minutes at RT and then centrifuged ($12,000 \times g$, 10 minutes, 4°C). The supernatant was carefully removed and the pellet was vortexed in 100 μ L of 75% ethanol and centrifuged ($12,000 \times g$, 10 minutes, 4°C). The supernatant was carefully removed and the RNA pellet was allowed to air-

dry. The pellet was then dissolved in 20 μ L of nuclease-free water, and RNA concentration, as well as the A260/A280 ratio, were assessed using NanoDrop™ 2000c microspectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA USA). The sample was immediately reversely transcribed.

RT and Q-PCR

Reverse transcription reaction was performed in triplicate by the protocol of Wiame et al. [4] using random primers and Maxima™ Reverse Transcriptase kit (Thermo Fisher Scientific Inc., Waltham, MA USA) as per manufacturer's instructions. The resulting cDNA sample was stored at -20°C until used.

Q-PCR of isolated cDNA was performed using SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix (Bio-Rad, Hercules, CA the USA) and carried out in CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA USA). The primers used for evaluation of specific gene expression are listed in Table 2. The expression of specific genes was compared to 18SrRNA as a reference gene and expressed as $\Delta\Delta C_t$.

Table 2 List of qPCR primers used throughout the study. “–” denotes primers designed using NCBI Primer-BLAST tool.

Gene	Forward primer	Reverse primer	Ref.
<i>DEFB2</i>	TGGTGAAGCTCCAGCCATC	ACATGTCGCACGTCTCTGAT	–
<i>IL17A</i>	TCCCACGAAATCCAGGATGC	GGATGTTGAGTTGACCATCAC	[5]
<i>TSLP</i>	CCCAGGCTATTCGGAAGTCA	ACGCCACAATCCTTGTAATTGTG	[6]
<i>IL13</i>	CGAGAAGACCCAGAGGATGCT	GGGCCACCTCGATTTTGG	[6]
<i>IL5</i>	AAGAGACCTTGGCACTGCTTTC	GGAACAGGAATCCTCAGAGTCTCA	[7]
<i>T1R3</i>	CAAAACCCAGACGACATCG	CATGCCAGGAACCGAGAC	[8]
<i>18SrRNA</i>	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC	[9]

NGS library preparation and sequencing

DNA was extracted from nasal swabs using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Samples were eluted with nuclease-free water, in a volume of 50 μ L. As a negative control for background 16S rRNA contamination the same water was used. DNA concentration was determined using a Qubit high-sensitivity (HS) assay kit (ThermoFisher, Waltham, USA). NGS libraries preparation was performed according to Illumina's “16S Metagenomic Sequencing Library Preparation”. Briefly, for 16S rDNA V3-V4 region amplification was used following forward (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and reverse (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') primers with default PCR program from the protocol. After each amplification or clean-up step, samples were controlled with gel electrophoresis or TapeStation. Accurate quantitation of the libraries was carried out using a NEBNext Library Quant Kit for Illumina (New England Biolabs, Ipswich, USA). Then, the samples were

pooled at an equimolar concentration and sequenced as paired-ends using the Illumina MiSeq platform (Illumina, San Diego, CA).

Bioinformatic analysis

After sequencing, the raw data for 133 samples were demultiplexed with the BaseSpace Online platform (Illumina, San Diego, CA). Fastq files for each sample were trimmed using trim_galore v. 1.18 with following parameters `--length 100, --max_n 0, -q 25, --phred33` (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The quality of reads before and after pre-processing was assessed using FastQC (v0.11.8) [10]. Further analysis steps were performed using the Qiime 2 (v. 2019.10) platform [11]. Amplicon sequence variant (ASV) table was produced through the use DADA2 plugin for paired-end reads [9]. ASV with a frequency lower than 118 in all samples was removed to denoise the data. Sampling depth used to diversity metrics calculation was set up to 3651 based on the alpha-rarefaction analysis. The taxonomic classification of ASV's was done with the classify-learn plugin with default parameters [12,13]. The database used as reference was Silva 132 [14]. The Analysis of the Composition of Microbiomes (ANCOM) was performed to identify taxa that were differentially abundant across groups [15]. Taxa occurring in less than 20 samples were removed to reduce noises in ANCOM. Dysbiosis indexes were calculated based on the relative abundance ratio of taxa involved to index.

Results

Microbial diversity

After preprocessing with DADA2 6 459 223 sequences for 133 samples were obtained. The number of sequences per sample was in a range from 3 651 to 181 880. The mean number of reading per sample was 48 565 and a median of 37 292. 1 680 ASV was identified. In ANCOM significant differences of abundance for phyla *Actinobacteria* and *Patescibacteria* between groups with and without asthma were identified. Both of them have W statistic equal to 1.

Table 3 Multivariate model of logistic regression analysis with asthma as the dependent variable and group of independent variables associated with asthma in the univariate model given in Table 1 of the manuscript. Step3 indicates the final model.

Variables in the Equation		P	OR	95%CI	
Step 1(1)	atopy	1E-04	5.20	2.27	11.91
	Constant	5E-01	0.85		
Step 2(2)	atopy	4E-05	8.03	2.96	21.77
	asthma-specific microbiome composition	5E-05	8.29	3.00	22.94
	Constant	4E-04	0.17		
Step 3(3)	reduced sweet taste perception	2E-02	3.09	1.18	8.07
	atopy	6E-05	7.96	2.88	22.00
	asthma-specific microbiome composition	7E-05	8.32	2.93	23.60
	Constant	6E-05	0.07		

Variable(s) entered on step 1: atopy

Variable(s) entered on step 2: asthma-specific microbiome composition

Variable(s) entered on step 3: reduced sweet taste perception

Table 4 The Level of taste perception according to gustometry results in asthmatics vs non-asthmatics. Reduced taste perception defined as 2nd or 3rd or 4th vs 1st strip was included into following analyses

	non-asthmatics (n=51)		asthmatics (n=82)		OR	95%CI		P
	N	%	N	%				
level of sweet taste perception								
1 st strip	20	39.2	17	21.0	ref			
2 nd strip	14	27.5	32	39.5	2.69	1.09	6.62	0.031
3 rd strip	14	27.5	26	32.1	2.18	0.87	5.46	0.095
4 th strip	3	5.9	6	7.4	2.35	0.51	10.86	0.273
level of sour taste perception								
1 st strip	6	11.8	12	14.8	ref			
2 nd strip	16	31.4	31	38.3	0.97	0.31	3.06	0.957
3 rd strip	24	47.1	32	39.5	0.67	0.22	2.03	0.476
4 th strip	5	9.8	6	7.4	0.60	0.13	2.80	0.515
level of salty taste perception								
1 st strip	8	15.7	22	27.2	ref			
2 nd strip	17	33.3	24	29.6	0.51	0.19	1.42	0.200
3 rd strip	21	41.2	22	27.2	0.38	0.14	1.04	0.060
4 th strip	5	9.8	13	16.0	0.95	0.25	3.51	0.933
level of bitter taste perception								
1 st strip	15	29.4	17	21.0	ref			
2 nd strip	17	33.3	25	30.9	1.30	0.51	3.28	0.582
3 rd strip	17	33.3	29	35.8	1.51	0.60	3.76	0.382
4 th strip	2	3.9	10	12.3	4.41	0.83	23.42	0.081
reduced sweet taste perception ^a	31	60.8	64	79.0	2.43	1.12	5.28	0.025
reduced bitter taste perception ^a	36	70.6	64	79.0	1.57	0.70	3.51	0.273
significantly reduced sweet taste perception ^b	3	5.9	6	7.4	1.28	0.31	5.36	0.736
significantly reduced bitter taste perception ^b	2	3.9	10	12.3	3.45	0.72	16.44	0.120

^a reduced taste perception: 2nd or 3rd or 4th vs 1st strip

^b significantly reduced taste perception: 4th vs other strips

ref- reference category of categorized variable

Table 5 Multivariate model of logistic regression analysis with asthma-specific microbiome composition as the dependent variable and group of independent variables associated with asthma-specific microbiome composition in the univariate model given in Table S4. Step3 indicates the final model.

Variables in the Equation		P	OR	95%CI	
Step 1(1)	reduced Shannon index	0.0011	3.80	1.70	8.49
	Constant	0.5470	1.20		
Step 2(2)	reduced Shannon index	0.0011	3.90	1.72	8.86
	Staphylococcus >0 of relative frequency	0.0555	3.60	0.97	13.33
	Constant	0.9382	0.98		
Step 3(3)	sweets consumption>median	0.0287	2.64	1.11	6.28
	reduced Shannon index	0.0017	3.83	1.65	8.87
	Staphylococcus >0 of relative frequency	0.0330	4.25	1.12	16.08
	Constant	0.2501	0.65		

Variable(s) entered on step 1: reduced Shannon index

Variable(s) entered on step 2: Staphylococcus >0 of relative frequency

Variable(s) entered on step 3: sweets consumption>median

Table 6 Clinical characteristics of the study population by reduced Shannon index. Data are presented by number (%) of observation, otherwise indicated. Odds ratios with 95% confidence intervals from univariate logistic regression analysis are given.

	others (n=44)		reduced Shannon index (n=89)		P	OR	95%CI	
	N	%	N	%				
age (years), median(quartiles Q1-Q3)	6(5-7)		6(5-7)		0.9172	1.01	0.81	1.27
male gender	27	61.4	54	60.7	0.9389	0.97	0.46	2.04
preterm delivery	7	15.9	20	22.5	0.3782	1.53	0.59	3.96
natural delivery	21	47.7	42	47.2	0.9535	0.98	0.47	2.02
older siblings in family	16	36.4	30	33.7	0.7620	0.89	0.42	1.89
family history of allergy	34	77.3	54	60.7	0.0600	0.45	0.20	1.03
food allergy in infancy	17	38.6	41	46.1	0.4168	1.36	0.65	2.83
atopic dermatitis	3	7.9	6	7.7	0.9695	0.97	0.23	4.12
atopy	17	39.5	37	44.0	0.6266	1.20	0.57	2.54
environmental tobacco smoke (ETS)*	10	22.7	8	9.0	0.0346	0.34	0.12	0.92
BMI (kg/m ²), median(quartiles Q1-Q3)	15(14.4-17.3)		16(14.8-17.3)		0.7179	1.03	0.88	1.20
SN-5 (points), median(quartiles Q1-Q3)	3.6(2.8-4.2)		3.6(2.8-4.2)		0.8256	1.04	0.75	1.44
SN5>50 percentile	23	52.3	49	55.1	0.7618	1.12	0.54	2.31
SN5>75 percentile	10	22.7	21	23.6	0.9113	1.05	0.45	2.48
reduced sweet taste perception	34	77.3	61	69.3	0.3391	0.66	0.29	1.54
reduced bitter taste perception	36	81.8	64	72.7	0.2536	0.59	0.24	1.46
total number of antibiotic courses/year, median(quartiles Q1-Q3)	1.5(0.8-2.7)		2.2(1.4-3.2)		0.0831	1.25	0.97	1.61
antibiotic courses/year <25 percentile	18	40.9	17	19.1		ref.		
25-50 percentile	10	22.7	25	28.1	0.0536	2.65	0.99	7.11
50-75 percentile	8	18.2	23	25.8	0.0363	3.04	1.07	8.63
>75 percentile	8	18.2	24	27.0	0.0292	3.18	1.12	8.98
antibiotic courses/year>median	17	38.6	53	59.6	0.0244	2.34	1.12	4.90
sweets consumption (episodes per week), median(quartiles Q1-Q3)	6(3.5-7)		6(4-7)		0.2581	1.06	0.96	1.18
sweets consumption <25percentil	16	36.4	27	30.3		ref.		
25-50 percentile	10	22.7	20	22.5	0.7338	1.19	0.45	3.15
50-75 percentile	14	31.8	29	32.6	0.6511	1.23	0.50	2.98
>75 percentile	4	9.1	13	14.6	0.3156	1.93	0.54	6.93
sweets consumption>median	18	40.9	42	47.2	0.4937	1.29	0.62	2.68
pathogens, relative frequency>0								
<i>Staphylococcus</i>	8	18.2	18	20.2	0.7799	1.14	0.45	2.88
<i>Haemophilus</i>	44	100.0	87	97.8	-	-	-	-
<i>Moraxella</i>	14	31.8	34	38.2	0.4713	1.32	0.62	2.85
<i>Pseudomonas</i>	8	18.2	18	20.2	0.7799	1.14	0.45	2.88
pathogens, relative frequency>median								
<i>Staphylococcus</i>	2	25.0	11	61.1	0.1022	4.71	0.73	30.28
<i>Haemophilus</i>	22	50.0	43	49.4	0.9505	0.98	0.47	2.02
<i>Moraxella</i>	4	28.6	20	58.8	0.0637	3.57	0.93	13.72
<i>Pseudomonas</i>	1	12.5	13	72.2	0.0149	18.20	1.76	188.07

ref- reference category of categorized variable

*One or more smoking person at home

Table 7 Log-transform expressions were included in multifactorial ANOVA. Interactions with potential confounders are shown.

	others	asthma-specific composition	between-groups	P-level		
	median, n	median, n		ANOVA interactions with		
				reduced Shannon index	asthma	atopy
DAFB2	22,07, n=29	3,335, n=77	0.0035	0.886	0.583	0.174
IL-13	0,004, n=20	0,001, n=43	0.0176	0.444	0.443	0.905
IL-5	0,0009, n=5	9,6e-005, n=17	0.031	-	-	-

the symbol “-” indicates that interaction can’t be calculated due to small sample size

Table 8 Log-transform expressions were included in multifactorial ANOVA. Interactions with potential confounders are shown.

	others	reduced Shannon index	between-groups	P-level		
	median, n	median, n		ANOVA interactions with		
				asthmatic composition	asthma	atopy
IL-17	0,017, n=35	0,044, n=71	0.0487	0.571	0.655	0.481
T1R3	0,072, n=38	0,275, n=79	0.0199	0.538	0.759	0.094
TSLP	0,001, n=11	0,0003, n=31	0.0381	-	-	-

the symbol “-” indicates that interaction can’t be calculated due to small sample size

Figure E1

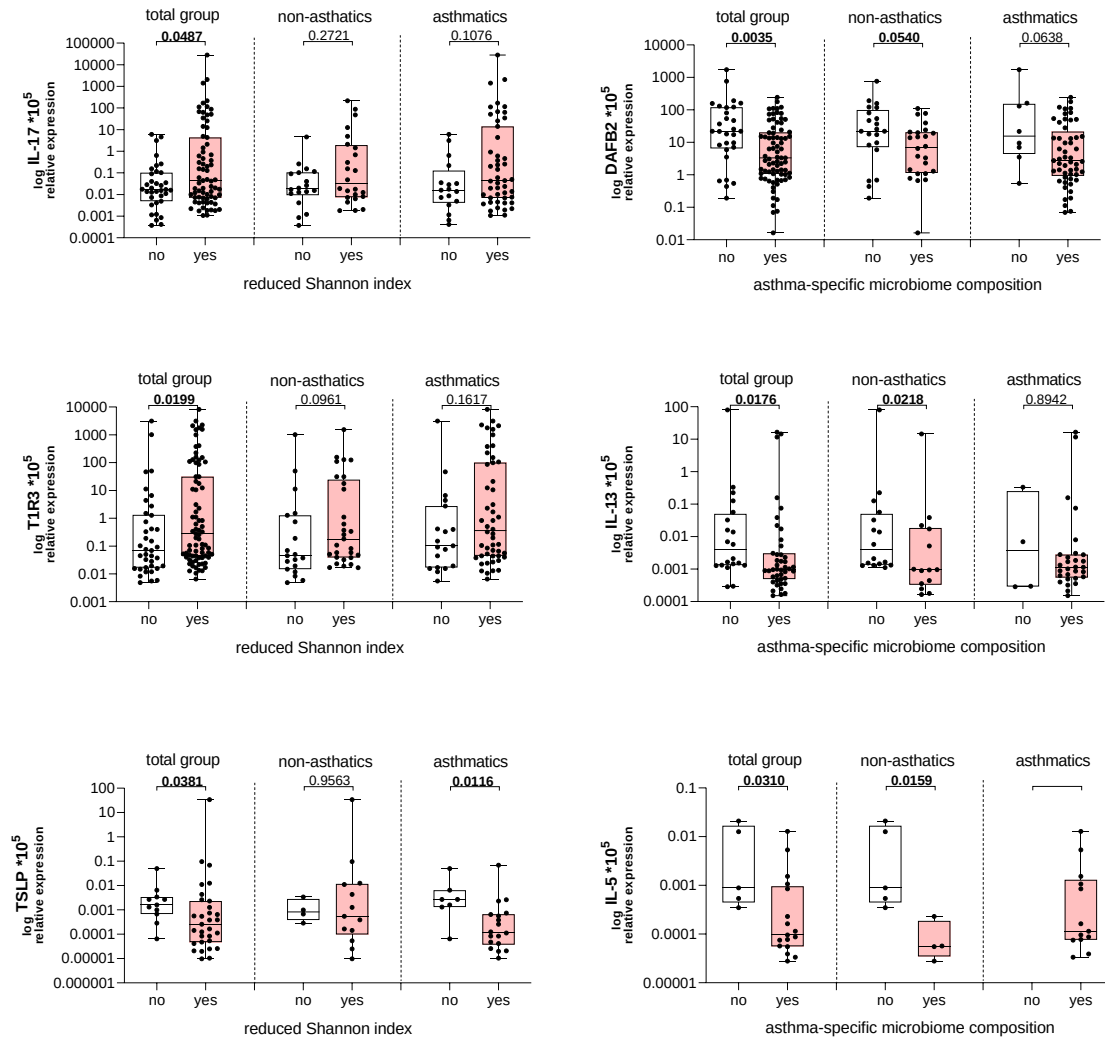


Figure E1 Relative expression of various molecules in the nasal mucosa of children with chronic rhinosinusitis and with vs without asthma-specific composition (right panel) and with vs without reduced Shannon index (left panel). The visualizations of subgroups analyses are shown. Data are presented as individual values (points), lower-upper quartiles (boxes) and min-max values (whiskers).

Figure E2

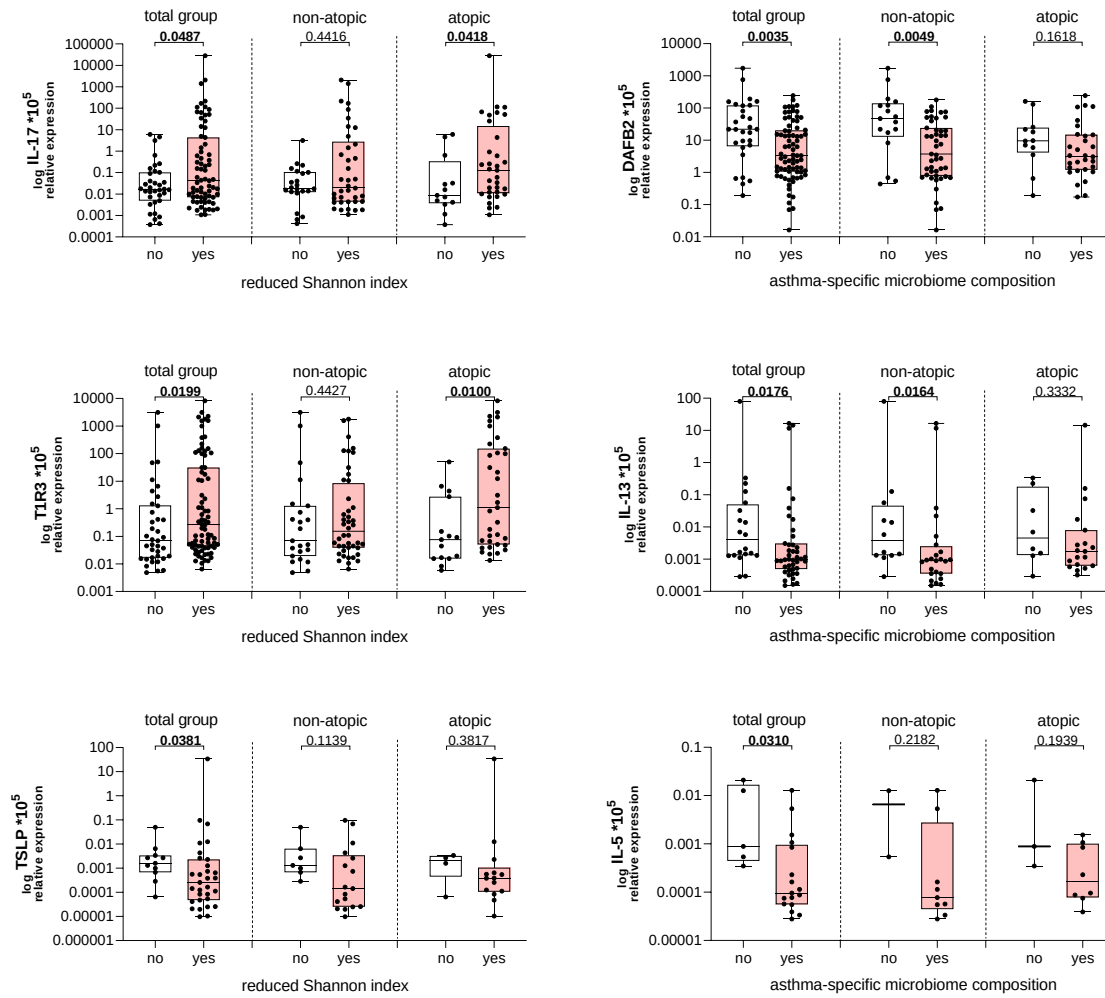


Figure E2 expression of various molecules in the nasal mucosa of children with chronic rhinosinusitis and with vs without asthma-specific composition (right panel) and with vs without reduced Shannon index (left panel). The visualizations of subgroups analyses are shown. Data are presented as individual values (points), lower-upper quartiles (boxes) and min-max values (whiskers).

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