

Examination of host genetic effects on nasal microbiome composition



Haritz Irizar, PhD,^a Yoojin Chun, MS,^a Zoe Arditi, BA,^a Anh Do, PhD,^a Galina Grishina, MS,^b Alexander Grishin, PhD,^b Alfin Vicencio, MD,^b and Supinda Bunyavanich, MD, MPH^{a,b} *New York, NY*

Background: Genetic predisposition increases risk for asthma, and distinct nasal microbial compositions are associated with asthma. Host genetics might shape nasal microbiome composition.

Objective: We examined associations between host genetics and nasal microbiome composition.

Methods: Nasal samples were collected from 584 participants from the Mount Sinai Health System, New York. Seventy-seven follow-up samples were collected from a subset of 40 participants. 16S rRNA sequencing and RNA sequencing were performed on nasal samples. Beta diversity was calculated, variant calling on RNA sequencing data was performed, and genetic relatedness between individuals was determined. Using linear regression models, we tested for associations between genetic relatedness and nasal microbiome composition.

Results: The median age of the cohort was 14.6 (interquartile range 11.2-19.5) years, with participants representing diverse ancestries and 52.7% of the cohort being female. For participants who provided follow-up samples, the median time between samples was 5.1 (interquartile range 1.4-7.2) months. Nasal microbiome composition similarity as reflected by beta diversity was significantly higher within subjects over time versus between subjects (coefficient = 0.091, $P = 2.84 \times 10^{-7}$). There was no significant association between genetic relatedness and beta diversity (coefficient = -0.05, $P = .29$). Additional analyses exploring the relationship between beta diversity and genetic variance yielded similar results.

Conclusion: Host genetics has little influence on nasal microbiome composition. (*J Allergy Clin Immunol* 2022;150:1232-6.)

Key words: Nasal microbiome, host genetics, beta diversity, genetic relatedness, heritability

INTRODUCTION

A plethora of microbial communities resides across human tissues and anatomic sites, with many exerting an influence on human physiology.¹⁻³ As the importance of the microbiome on human health has been increasingly appreciated, there has been growing interest in understanding what factors, both environmental and from the host, influence the composition of microbial communities. A clearer picture of the degree to which the environment and host genetics influence the microbiome could advance our understanding of how these communities are shaped and how amenable they may be to therapeutic intervention.

Several studies have explored the effect of host genetics on the microbiome, mainly in the gut, with conflicting results. Some studies comparing gut microbial communities in monozygotic twins, dizygotic twins, and unrelated individuals found significant associations between genetic relatedness and gut microbial composition.^{4,5} Other studies, including both twin and population studies, have not observed such associations.^{6,7} Among studies that investigated host genetic effects on the abundance of specific gut taxa, some identified significant effects,⁸⁻¹⁰ while a population cohort study⁷ found none.

The nasal microbiome is altered in respiratory diseases such as asthma,^{11,12} allergic rhinitis, and atopy.^{13,14} These conditions are also shaped by genetics.¹⁵ Previous studies of host genetic effects on nasal microbiome composition have yielded conflicting results, with a twin study reporting no significant relationships¹⁶ and 2 population studies^{17,18} reporting otherwise.

Here we present a population-based study aimed at further exploring the heritability of the nasal microbiome.

RESULTS AND DISCUSSION

Fig 1 provides an overview of the study design and methods.

Study population and design

A total of 584 subjects, including 370 subjects with asthma and 214 healthy controls, were recruited from the Mount Sinai Health System, New York, for the Airway in Asthma (ARIA) study.^{14,19} The study was approved by the Mount Sinai institutional review board, and participants or parents of minors provided written informed consent. Subjects with asthma had persistent asthma according to the Expert Panel Report 3 guidelines of the National Heart, Lung, and Blood Institute,²⁰ and control subjects had no personal or family history of asthma (see the Methods in this article's Online Repository at www.jacionline.org). The median age of the cohort was 14.6 (interquartile range 11.2-19.5) years, 52.5% of subjects were female, and self-identified race/ethnicity included 10.1% Asian, 17.3% Black, 28.0% Latino, 5.1% mixed,

From ^athe Department of Genetics and Genomic Sciences and ^bthe Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York.

The study was supported by the National Institutes of Health (R01 AI118833). This work was supported in part through the computational resources and staff expertise provided by Scientific Computing at the Icahn School of Medicine at Mount Sinai.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication February 14, 2022; revised May 24, 2022; accepted for publication June 1, 2022.

Available online June 16, 2022.

Corresponding author: Supinda Bunyavanich, MD, MPH, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, New York, NY 10029. E-mail: supinda@post.harvard.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2022 American Academy of Allergy, Asthma & Immunology
<https://doi.org/10.1016/j.jaci.2022.06.004>

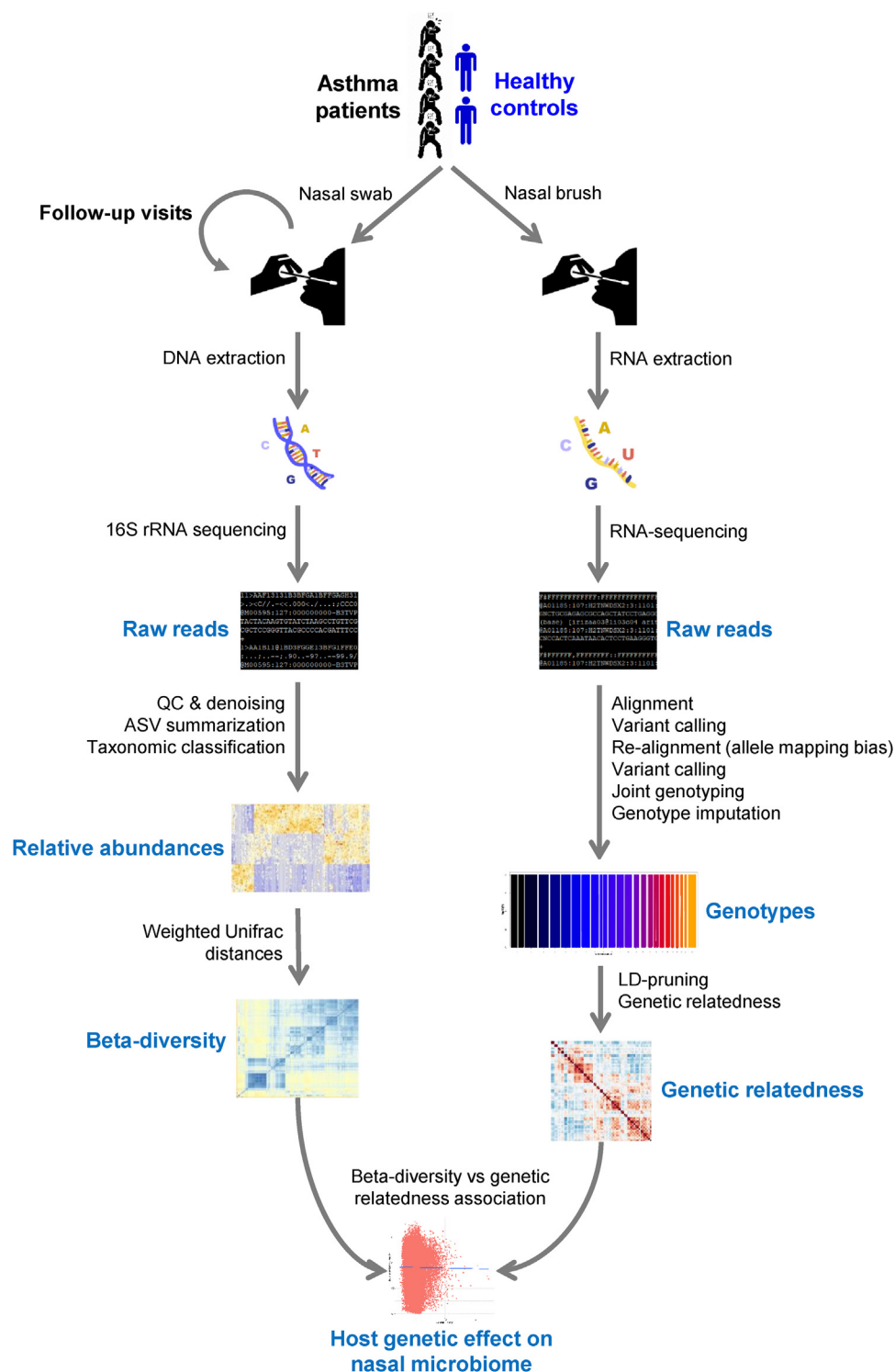


FIG 1. Schematic of the study workflow.

and 38.1% White. For participants who provided follow-up samples, the median time between samples was 5.1 (interquartile range 1.4-7.2) months (Table I).

Nasal swab samples were collected from all 584 subjects for DNA isolation and 16S rRNA sequencing. Forty subjects returned for 1 or more follow-up visits, during which 77 additional nasal

swab samples in total were collected. For 365 of the 584 subjects, a nasal brushing was collected from the contralateral nare for RNA isolation and sequencing, yielding a subsample for genetic analyses (Table I). Nucleic acid isolations, sequencing, sequence data processing, and quality control were performed using previously described protocols.¹⁴

TABLE I. Characteristics of the ARIA cohort and of the subsample examined for genetic relatedness

Characteristic	ARIA cohort	Genetic subsample
	(N = 584)	(n = 365)
Age at baseline (years), median [IQR]	14.61 [11.22-19.52]	14.42 [11.21-17.83]
Female sex	307 (52.6)	194 (53.2)
Race/ethnicity, self-reported		
Asian	59 (10.1)	29 (7.95)
Black	101 (17.29)	56 (15.34)
Latino	163 (27.91)	100 (27.4)
Multiple races	30 (5.14)	26 (7.12)
White	223 (38.18)	149 (40.82)
Not reported	8 (1.37)	5 (1.37)
No. of follow-up visits		
0	544 (93.15)	352 (96.44)
1	24 (4.11)	10 (2.74)
2	9 (1.54)	2 (0.55)
3	3 (0.51)	1 (0.27)
4	1 (0.17)	0
5	2 (0.34)	0
12	1 (0.17)	0
Time between visits (months), median [IQR]	5.06 [1.38-7.16]	8.05 [5.16-23.75]
Asthma	370 (63.36)	200 (54.79)
Allergic rhinitis	186 (48.44)	112 (30.68)

Data are presented as number (%) unless otherwise indicated. *IQR*, Interquartile range.

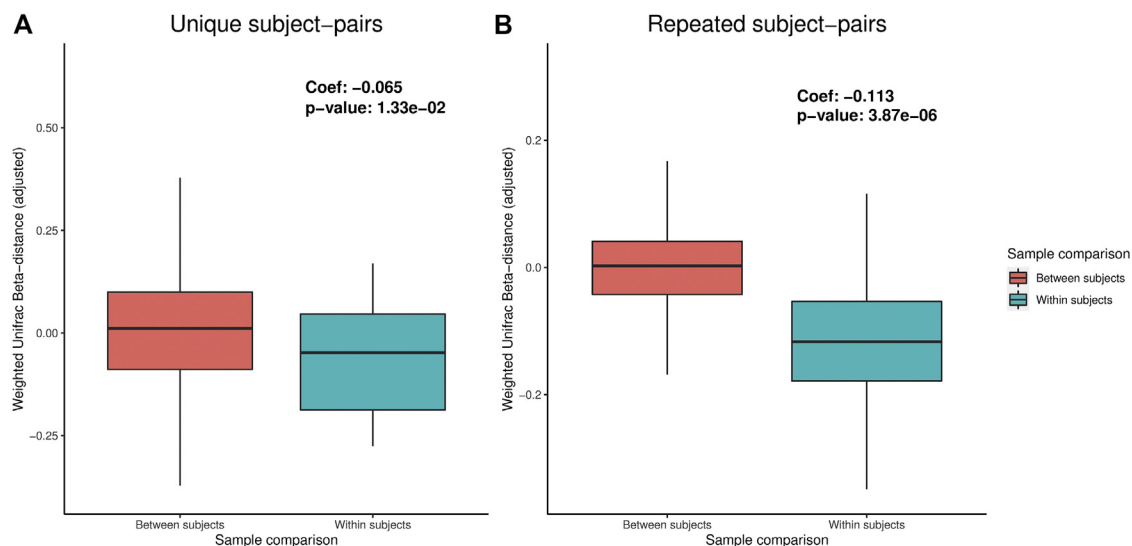


FIG 2. Nasal microbiome similarity by type of sample comparison (between subjects or within subjects) for (A) unique subject pairs and (B) repeated subject pairs. The coefficients and *P* values correspond to the between-subject vs within-subject effect on beta diversity obtained in the regression adjusted for the following covariates: asthma status, active allergic rhinitis, sequencing batch, age, and, for the repeated subject-pair data, subject pair. (A) Beta diversity values from 24 within-subject comparisons were compared to beta diversity values from 149,819 between-subject comparisons. (B) Beta diversity values from 163 within-subject comparisons were compared to beta diversity values from 68,124 between-subject comparisons.

Nasal microbiome composition is more similar within subject over time than between subjects

Leveraging the participants who contributed 2 or more nasal swabs over time, we first aimed to test whether nasal microbiome composition as captured by beta diversity (measured as UniFrac-weighted beta diversity values²¹) of samples from the same individual over time is more similar than from samples collected from different individuals. Two regression models adjusted for

covariates were fit: one with data from subject pairs with unique pairings in the data set, and the other with data from subject pairs with more than 1 pairing in the data set (for details, see the Methods, Fig E1, and Table E1 in the Online Repository at www.jacionline.org). In both models, beta diversity was the dependent variable, and “subject match” was the predictor of interest.

The results of both the standard linear regression on unique subject pairs (coefficient = -0.065, *P* = .013) and of the mixed

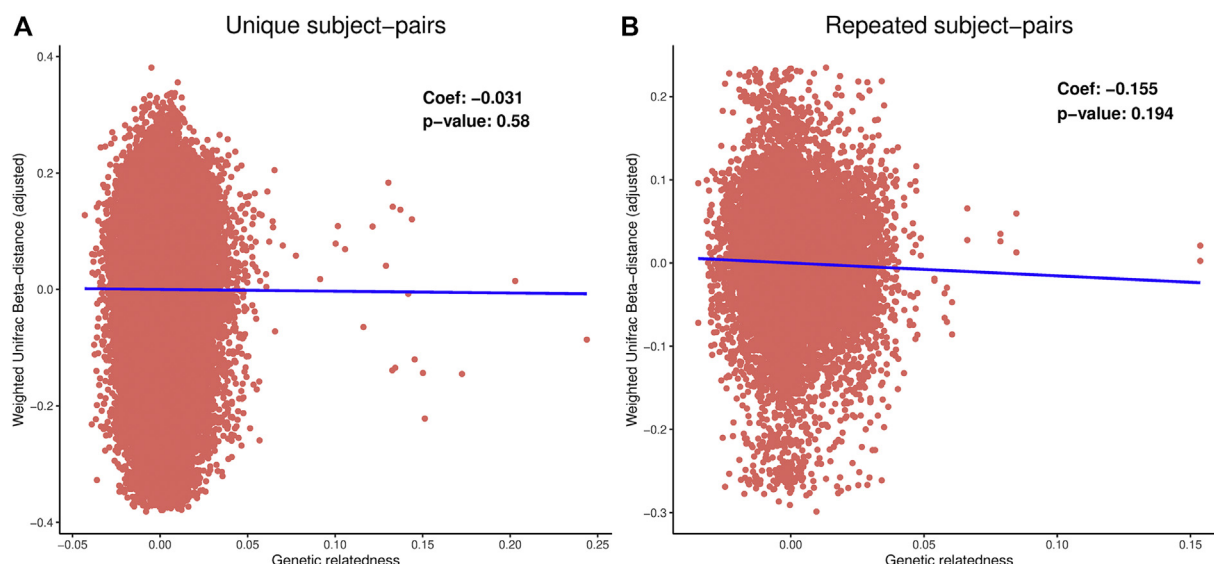


FIG 3. Relationship between genetic relatedness between individuals and beta diversity between nasal microbiome samples in (A) unique subject pairs and (B) repeated subject pairs. The coefficients and *P* values are for the association between genetic relatedness and beta diversity, adjusted for the following covariates: asthma status, active allergic rhinitis, sequencing batch, age, and, for the repeated subject pair data, subject pair. (A) Beta diversity values from 62,120 sample comparisons and genetic relatedness values from 62,120 subject comparisons were used. (B) Beta diversity values from 12,523 sample comparisons and genetic relatedness values from 5,029 subject comparisons were used.

effects regression on repeated subject pairs (coefficient = -0.113 , $P = 3.87 \times 10^{-6}$) showed significantly lower average beta diversity among nasal samples collected from the same individual (within subjects) over time than between samples collected from different individuals (between subjects) (Fig 2). A meta-analysis of these results revealed a significant decrease of nearly 0.1 in beta diversity (coefficient = -0.091 , $P = 4.02 \times 10^{-7}$), supporting lower dissimilarity (ie, greater similarity) in nasal microbiome composition within subjects than between subjects.

No association between genetic relatedness and nasal microbiome composition

To evaluate the extent to which the higher within-subject microbiome compositional similarity observed is driven by host genetics, we calculated ancestry-adjusted genetic relatedness between subjects and assessed the association between genetic relatedness and beta diversity. Variant calling was performed on RNA sequencing data from the genetic subsample (Table I) to obtain, after quality control, genotypes for 493,722 loci with good genomic coverage (see Fig E2 in the Online Repository at www.jacionline.org). After imputation and quality control, genotypes for 2.6 million loci remained for analysis. The distribution of the samples along principal components 1 and 3 generated from the imputed genotypes corresponded with self-reported race/ethnicity (see Fig E3 in the Online Repository). The genotypes were then used to create a genetic ancestry-adjusted genetic relatedness matrix between all pairs of subjects.

Two regression models adjusted for covariates were then fit: one using data from unique subject pairs, and the other using data from repeated subject pairs (see Methods, Fig E1, and Table E1 in the Online Repository), with beta diversity as the dependent variable and genetic relatedness as the predictor of interest. Neither the standard regression performed on unique subject-pair data

(coefficient = -0.031 , $P = .58$) nor the mixed effects regression of repeated subject-pair data (coefficient = -0.155 , $P = .19$) revealed a significant association between genetic relatedness and nasal microbiome composition (Fig 3). A meta-analysis of the individual regression results confirmed the lack of significant association (coefficient = -0.053 , $P = .29$).

Additional analyses were then performed to test the main results. First, the top 3 principal coordinates from multidimensional scaling on the matrix of beta diversities were used as outcomes in linear mixed models with the top 10 genetic principal components as predictors (Table E1). There were no significant associations between any of the microbial principal coordinates and genetic principal components. Second, we performed PERMANOVA analyses²² to test for associations between any of the 10 genetic principal components and variance in the beta diversity matrix (Table E1). These analyses also revealed no significant associations. Finally, we performed a multiple regression on distance matrices²³ to evaluate the association between beta diversity and genetic relatedness adjusted for covariates (Table E1). This also revealed no significant association between beta diversity and genetic relatedness (coefficient = -0.032 , $P = .48$).

The findings from this study suggest that the composition of the nasal microbiome is largely driven by environmental factors rather than the host's genetics. These findings are consistent with a previous twin study of 46 monozygotic and 43 dizygotic twins.¹⁶ After unsupervised clustering of nasal microbiome profiles, similar levels of concordance were observed in monozygotic and dizygotic twins, as well as similar within-twin pairwise beta diversity, suggesting that the composition of the nasal microbiome is environmentally driven.¹⁶ However, our results also contrast with 2 previous population-based studies that used a similar analytical approach on smaller samples ($n = 93$ ¹⁷ and $n = 144$ ¹⁸ vs $n = 365$ in our genetic analysis). In both studies, a negative correlation was found between nasal beta diversity

and genetic relatedness.^{17,18} One of these studies was performed in a genetically isolated sample of 144 Hutterite adults, and correlation between genetic relatedness and nasal beta diversity was based on unadjusted analyses.¹⁸ The other study of 93 participants used covariate-adjusted analyses to assess the correlation between genetic relatedness and beta diversity in 15 body sites and observed a significant correlation for the anterior nares.¹⁷ However, the reported correlation, $R = 0.086$,¹⁷ implies a shared variance of only 0.74%. Thus, while they observed a statistically significant association between genetic relatedness and nasal microbiome beta diversity, the effect size was small. The enhancements of our study include a substantially larger sample from a diverse population, a genetic relatedness metric adjusted for genetic ancestry effects, regression-based analyses adjusted for potential confounders, and complementary analyses using alternative models that support our main findings.

Nonetheless, our study has several limitations, including the lack of environmental measures to directly demonstrate environmental influence rather than the absence of genetic effects. Measuring the comprehensive effects of environmental influences (eg, the exposome) carries its own challenges.²⁴ However, a future study in which the degree of environmental similarity between individuals is captured will be necessary if the contribution of environment versus genetics on microbiome composition, nasal or otherwise, is to be definitively determined, especially in ethnically diverse samples such as ours, where relevant environmental factors can be correlated with ancestry. Although we did not detect an effect of overall host genetics on nasal microbiome composition, it is possible that specific host genetic variants are associated with the abundance of particular microbial taxa. It is also possible that, as has been observed with other traits,²⁵ heritability estimates change across the life-span, and results could differ in a younger or older sample. Continued exploration of this area has the potential to generate valuable insight into host–nasal microbiome relationships and mechanisms that contribute to shaping the airway microbiome.

Key messages

- Most of the research exploring the effects of host genetics on microbiome composition has been done in the gut, with the few studies performed in the nasal airway showing conflicting results.
- This study of host genetics effects on airway microbiome composition showed that nasal microbiome composition is more similar within subjects over time than between subjects.
- We found no significant associations between genetic relatedness and nasal microbiome composition, supporting that the composition of the nasal microbiome is largely driven by environmental factors.

REFERENCES

- Hanski I, Herten L von, Fyhrquist N, Koskinen K, Torppa K, Laatikainen T, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci U S A* 2012;109:8334-9.
- Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med* 2017;9:eaah4680.
- Ho HE, Chun Y, Jeong S, Jumreornvong O, Sicherer SH, Bunyavanich S. Multidimensional study of the oral microbiome, metabolite, and immunologic environment in peanut allergy. *J Allergy Clin Immunol* 2021;148:627-32.e3.
- Zoetendal EG, Ak AD. The host genotype affects the bacterial community in the human gastrointestinal tract. *Microb Ecol Health Dis* 2001;13:129-34.
- Stewart JA, Chadwick VS, Murray A. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J Med Microbiol* 2005;54:1239-42.
- Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature* 2012;486:222-7.
- Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 2018;555:210-5.
- Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, et al. Genetic determinants of the gut microbiome in UK twins. *Cell Host Microbe* 2016;19:731-43.
- Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat Genet* 2016;48:1413-7.
- Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat Genet* 2021;53:156-65.
- Depner M, Ege MJ, Cox MJ, Dwyer S, Walker AW, Birzele LT, et al. Bacterial microbiota of the upper respiratory tract and childhood asthma. *J Allergy Clin Immunol* 2017;139:826.
- Fazlollahi M, Lee TD, Andrade J, Oguntuyo K, Chun Y, Grishina G, et al. The nasal microbiome in asthma. *J Allergy Clin Immunol* 2018;142:834-43.e2.
- Ta LDH, Yap GC, Tay CJX, Lim ASM, Huang CH, Chu CW, et al. Establishment of the nasal microbiota in the first 18 months of life: correlation with early-onset rhinitis and wheezing. *J Allergy Clin Immunol* 2018;142:86-95.
- Chun Y, Do A, Grishina G, Arditi Z, Ribeiro V, Grishin A, et al. The nasal microbiome, nasal transcriptome, and pet sensitization. *J Allergy Clin Immunol* 2021;148:244-9.e4.
- Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008;8:169-82.
- Liu CM, Price LB, Hungate BA, Abraham AG, Larsen LA, Christensen K, et al. *Staphylococcus aureus* and the ecology of the nasal microbiome. *Sci Adv* 2015;1:e1400216.
- Blekhan R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, et al. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol* 2015;16:191.
- Igartua C, Davenport ER, Gilad Y, Nicolae DL, Pinto J, Ober C. Host genetic variation in mucosal immunity pathways influences the upper airway microbiome. *Microbiome* 2017;5:16.
- Do AN, Chun Y, Grishina G, Grishin A, Rogers AJ, Raby BA, et al. Network study of nasal transcriptome profiles reveals master regulator genes of asthma. *J Allergy Clin Immunol* 2021;147:879-93.
- National Institutes of Health; National Heart, Lung, and Blood Institute. Guidelines for the diagnosis and management of asthma 2007 (EPR-3). Published September 2012. Available at: <https://www.nhlbi.nih.gov/health-topics/guidelines-for-diagnosis-management-of-asthma>. Accessed February 2, 2022.
- Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol* 2007;73:1576-85.
- Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 2001;26:32-46.
- Lichstein JW. Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecol* 2007;188:117-31.
- Siroux V, Agier L, Slama R. The exposome concept: a challenge and a potential driver for environmental health research. *Eur Respir Rev* 2016;25:124-9.
- BIOS Consortium, van Dongen J, Nivard MG, Willemsen G, Hottenga JJ, Helmer Q, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat Commun* 2016;7:11115.