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# Breath volatilome as a non-invasive reflection of gut microbiota-driven health and disease

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**By using integrated multi-omics and developing breath collection platform in gnotobiotic mice, Hernandez-Leyva et al.[1] provide mechanistic evidence that exhaled breath volatilome can be used as a non-invasive procedure to predict the abundance of the asthma-associated species *Eubacterium siraeum* in children.**

The earliest conceptual link between breath analysis and disease dates back to antiquity, when Hippocrates described disease-associated breath odors.[2] The first comprehensive analysis of volatile organic compounds (VOCs) in human breath using modern analytical tools is generally attributed to the study of Pauling and colleagues in 1971, who applied gas chromatography with a flame ionization detector (GC-FID) and established the chemical complexity of exhaled breath by demonstrating that human breath contains more than 250 identified volatile compounds.[3] This paper is widely considered the foundational work that established modern "breathomics." A major step toward using breath as a non-invasive clinical diagnostic tool in the context of diseases of the respiratory system arrived in the 1990s. One study validated breath nitric oxide (NO) as a non-invasive biomarker of airway inflammation in asthma,[4] and another one demonstrated that breath VOC profiles could discriminate between patients with lung cancer and healthy controls.[5] Evidence from animal and human studies indicates a bidirectional gut-airway axis: the gut microbiome modulates respiratory immunity, while respiratory infections and chronic airway inflammation can alter its composition and metabolic activity, with potential long-term effects on respiratory health.[6] Correlation analyses recently enabled to find significant relation between volatile compounds in breath and the presence of specific gut bacteria in humans.[7,8] The proof of concept that changes of gut microbiota associated with asthma are driving changes in breath VOC profile was needed to propose breath volatilome as a diagnostic tool in this context.

Hernandez-Leyva et al.[1] have filled this gap by providing experimental evidence for a causal biological role of gut microbial dysbiosis in asthma-related volatilome changes occurring in children, following a sequence of procedures summarized in Figure 1. As a first step, they integrated advanced multivariate statistics and interpretable machine learning to dissect the relationship between gut microbiota composition (assessed upon whole metagenomic sequencing of fecal communities) and the breath volatilome (assessed by gas chromatography-mass spectrometry [GC-MS]) in both healthy children and conventionalized mice. Multivariate analyses such as Procrustes analyses (protest function), PERMANOVA (adonis2 function), and interpretable machine learning models with Shapley additive explanations (SHAP values) establish robust, quantitative associations between gut microbiota composition and breath VOC profiles, identifying and quantifying the direction and magnitude of individual taxa contributions to specific VOC predictions (i.e., *Roseburia*-isoprene, *Bacteroides ovatus*-acetate relationships in human; *Bifidobacterium pseudolongum*-acetic acid, *Akkermansia muciniphila*-benzene and p−cymene relationships in mice). Of the five breath VOCs best explained by gut microbiome composition, several compounds, such as 4-methyl-2-pentanone, benzene, and levomenthol, shared a relatively high variance in both human and mouse breath, explained by the gut microbiome characteristics. A second step was devoted to demonstrate that breath VOCs were shaped by diet, host sex, and human microbial origin in germ-free mice colonized with a fecal specimen from a healthy human donor and fed a control versus high-fat diet. This experiment showed that the fat content of the diet significantly alters breath VOC profiles in gnotobiotic mice, despite minor effect on the fecal microbiota composition. Crucially, the study moved beyond correlation and prediction by incorporating gnotobiotic monocolonization experiments and anaerobic culture headspace analyses, experimentally testing whether specific microbes are sufficient to alter breath VOC signatures and directly produce the detected compounds. Monocolonization studies demonstrated that several VOCs found in the culture headspace of five common gut anaerobic commensals (*Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, *Collinsella aerofaciens*, *Escherichia coli, Ruminococcus torques*) were enriched in the exhaled breath of mice colonized with the same microbes. The last objective was to evaluate whether the breath volatilome might be used as a biomarker for disease-associated gut microbial dysbiosis. By comparing data obtained in healthy children versus children presenting asthma, they showed that the breath VOCs that best distinguish healthy individuals from those with asthma also predict the relative abundance of *Eubacterium*

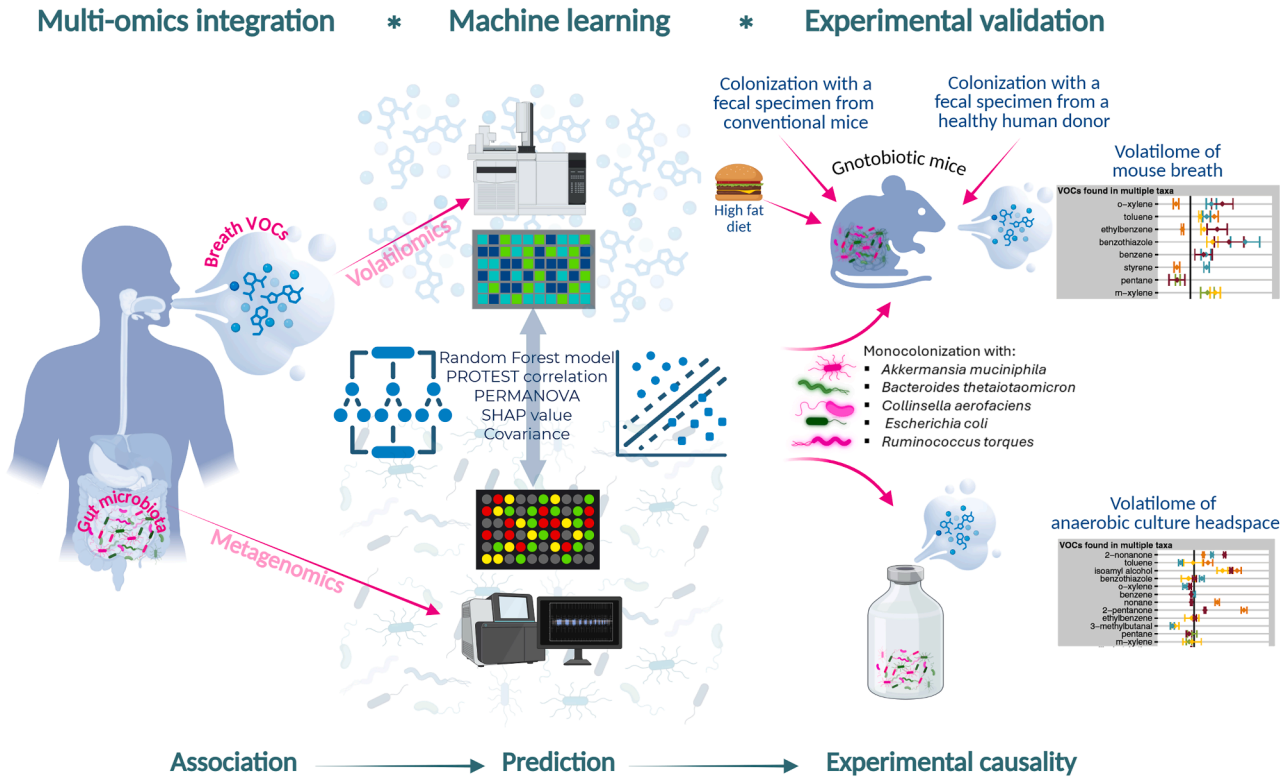


**Figure 1. Integrated multi-omics framework linking gut microbiota to the breath volatilome**

This schematic illustrates a system-level approach combining metagenomics, breath volatile organic compounds (VOCs) metabolomics, and machine learning to identify associations between gut microbial composition and the host breath volatilome in pediatric healthy population and conventionalized mice. Procrustes analysis with PROTEST correlation reveals global co-variation between microbiome structure and VOC profiles, while Random Forest models with Shapley additive explanations (SHAP) interpretation identify microbial taxa contributing to volatilomic signatures. Experimental validation is performed in germ-free mice colonized with conventional mice microbiota, human microbiota, or individual bacterial species, with volatilome measurements from mouse breath and anaerobic bacterial cultures enabling assessment of microbial contributions to host breath VOC profiles. Created in BioRender. Neyrinck, A. (2026) https://BioRender.com/gl7c5kr.

*siraeum*, a known marker of dysbiosis in asthma.

Altogether, Hernandez-Leyva et al.[1] provide direct mechanistic evidence that gut microbiota composition and metabolism shape the host exhaled breath volatilome. They demonstrate that volatilomic signatures can predict the abundance of disease-associated bacterial species, supporting microbiome-based breath diagnostics. For sure, the proof of principle should be confirmed in larger cohorts, including adults with asthma, as the gut microbiome and nutritional characteristics of humans change with age and living conditions. In addition, breath VOC profile is largely influenced by the exposure to xenobiotics (environmental pollutants), smoking status, dietary habits, and the existence of co-morbidities, all of which must be taken into account for the appropriate interpretation of breathomics data. Hernandez-Leyva et al.[1] emphasize the diet as a critical factor in microbiome-based breath diagnostics. The interest of breath volatilome to better understand the influence of diet on microbiome and health is supported by previous data obtained in healthy human volunteers, demonstrating notably that the fermentative capacity of the gut microbiome toward dietary fiber can be revealed upon the kinetics of release of specific VOCs in breath.[8–10]

In conclusion, the data provided by Hernandez-Leyva et al.[1] support the interest of the evaluation of breath volatilome as a non-invasive procedure to unravel the contribution of the gut microbiota in health and disease in humans, but also as a procedure helping to understand the potential interaction of bioactive volatile compounds with host physiology in preclinical studies in mice.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

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