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Short-chain fatty acids and volatile organic compound pattern examination as a diagnostic marker for evaluating Colorectal Cancer and Inflammatory Bowel Diseases

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Abstract

CRC and IBD have been linked to the volatolome, which is a mixture of volatile organic compounds (VOCs). The volatolome seems to have promise as a non-invasive biomarker for the identification of CRC and IBD. Multiple researches have been conducted on the volatolome's potential, utilising either chemical analysis or pattern-recognition approaches to determine its potential. The existing literature on the potential of the volatolome as a cancer and inflammatory bowel disease (IBD) biomarker was examined. The survey examines 23 journal papers that were obtained electronically through major scientific databases such as PubMed, Google Scholar, Scopus, IEEE, and Science Direct, which were searched using sets of keywords. The papers were obtained through major scientific databases such as PubMed, Google Scholar, Scopus, IEEE, and Science Direct, which were searched using sets of keywords. Publication of the articles was made possible by the use of major scientific databases such as Pubmed and Google Scholar, as well as the IEEE and Science Direct. VOC analyses appear promising for future screening of CRC and IBD, with potentially improved test performances allowing for earlier detection of IBD and CRC and consequently earlier initiation of treatment, potentially reducing morbidity and mortality rates as well as lower rates of (unnecessary) colonoscopies, according to the studies included.

Keywords -Volatile organic compound, Short-chain fatty acids, diagnostic marker, Colorectal Cancer

1.0. Introduction

Celiac disease (CD) and ulcerative colitis (UC) are the most prevalent chronic inflammatory bowel diseases (CIBDs) that affect the colon, with Crohn's disease being the most frequent (IBD). However, despite the fact that the majority of individuals with ulcerative colitis and Crohn's disease experience symptoms mostly in the gastrointestinal tract, both ailments are considered systemic illnesses that commonly affect other organs [1].

Affections that manifest themselves outside of the gastrointestinal system are referred to as extra intestinal symptoms, and they are not always associated with the underlying bowel ailment. In addition to the digestive system, extra intestinal disease may impact practically every organ system in the human body. The skin, eyes, joints, biliary tract, and lungs are among the organs that are most often impacted by this condition [2]. Some symptoms, such as oral lesions, gallstones, pancreatitis, nephrolithiasis, and amyloidosis, are more often

associated with CD than with UC, while others, such as nephrolithiasis and amyloidosis, are more frequently associated with the latter. In addition to these symptoms, people with CD and UC also have cutaneous and ocular signs in similar proportions, according to the CDC.

Extensive extraintestinal organ involvement in inflammatory bowel disease (IBD) can be caused by a variety of factors, and it can be difficult to distinguish between true extra intestinal manifestations (EIMs), which are caused by the disease itself, and secondary extra intestinal symptoms, which can be caused by malnutrition, chronic inflammation, or treatment side effects. However, some of these EIMs may not be associated with disease activity (for example, primary sclerosing cholangitis and ankylosing spondylitis), but in general, EIMs are associated with the clinical course of IBD and may have a significant impact on patient life, morbidity, and even death in these patients, among other factors.

On average, one in every three Americans is thought to be at risk for developing colorectal cancer at some point in their lives. Across Europe, it is the second leading cause of cancer-related death; in the United States, however, it is the third major cause of cancer-related mortality. Colonoscopy is the gold standard for diagnosing colorectal cancer, but it is too costly for widespread screening [3].

Furthermore, colonoscopy is despised by many individuals since it is an invasive treatment. Faecal immunochemical blood (FIT) tests are the most frequently used non-invasive screening tools, with high specificity but a wide range of sensitivity (61-91 percent). Adherence to screening programmes is only rarely achieved in the target group, with only 50 to 70 percent of the target group adhering to the screening programme. The use of volatile organic compounds (VOCs) in breath to identify a patient's disease status may be an effective and non-invasive method of detecting people with CRC [4].

In the context of clinical and nutritional state, intermediate products of metabolism may give helpful diagnostic assistance for monitoring metabolic illness, such as chronic inflammatory disease and gastrointestinal disease, utilising non-invasive approaches like blood testing. True, it is possible to design specific metabolic profiles for volatile organic compounds (VOCs) with low molecular weight that are divided into the gaseous phase by the alveolar circulation and exhibit themselves as vapours in exhaled breath. Breath analysis has evolved into a non-invasive and sensitive medical diagnostic technique that may be used to conduct quick evaluations due to the development of more powerful analytical technologies that enable the detection of volatile metabolites present in the breath in small amounts [5].

A diversified variety of diagnostic procedures and techniques have been developed and put into use for a wide range of infectious and non-infectious ailments throughout the years. Improvements in diagnostic tools are becoming increasingly recognised as essential components of a personalised approach that takes into account the identification of individuals at risk for developing diseases, the interpretation of diagnostic tests, the provision of prognostic information, and the prediction and monitoring of the efficacy of therapeutic interventions. A new frontier of diagnostic approaches based on the detection of disease-associated volatile organic compounds will be described in this article (VOCs) [6].

Various forms of cancer have been researched for their presence of volatile organic compounds (VOCs), which represent changes in the pathophysiology and metabolic processes of the disease. Cancer-associated volatile organic compounds (VOC) are released from the afflicted tissue into the faeces or blood circulation, where they are exhaled in the

breath or expelled in the urine, respectively. Several studies have revealed that volatile organic compounds (VOCs) released from various substrates, including faeces, urine, exhaled air, and blood, may serve as biomarkers for colorectal cancer. As a relatively fresh and non-invasive screening method for CRC, VOC analysis is projected to become an increasingly popular population-based screening tool in the near future.

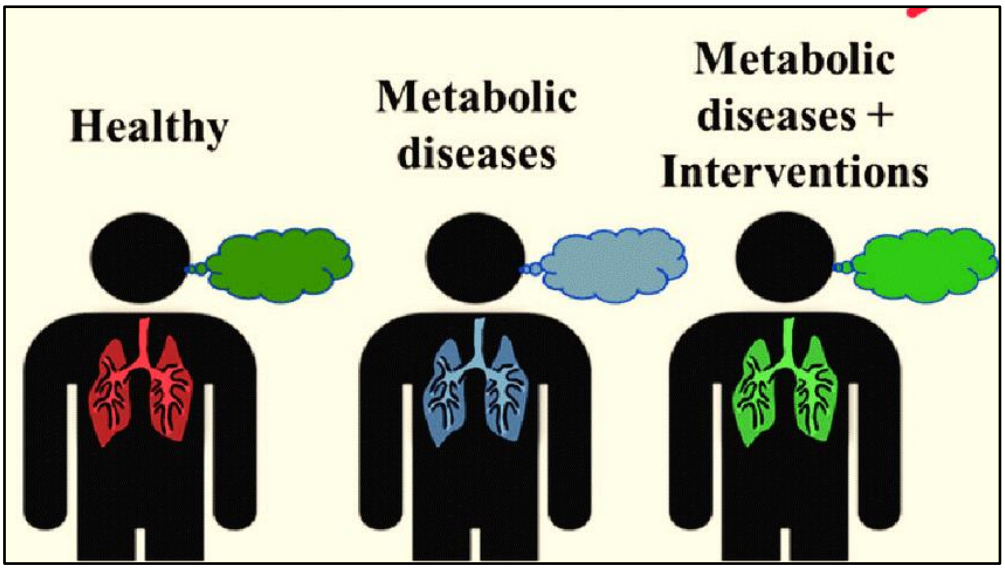

Figure 1 - A schematic demonstrates the possible breath VOC pattern changes from a healthy 78 individual to a patient with metabolic diseases, and to the patient who went through the dietary interventions of the metabolic diseases. Different colours of the breath indicate different breath VOC patterns.

In view of these compelling rationales, a series of clinical studies have assessed VOC analysis for screening CRC. Unfortunately, there was no diagnostic meta-analysis to integrate these results and derive conclusions. Recognizing that individual study might be unable to obtain sufficient data to affect practice on their own, we sought to objectively assess the potential role of VOC analysis as a new screening tool for CRC. We; therefore, did a systematic review and meta- analysis of observational studies to compare CRC patients with healthy controls (HC) on the VOC analysis [7].

2.0 Biomarkers or diagnostic markers for colorectal cancer

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator ofnormal biological processes, pathogenic processes, or pharmacologic response to a therapeutic intervention.” The World Health Organisation (WHO) has sought to define biomarkers further as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”. Using this board definition of a biomarker, clinical signs such as height and weight could be considered as biomarkers, however, the term is now typically shorthand for “molecular biomarker.” Molecular biomarkers can themselves take many forms, and as a consequence there are many strategies available for their discovery and validation [8]. When considering malignancy any measurable specific molecular alteration of a cancer cell either at the DNA, RNA, protein, or

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metabolite level can be referred to as a cancer biomarker. An ideal biomarker for cancer would have applications in determiningpredisposition, early detection/screening, assessment of prognosis, and predicting drug response or amenability to therapy. No one single biomarker could meet all these needs and socombinations of specific applications of biomarkers are often performed.

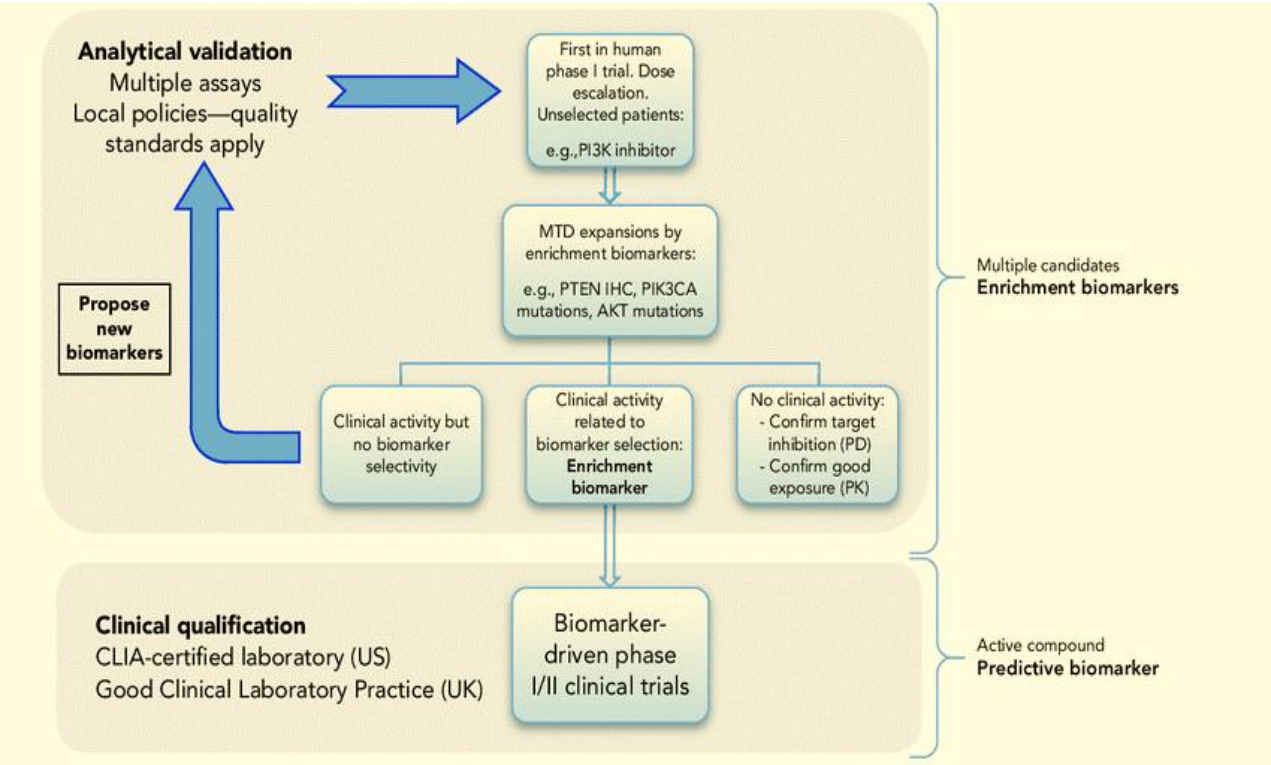

Figure 2 – Screening biomarker roadmap

There has been a great deal of work recently to find and define the role for non-invasive biomarkers, particularly for cancer. In order to prevent disorganized and uncoordinated biomarker research, Cancer Research UK's Biomarker Discovery and Development Committee has suggested 'roadmaps', which define a succinct research pathway. These are broadly divided into four chronological sections. 1) Rationale: does the envisioned biomarker address an unmet clinical need 2) Biomarker assay development: the assay must be accurate and reproducible.

The assay should be simple and cost effective, and performed to good clinical laboratory practice standards (as laid out by the British Association of Research Quality Assurance). 3) Biomarker discovery: the distribution of the biomarker in an appropriate sample population should be defined, and a retrospective analysis of the relationship between the biomarker and clinical outcome should be performed. 4) Biomarker clinical qualification: the relationship between the biomarker and clinical outcome should initially be assessed in a large retrospective analysis. Then, a large, prospective randomized study should be performed to assess the impact of thebiomarker on clinical outcome [9].

3.0. Short-chain fatty acids as a biomarker or diagnostic marker and their connection with diseases

This is well that people cannot digest the bulk of dietary fibres due to the absence of associated enzymes, and the non-digestible carbohydrates are fermented in the large intestine by the colonic/gut microbiota. SCFAs, saturated aliphatic organic acids, become the primary category of metabolites that are produced by the gut microbial population as a consequence of fermentation of dietary fibers. SCFAs consist of one to six carbons, and acetate (C2), propionate (C3), and butyrate (C4) have been described as the most prevalent ones by comprising 90–95 percent of the SCFA present in the colon. Previous research has found that the production of SCFAs is regulated by numerous variables, including the pattern of food consumption, antibiotics treatment, and microbial populations. In particular, carbohydrates are the major source of SCFAs synthesis; however, amino acids, such as valine, leucine, and isoleucine from the protein breakdown are also involved in the formation of branched SCFAs such as isobutyrate, isovalerate, and 2-methyl butyrate. Earlier investigations have elucidated the metabolic pathways of many SCFAs [10].

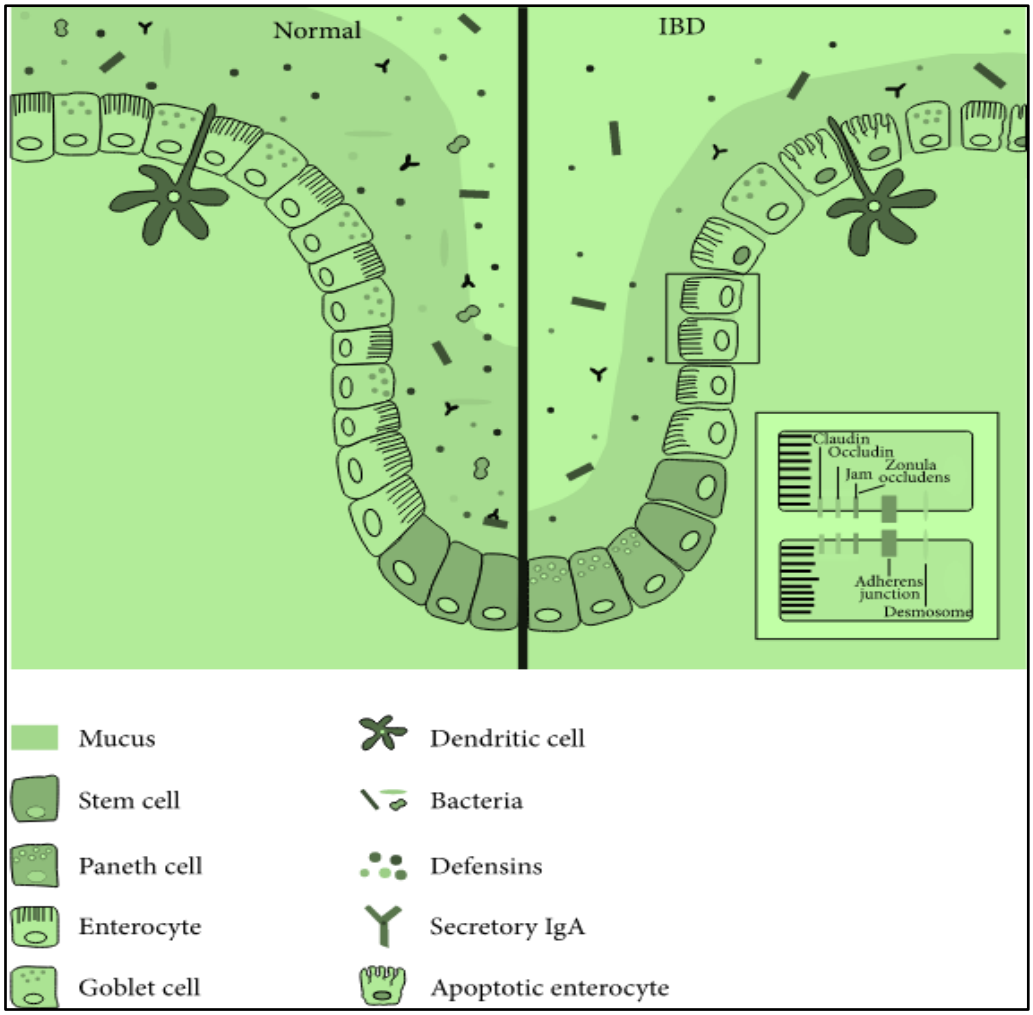

Figure 3 - Intestinal barrier alterations in IBD. Alterations and disruptions to the intestinal barrier in patients with inflammatory bowel disease (IBD) enable translocation of bacteria and bacterial metabolites into the bowel wall

For example, two primary routes for synthesizing acetate are begun from formic acid by the Wood–Ljungdahl pathway or from hydrogen and carbon dioxide by acetogenic bacteria. In

thecase of propionate production, three pathways, engaged in succinate, acrylate, and propanediol synthesis, are considered as main routes. Among them, propionate generation via succinate pathway is dominating, and numerous Firmicutes and Bacteroidetes were discovered to be engaged in succinate pathways, and their richness was also connected to the amount of propionate in fecal samples [11].

Furthermore, epidemiological studies have reported that the gut microbiome might contribute in host-signalling processes through the production of microbial metabolites, as well as employ SCFAs as energy sources to fuel the host's cell metabolism. Twenty SCFAs were also shown to have an effect on host physiology and to have the ability to control the development of metabolic syndrome by integrating into glucose and lipid metabolism, respectively. Additionally, SCFAs serve critical roles in the preservation of gut barrier function by serving as fuel for intestinal epithelial cells, which may result in an increase in mucin manufacturing, which may help to avoid the onset and development of gut disorders. It has been observed that butyric acids are particularly effective at preventing bacterial adherence and improving the integrity of tight junctions [12].

Furthermore, during the inflammatory processes of the host, the immune modulating role of the microbial population that generates SCFAs has been characterized. However, it has been shown that butyrate and propionate may help to promote the differentiation of T-regulatory cells into effector and regulatory T cells by inhibiting the activity of histone deacetylase. The decreased incidence of intestinal inflammation, as a result, may have a favourable effect on increasing host immunological tolerance as well as lowering the chance of developing chronic inflammatory bowel disease and colon cancer [13].

4.0. Volatile organic compounds identify patients with colorectal cancer

The availability of an effective and reliable colorectal cancer screening tool is of paramount importance in the health service plans of Western countries to permit early diagnosis and/or identification of precursor polyps. Improved tests are required that are able consistently to show high sensitivity and specificity for these diagnoses, and that are easy to perform and capable of engendering high patient compliance [14]. Recent advances in molecular biology in colorectal cancer have focused on several compounds, although some have displayed inadequate sensitivity and specificity in the discrimination between normal controls and patients with either established cancer or precursor lesions [15].

The application of metabolomics for such screening purposes is designed to define specific chemical fingerprints of cellular function that act as a snapshot of active cellular physiology, reflecting its metabolic profile, but that are not defined by conventional assessments of RNA expression or proteomic cellular analyses. The exact interplay between this system biology and its functional genomics, integrating proteomic and genetic with end-product metabolic information as an early cancer screening tool, is at present not completely known. In recent years several studies have assessed the capacity of breath analysis to diagnose lung cancer, asbestos exposure, breast cancer, malignant melanoma, aerodigestive squamous cell carcinoma and hepatocellular carcinoma [16].

Patients with colorectal cancer have a different selective VOC pattern compared with healthy controls, based on analysis of 15 of 58 specific compounds in exhaled breath samples. Each disease appears to have a specific VOC profile, suggestive of several different derangements in metabolic pathways]17]. A range of different analytical methods have been used for

breath analysis, including cross-reactive species nano sensor array technology linked to GC-MS, different solidphase absorbents for marker microextraction, ion mobility spectrometric techniques designed to increase the detection threshold of VOCs, colorimetric analysis of chemically sensitive compounds impregnated into disposable cartridges, and various techniques to detect non-polar molecules within the exhaled breath condensate [18].

Further differences may be detected as variations exist between the ratios of exogenous VOCs, which are adsorbed from the environment, to endogenous VOCs generated by cellular biochemical processes in the body; the biochemical pathways of some compounds appearing in exhaled breath are not completely understood at present. Most studies have focused on single VOC agents or collation of a few VOCs as specific biomarkers, although some have adopted a discriminant function analytical model of multiple compound clusters derived from available spectral libraries to predict membership within a particular disease group. Peng and colleagues, who assessed a mixture of tumors including lung, breast, colorectal and prostate lesions, and measured exhaled condensates with nanosensors [19]. In this latter study, there was accurate discrimination between normal and 'cancerous' breath, and between the breath analyses of some cancer types, irrespective of age or sex [20].

Levels of some specific VOCs such as 1, 3-dimethylbenzene, 1, 2-pentadiene, cyclohexane, methylcyclohexane and 4-methyloctane were higher in patients with colorectal cancer than in controls (average concentrations for patients with colorectal were about double). These other VOCs showed variable profiles in different cancers and variable correlation with one another, suggesting that it is the pattern of VOCs rather than a single VOC that is more likely to be representative of the metabolic derangements evident in colorectal cancer. To keep the samples as homogeneous as possible, patients with severe chronic obstructive pulmonary disease, inflammatory bowel disease, decompensated diabetes or a previous history of other malignancies were excluded from this preliminary study [21].

Currently there is no accepted working protocol describing the monitoring procedure, combinations of VOCs to be assessed and the best statistical method for group discrimination. Future work on VOCs will assess the predictive value of different compound patterns in polyp detection, investigate breath profile assessment after colorectal cancer resection as a potential means of monitoring disease recurrence, and compare VOC profiles in colorectal cancer with those in inflammatory bowel diseases and other gastrointestinal tract cancers. [22]

The present findings further support the value of breath testing as a screening tool. The next step will be to increase the number of subjects involved in order to provide a simpler algebraic formula that will make this evaluation easier, to identify a diagnostic marker and to improve the performance of the statistical method when applied to samples in a blinded fashion. The methodology could also be improved by the use of an electronic nose [23].

5.0. Conclusions and future directions

Analyses of plasma/fecal SCFAs and breath VOC profiles have been considered as promising and minimuminvasive/non-invasive tools for evaluating changes in human metabolism in response to a variety of dietary interventions in randomized clinical trials. The biological implications of these small molecular metabolites to the gut microbial metabolism and human metabolic network are a fascinating area of research, which highlighted the need for more comprehensive studies in the future [24]. In this review, we investigated the association

between diets and fecal SCFAs in patients with MetS or at high risk of MetS, and diet-induced changes of the gut microbiome and their production of SCFAs are also discussed. Some interesting clinical evidence that proved the usefulness of these metabolite biomarkers in reflecting the effectiveness of investigated dietary interventions was discovered in these microbe-gut-host health nexus studies [25].

In addition, we highlighted the possibility of breath VOCs analysis to examine the dietary intervention effect on human metabolism. However, several factors, including the impact of food components, time, and dose-response of these dietary interventions to these metabolite biomarkers, and the response stability need to be further investigated between individuals with diverse genetic profiles. Moreover, the use of many emerging MS-based analytical platforms, such as secondary electrospray ionization (SESI),26,89,90 proton transfer reaction (PTR),91 or selected ion flow tube mass spectrometry.92 have become popular recently [26].

These breath analysis techniques have been reported to have various advantages in facilitating rapid data acquisition based on real-time and online analysis of VOCs. Notably, PTR-MS and SESI-MS have gained attention based on the high sensitivity at trace levels of part per billion (ppb) or a detection limit of 0.2 parts per trillion (ppt), respectively [27,28].

This merit enables the determination of potential biomarkers by fast detection and identification of microbial VOCs inthe headspace of in vitro culture in non-invasive and spontaneously responsive manners. Therefore, these recently invented MS-based tools can be well-suited for breathomics analysis, and can be further considered for robust and reproducible analytical measurements in the nutrient intervention trials. Moving forward, we believe plasma/fecal SCFA analysis and human exhaled VOC analysis can be one of the most effective and powerful tools for exploring the impact of dietary intervention on responsive metabolic alteration towards the development of personalized nutrition [29,30].

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