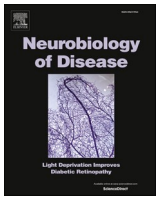
Neurobiology of Disease 164 (2022) 105614

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

Using integrated meta-omics to appreciate the role of the gut microbiota in epilepsy

Lucia Boeri [a], Francesca Donnaloja [a], Marzia Campanile [a], Lorenzo Sardelli [a], Marta Tunesi [a], Federica Fusco [a], Carmen Giordano [a,*], Diego Albani [b]

[a] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milan, Italy
[b] Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, via Mario Negri 2, 20156 Milan, Italy

ARTICLE INFO

Keywords:
Multi-omics
Epilepsy
Gut microbiota
Stool sampling
Extraction methods
Metagenomics
Metatranscriptomics
Metaproteomics
Metabolomics

ABSTRACT

The way the human microbiota may modulate neurological pathologies is a fascinating matter of research. Epilepsy is a common neurological disorder, which has been largely investigated in correlation with microbiota health and function. However, the mechanisms that regulate this apparent connection are scarcely defined, and extensive effort has been conducted to understand the role of microbiota in preventing and reducing epileptic seizures. Intestinal bacteria seem to modulate the seizure frequency mainly by releasing neurotransmitters and inflammatory mediators. In order to elucidate the complex microbial contribution to epilepsy pathophysiology, integrated meta-omics could be pivotal. In fact, the combination of two or more meta-omics approaches allows a multifactorial study of microbial activity within the frame of disease or drug treatments. In this review, we provide information depicting and supporting the use of multi-omics to study the microbiota-epilepsy connection. We described different meta-omics analyses (metagenomics, metatranscriptomics, metaproteomics and metabolomics), focusing on current technical challenges in stool collection procedures, sample extraction methods and data processing. We further discussed the current advantages and limitations of using the integrative approach of multi-omics in epilepsy investigations.

1. Introduction

The term microbiota-gut-brain axis (MGBA) was coined to describe the complex interconnection between the microbial community residing in the intestine and the brain. In the last decades it became evident that many neurological disorders (e.g., depression, anxiety, Parkinson's and Alzheimer's diseases, schizophrenia, autism spectrum disorder, multiple sclerosis, hepatic encephalopathy, migraine and epilepsy) show alterations of the gut microbiota composition, defined as dysbiosis (Liang et al., 2018; Pittman, 2020; Strandwitz, 2018). Epilepsy is one of the most common chronic neurological diseases and it was estimated that 45.9 million people were affected worldwide in 2016 (Beghi et al., 2019). The disease is characterized by the occurrence of epileptic seizures that drastically decrease the quality of life. Although a lot of research aimed at identifying the causes of the disease, the pathophysiology of epilepsy remains unclear in approximately 50% of the cases (Beghi, 2020). Moreover, 1/6 of epileptic patients develop drug-resistant epilepsy (DRE), the refractory form of the disease (Giussani et al., 2016). Metataxonomic analyses on microbiome biodiversity showed close correlation between gut microbiota and epilepsy (Gong et al., 2021; Gong et al., 2020; Peng et al., 2018). As an example, decreased alpha-diversity and changes in specific phyla abundance (e.g., increased Actinobacteria) have been observed in epileptic adults and DRE children compared to healthy controls (Gong et al., 2021; Gong et al., 2020; Lee et al., 2020; Şafak et al., 2019), with DRE patients having higher levels of rare bacteria (Peng et al., 2018). Differences in microbiota composition were also observed among patients experiencing four or less seizures per year and those experiencing a higher number of seizure events (Peng et al., 2018). Interestingly, children's microbiota profile appears to be correlated to the efficacy of ketogenic diet (KD) treatment (Zhang et al., 2018a), a current therapy for refractory epileptic patients, that drives the reduction in pediatric epilepsy-associated genera (Gong et al., 2021; Ułamek-Kozioł et al., 2019). Indeed, an increase in relative abundance of Ruminococcaceae,

* Corresponding author.
E-mail addresses: lucia.boeri@polimi.it (L. Boeri), francesca.donnaloja@polimi.it (F. Donnaloja), marzia.campanile@polimi.it (M. Campanile), lorenzo.sardelli@polimi.it (L. Sardelli), marta.tunesi@polimi.it (M. Tunesi), federica.fusco@polimi.it (F. Fusco), carmen.giordano@polimi.it (C. Giordano), diego.albani@marionegri.it (D. Albani).

https://doi.org/10.1016/j.nbd.2022.105614
Received 4 October 2021; Received in revised form 31 December 2021; Accepted 5 January 2022
Available online 10 January 2022

Lachnospiraceae and Rikenellaceae was observed in children unresponsive to KD (Zhang et al., 2018b). Other evidence to support the role of gut microbiota in disease pathophysiology came from the beneficial effects of probiotics or antibiotics treatment in DRE patients (Braakman and van Ingen, 2018; Gómez-Eguílaz et al., 2018).

Fecal microbiota transplantation (FMT) is a promising approach to treat epilepsy and reduce seizures (He et al., 2017). The study of FMT in animal models demonstrated the importance of the role of gut microbiota in seizure occurrence. Indeed, the FMT from epileptic to healthy mice showed an increasing neuronal excitability and susceptibility to seizures in recipient healthy animals (Mengoni et al., 2021). Using rat models it was demonstrated that FMT determines taxonomic alterations in beta diversity, and a reduction in absence seizures respect to untreated controls (Citraro et al., 2021). The connection between gut microbiota and brain involves different pathways, where bacteria may play a role in modulating body levels of molecules such as neurotransmitters (Rana and Musto, 2018; Wang and Wang, 2016; Yano et al., 2015), neurotrophic factors (Iughetti et al., 2018; Maqsood and Stone, 2016) and inflammatory signals (Al Bander et al., 2020; Boeri et al., 2021) (Fig. 1).

In the epileptic seizure context, characterized by a transient neuronal hyperexcitability, the abnormal brain activity is thought to be caused by an imbalance of excitatory/inhibitory signaling. The primary inhibitory neurotransmitter of central nervous system, γ-aminobutyric acid (GABA), is also produced by intestinal bacteria. Although the ability of GABA to cross the blood-brain-barrier (BBB) is still debated (Hepsomali et al., 2020), it is interesting to note that decreased levels of *Bacteroides* genera, to which the majority of GABA synthesis in the gut may be attributable (Otaru et al., 2021), have been observed in children with intractable epilepsy (Lee et al., 2020). Moreover, brain serotonin (5-HT) level has been inversely correlated with seizure frequency and susceptibility in human and animal models (Bagdy et al., 2007; Ribot et al., 2017). It seems that gut microbiota indirectly modulates brain 5-HT levels by metabolizing tryptophan, which is a serotonin precursor able to cross the BBB (Kaur et al., 2019).

It has been demonstrated that prebiotic administration elevates central brain-derived neurotrophic factor (BDNF) synthesis in adult male rats (Savignac et al., 2013), and Iughetti et al. (2018) well reviewed the relationship between serum BDNF levels and epilepsy in human, suggesting BDNF as potential therapeutic target (Iughetti et al., 2018).

Pre-clinical data suggest a causal relationship between inflammatory state and epileptic condition (Devinsky et al., 2013; Rana and Musto, 2018; Vezzani and Granata, 2005). The administration of lipopolysaccharides (LPS), pro-inflammatory molecules released from Gram negative bacteria, have been shown to increase seizure susceptibility in animal models (Ho et al., 2015; Kovács et al., 2014) whereas anti-inflammatory molecules as short-chain fatty acids (SCFA) resulted increased in DRE children after KD therapy (Gong et al., 2021; Mirzaei et al., 2021), suggesting a beneficial effect on epileptic patients. It has

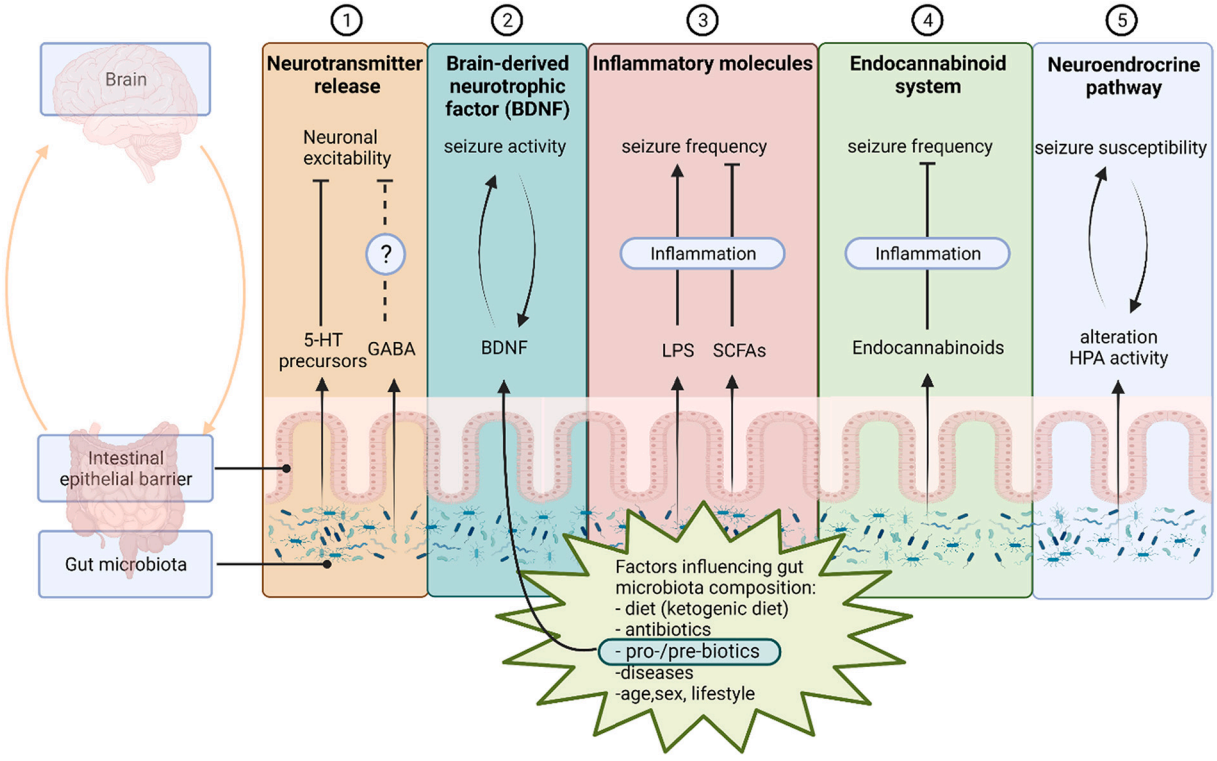


Fig. 1. Molecular pathways possibly involved in gut microbiota interaction with epilepsy.
Intestinal bacteria are thought to influence the pathophysiology of epilepsy through different mechanisms: 1) GABA and 5-HT neurotransmitters are strong modulators of neuronal excitability, therefore their brain concentration may be fundamental in regulating seizure events. It has been hypothesized that gut bacteria modulate their brain levels by peripherally producing GABA and metabolizing 5-HT precursors. 2) Upon probiotics administration gut microbiota could influence BDNF levels, which appear to correlate with epilepsy severity. Seizures could in turn affect BDNF production, suggesting a mutual interaction between BDNF and epilepsy. 3) LPS and SCFAs are pro-inflammatory and anti-inflammatory molecules, respectively, and their blood levels are tightly determined by microbiota composition. As inflammation plays a key role in seizures frequency, these bacterial products could act on epilepsy features targeting inflammatory pathways. 4) The endocannabinoid (EC) system is well known as inflammation suppressor. Gut microbiota could be able to influence the expression of EC receptors and EC agonists, suppressing inflammation and affecting seizures events. 5) Microbiota-induced alterations of the HPA axis activity can enhance seizures rates possibly through corticosteroids action. Initial seizure insults could affect HPA activation too, resulting in a promotion of seizure susceptibility and highlighting the HPA-seizure crosstalk. 5-HT: serotonin; GABA: γ-aminobutyric acid; BDNF: brain-derived neurotrophic factor; HPA axis: hypothalamic-pituitary-adrenal axis; LPS: lipopolysaccharide; SCFAs: small chain fatty acids. Image created with BioRender.com.

been also hypothesized that microbiota may reduce the inflammatory response through the endocannabinoid pathway (Alger, 2004; Cani et al., 2016). Indeed, *Lactobacillus* strains enhance μ-opioid and cannabinoid receptors expression in intestinal epithelial cells (Rousseaux et al., 2007), and LPS modulates endocannabinoid receptors agonist synthesis in immune cells (Liu et al., 2003; Maccarrone et al., 2001; Zhu et al., 2011).

It is important to note that the presence of bacterial molecules in the bloodstream is strongly enhanced in leaky gut syndrome, characterized by weakening and increased permeability of the intestinal barrier (Camilleri, 2020; Mohammad and Thiemermann, 2021). Barrier function is in turn regulated by gut microbiota composition. As an example, *Akkermansia muciniphila*, *Bifidobacterium infantis* and *Escherichia coli* Nissle 1917 exerted positive effects on intestinal barrier integrity, whereas *E. coli* strain C25 led to tight junction disruption (Bergmann et al., 2013; Chelakkot et al., 2018; Ukena et al., 2007; Zareie et al., 2005). Therefore, the modulation of barrier permeability by gut microbiota is another mechanism of interest in microbiota-gut-epilepsy axis. Mazarati et al., studied how the lateral fluid percussion injury and post- traumatic epilepsy were associated with the intestinal permeability and LPS concentration into the bloodstream (endotoxemia) (Mazarati et al., 2021). They showed that endotoxemia had a complex role in post-traumatic epilepsy development and was correlated with the severity of both acute and chronic post-traumatic motor dysfunction. Indeed, acute post-brain injury endotoxemia in rats was associated with late neuroprotection from chronic epilepsy, while late endotoxemia has been associated to both anti- and pro-epileptic effects (Mazarati et al., 2021).

It is documented that dysbiosis leads to hypothalamic pituitary adrenal (HPA) axis dysregulation (Farzi et al., 2018). Interestingly, HPA axis activation is associated with increased seizures susceptibility (Hooper et al., 2018; Joëls, 2009) and adrenocorticotropic hormone (ACTH) treatment in infants with West syndrome affects microbiota shape (Xu et al., 2021).

Giving the available clinical and preclinical data, the relation between epilepsy and microbiota is reasonable but complex and the new frontier of meta-omics analyses could be fundamental to deepen the molecular and genetic basis of this interaction. While metagenomic studies were fundamental for microbial taxonomic characterization in epilepsy (Tilocca et al., 2020), other meta-omics techniques (metatranscriptomics, metaproteomics and metabolomics) have not been fully exploited yet in this field (Table 1). An integrative meta-omics approach (multi-omics) could undoubtedly lead to a better understanding of the molecular mechanisms underlying microbiota-epilepsy linkage and help drug development.

The multi-omics approach is still a challenge independently from the field of application. The main limitations are related to technical issues about not only the analysis methods but also the sample manipulation and preparation for meta-omics analysis. We will discuss the main technical aspects that should be taken into account when moving to a multi-omics approach, and highlight the importance of facing these challenges to study epilepsy also thanks to microbiota multifactorial analyses.

Table 1
Summary of studies investigating the association between gut microbiota and epilepsy using meta-omics analyses.

Meta-omics	Technique	Subject	Stool sampling and storage	Sample extraction method	Reference
Single-omics					
Metagenomics	16S rRNA sequencing	Human	ND	MoBio Power® Soil Kit	(Xie et al., 2017)
Metagenomics	16S rRNA sequencing	Human	Frozen at −80 °C within 2 h	QIAmp® DNA Stool Mini Kit	(Zhang et al., 2018a)
Metagenomics	16S rRNA sequencing	Human	Immediately frozen at −80 °C	FastDNA™ SPIN Kit for Soil	(Peng et al., 2018)
Metagenomics	16S rRNA sequencing	Human	Frozen at −80 °C within 2 h	E.Z.N.A.® Soil DNA Kit	(Gong et al., 2020)
Metagenomics	16S rRNA sequencing	Human	Frozen at −80 °C within 30 min	E.Z.N.A.® Soil DNA Kit	(Huang et al., 2019)
Metagenomics	16S rRNA sequencing	Human	SB-01 stool sampling kit. Feces kept at 4 °C until the freezing at −80 °C in the lab	Homogenization, bead-beating, centrifugation at 14,000*g* for 10 min	(Lee et al., 2020)
Metagenomics	16S rRNA sequencing	Human	Stored at −80 °C until DNA extraction	GeneMATRIX Tissue & Bacterial DNA Purification kit	(Şafak et al., 2019)
Metagenomics	16S rRNA sequencing	Human	Frozen at −80 °C within 12 h	OMINgene-Gut	(Lee et al., 2021)
Metagenomics	16S rRNA sequencing	Rat	Immediately frozen at −80 °C	QIAamp® DNA Stool Mini Kit	(Citraro et al., 2021)
Metagenomics	16S rRNA sequencing	Dog	Feces kept at 4 °C until the freezing at −80 °C in the lab	Custom extraction protocol based on bead-beating and Qiagen reagents	(Muñana et al., 2020)
Metagenomics	Shotgun sequencing	Human	FLOQSwab® kept at 4 °C and transferred to −70 °C within few hours	PowerMicrobiome™ RNA Isolation Kit	(Lindefeldt et al., 2019)
Metabolomics	GC	Human	Immediately frozen at −20 °C, delivered to the laboratory within 48 h and stored at −80 °C	Liquid-liquid extraction	(Ferraris et al., 2021)
Metabolomics	NMR + LC-MS/MS	Human + Rat	Fresh fecal samples were analyzed within 24 h	Methanol-based solid phase extraction cartridge	(Yoo et al., 2014)
Multi-omics					
Metagenomics + metabolomics	16S rRNA sequencing + HPLC-UV	Human	Frozen at −80 °C within 2 h	CTAB/SDS method + liquid-liquid extraction	(Gong et al., 2021)
Metagenomics + metabolomics	16S rRNA sequencing + GC/MS, LC/MS and LC/MS/MS	Mouse	Immediately snap frozen in liquid nitrogen and stored at −80 °C	MoBio Power® Soil Kit + organic aqueous solvents-based extraction	(Olson et al., 2018)
Metagenomics + metabolomics	16S rRNA sequencing + GC/MS	Mouse	Immediately processed for DNA extraction and GC/MS	DNeasy Power ® Soil Kit + liquid-liquid extraction	(Eor et al., 2021)

ND: not defined; CTAB/SDS: cetyltrimethyl ammonium bromide/sodium dodecyl sulfate; NMR: nuclear magnetic resonance; LC: liquid chromatography; GC: gas chromatography; MS: mass spectroscopy; HPLC-UV: high-performance liquid chromatography with ultraviolet spectrophotometer; UHPLC-Q-TOF-MS: ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry.

2. Meta-omics for human microbiota characterization

2.1. Metagenomics

Metagenomic analyses provide accurate and quantitative description of microbial composition (Sirangelo, 2018; Tilocca et al., 2020). It is currently based on next-generation sequencing (NGS) techniques, which can be used for sequencing either the whole genome (shotgun metagenomics) or a single amplicon (16S rRNA gene sequencing).

The shotgun sequencing produces informative details about the microbial population and relative function. The alignment of the whole microbial DNA to reference genomes allows to identify all the organisms of database-collected bacterial community at the species/strain levels, and their gene-related functions and variants (Knight et al., 2018; Sirangelo, 2018; Weinstock, 2012). Despite many advantages, some challenging step during DNA preparation, discussed further below, and post-analytical processing procedures make this technique still to be improved. The last issue is about the high-level of expertise and high-cost machinery necessary to process the huge amount of data (Zhang et al., 2019). It is exacerbated by the reference databases used to extract list of genes and number of matched DNA reads (Sirangelo, 2018; Tilocca et al., 2020; Weinstock, 2012). The lack of some matching reference sequences makes the complete definition of the genome map and relative function difficult (Weinstock, 2012). This last limit can be overcome by the gene de novo prediction of shotgun sequencing but this approach increases the time and costs of post-analytical processing even more. Another technical aspect that limits shotgun metagenomics is that it does not distinguish between viable and dead cells (Heintz-Buschart et al., 2016). Indeed, this technique cannot offer accurate relative functional information (Mitra et al., 2013).

16S rRNA gene sequencing is a metagenomic technique, which is used specifically for taxonomic profiling. It is widely used since it is time- and cost-effective and cheaper than shotgun sequencing. It is based on the amplification of specific hypervariable regions (V1-V9) of the 16S rRNA gene characterizing each bacteria species (Cao et al., 2017; Knight et al., 2018). Until a few years ago, the only methods for data comparison with the available taxa databases were based on the DNA reads clustering into operational taxonomic units (OTU), which collect groups of organisms with 16S rRNA gene sequences identity of at least 97% (Tilocca et al., 2020; Weinstock, 2012). Sequences clustering is used to take microbial community census and define the bacterial composition profile (Sirangelo, 2018). Recently, amplicon sequencing variants (ASV) have been proposed as an alternative to OTUs. ASV methods allow the analysis of single-nucleotide differences instead of user-created OTU differences. Indeed, ASV-based clustering may resolve the bias of OTU-generated clusters down to the taxonomic level of species (Callahan et al., 2017; Prodan et al., 2020). Despite the resolution and accuracy of the ASV approach, recent evidence showed the risk of the ASV-based analysis to lead to genome splitting into separate clusters and thus, the need to improve the methods to not generate conflicting inferences about the ASVs from the same genome (Schloss, 2021). Respect to shotgun metagenomics, 16S rRNA sequencing is equally unable to discriminate viable taxa but still shows more technical troubles to achieve species-level accuracy in taxonomic profiling. Under this perspective, 16S rRNA gene sequencing may be considered even more limited than shotgun metagenomics in terms of collection of functional and genetic information (Tilocca et al., 2020; Weinstock, 2012).

Since both metagenomic approaches are based on the use of reference genome databases, the lack of references from unusual bacteria is a challenging aspect during bioinformatic processing (Angiuoli et al., 2011). Indeed, many resources have been employed to complete reference databases, and improve software efficiency and computational algorithms (Sirangelo, 2018; Weinstock, 2012).

To date, metagenomics represents the meta-omics analysis mostly used to study the role of gut microbiota in epilepsy. It is able to assess the microbiome biodiversity and relative abundance in relation to treatments or pathological evidence (Table 1). For instance, 16S rRNA sequencing has been frequently used to analyze taxonomic changes after FTM or KD (Citraro et al., 2021; Gong et al., 2021). The work of Lindefeldt et al., is an elegant example of how shotgun metagenomics can help in defining new bacterial and molecular targets (Lindefeldt et al., 2019). Authors selected children with therapy-resistant epilepsy and analyzed their taxonomic and functional profiles before and after 3 months of KD. Thanks to the shotgun sequencing approach, they identified seven pathways of carbohydrate metabolism, which are attributable to *Bifidobacteria* and *Escherichia,* and could contribute to the beneficial response of patients to KD. Overall, metagenomics is a powerful tool to provide genetic and functional information about the human microbiota but its inability in discriminating between dead or viable cells (Heintz-Buschart et al., 2016; Mitra et al., 2013) and providing data about bacterial activity introduces the need of the integration of metagenomic data with other meta-omics analyses.

2.2. Metatranscriptomics

Metatranscriptomics allows to sequence bacterial transcriptome and elaborate the relative gene expression profile and thus, functional activity (Bashiardes et al., 2016; Knight et al., 2018; Urich et al., 2008). It is based on the extraction and sequencing of mRNA from a microbial ecosystem. As metagenomics, mRNA sequencing usually involves NGS techniques (RNA-seq) with additional challenging steps: (1) extraction of sufficient high-quality mRNA (Reck et al., 2015); (2) efficient reverse transcription of mRNA to cDNA (Gosalbes et al., 2011); (3) selective removal of other abundant RNAs (i.e rRNA and tRNA that approximately represent 98% of total RNA sample) to not obscure the high-informative mRNA (Morgan and Huttenhower, 2014; Zhang et al., 2019). As metagenomics, the bioinformatic processing is based on the sequences alignment to specific reference databases, which are limited even more then for metagenomic analyses (Sirangelo, 2018; Tilocca et al., 2020). The detection of mRNA in bacterial samples identify the gene expression profile but does not provide the relative protein translation (Zhang et al., 2019), whose quantification requires non-genomic analysis, such as metaproteomics.

2.3. Metaproteomics

Metaproteomics and metabolomics are probably the most informative tools available to define the microbial activity and function. They clarify functional aspects involved in a physiological state even in comparison with pathologic conditions (Zhu et al., 2021). Metaproteomics uses analytical techniques based on mass spectrometry (MS) to provide information about the differential microbial protein expression based on time/treatment conditions, protein molar quantitation, post-translational modification, and partitioning in subcellular structures (Hettich et al., 2013; Wang et al., 2020). The performance of this analysis is highly dependent on the stool sample storage and extraction (Issa Isaac et al., 2019) and the lack of universal guidelines/standardized protocols makes the process even more complex (Heyer et al., 2019). Indeed, the high contamination of host proteins or indigested food not only can saturate MS spectra but also generate background noise, which may alter the results (Mayers et al., 2017). In addition, it is worth noting that metaproteomics is a low-depth analysis that limits protein detection to 10–20% of expressed proteins (Zhang et al., 2019). As in case of genomic data, the number of identified proteins depends on the quality of the protein database used. While large databases require high computational time and large number of peptides, small databases may exclude large amount of detected proteins (Chatterjee et al., 2016; Jagtap et al., 2013). A possible strategy to overcome the database-related challenges is represented by an iterative method that involves a preliminary search from large databases and an in-depth analysis from smaller databases (Issa Isaac et al., 2019).

In short, metaproteomics is a key technology to identify microbial

functional patterns but it remains poorly exploited due to the several challenging technicalities: (1) the lack of standardized protocols for sample preparation; (2) the inability of MS to measure low-abundance peptides (Zhang et al., 2018b); (3) the inadequacy of the current reference databases of microbial proteins (Sirangelo, 2018); (4) and the need of implemented bioinformatic tools (Heyer et al., 2017; Zhang et al., 2018a).

2.4. Metabolomics

Metabolomics integrates quantitative and qualitative analyses of all the small molecules (<1500 Da) originating from microbial symbionts, hosts and environmental intake (Martias et al., 2021; Smirnov et al., 2016). Via nuclear magnetic resonance (NMR) spectroscopy and MS, metabolomics shows an instantaneous snapshot of the physiology of the cells (Luan et al., 2019). Metabolomics could be applied to targeted analyses referring to small specific set of known compounds such as lipids, amino acids, carbohydrates or to untargeted analyses also known as "metabolite fingerprinting" to reach complete metabolic comparison (Cambiaghi et al., 2017; Smirnov et al., 2016). Even though metabolomics is an extraordinary tool to investigate the bacterial role in several pathologies - such as human intestinal disorders, neurodegenerative diseases and aging -, it results extremely limited by hard discrimination between host and bacterial metabolites (Nicholson et al., 2012), difficulty in associating the relative phylogenetic origin, and inadequate reference databases (Smirnov et al., 2016; Zhang et al., 2019). In this context, the choice of adequate analytical tools and pipelines (i.e. sample handling, selection of appropriate equipment and statistical evaluation) represents a crucial step to enable meaningful biological interpretation (Smirnov et al., 2016). Due to the enormous amount and diversity of metabolites detected in a stool sample and the heterogeneity of the current databases, data analyses may require user's manual integration of multiple databases (Cambiaghi et al., 2017). This manual integration represents a further time-consuming and challenging step with high-risk of user-related errors.

In the study of microbiota-epilepsy connection metabolomics is emerging and has been mostly applied to measure a specific set of compounds, such as SCFAs (Eor et al., 2021; Ferraris et al., 2021; Gong et al., 2021). A recent work in this field introduced metabolomics also to profile the entire metabolite fingerprint in response to KD, but authors focused on mouse models and not yet on human beings (Olson et al., 2018; Yoo et al., 2014), the next now at-hand level.

2.5. Multi-omics

Nowadays several studies integrated various -omics techniques to

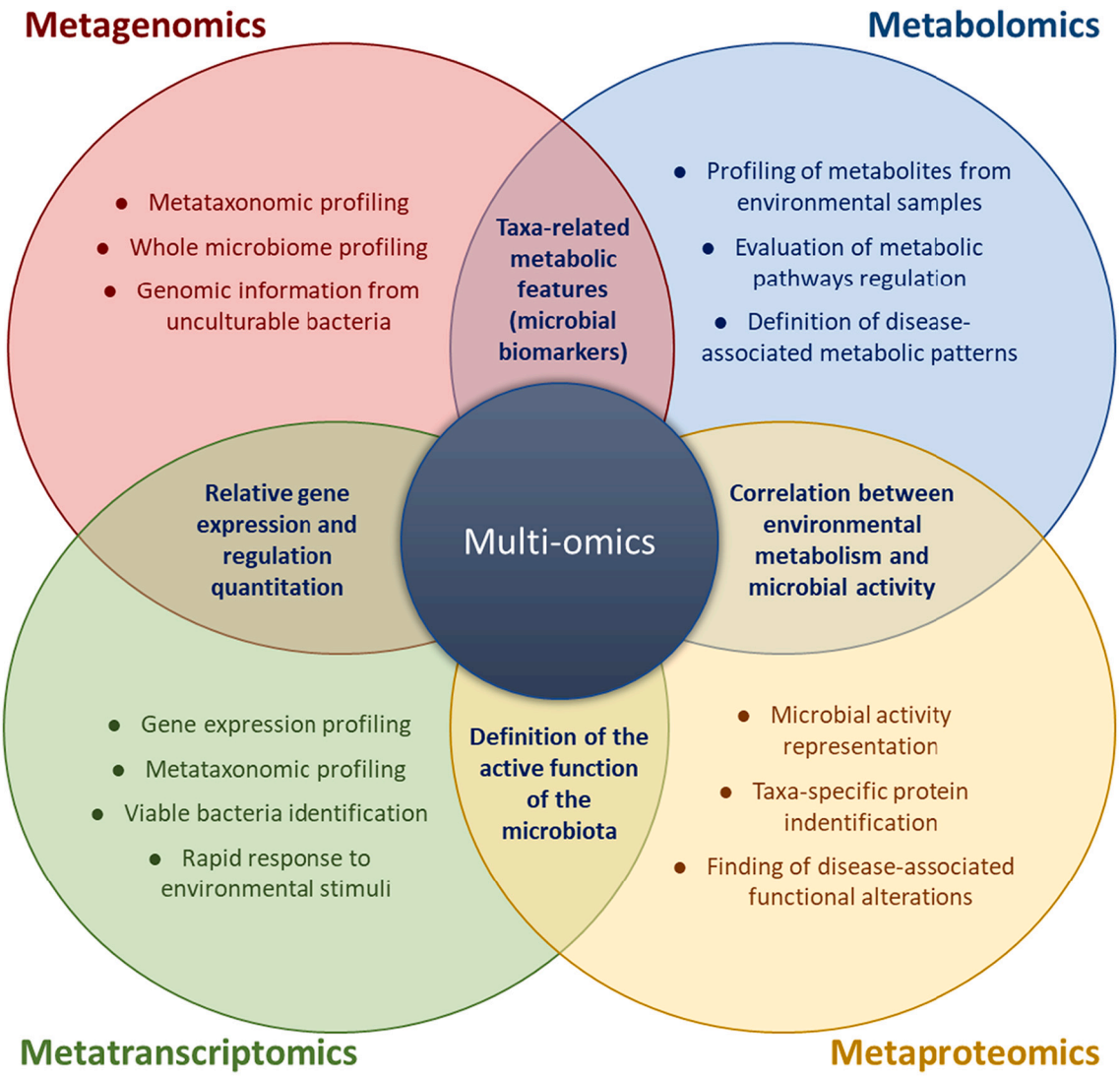


Fig. 2. Graphical representation of advantages in using meta-omics analyses, separately or in combination (multi-omics).

examine the complex microbiota environment. In this context, multi-omics combines two or more meta-omics approaches to: (1) investigate the transcript/gene ratio that is an index of activation/repression of gene expression; (2) provide detailed molecular description of microbiota-host interaction; (3) and reveal the bilateral flow of information that underlies different pathological conditions (Knight et al., 2018; Tilocca et al., 2020) (Fig. 2).

For instance, metagenomics, required to investigate composition and gene-related functional capacity of the microbiota, may be combined with metatranscriptomics to define which genes are induced/repressed or with metaproteomics to identify and quantify the expressed proteins in relation to each bacterial species. Heintz-Buschart et al. (2016) combined metagenomics, metatranscriptomics and metaproteomics to characterize the gut microbiota of patients with type 1 diabetes mellitus (T1DM) (Heintz-Buschart et al., 2016). The definition of the genomic and functional profile of the gut microbiota provided inter-individual functional differences and allowed the elucidation of the bacterial genes that may have a role in the metabolic transformations relevant to the T1DM pathophysiology. Metagenomics can be also integrated with metabolomics to characterize the ongoing physiological or pathological process. This integrative approach defines co-variation between the microbiota composition and the metabolic response, showing the impact of pathological or pharmacological conditions (Zhang et al., 2019). In the microbiota-epilepsy connection investigation, this integrative approach is the only multi-omics combination used so far. It demonstrated how taxonomic distribution and metabolites production change in response to KD and in relation to seizure susceptibility (Olson et al., 2018). The emerging importance of this multi-omics approach is confirmed by several studies in different clinical fields, such as metabolic and other neurodegenerative disorders (Olson et al., 2018). For instance, whole-genome shotgun sequencing and metabolomics (capillary electrophoresis time-of-fight MS) were combined to identify cases of gut intramucosal carcinoma since they show a significant alteration in metagenomic and metabolomic profiles respect to healthy patients (Yachida et al., 2019). In the field of neurodegenerative diseases, this approach showed Parkinson's disease-specific differential patterns of metabolic features (i.e., sulfur co-metabolism) that could open up routes towards a better understanding and prediction of phenotypic variability in this severe disease (Hertel et al., 2019; Tan et al., 2021).

With the potential of multi-omics clear, it becomes pivotal the definition of standardized protocols for the sample preparation, which is, along with post-analytical processing, the most challenging step in each meta-omics analysis. In particular, meta-omics studies on gut microbiota are strongly influenced by both sampling procedures and molecule extraction methods (Costea et al., 2017).

3. The importance of stool sampling methods

Optimizing the sampling procedure is the first and probably most practical issue while characterizing the microbiota by meta-omics techniques. The management of the samples can be faced in two interconnected viewpoints: the sampling and the storage procedures.

The human microbiota can be collected by stools, endoscopic procedures, aspiration of intestinal fluid, capsule endoscopy, among others, described in detail elsewhere (Million et al., 2020; Yang et al., 2010). Surgical sampling such as biopsy is the most reliable procedure, as it minimizes possible contamination, for example by materials derived from non-collecting sites. However, it is invasive and challenging, requiring preparation steps that may damage microbiota, like antibiotic administration (Tang et al., 2020; Tuohy and Scott, 2015). Differently, stool sampling remains one of the most used methods to collect microbiota samples thanks to its simplicity, repeatability on daily basis, affordability and non-invasiveness. However, stool samples provide information only about the more transient luminal bacteria of the large intestine (i.e., caecum and colon), failing in recapitulating the complex spatial organization of gut microbiota communities (Donaldson et al., 2015; Geerlings et al., 2018; Jones et al., 2018; Rossen et al., 2015; Tang et al., 2020; Zhang et al., 2014).

In the human gut, anaerobic bacteria are 100–1000 times more numerous than aerobic ones (Bellali et al., 2019), but the fact that extreme oxygen sensitive bacteria die after a few minutes of exposure to atmospheric oxygen concentrations (Loesche, 1969) poses some challenges for sample collection. To maintain the populations of obligate anaerobes, the delay between collection and processing should not exceed a few minutes. Once in the lab, an anaerobic chamber ensuring an oxygen concentration below 1% should be required for all manipulations. Unfortunately, among the meta-omics studies, only few works consider anaerobiosis during stool sampling (Costea et al., 2017; Yoo et al., 2014). Most of them do not pay sufficient attention to preserve anaerobic population viability with the risk of misinterpreting the data dependent on microbial composition (Martínez et al., 2019). In order to facilitate the preservation of anaerobes in the sample preparation for meta-omics, the commercial kit GutAlive could be used to recapitulate higher levels of diversity since it maintains the viability of obligate anaerobes up to 72 h with an anaerobic reagent to be combined with water (Martínez et al., 2019). However, its capability to preserve all the anaerobic bacteria in the stool is currently unknown. As anaerobic incubation systems, jars or plastic bags could be also used (e.g., GasPak™, Becton Dickinson), coupled with a gas mixture and an oxygen indicator (Lagier et al., 2015; McDonald et al., 2013). Alternatives could be evacuation-exchange systems, where air is removed by suction and filling with an anaerobic atmosphere (e.g., 80% N_2, 10% H_2, and 10% CO_2 or 90% N_2, 5% H_2, and 5% CO_2). However, both systems are not suitable for extreme oxygen sensitive bacteria, because a few hours could be required before anaerobiosis (Ingala et al., 2018; Tang et al., 2020).

Storage greatly influences the quality, consistency, reliability, and reproducibility of the results for high-throughput microbiota research. Intra-individual variation can bias the results, but in the case of stools, homogenization contributes to its reduction (Wu et al., 2010). However, after homogenization, the information related to stool structure are neglected, the relative abundance of some bacterial families and genera may be altered and differences in microbial composition and ratio between soluble and insoluble fractions were noticed (Bircher et al., 2020; Song et al., 2016). In the context of metagenomics analysis, Maghini et al. (2021) suggested not to homogenize the fecal sample but to maintain stool integrity and use biopsy punches with plungers to aliquot (Maghini et al., 2021). However, most of the meta-omics-based studies include stool samples preparation steps by homogenizing, preferring a reduced intra-individual variability to the stool structure-dependent information (Cortes et al., 2019; Costea et al., 2017; Han et al., 2021; Reck et al., 2015).

For sample shipment to the laboratory, compliance with delivery instructions is essential to avoid temperature fluctuations, maintain cell viability, and prevent shifts in microbial composition. The Human Microbiome Project recommended to immediately freeze the stool sample with liquid nitrogen or dry ice, and then store it at −80 °C (The Human Microbiome Project Consortium, 2012). This storage method is the Gold Standard for each meta-omics analysis. Storage at −80 °C (e.g., after adding 10% glycerol as a cryoprotectant) also ensures the stability and viability of the microbial community up to two years, while storage at −20 °C only for a few months (Mathieson et al., 2016). However, the procedure of stool sampling is often done by patients at their own home where sophisticated technologies are not available. In this context, shared guidelines about the maximum acceptable times as a function of temperature have not been established yet, but many method comparisons highlighted the need to control these parameters especially in the perspective of performing meta-omics analyses (Angebault et al., 2018; Cardona et al., 2012; Dominianni et al., 2014). The strategies to store fecal samples at home are three: (1) immediately freezing at −20 °C; (2) storage in the fridge at 4 °C; (3) mixing with stabilizing buffers and storage at room temperature. (1) The immediate freezing at −20 °C

seems to sufficiently preserve DNA and RNA integrity, as well as the bacterial composition (Cardona et al., 2012). (2) Refrigeration at 4 °C up to 3 days does not significantly impact on biodiversity and DNA extraction efficacy. (3) RNALater®, DNA/RNA Shield™ and OMNIgene®·GUT are the reagents most tested as stabilizers at room temperature (Angebault et al., 2018; Choo et al., 2015; Dominianni et al., 2014; Kazantseva and Kallastu, 2020; Reck et al., 2015). They seem to be a good alternative if the refrigeration is not possible. The combination of the second and the third storage method (the use of stabilizers at 4 °C) seems to be the most practical and efficient condition maintaining biodiversity and DNA integrity (Reck et al., 2015). In absence of stabilizers, storage at room temperature is possible just for few hours (up to 3) since it promotes unphysiological bacterial growth and DNA and RNA degradation (Cardona et al., 2012; Dominianni et al., 2014). Storage procedures for further metabolomic and metaproteomic analyses are even less defined. Indeed, fecal samples are generally collected, transferred on ice and frozen at-80 °C within few hours to avoid protein and metabolite degradation (Cortes et al., 2019; García-Durán et al., 2021; Han et al., 2021; Martias et al., 2021; Zhang et al., 2018b).

4. The influence of sample extraction methods on meta-omics success

The output quality of meta-omics is strictly dependent on the extraction reagents and methods. Each meta-analysis requires specific precautions during extraction procedures to get reproducible, representative and reliable results (Cortes et al., 2019; Costea et al., 2017; Hosseinkhani et al., 2021). In the last few years, many methods comparisons discussed which elements – such as the extraction solvent and lysis protocol – influence the extraction efficiency in terms of concentration, quality and taxonomic coverage of the material of interest (i.e. DNA, RNA, proteins and metabolites) (Angebault et al., 2018; García-Durán et al., 2021; Lim et al., 2018; Reck et al., 2015; Zhang et al., 2018b).

In metagenomics studies, DNA has to be processed to minimize the fragmentation and maximize the concentration. The better is the DNA quality, the greater is the taxonomic representation, and the easier is the processing of repetitive genomes (Costea et al., 2017; Maghini et al., 2021). In addition to stool collection methods, the main factors that influence the DNA extraction outcome are: (1) the stabilizing reagents used for stool sampling; (2) the methods of cell lysis; (3) the contamination of non-bacterial DNA; (4) the presence of DNA amplification inhibitors. (1) The most used stabilizers in DNA extraction are Tris-EDTA buffer, Stratec stool collection DNA stabilizer, RNAlater, DNA/RNA Shield™ reagent and OMNIgene Gut (Chen et al., 2020; Choo et al., 2015; Song et al., 2018; Wegl et al., 2021). Depending on the reagent type, the relative abundance of the gut microorganisms changes and thus, the choice of the DNA stabilizer becomes pivotal (Choo et al., 2015). Among these stabilizers, the OMNIgene Gut is the reagent most validated and used, since it guarantees a good DNA extraction rate and shows the smallest differences in relative abundance of microorganisms (Choo et al., 2015). The Stratec stool collection and DNA/RNA Shield™ reagents provide a similar extraction efficiency to OMNIgene Gut (Chen et al., 2020; Wegl et al., 2021), while the Tris–EDTA buffer seems to be the less efficient and determines the highest change in relative abundance respect to the gold standard of stool collection methods (Choo et al., 2015). The RNAlater is a very used DNA stabilizer but it showed worrying discrepancy among the literature data on DNA extraction efficiency and taxonomic coverage (Angebault et al., 2018; Choo et al., 2015), making the use of this reagent less attractive for DNA extraction. (2) The protocol of lysis is a key element in the extraction procedures of all meta-omics. In metagenomics, the cell disruption method influences the DNA concentration, quality and fragmentation. As a consequence, it also determines the taxonomic coverage, largely improved by the presence of high molecular weight DNA (HMWDNA) (Maghini et al., 2021). The mechanical disruption by bead-beating is the method commonly preferred to lyse microbiota samples, since it breaks even the harder cell walls of Gram-positive bacteria (Costea et al., 2017; Lim et al., 2020; Lim et al., 2018). However, the bead-beating-based lysis provides high level of fragmented DNA thus affecting the taxonomic coverage. Maghini et al. (2021) elaborated an interesting extraction protocol in which they substituted the common mechanical lysis with a gentler enzymatic cell disruption (Maghini et al., 2021). Adding size selection by Solid Phase Reversible Immobilization (SPRI) beads, they set a protocol to obtain high quality of HMWDNA. (3) During DNA extraction from stool samples, it is necessary to eliminate host and non-bacterial DNA. It could be removed using different strategies: disruption of mammalian cells (e.g., osmotic or chaotropic lysis), which are generally more susceptible than bacteria; elimination of contaminating DNA by targeting the methylation sites on eukaryotic DNA (e.g., NEBNext® Microbiome DNA Enrichment kit) or by bioinformatics post-analytical processing; capture of bacterial DNA with specific probes (Bachmann et al., 2018). (4) The presence of DNA amplification inhibitors is a determinant factor in the amplification step necessary for the library preparation. In this regard, it is pivotal to remove the stool-derived contaminants, such as polysaccharides and bile salts (Schrader et al., 2012), and nowadays it is possible using most of the commercial kits commonly used for DNA extraction. Considering all the factors challenging the DNA extraction from stool samples, Costea et al. (2017) elegantly compared all the best-performing DNA extraction methods and provided a modified protocol based on the commercial QIAamp Stool Mini kit (Qiagen) (Costea et al., 2017). This new protocol guarantees high quality of DNA, high taxonomic coverage and the possibility to be easily implemented.

Metatranscriptomics requires even more precautions respect to metagenomics since RNA is more exposed to degradation due to its short half-life and sensibility to enzymatic degradation (Deutscher, 2006). In order to achieve reliable gene expression data, extracted RNA has to be highly purified, integral, enriched and representative of the microbial biodiversity (Reck et al., 2015). The factors that most influence the RNA extraction are: (1) RNA stabilizing buffers; (2) methods of cell lysis; (3) contamination of non-bacterial RNA and bacterial non-messenger RNA; (4) presence of RNA and DNA amplification inhibitors or degradative enzymes. To examine the quality of RNA extraction, RNA is evaluated by observing the concentration, the 260/280 and 260/230 ratios for protein and contaminants purity, the RNA integrity number (RIN) and the taxonomic profiles. Reck et al. (2015) compared the best-performing RNA extraction methods for metatranscriptomics and showed that the reagent that best stabilizes RNA and reduces its degradation during stool sampling procedures is RNAlater maintained at 4 °C (Reck et al., 2015). Unfortunately, RNAlater seems to reduce the number of functional COG (cluster of orthologous group) and thus, the representation of the functional profile of microbiota. For this purpose, the stabilizing buffer RNA Protect seems to be more conservative. Reck et al. concluded that, among the extraction methods evaluated, the commercial Powermicrobiome RNA Isolation Kit (MoBio) combined with mechanical cell lysis with a bead beater seems to be the most efficient in terms of RNA quality and quantity (Reck et al., 2015). As in case of DNA extraction, the last aspects affecting the RNA extraction rate are easily addressed by commercial kits. While the removal of amplification inhibitors and degradative enzymes are standard steps in most of the current commercial kit of RNA extraction, bacterial rRNA or host RNA should be specifically removed by focused kits (e.g., Ribo-zero Magnetic kit, Epicentre) or bioinformatics post-analytical processing (Bashiardes et al., 2016).

Metaproteomics and metabolomics need high-concentration of high-quality proteins and metabolites, respectively. The analysis success is strictly related to the rate of protein or metabolite identification (Peters et al., 2019). For both meta-analyses, the extraction buffer and the lysis method are the aspects more influencing the results in terms of repeatability, recovery and functional coverage. In case of metaproteomics, the best protein extraction methods in literature involve the use of a sodium

dodecyl sulfate (SDS)-based buffer and the combination of mechanical and physical lysis methods (ultrasonication and bead-beating with glass beads <0.75 mm) (Cortes et al., 2019; García-Durán et al., 2021; Zhang et al., 2018b). The extraction method from fecal samples for metabolomics is still poorly defined. As in proteomics, sonication and bead-beating are the most used methods for cell disruption. Instead, the choice of the extraction buffer is more challenging. Some research group evaluated the extraction efficiency of single-phase and double-phase solvents, such as methanol, ethanol, acetonitrile, chloroform, and methyl tert-butyl ether (MTBE) (Hosseinkhani et al., 2021; Martias et al., 2021). MTBE seems to be the best buffer in terms of extraction recovery, identification and coverage (Hosseinkhani et al., 2021). However, as emerged by the latest works on both metaproteomics and metabolomics, the extraction buffer choice still selectively influences the representation of the taxonomic profile (García-Durán et al., 2021; Hosseinkhani et al., 2021).

5. Current challenges for the multi-omics approach in the study of microbiota-epilepsy correlation

In the last few years, the great potential of integrated meta-omics analyses has been stimulating many research groups to overcome the technical challenges towards the elaboration of standard procedures for microbiota sampling and processing, and the implementation of bioinformatic tools for post-analytical processing (García-Durán et al., 2021; Han et al., 2021; Maghini et al., 2021; Sedlar et al., 2017).

Summarily, the unresolved technical issues impairing the sample preparation are still two: the oxygen concentration during stool collection and the stabilizing reagents used to preserve the sample. Indeed, while the stool sampling conditions should consider anaerobiosis maintenance to preserve the anaerobic bacterial population to the best, the stabilizing buffers should poorly affect the microbial composition and be more versatile to various meta-omics techniques. In this regard, the stabilizer AllProtect Tissue Reagent (Qiagen) seems to be the ideal reagent for multi-omics approaches since it is efficient in stabilizing DNA, RNA, proteins and metabolites (Reck et al., 2015). However, further studies have to be done to clarify its impact on the biodiversity and relative abundance.

The post-analytical processing is a relevant challenge in each meta-omic analysis and becomes a great limit in the multi-omics approach. The increased complexity and diversity of collected data make the data integration, their correlation and interpretation not trivial (Zhang et al., 2019). Data analysis requires advanced statistics with large numbers of comparisons and statistical analyses, large investment of time and skilled human resource (Hasin et al., 2017; Huang et al., 2017; Knight et al., 2018; Zhang et al., 2019). Moreover, the data correlation process requires high-power calculation, the development of pipelines to integrate these data, and a large computational storage space (Segata et al., 2013). In line with this, many resources have been employed in the development of a software system able to support integrative multi-omics and machine learning represents a promising strategy to investigate microbiota-dependent mechanisms (Huang et al., 2017; Patel-Murray et al., 2020; Reel et al., 2021; Yuan et al., 2021).

Despite these issues, the extremely promising results so far achieved has opened its application in clinical fields to pursue custom therapeutic approaches. In the context of epilepsy, the recent advent of meta-omics techniques has stimulated the research in deeply enquiring the microbiota-brain connection. For instance, metagenomic analyses have been instrumental to reveal gut microbiota changes related to disease type and severity. Meta-omics techniques, used separately or in combination, could become fundamental to deepen key molecular pathways at the base of the microbiota-epilepsy connection. For instance, the combination of all the meta-omics could clarify in deeper detail the mechanisms at the basis of the promotion of GABA biosynthesis by *Bacteroides* and its relevance in epileptic patients (Olson et al., 2018; Otaru et al., 2021). In respect to experimental evidence exploiting metagenomics and metabolomics (Olson et al., 2018), the full integrated multi-omics approach could show for instance bacterial abundance variations (metagenomics) in association with GABA production (metabolomics) and glutamate decarboxylase (GAD) expression (metatranscriptomics and metaproteomics) patterns. Multi-omics could be also useful to deepen the mechanism by which the ACTH treatment for epileptic encephalopathy (West syndrome) was able to change microbiota abundance, via the HPA axis (Xu et al., 2021). Metatranscriptomics, metaproteomics and metabolomics could add wide information about times and pathways of the bacterial response.

As already introduced before, the fecal microbiota transplantation (FMT) is an emerging approach for the treatment of several pathologies and the study of the functional connection between microbiota and the host. Some recent works assessed the effects of FMT from epileptic animals to healthy recipients. For instance, Mengoni et al. analyzed the host response to FTM and measured how the treatment influenced the neuronal excitability in the brain and thus, the seizure threshold (Mengoni et al., 2021). In this work they did not consider the behaviour of the microbiota transplanted after the treatment. So far, only one work analyzed the taxonomic composition of the gut microbiota before and after the FMT (Citraro et al., 2021). An integrative meta-omics approach could show how microbiota directly influences the neuronal excitability by identifying specific molecular pathways and metabolic profiles involved. Even if it is quite accepted that the microbiota plays a role in seizure treatment success (Mengoni et al., 2021; Zhang et al., 2018a), the molecular mechanisms underlying its role in the treatment of drug-resistance patients is less evident (Chatzikonstantinou et al., 2021). A multi-omics approach could help in defining the microbial contribution to this resistance under different molecular points of view, evaluating genomic, expression and functional patterns of the microbiota in response to specific drug treatments. The multi-omics approach to clarify microbiota role in epilepsy and other neurodegenerative disorders could take advantage from peculiar in vitro models, as organs-on-a-chip, which may recapitulate in simplified but informative manner the MGB axis and allow controlled extraction of molecular material also for meta-omics analyses (Liu et al., 2019; Raimondi et al., 2020; Raimondi et al., 2019; Sardelli et al., 2021).

6. Conclusion

In recent years, the gut microbiota has been often associated to epilepsy pathophysiology, but its exact role has still to be clarified. It has been well established that dysbiotic conditions correlate with epilepsy drug resistant pathology and, by modulating the intestinal microbial population, it is possible to guide the seizure frequency and the patient's response to anti-epileptic drugs. Unfortunately, the microbial mechanisms that impact on the seizure frequency and severity are still far from being elucidated. Multi-omics is an integrated science that could allow the characterization of the microbial population and its interaction with the host in terms of taxonomic, genomic, functional, and metabolic features. Even if many attempts have been made towards a multi-omics approach, many other efforts need to be made to make this integrated analysis a common experimental procedure.

In this review, we summarized the potential of using integrated meta-omics for studying microbiota-host interaction. We showed both advantages and limits of each meta-omic analysis, paying particular attention to the technical aspects challenging the stool collection, sample extraction and data analysis. Even if a multi-omics approach integrating all the meta-omics still requires a great deal of efforts, the combination of two meta-omics seems at hand and it is currently used for investigating different pathologies. The application of the same integrative approach in epilepsy could help in identifying the molecular pathways and bacterial biomarkers that are key elements in the microbiota-epilepsy connection.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgements

The Authors thank the project: "MINERVA" (Grant agreement No. 724734 under the European Research Council (ERC)-European Union's Horizon 2020 Research and Innovation Programs). The work reflects the authors' views only.

References

Al Bander, Z., Nitert, M.D., Mousa, A., Naderpoor, N., 2020. The gut microbiota and inflammation: An overview. Int. J. Environ. Res. Public Health 17, 1–22. https://doi.org/10.3390/ijerph17207618.

Alger, B.E., 2004. Endocannabinoids and their implications for epilepsy. Epilepsy Curr. 4, 169. https://doi.org/10.1111/J.1535-7597.2004.04501.X.

Angebault, C., Ghozlane, A., Volant, S., Botterel, F., D'Enfert, C., Bougnoux, M.E., 2018. Combined bacterial and fungal intestinal microbiota analyses: impact of storage conditions and DNA extraction protocols. PLoS One 13. https://doi.org/10.1371/journal.pone.0201174.

Angiuoli, S.V., White, J.R., Matalka, M., White, O., Fricke, W.F., 2011. Resources and costs for microbial sequence analysis evaluated using virtual machines and cloud computing. PLoS One 6. https://doi.org/10.1371/journal.pone.0026624.

Bachmann, N.L., Rockett, R.J., Timms, V.J., Sintchenko, V., 2018. Advances in clinical sample preparation for identification and characterization of bacterial pathogens using metagenomics. Front. Public Health. https://doi.org/10.3389/fpubh.2018.00363.

Bagdy, G., Kecskemeti, V., Riba, P., Jakus, R., 2007. Serotonin and epilepsy. J. Neurochem. https://doi.org/10.1111/j.1471-4159.2006.04277.x.

Bashiardes, S., Zilberman-Schapira, G., Elinav, E., 2016. Use of metatranscriptomics in microbiome research. Bioinform. Biol. Insights 10, 19–25. https://doi.org/10.4137/BBI.S34610.

Beghi, E., 2020. The epidemiology of epilepsy. Neuroepidemiology. https://doi.org/10.1159/000503831.

Beghi, E., Giussani, G., Abd-Allah, F., Abdela, J., Abdelalim, A., Abraha, H.N., Adib, M. G., Agrawal, S., Alahdab, F., Awasthi, A., Ayele, Y., Barboza, M.A., Belachew, A.B., Biadgo, B., Bijani, A., Bitew, H., Carvalho, F., Chaiah, Y., Daryani, A., Do, H.P., Dubey, M., Endries, A.Y.Y., Eskandarieh, S., Faro, A., Farzadfar, F., Fereshtehnejad, S.M., Fernandes, E., Fijabi, D.O., Filip, I., Fischer, F., Gebre, A.K., Tsadik, A.G., Gebremichael, T.G., Gezae, K.E., Ghasemi-Kasman, M., Weldegwergs, K.G., Degefa, M.G., Gnedovskaya, E.V., Hagos, T.B., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hassen, H.Y., Hay, S.I., Jakovljevic, M., Kasaeian, A., Kassa, T.D., Khader, Y.S., Khalil, I., Khan, E.A., Khubchandani, J., Kisa, A., Krohn, K.J., Kulkarni, C., Nirayo, Y.L., Mackay, M.T., Majdan, M., Majeed, A., Manhertz, T., Mehndiratta, M.M., Mekonen, T., Meles, H.G., Mengistu, G., Mohammed, S., Naghavi, M., Mokdad, A.H., Mustafa, G., Irvani, S.S.N., Nguyen, L.H., Nichols, E., Nixon, M.R., Ogbo, F.A., Olagunju, A.T., Olagunju, T.O., Owolabi, M.O., Phillips, M.R., Pinilla-Monsalve, G.D., Qorbani, M., Radfar, A., Rafay, A., Rahimi-Movaghar, V., Reinig, N., Sachdev, P.S., Safari, H., Safari, S., Safiri, S., Sahraian, M.A., Samy, A.M., Sarvi, S., Sawhney, M., Shaikh, M.A., Sharif, M., Singh, G., Smith, M., Szoeke, C.E.I., Tabarés-Seisdedos, R., Temsah, M.H., Temsah, O., Tortajada-Girbés, M., Tran, B.X., Tsegay, A.A.T., Ullah, I., Venketasubramanian, N., Westerman, R., Winkler, A.S., Yimer, E.M., Yonemoto, N., Feigin, V.L., Vos, T., Murray, C.J.L., 2019. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the global burden of disease study 2016. Lancet Neurol. 18, 357–375. https://doi.org/10.1016/S1474-4422(18)30454-X.

Bellali, S., Lagier, J.C., Raoult, D., Khalil, J.B., 2019. Among live and dead bacteria, the optimization of sample collection and processing remains essential in recovering gut microbiota components. Front. Microbiol. 10, 1–9. https://doi.org/10.3389/fmicb.2019.01606.

Bergmann, K.R., Liu, S.X.L., Tian, R., Kushnir, A., Turner, J.R., Li, H.L., Chou, P.M., Weber, C.R., De Plaen, I.G., 2013. Bifidobacteria stabilize claudins at tight junctions and prevent intestinal barrier dysfunction in mouse necrotizing enterocolitis. Am. J. Pathol. 182, 1595–1606. https://doi.org/10.1016/j.ajpath.2013.01.013.

Bircher, L., Schwab, C., Geirnaert, A., Greppi, A., Lacroix, C., 2020. Planktonic and sessile artificial colonic microbiota harbor distinct composition and reestablish differently upon frozen and freeze-dried long-term storage. mSystems 5, 1–18. https://doi.org/10.1128/msystems.00521-19.

Boeri, L., Perottoni, S., Izzo, L., Giordano, C., Albani, D., 2021. Microbiota-host immunity communication in neurodegenerative disorders: bioengineering challenges for in vitro modeling. Adv. Healthc. Mater. 10, 2002043. https://doi.org/10.1002/ADHM.202002043.

Braakman, H.M.H., van Ingen, J., 2018. Can epilepsy be treated by antibiotics? J. Neurol. 2658 (265), 1934–1936. https://doi.org/10.1007/S00415-018-8943-3.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639. https://doi.org/10.1038/ISMEJ.2017.119.

Cambiaghi, A., Ferrario, M., Masseroli, M., 2017. Analysis of metabolomic data: tools, current strategies and future challenges for omics data integration. Brief. Bioinform. 18, 498–510. https://doi.org/10.1093/bib/bbw031.

Camilleri, M., 2020. The Leaky Gut: Mechanisms, Measurement and Clinical Implications in Humans, 68, pp. 1516–1526. https://doi.org/10.1136/gutjnl-2019-318427.The.

Cani, P.D., Plovier, H., Van Hul, M., Geurts, L., Delzenne, N.M., Druart, C., Everard, A., 2016. Endocannabinoids-at the crossroads between the gut microbiota and host metabolism. Nat. Rev. Endocrinol. 12, 133–143. https://doi.org/10.1038/nrendo.2015.211.

Cao, Y., Fanning, S., Proos, S., Jordan, K., Srikumar, S., 2017. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Front. Microbiol. 0, 1829. https://doi.org/10.3389/FMICB.2017.01829.

Cardona, S., Eck, A., Cassellas, M., Gallart, M., Alastrue, C., Dore, J., Azpiroz, F., Roca, J., Guarner, F., Manichanh, C., 2012. Storage conditions of intestinal microbiota matter in metagenomic analysis. BMC Microbiol. 121 (12), 1–8. https://doi.org/10.1186/1471-2180-12-158.

Chatterjee, S., Stupp, G.S., Park, S.K.R., Ducom, J.C., Yates, J.R., Su, A.I., Wolan, D.W., 2016. A comprehensive and scalable database search system for metaproteomics. BMC Genomics 17. https://doi.org/10.1186/s12864-016-2855-3.

Chatzikonstantinou, S., Gioula, G., Kimiskidis, V.K., McKenna, J., Mavroudis, I., Kazis, D., 2021. The gut microbiome in drug-resistant epilepsy. Epilepsia Open 6, 28. https://doi.org/10.1002/EPI4.12461.

Chelakkot, C., Choi, Y., Kim, D.K., Park, H.T., Ghim, J., Kwon, Y., Jeon, J., Kim, M.S., Jee, Y.K., Gho, Y.S., Park, H.S., Kim, Y.K., Ryu, S.H., 2018. *Akkermansia muciniphila*-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. Exp. Mol. Med. 50 https://doi.org/10.1038/emm.2017.282 e450-11.

Chen, C.C., Wu, W.K., Chang, C.M., Panyod, S., Lu, T.P., Liou, J.M., Fang, Y.J., Chuang, E.Y., Wu, M.S., 2020. Comparison of DNA stabilizers and storage conditions on preserving fecal microbiota profiles. J. Formos. Med. Assoc. 119, 1791–1798. https://doi.org/10.1016/j.jfma.2020.01.013.

Choo, J.M., Leong, L.E., Rogers, G.B., 2015. Sample storage conditions significantly influence faecal microbiome profiles. Sci. Rep. 5 https://doi.org/10.1038/srep16350.

Citraro, R., Lembo, F., De Caro, C., Tallarico, M., Coretti, L., Iannone, L.F., Leo, A., Palumbo, D., Cuomo, M., Buommino, E., Nesci, V., Marascio, N., Iannone, M., Quirino, A., Russo, R., Calignano, A., Constanti, A., Russo, E., De Sarro, G., 2021. First evidence of altered microbiota and intestinal damage and their link to absence epilepsy in a genetic animal model, the WAG/Rij rat. Epilepsia 62, 529–541. https://doi.org/10.1111/epi.16813.

Cortes, L., Wopereis, H., Tartiere, A., Piquenot, J., Gouw, J.W., Tims, S., Knol, J., Chelsky, D., 2019. Metaproteomic and 16S rRNA gene sequencing analysis of the infant fecal microbiome. Int. J. Mol. Sci. 20 https://doi.org/10.3390/ijms20061430.

Costea, P.I., Zeller, G., Sunagawa, S., Pelletier, E., Alberti, A., Levenez, F., Tramontano, M., Driessen, M., Hercog, R., Jung, F.E., Kultima, J.R., Hayward, M.R., Coelho, L.P., Allen-Vercoe, E., Bertrand, L., Blaut, M., Brown, J.R.M., Carton, T., Cools-Portier, S., Daigneault, M., Derrien, M., Druesne, A., De Vos, W.M., Finlay, B. B., Flint, H.J., Guarner, F., Hattori, M., Heilig, H., Luna, R.A., Van Hylckama Vlieg, J., Junick, J., Klymiuk, I., Langella, P., Le Chatelier, E., Mai, V., Manichanh, C., Martin, J.C., Mery, C., Morita, H., O'toole, P.W., Orvain, C., Patil, K. R., Penders, J., Persson, S., Pons, N., Popova, M., Salonen, A., Saulnier, D., Scott, K. P., Singh, B., Slezak, K., Veiga, P., Versalovic, J., Zhao, L., Zoetendal, E.G., Ehrlich, S. D., Dore, J., Bork, P., 2017. Towards standards for human fecal sample processing in metagenomic studies. Nat. Biotechnol. 35, 1069–1076. https://doi.org/10.1038/nbt.3960.

Deutscher, M.P., 2006. Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res. 34, 659–666. https://doi.org/10.1093/NAR/GKJ472.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N.C., Rogawski, M.A., 2013. Glia and epilepsy: excitability and inflammation. Trends Neurosci. https://doi.org/10.1016/j.tins.2012.11.008.

Dominianni, C., Wu, J., Hayes, R.B., Ahn, J., 2014. Comparison of methods for fecal microbiome biospecimen collection. BMC Microbiol. 14 https://doi.org/10.1186/1471-2180-14-103.

Donaldson, G.P., Lee, S.M., Mazmanian, S.K., 2015. Gut biogeography of the bacterial microbiota. Nat. Rev. Microbiol. 14, 20–32. https://doi.org/10.1038/nrmicro3552.

Eor, J.Y., Son, Y.J., Kim, J.Y., Kang, H.C., Youn, S.E., Kim, J.H., Kim, S.H., 2021. Neuroprotective effect of both synbiotics and ketogenic diet in a pentylenetetrazol-induced acute seizure murine model. Epilepsy Res. 174 https://doi.org/10.1016/j.eplepsyres.2021.106668.

Farzi, A., Fröhlich, E.E., Holzer, P., 2018. Gut microbiota and the neuroendocrine system. Neurotherapeutics 15, 5. https://doi.org/10.1007/S13311-017-0600-5.

Ferraris, C., Meroni, E., Casiraghi, M.C., Tagliabue, A., De Giorgis, V., Erba, D., 2021. One month of classic therapeutic ketogenic diet decreases short chain fatty acids production in epileptic patients. Front. Nutr. 8 https://doi.org/10.3389/FNUT.2021.613100.

García-Durán, C., Martínez-López, R., Zapico, I., Pérez, E., Romeu, E., Arroyo, J., Hernáez, M.L., Pitarch, A., Monteoliva, L., Gil, C., 2021. Distinct human gut microbial taxonomic signatures uncovered with different sample processing and microbial cell disruption methods for metaproteomic analysis. Front. Microbiol. 12 https://doi.org/10.3389/FMICB.2021.618566.

Geerlings, S., Kostopoulos, I., de Vos, W., Belzer, C., 2018. *Akkermansia muciniphila* in the human gastrointestinal tract: when, where, and how? Microorganisms 6, 75. https://doi.org/10.3390/microorganisms6030075.

Giussani, G., Canelli, V., Bianchi, E., Franchi, C., Nobili, A., Erba, G., Beghi, E., Agostoni, E., Airoldi, L., Basso, F., Carpanelli, M., Di Stefano, M., Magnoni, A., Martinelli, O., Rigamonti, A., Salmaggi, A., Stanzani, L., Volpe, C., Zanotta, N.,

Zucca, C., Agudio, M., Arienti, F., Arrigoni, G.R., Balestra, G., Ballabio, P., Balossi, S. M., Baratti, M.R., Barteselli, F.E., Bellani, F., Bellani, M., Bellini, G., Beretta, P.A., Beretta, R., Bergamini, M., Bertella, A., Bertolini, F., Bevilacqua, R., Bianchi, M.A., Bigiolli, P., Binda, G., Biondelli, G., Bodega, L., Bolis, E.G., Bolla, M.V.C., Bonacina, C.A., Bonfanti, M., Borghetti, F., Brambilla, R., Brancaleone, W., Brusadelli, F., Bub, S., Caccia, G., Caglio, M., Rossi, C.C., Capra, D., Carone, D., Carì, G., Carrera, S., Cavenago, R., Cecchetti, M.L., Centonze, B.A., Cereghini, F., Cerrone, C., Ciappetta, M.P., Cogliati, B., Colombano, O., Colombo, A., Colombo, D., Corti, M.L., Cremonini, N., Crippa, M., Crippa, R.A., Crotta, M., Curto, S., Daielli, P., De Gilio, D., De Pascalis, G.P., Decet, E., Morte, L. Della, Duvia, F., Favorito, A., Ferrario, R., Fezzi, L., Finzi, A.L., Fiorentino, B.U., Fornaciari, A., Franceschetti, S., Fulconis, F., Fumagalli, G., Fumagalli, M., Galbiati, G., Galimberti, C.A., Gecchele, D., Ghiazza, B., Ghislanzoni, P., Gioffredi, L., Hassibi, T., Julita, S., Leone, G., Levi, C., Locatelli, G., Locati, A.A., Longhi, E.G., Lukacova, I., Manfroi, M. G., Manzoni, P., Marcolini, C., Minicucci, F., Martini, M.S., Masperi, R., Mayan, S., Mazzoleni, V., Menga, A., Merlini, F., Messina, E., Micheli, M., Micò, R., Millul, A., Montini, B., Palazzuolo, A., Panzeri, S.P., Passoni, G., Pensotti, L., Petrone, M.M., Pezzuto, R., Pigazzini, E., Pirovano, N., Pontiggia, M., Pozzi, R., Quadrelli, C., Rampello, F., Realini, M., Righetti, G., Ripa, C., Rivetta, S., Romeo, A., Rota, C., Rusconi, T., Santarpia, A., Santi, I., Sciani, L., Scoccimarro, G., Scopinaro, E., Semerano, A.G., Sferco, D., Sirtori, A.E., Spandri, A., Spinelli, B., Stefanoni, F., Taiana, R., Tamagnini, L., Tassi, L., Tentori, G., Teodoro, A., Tinterova, K., Tocchetti, A., Todeschini, M., Trezza, L., Valsecchi, V., Vanini, R., Vercelloni, C.F., Villella, A., Viola, M., Vismara, P., Zoboli, D.M., Montesano, M., Romi, S., 2016. A population-based study of active and drug-resistant epilepsies in northern Italy. Epilepsy Behav. 55, 30–37. https://doi.org/10.1016/j.yebeh.2015.11.021.

Gómez-Eguílaz, M., Ramón-Trapero, J.L., Pérez-Martínez, L., Blanco, J.R., 2018. The beneficial effect of probiotics as a supplementary treatment in drug-resistant epilepsy: a pilot study. Benefic. Microbes 9, 875–881. https://doi.org/10.3920/BM2018.0018.

Gong, X., Liu, X., Chen, C., Lin, J., Li, A., Guo, K., An, D., Zhou, D., Hong, Z., 2020. Alteration of gut microbiota in patients with epilepsy and the potential index as a biomarker. Front. Microbiol. 11 https://doi.org/10.3389/FMICB.2020.517797.

Gong, X., Cai, Q., Liu, X., An, D., Zhou, D., Luo, R., Peng, R., Hong, Z., 2021. Gut flora and metabolism are altered in epilepsy and partially restored after ketogenic diets. Microb. Pathog. 155, 104899 https://doi.org/10.1016/j.micpath.2021.104899.

Gosalbes, M.J., Durbán, A., Pignatelli, M., Abellan, J.J., Jiménez-Hernández, N., Pérez-Cobas, A.E., Latorre, A., Moya, A., 2011. Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 6. https://doi.org/10.1371/journal.pone.0017447.

Han, S., Van Treuren, W., Fischer, C.R., Merrill, B.D., Defelice, B.C., Sanchez, J.M., Higginbottom, S.K., Guthrie, L., Fall, L.A., Dodd, D., Fischbach, M.A., Sonnenburg, J. L., 2021. A metabolomics pipeline enables mechanistic interrogation of the gut microbiome. bioRxiv 595. https://doi.org/10.1038/S41586-021-03707-9, 2021.05.25.445684.

Hasin, Y., Seldin, M., Lusis, A., 2017. Multi-omics approaches to disease. Genome Biol. https://doi.org/10.1186/s13059-017-1215-1.

He, Z., Cui, B.T., Zhang, T., Li, P., Long, C.Y., Ji, G.Z., Zhang, F.M., 2017. Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease: the first report. World J. Gastroenterol. 23, 3565–3568. https://doi.org/10.3748/wjg.v23.i19.3565.

Heintz-Buschart, A., May, P., Laczny, C.C., Lebrun, L.A., Bellora, C., Krishna, A., Wampach, L., Schneider, J.G., Hogan, A., De Beaufort, C., Wilmes, P., 2016. Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes. Nat. Microbiol. 2 https://doi.org/10.1038/nmicrobiol.2016.180.

Hepsomali, P., Groeger, J.A., Nishihira, J., Scholey, A., 2020. Effects of oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: a systematic review. Front. Neurosci. https://doi.org/10.3389/fnins.2020.00923.

Hertel, J., Harms, A.C., Heinken, A., Baldini, F., Thinnes, C.C., Glaab, E., Vasco, D.A., Pietzner, M., Stewart, I.D., Wareham, N.J., Langenberg, C., Trenkwalder, C., Krüger, R., Hankemeier, T., Fleming, R.M.T., Mollenhauer, B., Thiele, I., 2019. Integrated analyses of microbiome and longitudinal metabolome data reveal microbial-host interactions on sulfur metabolism in Parkinson's disease. Cell Rep. 29, 1767–1777.e8. https://doi.org/10.1016/j.celrep.2019.10.035.

Hettich, R.L., Pan, C., Chourey, K., Giannone, R.J., 2013. Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. Anal. Chem. 85, 4203–4214. https://doi.org/10.1021/ac303053e.

Heyer, R., Schallert, K., Zoun, R., Becher, B., Saake, G., Benndorf, D., 2017. Challenges and perspectives of metaproteomic data analysis. J. Biotechnol. https://doi.org/10.1016/j.jbiotec.2017.06.1201.

Heyer, R., Schallert, K., Büdel, A., Zoun, R., Dorl, S., Behne, A., Kohrs, F., Püttker, S., Siewert, C., Muth, T., Saake, G., Reichl, U., Benndorf, D., 2019. A robust and universal metaproteomics workflow for research studies and routine diagnostics within 24 h using phenol extraction, fasp digest, and the metaproteomeanalyzer. Front. Microbiol. 10 https://doi.org/10.3389/fmicb.2019.01883.

Ho, Y.H., Te Lin, Y., Wu, C.W.J., Chao, Y.M., Chang, A.Y.W., Chan, J.Y.H., 2015. Peripheral inflammation increases seizure susceptibility via the induction of neuroinflammation and oxidative stress in the hippocampus. J. Biomed. Sci. 22, 1–14. https://doi.org/10.1186/s12929-015-0157-8.

Hooper, A., Paracha, R., Maguire, J., 2018. Seizure-induced activation of the HPA axis increases seizure frequency and comorbid depression-like behaviors. Epilepsy Behav. 78, 124–133. https://doi.org/10.1016/j.yebeh.2017.10.025.

Hosseinkhani, F., Dubbelman, A.-C., Karu, N., Harms, A.C., Hankemeier, T., 2021. Towards standards for human fecal sample preparation in targeted and untargeted LC-HRMS studies. Metabolites 11, 364. https://doi.org/10.3390/metabo11060364.

Huang, S., Chaudhary, K., Garmire, L.X., 2017. More is better: recent progress in multi-omics data integration methods. Front. Genet. https://doi.org/10.3389/fgene.2017.00084.

Huang, C., Li, Y., Feng, X., Li, D., Li, X., Ouyang, Q., Dai, W., Wu, G., Zhou, Q., Wang, P., Zhou, K., Xu, X., Li, S., Peng, Y., 2019. Distinct gut microbiota composition and functional category in children with cerebral palsy and epilepsy. Front. Pediatr. 7 https://doi.org/10.3389/fped.2019.00394.

Ingala, M.R., Simmons, N.B., Wultsch, C., Krampis, K., Speer, K.A., Perkins, S.L., 2018. Comparing microbiome sampling methods in a wild mammal: fecal and intestinal samples record different signals of host ecology. Evolution. Front. Microbiol. 0, 803. https://doi.org/10.3389/FMICB.2018.00803.

Issa Isaac, N., Philippe, D., Nicholas, A., Raoult, D., Eric, C., 2019. Metaproteomics of the human gut microbiota: challenges and contributions to other OMICS. Clin. Mass Spectrom. 14, 18–30. https://doi.org/10.1016/J.CLINMS.2019.06.001.

Iughetti, L., Lucaccioni, L., Fugetto, F., Predieri, B., Berardi, A., Ferrari, F., 2018. Brain-derived neurotrophic factor and epilepsy: a systematic review. Neuropeptides 72, 23–29. https://doi.org/10.1016/j.npep.2018.09.005.

Jagtap, P., Goslinga, J., Kooren, J.A., Mcgowan, T., Wroblewski, M.S., Seymour, S.L., Griffin, T.J., 2013. A two-step database search method improves sensitivity in peptide sequence matches for metaproteomics and proteogenomics studies. Proteomics 13, 1352–1357. https://doi.org/10.1002/pmic.201200352.

Joëls, M., 2009. Stress, the hippocampus, and epilepsy. Epilepsia 50, 586–597. https://doi.org/10.1111/J.1528-1167.2008.01902.X.

Jones, R.B., Zhu, X., Moan, E., Murff, H.J., Ness, R.M., Seidner, D.L., Sun, S., Yu, C., Dai, Q., Fodor, A.A., Azcarate-peril, M.A., Shrubsole, M.J., 2018. Inter-niche and inter-individual variation in gut microbial community assessment using stool, rectal swab, and mucosal samples. Sci. Rep. 1–12 https://doi.org/10.1038/s41598-018-22408-4.

Kaur, H., Bose, C., Mande, S.S., 2019. Tryptophan metabolism by gut microbiome and gut-brain-axis: an in silico analysis. Front. Neurosci. 13, 1365. https://doi.org/10.3389/fnins.2019.01365.

Kazantseva, J., Kallastu, A., 2020. Optimisation of Sample Storage and DNA Extraction for Human Gut Microbiome Studies Center of Food and Fermentation Technologies https://orcid.org/0000-0001-6108-5499. Esther Malv Center of Food and Fermentation Technologies Aleksei Kaleda Center of Food and Fermentation Technologies. https://doi.org/10.21203/rs.3.rs-136572/v1.

Knight, R., Vrbanac, A., Taylor, B.C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolek, T., McCall, L.-I., McDonald, D., Melnik, A.V., Morton, J.T., Navas, J., Quinn, R.A., Sanders, J.G., Swafford, A.D., Thompson, L.R., Tripathi, A., Xu, Z.Z., Zaneveld, J.R., Zhu, Q., Caporaso, J.G., Dorrestein, P.C., 2018. Best practices for analysing microbiomes. Nat. Rev. Microbiol. 167 (16), 410–422. https://doi.org/10.1038/s41579-018-0029-9.

Kovács, Z., Dobolyi, Á., Juhász, G., Kékesi, K.A., 2014. Lipopolysaccharide induced increase in seizure activity in two animal models of absence epilepsy WAG/Rij and GAERS rats and long evans rats. Brain Res. Bull. 104, 7–18. https://doi.org/10.1016/j.brainresbull.2014.03.003.

Lagier, J.C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., Raoult, D., 2015. Current and past strategies for bacterial culture in clinical microbiology. Clin. Microbiol. Rev. 28, 208–236. https://doi.org/10.1128/CMR.00110-14.

Lee, K., Kim, N., Shim, J.O., Kim, G.-H., 2020. Gut bacterial dysbiosis in children with intractable epilepsy. J. Clin. Med. 10, 5. https://doi.org/10.3390/jcm10010005.

Lee, H., Lee, S., Lee, D.H., Kim, D.W., 2021. A comparison of the gut microbiota among adult patients with drug-responsive and drug-resistant epilepsy: An exploratory study. Epilepsy Res. 172 https://doi.org/10.1016/J.EPLEPSYRES.2021.106601.

Liang, S., Wu, X., Jin, F., 2018. Gut-brain psychology: rethinking psychology from the microbiota–gut–brain axis. Front. Integr. Neurosci. https://doi.org/10.3389/fnint.2018.00033.

Lim, M.Y., Song, E.J., Kim, S.H., Lee, J., Do Nam, Y., 2018. Comparison of DNA extraction methods for human gut microbial community profiling. Syst. Appl. Microbiol. 41, 151–157. https://doi.org/10.1016/j.syapm.2017.11.008.

Lim, M.Y., Park, Y.S., Kim, J.H., Do Nam, Y., 2020. Evaluation of fecal DNA extraction protocols for human gut microbiome studies. BMC Microbiol. 20 https://doi.org/10.1186/s12866-020-01894-5.

Lindefeldt, M., Eng, A., Darban, H., Bjerkner, A., Zetterström, C.K., Allander, T., Andersson, B., Borenstein, E., Dahlin, M., Prast-Nielsen, S., 2019. The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy. NPJ Biofilms Microbiom. 5. https://doi.org/10.1038/s41522-018-0073-2.

Liu, J., Bátkai, S., Pacher, P., Harvey-White, J., Wagner, J.A., Cravatt, B.F., Gao, B., Kunos, G., 2003. Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet-activating factor. J. Biol. Chem. 278, 45034–45039. https://doi.org/10.1074/jbc.M306062200.

Liu, J., Sternberg, A.R., Ghiasvand, S., Berdichevsky, Y., 2019. Epilepsy-on-a-chip system for antiepileptic drug discovery. IEEE Trans. Biomed. Eng. 66, 1231. https://doi.org/10.1109/TBME.2018.2871415.

Loesche, W.J., 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18, 723–727. https://doi.org/10.1128/aem.18.5.723-727.1969.

Luan, H., Wang, X., Cai, Z., 2019. Mass spectrometry-based metabolomics: targeting the crosstalk between gut microbiota and brain in neurodegenerative disorders. Mass Spectrom. Rev. https://doi.org/10.1002/mas.21553.

Maccarrone, M., Bari, M., Salvati, S., Finazzi-Agrò, A., De Petrocellis, L., Fezza, F., Di Marzo, V., 2001. Lipopolysaccharide downregulates fatty acid amide hydrolase expression and increases anandamide levels in human peripheral lymphocytes. Arch. Biochem. Biophys. 393, 321–328. https://doi.org/10.1006/abbi.2001.2500.

Maghini, D.G., Moss, E.L., Vance, S.E., Bhatt, A.S., 2021. Improved high-molecular-weight DNA extraction, nanopore sequencing and metagenomic assembly from the human gut microbiome. Nat. Protoc. 16, 458–471. https://doi.org/10.1038/s41596-020-00424-x.

Maqsood, R., Stone, T.W., 2016. The gut-brain axis, BDNF, NMDA and CNS disorders. Neurochem. Res. https://doi.org/10.1007/s11064-016-2039-1.

Martias, C., Baroukh, N., Mavel, S., Blasco, H., Lefèvre, A., Roch, L., Montigny, F., Gatien, J., Schibler, L., Dufour-Rainfray, D., Nadal-Desbarats, L., Emond, P., 2021. Optimization of sample preparation for metabolomics exploration of urine, feces, blood and saliva in humans using combined NMR and UHPLC-HRMS platforms. Molecules 26, 4111. https://doi.org/10.3390/molecules26144111.

Martínez, N., Hidalgo-Cantabrana, C., Delgado, S., Margolles, A., Sánchez, B., 2019. Filling the gap between collection, transport and storage of the human gut microbiota. Sci. Rep. 9, 1–8. https://doi.org/10.1038/s41598-019-44888-8.

Mathieson, W., Sanchez, I., Mommaerts, K., Frasquilho, S., Betsou, F., 2016. An independent evaluation of the CryoXtract instruments' CXT350 frozen sample Aliquotter using tissue and fecal biospecimens. Biopreserv. Biobank. 14, 2–8. https://doi.org/10.1089/bio.2015.0016.

Mayers, M.D., Moon, C., Stupp, G.S., Su, A.I., Wolan, D.W., 2017. Quantitative metaproteomics and activity-based probe enrichment reveals significant alterations in protein expression from a mouse model of inflammatory bowel disease. J. Proteome Res. 16, 1014–1026. https://doi.org/10.1021/acs.jproteome.6b00938.

Mazarati, A., Medel-Matus, J.S., Shin, D., Jacobs, J.P., Sankar, R., 2021. Disruption of intestinal barrier and endotoxemia after traumatic brain injury: implications for post-traumatic epilepsy. Epilepsia 62, 1472–1481. https://doi.org/10.1111/epi.16909.

McDonald, J.A.K., Schroeter, K., Fuentes, S., Heikamp-deJong, I., Khursigara, C.M., de Vos, W.M., Allen-Vercoe, E., 2013. Evaluation of microbial community reproducibility, stability and composition in a human distal gut chemostat model. J. Microbiol. Methods 95, 167–174. https://doi.org/10.1016/j.mimet.2013.08.008.

Mengoni, F., Salari, V., Kosenkova, I., Tsenov, G., Donadelli, M., Malerba, G., Bertini, G., Del Gallo, F., Fabene, P.F., 2021. Gut microbiota modulates seizure susceptibility. Epilepsia 62, e153–e157. https://doi.org/10.1111/epi.17009.

Million, M., Armstrong, N., Khelaifia, S., Guilhot, E., Richez, M., Lagier, J.-C., Dubourg, G., Chabriere, E., Raoult, D., 2020. The antioxidants glutathione, ascorbic acid and uric acid maintain butyrate production by human gut Clostridia in the presence of oxygen in vitro. Sci. Report. 101 (10), 1–11. https://doi.org/10.1038/s41598-020-64834-3.

Mirzaei, R., Bouzari, B., Hosseini-Fard, S.R., Mazaheri, M., Ahmadyousefi, Y., Abdi, M., Jalalifar, S., Karimitabar, Z., Teimoori, A., Keyvani, H., Zamani, F., Yousefimashouf, R., Karampoor, S., 2021. Role of microbiota-derived short-chain fatty acids in nervous system disorders. Biomed. Pharmacother. 139, 111661 https://doi.org/10.1016/j.biopha.2021.111661.

Mitra, S., Förster-Fromme, K., Damms-Machado, A., Scheurenbrand, T., Biskup, S., Huson, D.H., Bischoff, S.C., 2013. Analysis of the intestinal microbiota using SOLiD 16S rRNA gene sequencing and SOLiD shotgun sequencing. BMC Genomics 14. https://doi.org/10.1186/1471-2164-14-S5-S16.

Mohammad, S., Thiemermann, C., 2021. Role of metabolic endotoxemia in systemic inflammation and potential interventions. Front. Immunol. 11, 1–16. https://doi.org/10.3389/fimmu.2020.594150.

Morgan, X.C., Huttenhower, C., 2014. Meta'omic analytic techniques for studying the intestinal microbiome. Gastroenterology 146. https://doi.org/10.1053/j.gastro.2014.01.049.

Muñana, K.R., Jacob, M.E., Callahan, B.J., 2020. Evaluation of fecal Lactobacillus populations in dogs with idiopathic epilepsy: a pilot study. Anim. Microbiome 2. https://doi.org/10.1186/s42523-020-00036-6.

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., Pettersson, S., 2012. Host-gut microbiota metabolic interactions. Science 80. https://doi.org/10.1126/science.1224203.

Olson, C.A., Vuong, H.E., Yano, J.M., Liang, Q.Y., Nusbaum, D.J., Hsiao, E.Y., 2018. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. Cell 173, 1728–1741.e13. https://doi.org/10.1016/j.cell.2018.04.027.

Otaru, N., Ye, K., Mujezinovic, D., Berchtold, L., Constancias, F., Cornejo, F.A., Krzystek, A., de Wouters, T., Braegger, C., Lacroix, C., Pugin, B., 2021. GABA production by human intestinal bacteroides spp.: prevalence, regulation, and role in acid stress tolerance. Front. Microbiol. 12 https://doi.org/10.3389/FMICB.2021.656895.

Patel-Murray, N.L., Adam, M., Huynh, N., Wassie, B.T., Milani, P., Fraenkel, E., 2020. A multi-omics interpretable machine learning model reveals modes of action of small molecules. Sci. Rep. 10 https://doi.org/10.1038/s41598-020-57691-7.

Peng, A., Qiu, X., Lai, W., Li, W., Zhang, L., Zhu, X., He, S., Duan, J., Chen, L., 2018. Altered composition of the gut microbiome in patients with drug-resistant epilepsy. Epilepsy Res. 147, 102–107. https://doi.org/10.1016/j.eplepsyres.2018.09.013.

Peters, D.L., Wang, W., Zhang, X., Ning, Z., Mayne, J., Figeys, D., 2019. Metaproteomic and metabolomic approaches for characterizing the gut microbiome. Proteomics 19, 1800363. https://doi.org/10.1002/PMIC.201800363.

Pittman, Q.J., 2020. A gut feeling about the ketogenic diet in epilepsy. Epilepsy Res. https://doi.org/10.1016/j.eplepsyres.2020.106409.

Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A.H., Nieuwdorp, M., Levin, E., 2020. Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. PLoS One 15. https://doi.org/10.1371/journal.pone.0227434.

Raimondi, M.T., Albani, D., Giordano, C., 2019. An organ-on-A-chip engineered platform to study the microbiota–gut–brain axis in neurodegeneration. Trends Mol. Med. https://doi.org/10.1016/j.molmed.2019.07.006.

Raimondi, I., Izzo, L., Tunesi, M., Comar, M., Albani, D., Giordano, C., 2020. Organ-on-a-chip in vitro models of the brain and the blood-brain barrier and their value to study the microbiota-gut-brain axis in neurodegeneration. Front. Bioeng. Biotechnol. https://doi.org/10.3389/fbioe.2019.00435.

Rana, A., Musto, A.E., 2018. The role of inflammation in the development of epilepsy. J. Neuroinflammation. https://doi.org/10.1186/s12974-018-1192-7.

Reck, M., Tomasch, J., Deng, Z., Jarek, M., Husemann, P., Wagner-Döbler, I., Consortium, O. behalf of C, 2015. Stool metatranscriptomics: a technical guideline for mRNA stabilisation and isolation. BMC Genomics 16. https://doi.org/10.1186/S12864-015-1694-Y.

Reel, P.S., Reel, S., Pearson, E., Trucco, E., Jefferson, E., 2021. Using machine learning approaches for multi-omics data analysis: a review. Biotechnol. Adv. https://doi.org/10.1016/j.biotechadv.2021.107739.

Ribot, R., Ouyang, B., Kanner, A.M., 2017. The impact of antidepressants on seizure frequency and depressive and anxiety disorders of patients with epilepsy: is it worth investigating? Epilepsy Behav. 70, 5–9. https://doi.org/10.1016/j.yebeh.2017.02.032.

Rossen, N.G., Fuentes, S., Boonstra, K., D'Haens, G.R., Heilig, H.G., Zoetendal, E.G., de Vos, W.M., Ponsioen, C.Y., 2015. The mucosa-associated microbiota of PSC patients is characterized by low diversity and low abundance of uncultured Clostridiales II. J. Crohn's Colitis 9, 342–348. https://doi.org/10.1093/ecco-jcc/jju023.

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., Chamaillard, M., Ouwehand, A., Leyer, G., Carcano, D., Colombel, J.F., Ardid, D., Desreumaux, P., 2007. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat. Med. 13, 35–37. https://doi.org/10.1038/nm1521.

Şafak, B., Altunan, B., Topçu, B., Eren Topkaya, A., 2019. The gut microbiome in epilepsy. Microb. Pathog. 139, 103853 https://doi.org/10.1016/j.micpath.2019.103853.

Sardelli, L., Perottoni, S., Tunesi, M., Boeri, L., Fusco, F., Petrini, P., Albani, D., Giordano, C., 2021. Technological tools and strategies for culturing human gut microbiota in engineered in vitro models. Biotechnol. Bioeng. 118, 2886–2905. https://doi.org/10.1002/BIT.27816.

Savignac, H.M., Corona, G., Mills, H., Chen, L., Spencer, J.P.E., Tzortzis, G., Burnet, P.W. J., 2013. Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-d-aspartate receptor subunits and d-serine. Neurochem. Int. 63, 756–764. https://doi.org/10.1016/j.neuint.2013.10.006.

Schloss, P.D., 2021. Amplicon sequence variants artificially split bacterial genomes into separate clusters. mSphere 6. https://doi.org/10.1128/msphere.00191-21.

Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors – occurrence, properties and removal. J. Appl. Microbiol. 113, 1014–1026. https://doi.org/10.1111/J.1365-2672.2012.05384.X.

Sedlar, K., Kupkova, K., Provaznik, I., 2017. Bioinformatics strategies for taxonomy independent binning and visualization of sequences in shotgun metagenomics. Comput. Struct. Biotechnol. J. 15, 48–55. https://doi.org/10.1016/J.CSBJ.2016.11.005.

Segata, N., Boernigen, D., Tickle, T.L., Morgan, X.C., Garrett, W.S., Huttenhower, C., 2013. Computational meta'omics for microbial community studies. Mol. Syst. Biol. https://doi.org/10.1038/msb.2013.22.

Sirangelo, T.M., 2018. Human gut microbiome analysis and multi-omics approach. Int. J. Pharma Med. Biol. Sci. 7, 52–57. https://doi.org/10.18178/ijpmbs.7.3.52-57.

Smirnov, K.S., Maier, T.V., Walker, A., Heinzmann, S.S., Forcisi, S., Martinez, I., Walter, J., Schmitt-Kopplin, P., 2016. Challenges of metabolomics in human gut microbiota research. Int. J. Med. Microbiol. https://doi.org/10.1016/j.ijmm.2016.03.006.

Song, S.J., Amir, A., Metcalf, J.L., Amato, K.R., Xu, Z.Z., Humphrey, G., Knight, R., 2016. Preservation methods differ in fecal microbiome stability. mSystems 1, e00021-16. https://doi.org/10.1128/mSystems.00021-16.Editor.

Song, E.J., Lee, E.S., Do Nam, Y., 2018. Progress of analytical tools and techniques for human gut microbiome research. J. Microbiol. https://doi.org/10.1007/s12275-018-8238-5.

Strandwitz, P., 2018. Neurotransmitter modulation by the gut microbiota. Brain Res. 1693, 128. https://doi.org/10.1016/J.BRAINRES.2018.03.015.

Tan, A.H., Chong, C.W., Lim, S.Y., Yap, I.K.S., Teh, C.S.J., Loke, M.F., Song, S.L., Tan, J. Y., Ang, B.H., Tan, Y.Q., Kho, M.T., Bowman, J., Mahadeva, S., Yong, H. Sen, Lang, A.E., 2021. Gut microbial ecosystem in Parkinson disease: new clinicobiological insights from multi-omics. Ann. Neurol. 89, 546–559. https://doi.org/10.1002/ana.25982.

Tang, Q., Jin, G., Wang, G., Liu, T., Liu, X., Wang, B., Cao, H., 2020. Current sampling methods for gut microbiota: a call for more precise devices. Front. Cell. Infect. Microbiol. https://doi.org/10.3389/fcimb.2020.00151.

The Human Microbiome Project Consortium, 2012. A framework for human microbiome research. Nat. 4867402 (486), 215–221. https://doi.org/10.1038/nature11209.

Tilocca, B., Pieroni, L., Soggiu, A., Britti, D., Bonizzi, L., Roncada, P., Greco, V., 2020. Gut–brain axis and neurodegeneration: state-of-the-art of meta-omics sciences for microbiota characterization. Int. J. Mol. Sci. 21, 1–20. https://doi.org/10.3390/ijms21114045.

Tuohy, K.M., Scott, K.P., 2015. The microbiota of the human gastrointestinal tract: a molecular view. In: Diet-Microbe Interactions in the Gut: Effects on Human Health and Disease. Elsevier Inc., pp. 1–15. https://doi.org/10.1016/B978-0-12-407825-3.00001-0

Ukena, S.N., Singh, A., Dringenberg, U., Engelhardt, R., Seidler, U., Hansen, W., Bleich, A., Bruder, D., Franzke, A., Rogler, G., Suerbaum, S., Buer, J., Gunzer, F., Westendorf, A.M., 2007. Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. PLoS One 2. https://doi.org/10.1371/journal.pone.0001308.

Ułamek-Kozioł, M., Czuczwar, S.J., Januszewski, S., Pluta, R., 2019. Ketogenic diet and epilepsy. Nutrients 11. https://doi.org/10.3390/NU11102510.

Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 3. https://doi.org/10.1371/journal.pone.0002527.

Vezzani, A., Granata, T., 2005. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia. https://doi.org/10.1111/j.1528-1167.2005.00298.x.

Wang, H.-X., Wang, Y.-P., 2016. Gut microbiota-brain axis. Chin. Med. J. 129, 2373–2380. https://doi.org/10.4103/0366-6999.190667.

Wang, Y., Zhou, Y., Xiao, X., Zheng, J., Zhou, H., 2020. Metaproteomics: a strategy to study the taxonomy and functionality of the gut microbiota. J. Proteome. https://doi.org/10.1016/j.jprot.2020.103737.

Wegl, G., Grabner, N., Köstelbauer, A., Klose, V., Ghanbari, M., 2021. Toward best practice in livestock microbiota research: a comprehensive comparison of sample storage and DNA extraction strategies. Front. Microbiol. 12, 322. https://doi.org/10.3389/FMICB.2021.627539/BIBTEX.

Weinstock, G.M., 2012. Genomic approaches to studying the human microbiota. Nat. 4897415 (489), 250–256. https://doi.org/10.1038/nature11553.

Wu, G.D., Lewis, J.D., Hoffmann, C., Chen, Y.Y., Knight, R., Bittinger, K., Hwang, J., Chen, J., Berkowsky, R., Nessel, L., Li, H., Bushman, F.D., 2010. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. BMC Microbiol. 10, 15–19. https://doi.org/10.1186/1471-2180-10-206.

Xie, G., Zhou, Q., Qiu, C.Z., Dai, W.K., Wang, H.P., Li, Y.H., Liao, J.X., Lu, X.G., Lin, S.F., Ye, J.H., Ma, Z.Y., Wang, W.J., 2017. Ketogenic diet poses a significant effect on imbalanced gut microbiota in infants with refractory epilepsy. World J. Gastroenterol. 23, 6164–6171. https://doi.org/10.3748/wjg.v23.i33.6164.

Xu, L., Chen, D., Zhao, C., Jiang, L., Mao, S., Song, C., Gao, F., 2021. Decreased abundance of Akkermansia after adrenocorticotropic hormone therapy in patients with west syndrome. BMC Microbiol. 21 https://doi.org/10.1186/s12866-021-02189-z.

Yachida, S., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., Watanabe, H., Masuda, K., Nishimoto, Y., Kubo, M., Hosoda, F., Rokutan, H., Matsumoto, M., Takamaru, H., Yamada, M., Matsuda, T., Iwasaki, M., Yamaji, T., Yachida, T., Soga, T., Kurokawa, K., Toyoda, A., Ogura, Y., Hayashi, T., Hatakeyama, M., Nakagama, H., Saito, Y., Fukuda, S., Shibata, T., Yamada, T., 2019. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat. Med. 256 (25), 968–976. https://doi.org/10.1038/s41591-019-0458-7.

Yang, N.W., Kim, J.M., Choi, G.J., Jang, S.J., 2010. Development and evaluation of the quick anaero-system-a new disposable anaerobic culture system. Korean J. Lab. Med. 30, 133–137. https://doi.org/10.3343/kjlm.2010.30.2.133.

Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., Hsiao, E.Y., 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161, 264. https://doi.org/10.1016/J.CELL.2015.02.047.

Yoo, D.H., Kim, I.S., Van Le, T.K., Jung, I.H., Yoo, H.H., Kim, D.H., 2014. Gut microbiota-mediated drug interactions between lovastatin and antibiotics. Drug Metab. Dispos. 42, 1508–1513. https://doi.org/10.1124/dmd.114.058354.

Yuan, L., Zhao, J., Sun, T., Shen, Z., 2021. A machine learning framework that integrates multi-omics data predicts cancer-related LncRNAs. BMC Bioinform. 22 https://doi.org/10.1186/s12859-021-04256-8.

Zareie, M., Riff, J., Donato, K., McKay, D.M., Perdue, M.H., Soderholm, J.D., Karmali, M., Cohen, M.B., Hawkins, J., Sherman, P.M., 2005. Novel effects of the prototype translocating *Escherichia coli*, strain C25 on intestinal epithelial structure and barrier function. Cell. Microbiol. 7, 1782–1797. https://doi.org/10.1111/j.1462-5822.2005.00595.x.

Zhang, Z., Geng, J., Tang, X., Fan, H., Xu, J., Wen, X., Ma, Z., Shi, P., 2014. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. ISME J. 8, 881–893. https://doi.org/10.1038/ismej.2013.185.

Zhang, X., Li, L., Mayne, J., Ning, Z., Stintzi, A., Figeys, D., 2018a. Assessing the impact of protein extraction methods for human gut metaproteomics. J. Proteome 180, 120–127. https://doi.org/10.1016/j.jprot.2017.07.001.

Zhang, Y., Zhou, S., Zhou, Y., Yu, L., Zhang, L., Wang, Y., 2018b. Altered gut microbiome composition in children with refractory epilepsy after ketogenic diet. Epilepsy Res. 145, 163–168. https://doi.org/10.1016/j.eplepsyres.2018.06.015.

Zhang, X., Li, L., Butcher, J., Stintzi, A., Figeys, D., 2019. Advancing functional and translational microbiome research using meta-omics approaches. Microbiome 7, 1–12. https://doi.org/10.1186/s40168-019-0767-6.

Zhu, C., Solorzano, C., Sahar, S., Realini, N., Fung, E., Sassone-Corsi, P., Piomelli, D., 2011. Proinflammatory stimuli control N-acylphosphatidylethanolamine-specific phospholipase D expression in macrophages. Mol. Pharmacol. 79, 786–792. https://doi.org/10.1124/mol.110.070201.

Zhu, X., Li, B., Lou, P., Dai, T., Chen, Y., Zhuge, A., Yuan, Y., Li, L., 2021. The relationship between the gut microbiome and neurodegenerative diseases. Neurosci. Bull. https://doi.org/10.1007/s12264-021-00730-8.