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Epigenetics of the molecular clock and bacterial diversity in bipolar disorder

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ABSTRACT

Objectives The gut microbiome harbors substantially more genetic material than our body cells and has an impact on a huge variety of physiological mechanisms including the production of neurotransmitters and the interaction with brain functions through the gut-brain-axis. Products of microbiota can affect methylation according to preclinical studies. The current investigation aimed at analyzing the correlation between gut microbiome diversity and the methylation of the clock gene *ARNTL* in individuals with Bipolar Disorder (BD).

Methods Genomic DNA was isolated from fasting blood of study participants with BD (n = 32). The methylation analysis of the *ARNTL* CG site cg05733463 was performed by bisulfite treatment of genomic DNA with the Epitect kit, PCR and pyrosequencing. Additionally, DNA was extracted from stool samples and subjected to 16S rRNA sequencing. QIIME was used to analyze microbiome data.

Results Methylation status of the *ARNTL* CpG position cg05733463 correlated significantly with bacterial diversity (Simpson index: $r = -0.389$, $p = 0.0238$) and evenness (Simpson evenness index: $r = -0.358$, $p = 0.044$). Furthermore, bacterial diversity differed significantly between euthymia and depression ($F(1,30) = 4.695$, $p = 0.039$).

Discussion The results of our pilot study show that bacterial diversity differs between euthymia and depression. Interestingly, gut microbiome diversity and evenness correlate negatively with methylation of *ARNTL*, which is known to regulate monoamine oxidase A transcription. We propose that alterations in overall diversity of the gut microbiome represent an internal environmental factor that has an epigenetic impact on the clock gene *ARNTL* which is thought to be involved in BD pathogenesis.

1. Introduction

Bipolar Disorder (BD) is a mood disorder, which is characterized by recurrent manic, depressive or mixed episodes. Even though many potential pathomechanisms of BD have been identified over the last decades, it is still largely unknown how these disease mechanisms interact with each other. Neurotransmitter dysbalances, neurotrophic factor imbalances, chronic inflammation, oxidative stress and disturbed circadian rhythms have been put forward as putative underlying mechanisms (Boland et al., 2012; Andreazza et al., 2008; Berk et al., 2011; Bengesser and Reininghaus, 2013; Tsai et al., 2012; Soczynska et al., 2011; Kapczinski 2011; Bengesser et al., 2016). Various genome wide association studies (GWAS), functional re-analyses of GWAS and hypothesis driven gene association studies have identified diverse susceptibility gene groups for BD like genes encoding for ion channel subunits and associated proteins (e.g. *ANK3*, *CACNA1C*) (Sklar et al., 2008; Lett et al., 2011), adhesion and migration proteins (e.g. *NCAN*) (Cichon et al., 2011), neurodevelopment (e.g. brain derived neurotrophic factor/ BDNF) (Mueller et al., 2006), clock genes (e.g. *ARNTL*) (Le-Niculescu et al., 2008) and lithium response pathways, just to mention an excerpt of the pathways. Interestingly, the first-line mood stabilizer lithium interferes with the molecular clock and the lithium-response has been associated with clock gene variants recently (Geoffroy et al., 2016). The renowned ConLiGen consortium (Consortium on Lithium Genetics) also revealed an association of the HLA antigen and inflammation genes with lithium treatment response and

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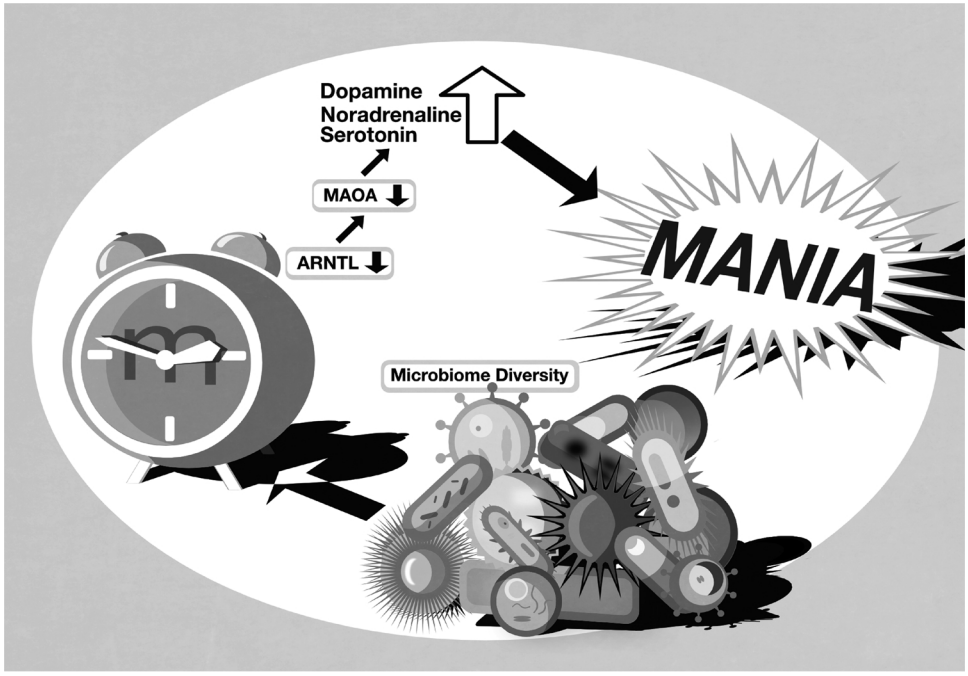

Fig. 1. Molecular Clock, Methylation, Mood and Microbiome: Fig. 1 depicts our original hypothesis about the concatenation between the microbiome of the gut, the molecular clock and the mood. The molecular circadian clock is connected with mood according to a model postulated earlier by Hampp et al., which is depicted in a simplified form in this Figure (Hampp, Ripperger et al. 2008; Hampp, Albrecht 2008). The clock gene *ARNTL* (*Aryl hydrocarbon receptor nuclear translocator-like gene*) encodes for a basic helix-loop-helix protein, which activates the transcription of *MAOA* that encodes for a neurotransmitter degrading enzyme according to *in-vitro* studies (Hampp et al., 2008; Hampp and Albrecht, 2008). Our own research group found that methylation of *ARNTL* differs significantly between patients with BD and controls (Bengesser et al., 2016). Methylation of *ARNTL* leads, according to our hypothesis, to silencing of *ARNTL* and decreased gene expression of *ARNTL*, which may result in reduced *MAOA* transcription and increased neurotransmitter levels in mania. The other way round we hypothesized that decreased methylation of *ARNTL* may lead to increased *ARNTL* gene expression and increased serotonin, noradrenaline and dopamine breakdown in depression according to our postulated model (Bengesser et al., 2016). Interestingly, methylation of DNA can be affected by metabolic products of microbiota (Shimizu, 2017). Thus, we suggested in our model that gut-microbiota affect methylation of *ARNTL*. The Fig. was designed by W. Krasser.

an inverse association of the polygenic score for schizophrenia with the lithium-response phenotype (Geoffroy et al., 2016; International Consortium on Lithium Genetics et al. 2018). Summarized, an orchestra of risk genes is mediating the vulnerability for BD. The interplay between this orchestra of risk gene variants and multiple gene-environment interactions is thought to mediate the disease outbreak. Even though heritability is as high as 80%, the gene-environment-interactions necessary to trigger affective episodes remain to be identified (Bengesser and Reininghaus, 2013).

Recently, the gut microbiome has been envisaged as a mediating disease mechanism in BD (Evans et al., 2017; Dickerson 2017; Rios et al., 2017). This is not surprising, because the gut microbiome includes more genetic material than our body cells and has an impact on a variety of physiological mechanisms including the production of neurotransmitters and the interaction with brain functions through the gut-brain-axis (Luczynski et al., 2016; Kelly 2017). The term “bacterial diversity" (alpha-diversity) refers to how many different species are present in a sample (within-sample diversity). The more different species there are present in one sample, the higher is the alpha-diversity in the gut (Evans et al., 2017). The Simpson index is a measure of bacterial diversity (alpha diversity). Besides species richness, the distribution of bacterial species (evenness) is of importance to characterize the diversity of a sample. Simpson’s evenness index is more sensitive to species evenness (Evans et al., 2017; Flowers et al., 2017).

To our best knowledge, there are only three original studies investigating the gut microbiome in individuals with BD (Evans et al., 2017; Flowers et al., 2017; Painold et al., 2018). Evans et al. compared the gut microbiota of patients with BD and healthy controls (HC). They found higher abundances of *Faecalibacterium*, and concluded that a higher representation is associated with a healthier state. In BD individuals, the representation of *Faecalibacterium* was related to better self-reported health outcomes (Evans et al., 2017). Flowers et al. reported on BD patients with or without atypical antipsychotics. Decreased species diversity was found in treated female patients compared to other females (Flowers et al., 2017). Our research group (Painold et al., 2018) found a negative correlation between microbial alpha-diversity and illness duration in BD. Further, we identified the phylum *Actinobacteria* (LDA = 4.82, P = 0.007) and the class *Coriobacteria* as significantly more abundant in BD when compared with HC, and *Ruminococcaceae* and *Faecalibacterium* as more abundant in HC when compared with BD individuals (Painold et al., 2018).

Epigenetic changes of human DNA and altered gene expression mediate gene-environment interactions and are putative mechanisms of the gut-brain-axis. Epigenetic modification of DNA, e.g. methylation of cytosines in CG rich regulatory targets, results from diverse gene-environment-interactions and regulates gene expression. Methylation of CG rich elements (CpG islands) in the 5′regulatory region of genes leads to silencing of transcription (Knippers, 2001). Epigenetic studies are still rare in the field of BD. Epigenome-wide association studies (EWAS) are even rare in the broad field of psychiatry. The first epigenome-wide methylation analysis in prefrontal cortical brain tissue of individuals with major psychosis (BD n = 35, Schizophrenia n = 35) by Mill and colleagues found epigenetic changes in loci of cell proliferation-, brain development-, glutamatergic- and GABAergic pathways (Mill et al., 2008). Epigenome-wide methylation analyses in peripheral blood of study participants are rare and contain only small sample sizes (Yongsheng et al. 2015). Nevertheless, there are rare hypothesis-driven methylation analyses that focused on a limited number of genes such as *KCNQ3*, *COMT*, *HTR1A*, *HTR2A*, *SERT* and *ARNTL* (Bengesser et al., 2016; Ghadirivasfi et al., 2011; Nohesara et al., 2011; Carrard et al., 2011, Sugawara 2011; Abdolmaleky et al., 2006). Specifically, the clock gene *ARNTL (Aryl hydrocarbon receptor nuclear translocator-like gene)* was hypermethylated at cg05733463 in study participants with BD compared to healthy controls (Bengesser et al., 2016). The CG site cg05733463 is associated with activating epigenetic marks in the UCSC genome browser (Kent et al., 2002), which underlines its function as a putative regulatory element of gene expression (11).

Epigenetic changes of *ARNTL* are of special interest in BD, because *ARNTL* encodes for a transcription factor of monoamine oxidase A (*MAOA*) according to a model postulated by Hampp et al. (Hampp et al., 2008; Hampp and Albrecht, 2008), which based on their *in-vitro* studies about the connection between the molecular clock and mood (see Fig.1). Thus, circadian rhythms and neurotransmitter degradation by *MAOA* are crosslinked and gene expression changes of *ARNTL* may

influence mood swings (Hampp et al., 2008; Hampp and Albrecht, 2008).

Several gene-environment interactions could principally influence the methylation of *ARNTL*. Among these, metabolites of the gut microbiome receive increasing interest as epigenetic factors regulating the gut-brain-axis (Stilling et al., 2014; Mischke and Plosch, 2016, Carbonero, 2017). Metabolites of the microbiota as well as nutrients processed in the gut can affect gene regulation according to previous literature. For example, polyphenols can affect epigenetic alterations (DNA methylation or histone acetylation) by interacting with the enzymes responsible for those epigenetic changes (Shimizu, 2017). Thus, there seems to be a concatenation between the gut microbiome and epigenetic gene regulation.

For this reason, the current pilot study explored a putative correlation between gut microbiome diversity and *ARNTL* DNA methylation in individuals with BD. We hypothesized that the diversity of the gut microbiome reflects an internal environmental factor that has an epigenetic impact on the clock gene *ARNTL* known to be affected in BD patients.

2. Materials and methods

2.1. Study participants

The study cohort included Caucasian Austrian individuals with BD (n = 32). The clinical characteristics of the study participants are depicted in Table 2. Recruited study participants with BD had been former in- or outpatients at the Department of Psychiatry and Psychotherapeutic Medicine of the Medical University of Graz. The diagnosis of BD was based on DSM-IV criteria and was verified by Standardized Clinical Interview for DSM-IV/ SCID-I. Patients had given written informed consent and were legally competent during their participation in the study. The study had been approved by the local ethics committee (Medical University of Graz) in compliance with the current revision of the Declaration of Helsinki, ICH guideline for Good Clinical Practice and current regulations (EK-number of the Austrian BIPFAT and BIPGEN study: 24–123 ex 11/12 and 23–199 ex 10/11). Exclusion criteria were the presence of chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, neurodegenerative and neuroinflammatory disorders (i.e. Alzheimer's, Huntington's, Parkinson's disorder and multiple sclerosis), haemodialysis and interferon-α-based immunotherapy. Further exclusion criteria were antibiotic or antifungal treatment in the previous two months, consumption of probiotics or prebiotics in the preceding two months, pregnancy and period of breastfeeding, inflammatory syndromes of the digestive tract, drug or alcohol abuse, and history of gastrointestinal surgery (other than appendectomy).

2.2. Microbiota analysis

2.2.1. DNA isolation and PCR amplification

Analysis of the gut microbiome was performed as described in the recently published original report (Painold et al., 2018). Stool samples were collected on the day of blood taking and the stool samples were immediately frozen at −20 °C before microbiome analysis with 16S rRNA sequencing. DNA was isolated from stool samples of study participants with BD (n = 32) with the PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc, CA, USA). DNA concentration was measured with Picogreen fluorescence. The V1–V2 region of the 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR). The primers GATTGCCAGCAGCCGCGGTAA and GGACTACCAGGGTATCT AAT and the Mastermix 16S Complete PCR Kit (Molzym, Bremen, Germany) were used for the PCR. The first PCR product was introduced to a second cycle of PCR with primers fusing the 16S primer sequence to the A and P adapters, required for Ion Torrent sequencing. A barcode sequence was included to multiplex up to 96 samples. Agarose gel electrophoresis was performed with the PCR products. Then the QIAquick (Qiagen, Hilden, Germany) gel extraction system was used for purification of the band with the expected length of 330 nucleotides. The concentration of the final PCR product was measured with Picogreen fluorescence.

2.2.2. Sequencing

Amplicons were pooled equimolarly and emulsion PCR was performed using the Ion Torrent One Touch 2.0 Kit (CatNr: 4480974, Thermo Fisher Scientific, MA, USA) according to manufacturer's protocols (Ion PGM Template OT2 200 Kit User Guide, CatNr: 4480974, Thermo Fisher Scientific). Afterwards the beads were isolated on the Ion ES station and loaded onto Ion Torrent 318 chips. Sequencing was done with the Ion Torrent PGM using the Ion 400BP Sequencing Kit (Thermo Fisher Scientific, MA, USA). Splitting of the sequences was performed by the barcode system and transferred to the Torrent suite server. Unmapped bam files were used as input for bioinformatics analysis.

2.2.3. Phylogenic analysis and bioinformatics

Phylogenic analysis and bioinformatics quality control was performed with DeconSeq (Schmieder and Edwards, 2011), Acacia tool (Bragg et al., 2012), Usearch algorithm (Edgar, 2010) and QIIME 1.8 (Caporaso et al., 2010).

2.2.4. Statistical analysis and visualization

Species richness, an indicator of how many different species are present in stool sample, was calculated with Simpson index and evenness with QIIME 1.8. Data were rarified to 8000 sequences per sample. All remaining statistical calculations were performed in IBM SPSS Statistics Version 22. Bioinformatics analysis was performed as described previously by Mörkl et al. (2017). Raw sequences were filtered for contaminating and artificial sequence fragments by DeconSeq (Schmieder and Edwards, 2011) and USEARCH (Edgar, 2010). Chimeric and low quality sequences were identified and removed using Acacia (Bragg et al., 2012). Phylogenetic analysis of the remaining high quality sequences was performed using QIIME 1.8 (Caporaseo et al. 2010). Finally, core diversity workflow of QIIME was used to compute the core set of diversity analysis.

2.3. Methylation analysis of ARNTL

Peripheral fasting blood was taken at 8:30 a.m. at the Department of Psychiatry and Psychotherapeutic Medicine of the Medical University of Graz. DNA isolation was performed with the salting out method from lymphocytes of patients with BD (n = 32) at the Diagnostic and Research Institute of Human Genetics of the Medical University of Graz. The DNA concentration was measured with NanoDrop© spectrometry.

Methylation of the CG site cg05733463 of the clock gene *ARNTL* was measured at the Institute of Neuropathology of the University of Bonn (Germany). Methylation status was analyzed by bisulfite treatment of DNA, PCR and pyrosequencing. Sodium bisulfite treatment was performed with the Epitect Bisulfite kit® (Qiagen) according to manufacturer's guidelines (2012). A DNA fragment containing the CG site cg05733463 was amplified from sodium bisulfite-converted DNA using the primers presented in Table 1. PyroMarkGold Reagents (Qiagen) were used for pyrosequencing and the Pyromark Q24 instrument (Qiagen) was used according to the manufacturer's instructions (2012). Pyrogram outputs were analyzed with the PyroMarkQ24 software and methylation of *ARNTL* was measured as described previously (Bengesser et al., 2016).

2.4. Statistics

2.4.1. Description of the sample and general results

The robust ANCOVA model (independent factor: affective phase,

Table 1
Primers used for the CG rich regions (manufactured by Biolegio®, Germany; designed by A. Waha). PS…pyrosequencing, PCR… Polymerase Chain Reaction, f…forward, r…reverse, BIOT…biotinylated.

PRIMERS			
CG rich position	**Primer ID**	Primer Sequence	Method
cg05733**463**	cg05733463-f	GGGAATTGTTTTTTTGGTTGTAGT	PCR
	cg05733463-r	CCCACAACACAAAATATTAATCAT (BIOT.)	PCR
	cg05733463-ps	TGTTTTTTTGGTTGTAGTTTAA	PS

dependent factor: bacterial diversity or evenness and covariates: age and sex) was used to analyze differences of bacterial diversity or evenness between depression (n = 13) and euthymia (n = 19). Similarly, differences of the epigenetic marker between both affective episodes were investigated with this model. Differences of the mentioned target parameters (bacterial diversity, bacterial evenness or methylation of *ARNTL*) between male (n = 25) and female study participants (n = 7) were also analyzed with the ANCOVA design with the covariate age. Homogeneity of the sample was measured with the Levene's test. Spearman correlation analysis was used to analyze correlations between clinical parameters (age, BDI, BMI, HAMD, YMRS) and bacterial diversity or methylation of *ARNTL*. The results are depicted in Table 2.

2.4.2. Correlation between bacterial diversity and methylation of ARNTL

SPSS version 22.0 was used for statistical analyses. The Kolmogorov–Smirnov test and the Shapiro-Wilk normality test showed that we had to reject the normality assumption. The correlation between the microbiome diversity marker Simpson Index and methylation of *ARNTL* was therefore analyzed with Spearman correlation. Similarly, Spearman correlation was used to analyze the correlation between the gut microbiome evenness marker Simpson evenness index and methylation of *ARNTL*.

3. Results

3.1. General description of the sample

Alpha-diversity measured by the Simpson index differed significantly between individuals with the current state of depression (n = 13) and euthymia (n = 19) in the current sample of study participants with BD ($F(1,30) = 4.695$, $p = 0.039$, Partial Eta$^2 = 0.144$). Descriptive statistics (mean average and standard deviation) is visualized in Fig. 2. In contrast, the methylation marker of *ARNTL* did not differ between depression and euthymia ($F(1,30) = 0.023$, $p = 0.881$, Partial Eta$^2 = 0.001$). Further general results and the clinical characteristics of the sample are depicted in Table 2. Additionally, the distribution of the Simpson index (A), Simpson evenness (B) and Methylation of *ARNTL* (C) in study participants with BD is shown in Fig. 3. The correlation between Simpson diversity index and the methylation marker of *ARNTL* is depicted in Fig. 4.

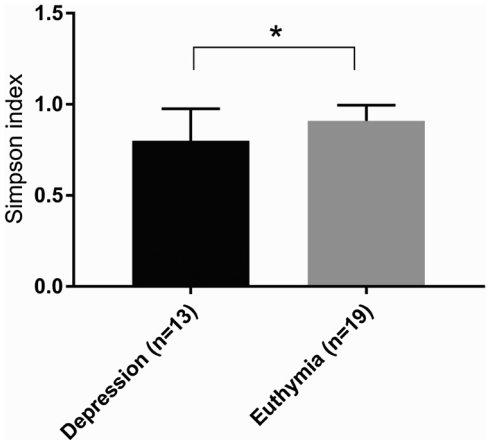

Fig. 2. Descriptive Statistics (mean average and standard deviation) of microbiome diversity in affective states of Bipolar Disorder: Simpson index (bacterial alpha-diversity) in depression and euthymia of study participants with Bipolar Disorder. The Simpson index is a measure of how many different bacterial species are present in the gut. * $p < 0.05$ (analyzed with ANCOVA with age and sex as covariates).

3.2. Correlation between ARNTL methylation and microbiome diversity

The methylation status (in %) of the *ARNTL* CpG position cg05733463 correlated significantly with bacterial diversity (Simpson index: $r = -0.389$, $p = 0.0238$) and evenness (Simpson evenness index: $r = -0.358$, $p = 0.044$) in individuals with BD (see Fig. 4).

Table 2
Characteristics of the study participants with Bipolar Disorder.

Characteristics of the study participants with Bipolar Disorder			
Clinical parameter	Descriptive statistics (M +/- SD)	Correlation or ANCOVA of clinical parameter with bacterial diversity marker	Correlation or ANCOVA of clinical parameter and Methylation marker of ARNTL
Study participants with BD	n = 32		
Depression (HAMD ≥ 10)	n = 13		
Euthymia (HAMD < 10)	n = 19	$F(1,30) = 4,695$, $p = 0.039$*	$F(1,30) = 0.023$, $p = 0.881$
Females with BD	n = 7	$F(1,31) = 0.031$, $p = 0.861$	$F(1,31) = 0.239$, $p = 0.629$
Males with BD	n = 25		
Age (average +/- SD)	41.67 (+/- 17.51)	$r = -0.341$, $p = 0.056$	$r = -0.046$, $p = 0.804$
BDI (average +/- SD)	14.73 (+/- 10.05)	$r = -0.283$, $p = 0.130$	$r = 0.251$, $p = 0.181$
HAMD (average +/- SD)	7.00 (+/- 4.35)	$r = -0.275$, $p = 0.128$	$r = 0.029$, $p = 0.875$
YMRS (average +/- SD)	0.81 (+/- 1.75)	$r = 0.038$, $p = 0.836$	$r = -0.119$, $p = 0.515$
BMI (average +/- SD)	27.99 (+/- 6.45)	$r = 0.108$, $p = 0.556$	$r = -0.165$, $p = 0.368$

Abbreviations: BD…Bipolar DisorderHAMD… Hamilton Depression Score, YMRS… Young Mania Rating Scale, BMI… Body Mass Index [kg/m^2]BDI… Beck's depression inventory scale, M… mean, SD…Standard Deviation. Statistics: ANCOVAs with the covariates age and sex were used to test differences between groups. Spearman's correlation was used to analyze correlations between clinical parameters (agedepression scale marker: BDI and HAMDmania scale marker: YMRSand BMI) and bacterial diversity or methylation of the ARNTL CG site cg05733463. Significant results ($p < 0.05$ are marked with * and are written in bold. The Simpson evenness marker did neither differ between affective episodes nor between females and males. Additionally, the evenness marker did not correlate with the clinical markers (age, BMI, BDI, HAMD and YMRS). For a better readability of the table, those results are not depicted here.

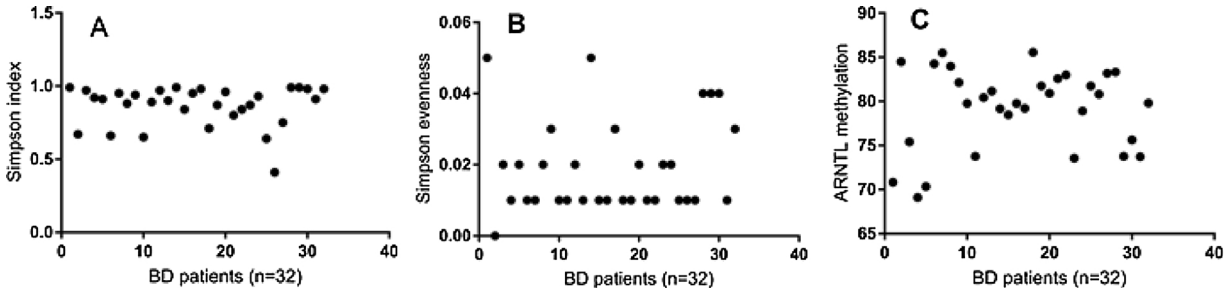

Fig. 3. Visualization of sample characteristics: Simpson index, Simpson evenness and Methylation of *ARNTL* in study participants with Bipolar Disorder (BD). The Simpson index is a measure of how many different bacterial species are present in the gut. The Simpson evenness index describes how evenly the species in the sample are distributed. The clock gene *ARNTL* (*Aryl hydrocarbon receptor nuclear translocator-like gene*) encodes for a basic helix-loop-helix protein, which activates the transcription of *MAOA* that encodes for a neurotransmitter degrading enzyme.

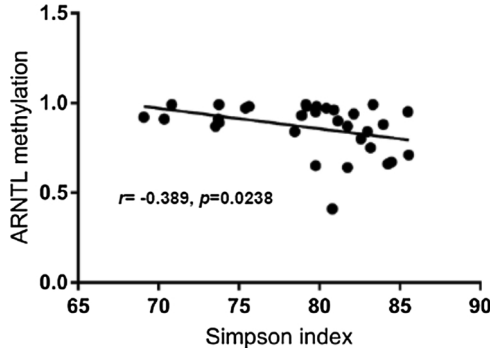

Fig. 4. Epigenetics of the clock gene ARNTL and gut microbiome diversity: Correlation between Methylation of *ARNTL* [%] and Simpson index.

4. Discussion

To the best of our knowledge, this is the first pilot study to show a correlation between CpG methylation status of the clock gene *ARNTL* and gut microbiome diversity as well as gut microbiome evenness in BD. Specifically, the methylation status at cg05733463 of the clock gene *ARNTL* correlated negatively with bacterial diversity and evenness: the lower the gut microbiome diversity, the higher the methylation. Via this mechanism, low microbiome diversity may lead to decreased *ARNTL* and *MAOA* gene expression and consequently to decreased breakdown and a pro-manic effect according to our postulated model (see Fig. 1).

The negative correlation between gut microbiome diversity and methylation of the *ARNTL* cg-site cg05733463 is of particular interest. According to the UCSC genome browser, this special position shows an activation of epigenetic marks such as histone acetylation (under physiological conditions), which underlines its important role as a regulatory element. An altered methylation at this site is likely to have an impact on the gene expression of *ARNTL* and, consequently, of other genes that are regulated by *ARNTL* (the original hypothesis is depicted in Fig. 1). It is of particular note that the clock gene *ARNTL* encodes a transcription factor of *MAOA*. Epigenetic silencing of *ARNTL* will therefore reduce MAOA activity according to our model (Bengesser et al., 2016) and, due to impaired degradation, enhance monoamine neurotransmitter levels (Hampp et al., 2008; Hampp and Albrecht, 2008). It can thus be envisaged that *ARNTL* gene expression and monoamine neurotransmitter degradation/activity are crosslinked with each other and have a bearing on mood swings typical for BD (Bengesser et al., 2016; Hampp 2008; Hampp and Albrecht, 2008).

Our study results about decreased bacterial diversity and evenness in depressive states of BD go in line with previous literature (Flowers et al., 2017; Nguyen et al., 2018; Painold et al., 2018), which was summarized in a recent review of Nguyen et al., highlighting distinct alterations of the gut microbiome in BD individuals (Nguyen et al., 2018). For instance, BD was shown to be associated with a reduced microbial diversity, and global community differences were found in comparison to non-psychiatric samples. However, the gut microbial diversity and evenness and subsequently gut microbiota metabolites could be connected to specific states of BD such as depression, euthymia and mania, which were not investigated in the studies so far as the current state of BD patients was not taken into account (Flowers, Evans et al., 2017; Evans et al., 2017). Nevertheless, this may be a crucial factor as the gut microbiome is of a highly dynamic nature and is also exhibiting daily cyclical fluctuations in composition (Zarrinpar et al., 2014; Evans 2017). These cyclical fluctuations may be of special relevance in the pathogenesis of BD (Maruani et al., 2018; Morgan 2013). Also fluctuations in circadian rhythms like reduced sleep, which is common in mania and depression, may explain unpredictable changes in the molecular clock. Furthermore, illness duration of BD was found to be inversely correlated with bacterial diversity (Painold et al., 2018), which could also be because of long-term alterations of the gut microbiota by psychopharmacological therapy (Morgan, Anderson et al. 2013). Additionally, gut microbiome diversity is associated with diverse environmental factors such as unbalanced diet, lack of physical activity, inflammation and oxidative stress, which could also influence epigenetic regulation (Xu and Knight, 2015; Alam et al., 2017; Morkl et al., 2017; Shimizu, 2017).

In our study, BD individuals with depression showed slightly lower microbiome diversity in comparison to euthymic individuals. Nevertheless, mood scores themselves (HAMD, BDI and YMRS) did neither correlate with the Simpson index nor with the Simpson evenness marker. The significantly decreased microbiome diversity in depression goes in line with the current literature depicting general microbiome alterations during depression (Winter et al., 2018). Nevertheless, the result is contradicting our expectation according to our postulated theory about the connection between the gut microbiome, the molecular clock and mood (see Fig.1). According to the negative correlation between Simpson index and the methylation of *ARNTL*, low gut-microbiome diversity should principally lead to high methylation of *ARNTL* and silencing, which should principally lead to decreased *ARNTL* and *MAOA* transcription (leading to low neurotransmitter breakdown and increased levels of serotonin, dopamine and noradrenalin). Nevertheless, the functional effect of *ARNTL* methylation on neurotransmitter degradation must be evaluated in more detail in future studies, because neurotransmitters are also degraded by the enzyme COMT (catechol-O-methyltransferase). Gene expression of *MAOA* could also be altered by epigenetic changes of *MAOA* itself, which has not yet been analyzed in BD (Bengesser and Reininghaus, 2013). Furthermore, patients with current states of mania must be recruited to evaluate our hypothesis completely. Based on decreased compliance in mania, the acquisition of stool samples from hypomanic BD patients takes time. Therefore, this report presents preliminary results of study participants with depression and euthymia.

Additionally, further research is absolutely necessary to investigate

metabolites of gut-bacteria in the stool samples or in the serum, because the metabolites themselves affect epigenetic changes in human DNA. Metabolites of gut-microbiota may even influence the permeability of the blood-brain-barrier (Logsdon et al., 2018). Thus, we suggest that besides gut microbiome diversity, either a high diversity of specific genera or specific gut microbial metabolites may be associated with mood swings. Bacterial diversity alone might not act as a main indicator for different mood states in BD disorder and to account for a "healthy state" of the gut microbiome. Alpha-diversity must be seen as a context-dependent parameter and not as a stand-alone indicator for gut dysbiosis. Specific bacterial metabolites could act as epigenetic regulators. Those metabolites may be of higher importance than diversity and evenness alone (Shimizu, 2017). Subsequently, this highlights the future need of the investigation of the gut-microbiota metabolites with modern methods of the prospering field of metabolomics.

The correlation between gut microbial diversity and *ARNTL* methylation may reflect one putative gene-environment concatenation between the gut microbiome and the pathomechanisms of BD. This could be another implication of the brain-gut-axis thought to play a role in a number of neuropsychiatric disorders (Stilling et al., 2014). We therefore suggest that the microbiome interacts with the circadian clock by inducing epigenetic changes in genes present in peripheral blood.

This study gives first results about a putative connection between the gut-microbiota and gene regulation of a major player of the molecular clock, which could putatively affect mood.

5. Limitations

This pilot study was performed in a rather small study sample, which included only study participants with BD in depressive and euthymic states. Hypomanic and manic study participants are not present in the current pilot study, because the compliance of participation is lower in the state of hypomania or mania and the patients have to give written informed consent in euthymia before participation in mania. Another limitation is the fact that depression was only investigated in patients with BD and not in unipolar depression. The latter will be investigated in further studies in the future. Methylation analysis was performed in peripheral blood and not in brain tissue based on ethical reasons. Nevertheless, previous literature indicates that the blood is a good mirror of ongoing mechanisms in the brain. BD patients remained on their usual pharmacotherapy (antidepressants and mood stabilizers) and as this was a naturalistic pilot-study, on their usual dietary patterns. Metabolic products of gut bacteria such as short chain fatty acids were not measured in this study.

6. Conclusion

Our results provide the first evidence that gut microbiome diversity and evenness correlate negatively with epigenetic alterations of the clock gene *ARNTL*, specifically methylation at the CpG site cg05733463. This site has been identified as a regulative target and *ARNTL* transcription correlates positively with *MAOA* transcription. The current findings open a new avenue of research by suggesting that alterations in gut microbiome represent an internal environmental factor that has an epigenetic impact on the clock gene *ARNTL*. Further studies are necessary to examine whether this relationship has a bearing on the pathomechanisms of BD.

Conflict of interest

I have no conflicts of interests to declare.

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References

Abdolmaleky, H.M., Cheng, K.H., Faraone, S.V., Wilcox, M., Glatt, S.J., Gao, F., Smith, C.L., Shafa, R., Aeali, B., Carnevale, J., Pan, H., Papageorgis, P., Ponte, J.F., Sivaraman, V., Tsuang, M.T., Thiagalingam, S., 2006. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. Hum. Mol. Genet. 15 (21), 3132–3145.

Alam, R., Abdolmaleky, H.M., Zhou, J.R., 2017. Microbiome, inflammation, epigenetic alterations, and mental diseases. Am. J. Med. Genet. B: Neuropsychiatric Genet. 174 (6), 651–660.

Andreazza, A.C., Kauer-Sant'anna, M., Frey, B.N., Bond, D.J., Kapczinski, F., Young, L.T., Yatham, L.N., 2008. Oxidative stress markers in bipolar disorder: a meta-analysis. J. Affect. Disord. 111 (2-3), 135–144.

Bengesser, S., Reininghaus, E., 2013. Genetics Of Bipolar Disorder. Peter-Lang.

Bengesser, S.A., Reininghaus, E.Z., Lackner, N., Birner, A., Fellendorf, F.T., Platzer, M., Kainzbauer, N., Tropper, B., Hormanseder, C., Queissner, R., Kapfhammer, H.P., Wallner-Liebmann, S.J., Fuchs, R., Petek, E., Windpassinger, C., Schnalzenberger, M., Reininghaus, B., Evert, B., Waha, A., 2016. Is the molecular clock ticking differently in bipolar disorder? Methylation analysis of the clock gene ARNTL. World J. Biol. Psychiatry 1–9.

Berk, M., Kapczinski, F., Andreazza, A.C., Dean, O.M., Giorlando, F., Maes, M., Yucel, M., Gama, C.S., Dodd, S., Dean, B., Magalhaes, P.V., Amminger, P., Mcgorry, P., Malhi, G.S., 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci. Biobehav. Rev. 35 (3), 804–817.

Boland, E.M., Bender, R.E., Alloy, L.B., Conner, B.T., Labelle, D.R., Abramson, L.Y., 2012. Life events and social rhythms in bipolar spectrum disorders: an examination of social rhythm sensitivity. J. Affect. Disord. 139 (3), 264–272.

Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P., Tyson, G.W., 2012. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nat. Methods 9 (5), 425–426.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., Mcdonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.

Carbonero, F., 2017. Human epigenetics and microbiome: the potential for a revolution in both research areas by integrative studies. Future Sci. OA 3 (3), FSO207–2017-0046 eCollection 2017 Aug.

Carrard, A., Salzmann, A., Malafosse, A., Karege, F., 2011. Increased DNA methylation status of the serotonin receptor 5HTR1A gene promoter in schizophrenia and bipolar disorder. J. Affect. Disord. 132 (3), 450–453.

Cichon, S., Muhleisen, T.W., Degenhardt, F.A., Mattheisen, M., Miro, X., Strohmaier, J., Steffens, M., Meesters, C., Herms, S., Weingarten, M., Priebe, L., Haenisch, B., Alexander, M., Vollmer, J., Breuer, R., Schmal, C., Tessmann, P., Moebus, S., Wichmann, H.E., Schreiber, S., Muller-Myhsok, B., Lucae, S., Jamain, S., Leboyer, M., Bellivier, F., Etain, B., Henry, C., Kahn, J.P., Heath, S., Bipolar Disorder Genome Study (Bigs) Consortium, Hamshere, M., O'donovan, M.C., Owen, M.J., Craddock, N., Schwarz, M., Vedder, H., Kammerer-Ciernioch, J., Reif, A., Sasse, J., Bauer, M., Hautzinger, M., Wright, A., Mitchell, P.B., Schofield, P.R., Montgomery, G.W., Medland, S.E., Gordon, S.D., Martin, N.G., Gustafsson, O., Andreassen, O., Djurovic, S., Sigurdsson, E., Steinberg, S., Stefansson, H., Stefansson, K., Kapur-Pojskic, L., Oruc, L., Rivas, F., Mayoral, F., Chuchalin, A., Babadjanova, G., Tiganov, A.S., Pantelejeva, G., Abramova, L.I., Grigoroiu-Serbanescu, M., Diaconu, C.C., Czerski, P.M., Hauser, J., Zimmer, A., Lathrop, M., Schulze, T.G., Wienker, T.F., Schumacher, J., Maier, W., Propping, P., Rietschel, M., Nothen, M.M., 2011. Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. Am. J. Hum. Genet. 88 (3), 372–381.

Dickerson, F., Severance, E., Yolken, R., 2017. The microbiome, immunity, and schizophrenia and bipolar disorder. Brain Behav. Immun. 62, 46–52.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than blast. Bioinformatics 26 (19), 2460–2461.

Evans, S.J., Bassis, C.M., Hein, R., Assari, S., Flowers, S.A., Kelly, M.B., Young, V.B., Ellingrod, V.E., Mcinnis, M.G., 2017. The gut microbiome composition associates with bipolar disorder and illness severity. J. Psychiatr. Res. 87, 23–29.

Flowers, S.A., Evans, S.J., Ward, K.M., Mcinnis, M.G., Ellingrod, V.L., 2017. Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. Pharmacotherapy: J. Hum. Pharmacol. Drug Therapy 37 (3), 261–267.

Geoffroy, P.A., Etain, B., Lajnef, M., Zerdazi, E.H., Brichant-Petitjean, C., Heilbronner, U., Hou, L., Degenhardt, F., Rietschel, M., Mcmahon, F.J., Schulze, T.G., Jamain, S., Marie-Claire, C., Bellivier, F., 2016. Circadian genes and Lithium response in bipolar disorders: associations with Ppargc1a (Pgc-1alpha) and rora. Genes Brain Behav. 15 (7), 660–668.

Ghadirivasfi, M., Nohesara, S., Ahmadkhaniha, H.R., Eskandari, M.R., Mostafavi, S., Thiagalingam, S., Abdolmaleky, H.M., 2011. Hypomethylation of the serotonin

receptor type-2A Gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. Am. J. Med. Genet. B: Neuropsychiatric Genet. 156 (5), 536–545.
Hampp, G., Albrecht, U., 2008. The circadian clock and mood-related behavior. Commun. Integr. Biol. 1 (1), 1–3.
Hampp, G., Ripperger, J.A., Houben, T., Schmutz, I., Blex, C., Perreau-Lenz, S., Brunk, I., Spanagel, R., Ahnert-Hilger, G., Meijer, J.H., Albrecht, U., 2008. Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. Curr. Biol.: CB 18 (9), 678–683.
International Consortium On Lithium Genetics (Conli + Gen), Amare, A.T., Schubert, K.O., Hou, L., Clark, S.R., Papiol, S., Heilbronner, U., Degenhardt, F., Tekola-Ayele, F., Hsu, Y.H., Shekhtman, T., Adli, M., Akula, N., Akiyama, K., Ardau, R., Arias, B., Aubry, J.M., Backlund, L., Bhattacharjee, A.K., Bellivier, F., Benabarre, A., Bengesser, S., Biernacka, J.M., Birner, A., Brichant-Petitjean, C., Cervantes, P., Chen, H.C., Chillotti, C., Cichon, S., Cruceanu, C., Czerski, P.M., Dalkner, N., Dayer, A., Del Zompo, M., Depaulo, J.R., Etain, B., Falkai, P., Forstner, A.J., Frisen, L., Frye, M.A., Fullerton, J.M., Gard, S., Garnham, J.S., Goes, F.S., Grigoroiu-Serbanescu, M., Grof, P., Hashimoto, R., Hauser, J., Herms, S., Hoffmann, P., Hofmann, A., Jamain, S., Jimenez, E., Kahn, J.P., Kassem, L., Kuo, P.H., Kato, T., Kelsoe, J., Kittel-Schneider, S., Kliwicki, S., Konig, B., Kusumi, I., Laje, G., Landen, M., Lavebratt, C., Leboyer, M., Leckband, S.G., Tortorella, A., Manchia, M., Martinsson, L., Mccarthy, M.J., Mcelroy, S., Colom, F., Mitjans, M., Mondimore, F.M., Monteleone, P., Nievergelt, C.M., Nothen, M.M., Novak, T., O'donovan, C., Ozaki, N., Osby, U., Pfennig, A., Potash, J.B., Reif, A., Reininghaus, E., Rouleau, G.A., Rybakowski, J.K., Schalling, M., Schofield, P.R., Schweizer, B.W., Severino, G., Shilling, P.D., Shimoda, K., Simhandl, C., Slaney, C.M., Squassina, A., Stamm, T., Stopkova, P., Maj, M., Turecki, G., Vieta, E., Volkert, J., Witt, S., Wright, A., Zandi, P.P., Mitchell, P.B., Bauer, M., Alda, M., Rietschel, M., Mcmahon, F.J., Schulze, T.G., Baune, B.T., 2018. Association of polygenic score for schizophrenia and HLA antigen and inflammation genes with response to Lithium in bipolar affective disorder: a genome-wide association study. JAMA Psychiatry 75 (1), 65–74.
Kapczinski, F., Dal-Pizzol, F., Teixeira, A.L., Magalhaes, P.V., Kauer-Sant'anna, M., Klamt, F., Moreira, J.C., De Bittencourt Pasquali, M.A., Fries, G.R., Quevedo, J., Gama, C.S., Post, R., 2011. Peripheral biomarkers and illness activity in bipolar disorder. J. Psychiatr. Res. 45 (2), 156–161.
Kelly, J.R., Minuto, C., Cryan, J.F., Clarke, G., Dinan, T.G., 2017. Cross talk: the microbiota and neurodevelopmental disorders. Front. Neurosci. 11, 490.
Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., Haussler, D., 2002. The human genome browser at UCSC. Genome Res. 12 (June (6)), 996–1006.
Knippers, R, 2001. Molekulare Genetik 8, pp. 1-586. Thieme.
Le-Niculescu, H., Mcfarland, M.J., Ogden, C.A., Balaraman, Y., Patel, S., Tan, J., Rodd, Z.A., Paulus, M., Geyer, M.A., Edenberg, H.J., Glatt, S.J., Faraone, S.V., Nurnberger, J.I., Kuczenski, R., Tsuang, M.T., Niculescu, A.B., 2008. Phenomic, convergent functional genomic, and biomarker studies in a stress-reactive genetic animal model of bipolar disorder and co-morbid alcoholism. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B (2), 134–166.
Lett, T.A., Zai, C.C., Tiwari, A.K., Shaikh, S.A., Likhodi, O., Kennedy, J.L., Mueller, D.J., 2011. Ank3, Cacna1c and ZNF804A gene variants in bipolar disorders and psychosis subphenotype. World J. Biol. Psychiatry 12 (August (5)), 392–397. https://doi.org/10.3109/15622975.2011.564655. Epub 2011 Jul 18.
Logsdon, A.F., Erickson, M.A., Rhea, E.M., Salameh, T.S., Banks, W.A., 2018. Gut reactions: how the blood-brain barrier connects the microbiome and the brain. Exp. Biol. Med. (Maywood) 243 (January (2)), 159–165. https://doi.org/10.1177/1535370217743766. Epub 2017 Nov 23.
Luczynski, P., Mcvey Neufeld, K.A., Oriach, C.S., Clarke, G., Dinan, T.G., Cryan, J.F., 2016. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int. J. Neuropsychopharmacol. 19 (8). https://doi.org/10.1093/ijnp/pyw020. Print 2016 Aug.
Maruani, J., Anderson, G., Etain, B., Lejoyeux, M., Bellivier, F., Geoffroy, P.A., 2018. The neurobiology of adaptation to seasons: relevance and correlations in bipolar disorders. Chronobiol. Int. 1–19.
Mill, J., Tang, T., Kaminsky, Z., et al., 2008. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am. J. Hum. Genet. 82 (3), 696–711. https://doi.org/10.1016/j.ajhg.2008.01.008.
Mischke, M., Plosch, T., 2016. The gut microbiota and their metabolites: potential implications for the host epigenome. Adv. Exp. Med. Biol. 902, 33–44.
Morgan, X.C., Segata, N., Huttenhower, C., 2013. Biodiversity and functional genomics in the human microbiome. Trends Genet. 29 (1), 51–58.
Morkl, S., Lackner, S., Muller, W., Gorkiewicz, G., Kashofer, K., Oberascher, A., Painold, A., Holl, A., Holzer, P., Meinitzer, A., Mangge, H., Holasek, S., 2017. Gut microbiota and body composition in anorexia nervosa inpatients in comparison to athletes, overweight, obese, and normal weight controls. Int. J. Eat. Disord. 50 (12), 1421–1431.
Mueller, D.J., De Luca, V., Sicard, T., King, N., Strauss, J., Kennedy, J.L., 2006. Brain-derived neurotrophic factor (BDNF) gene and rapid-cycling bipolar disorder: family-based association study. Br. J. Psychiatry 189 (October), 317–323.
Nohesara, S., Ghadirivasfi, M., Mostafavi, S., Eskandari, M.R., Ahmadkhaniha, H., Thiagalingam, S., Abdolmaleky, H.M., 2011. DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. J. Psychiatr. Res. 45 (11), 1432–1438.
Nguyen, T.T., Kosciolek, T., Eyler, L.T., Knight, R., Jeste, D.V., 2018. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. J. Psychiatr. Res. 99, 50–61.
Painold, A., Moerkl, S., Kashofer, K., Halwachs, B., Dalkner, N., Bengesser, S., Birner, A., Fellendorf, F., Platzer, M., Queissner, R., Schütze, G., Schwarz, G., Moll, N., Holzer, P., Holl, Ak., Kapfhammer, Hp., Gorkiewicz, G., Reininghaus, E.Z., 2018. A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. Bipolar Disord. 26 (July). https://doi.org/10.1111/bdi.12682.
Rios, A.C., Maurya, P.K., Pedrini, M., Zeni-Graiff, M., Asevedo, E., Mansur, R.B., Wieck, A., Grassi-Oliveira, R., Mcintyre, R.S., Hayashi, M.A.F., Brietzke, E., 2017. Microbiota abnormalities and the therapeutic potential of probiotics in the treatment of mood disorders. Rev. Neurosci. 28 (7), 739–749.
Schmieder, R., Edwards, R., 2011. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. PLoS One 6 (3) e17288.
Shimizu, M., 2017. Multifunctions of dietary polyphenols in the regulation of intestinal inflammation. J. Food Drug Anal. 25 (January (1)), 93–99. https://doi.org/10.1016/j.jfda.2016.12.003. Epub 2016 Dec 27.
Sklar, P., Smoller, J.W., Fan, J., Ferreira, M.A., Perlis, R.H., Chambert, K., Nimgaonkar, V.L., Mcqueen, M.B., Faraone, S.V., Kirby, A., De Bakker, P.I., Ogdie, M.N., Thase, M.E., Sachs, G.S., Todd-Brown, K., Gabriel, S.B., Sougnez, C., Gates, C., Blumenstiel, B., Defelice, M., Ardlie, K.G., Franklin, J., Muir, W.J., Mcghee, K.A., Macintyre, D.J., Mclean, A., Vanbeck, M., Mcquillin, A., Bass, N.J., Robinson, M., Lawrence, J., Anjorin, A., Curtis, D., Scolnick, E.M., Daly, M.J., Blackwood, D.H., Gurling, H.M., Purcell, S.M., 2008. Whole-genome association study of bipolar disorder. Mol. Psychiatry 13 (6), 558–569.
Soczynska, J.K., Kennedy, S.H., Woldeyohannes, H.O., Liauw, S.S., Alsuwaidan, M., Yim, C.Y., Mcintyre, R.S., 2011. Mood disorders and obesity: understanding inflammation as a pathophysiological nexus. Neuromolecular Med. 13 (2), 93–116.
Stilling, Rm, Dinan, Rg, Cryan, Jf, 2014. Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. Genes Brain Behav. 13 (January (1)), 69–86. https://doi.org/10.1111/gbb.12109.
Sugawara, H., Iwamoto, K., Bundo, M., Ueda, J., Miyauchi, T., Komori, A., Kazuno, A., Adati, N., Kusumi, I., Okazaki, Y., Ishigooka, J., Kojima, T., Kato, T., 2011. Hypermethylation of serotonin transporter gene in bipolar disorder detected by epigenome analysis of discordant monozygotic twins. Transl. Psychiatry 1, e24.
Tsai, S.Y., Chung, K.H., Wu, J.Y., Kuo, C.J., Lee, H.C., Huang, S.H., 2012. Inflammatory markers and their relationships with leptin and insulin from acute mania to full remission in bipolar disorder. J. Affect. Disord. 136 (1-2), 110–116.
Winter, G., Hart, R.A., Charlesworth, R.P.G., Sharpley, C.F., 2018. Gut microbiome and depression: what we know and what we need to know. Rev Neurosci. 29 (August (6)), 629–643. https://doi.org/10.1515/revneuro-2017-0072.
Xu, Z., Knight, R., 2015. Dietary effects on human gut microbiome diversity. Br. J. Nutr. (113 Suppl), S1–5.
Zarrinpar, A., Chaix, A., Yooseph, S., Panda, S., 2014. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metab. 20 (6), 1006–1017.