European Psychiatry 53 (2018) 37–45

Contents lists available at ScienceDirect

European Psychiatry

journal homepage: http://www.europsy-journal.com

Original article

Gut microbiome and magnetic resonance spectroscopy study of subjects at ultra-high risk for psychosis may support the membrane hypothesis

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ARTICLE INFO

Article history:
Received 28 February 2018
Received in revised form 7 May 2018
Accepted 25 May 2018
Available online xxx

Keywords:
Schizophrenia
Ultra-high risk
Microbiome
Magnetic resonance spectroscopy

ABSTRACT

Background: The microbiota-gut-brain axis and membrane dysfunction in the brain has attracted increasing attention in the field of psychiatric research. However, the possible interactive role of gut microbiota and brain function in the prodromal stage of schizophrenia has not been studied yet.
Methods: To explore this, we collected fecal samples and performed Magnetic Resonance Spectroscopy (MRS) scans in 81 high risk (HR) subjects, 19 ultra-high risk (UHR) subjects and 69 health controls (HC). Then we analyzed the differences in gut microbiota and choline concentrations in the anterior cingulate cortex (ACC).
Results: Presences of the orders *Clostridiales*,*Lactobacillales* and *Bacteroidales* were observed at increase levels in fecal samples of UHR subjects compared to the other two groups. The composition changes of gut microbiota indicate the increased production of Short Chain Fatty Acids (SCFAs), which could activate microglia and then disrupt membrane metabolism. Furthermore, this was confirmed by an increase of choline levels, a brain imaging marker of membrane dysfunction, which is also significantly elevated in UHR subjects compared to the HR and HC groups.
Conclusion: Both gut microbiome and imaging studies of UHR subjects suggest the membrane dysfunction in the brain and hence might support the membrane hypothesis of schizophrenia.

1. Introduction

Schizophrenia is a serious mental disorder that usually develops in late adolescence or early adulthood. Characterized by delusions, hallucinations and cognitive impairments, schizophrenia affects nearly 1% of the world's population [1]. In genetic analysis, monozygotic twins studies only show around 50% concordance rate which strongly suggests that genetic basis alone is insufficient in explaining the development of schizophrenia [2]. Therefore, this prompts the investigation of environmental risk factors. Notable environmental factors include perinatal complications, neuro-trauma, psycho-trauma, substance abuse, and migration among others. However, one of the most important and immediate environmental factor is the gut microbiota [3]. The human gut microbiome comprises of bacteria on the order of trillions in magnitude with between 3 and 10 million unique genes, dwarfing the human genome by a ratio of 150:1 [4–7]. This massive genetic repertoire which, unlike the human genome, is influenced by medication, probiotic or prebiotic supplementation or lifestyle changes, provides a means to better understand a variety of diseases and also to create low-cost rapid therapies with minimal side-effects.

The concept of the microbiota-gut-brain axis was proposed nearly a decade ago [8]. Considerable evidence now supports that

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http://dx.doi.org/10.1016/j.eurpsy.2018.05.011
0924-9338/

there is a bidirectional interaction between the nervous system and the enteric microbiota. Four main routes have been suggested: neural, endocrine, immune, and metabolic pathways [9,10].

Besides the relatively new microbiota-gut-brain axis concept, the origin of schizophrenia has many classic hypotheses, such as the dopamine (DA) hypothesis [11], serotonin (5-HT) hypothesis [12], glutamate hypothesis [13] and membrane hypothesis [14]. Thus, what are the relationships between microbiota-gut-brain axis and hypotheses of schizophrenia? The microbiota-gut-brain axis could be related to the classic neurotransmitter hypotheses due to the fact that microbes produce essential neurotransmitters such as DA, 5-HT and norepinephrine [15]. In fact, more than 90% of human 5-HT is produced in the gut where the microbiome promotes its synthesis and regulation [16,17]. In contrast, the role of the microbiota-gut-brain axis in the membrane hypothesis of schizophrenia is still unknown. The membrane hypothesis of schizophrenia is not only the most inclusive and promising hypothesis but also the biochemical basis of neurodevelopmental disorders. The core of the membrane hypothesis is that reduced synthesis and increased degradation of cell membranes, and the subsequent abnormal signal transmission are the causes of schizophrenia [14,18]. Furthermore, the membrane hypothesis also suggests that the schizophrenia is part of a systemic disease of membrane dysfunction instead of an independent brain disease, which happens to fit the key point of microbiota-gut-brain axis [14]. However, unlike the classic neurotransmitter hypotheses, the relationship with microbiota-gut-brain axis and membrane hypothesis is still lack of research. In order to fill this research gap, we simultaneously measured the composition of gut microbiota and the marker of cell membrane disruption in brain, which is choline [19]. As Schwarz et.al and Shen et.al have already conducted the gut microbiome study of first episode psychosis and schizophrenia [20,21], we proposed to study the gut microbiota and brain imaging features of schizophrenia-afflicted individuals by following the high-risk (HR) and ultra-high risk (UHR) subjects, thereby exploring the relationships of microbiota-gut-brain axis and membrane hypothesis as well as shedding light on a new perspective for schizophrenia research. Here, we report the baseline results of our longitudinal prospective observational study.

2. Methods

2.1. Participants

Eighty-one HR subjects, nineteen UHR subjects and sixty-nine health controls (HC) were recruited from November 2016 to May 2017 at the Second Xiangya Hospital, Central South University, Changsha, Hunan, China. All participants were 13–30 years old Han Chinese; the demographic characteristics are detailed in Table 1.

All HCs and HRs were screened for lifetime absence of all five-axis diagnoses of mental disorders using the DSM-IV-TR criteria [22]. In addition, all HCs were confirmed to have no family history of psychiatric disorder in their first-degree relatives. By contrast, the HRs were confirmed to have at least one of their first-degree relatives diagnosed with schizophrenia. All UHR subjects were screened by the Structured Interview for Prodromal Syndromes (SIPS) [23] and fulfilled one of the three subsets: Brief Intermittent Psychotic Syndrome (BIPS), Attenuated Positive Symptom Syndrome (APSS) and Genetic Risk and Deterioration Syndrome (GRDS). Exclusion criteria for all subjects included: gastrointestinal and endocrine diseases (including constipation and diarrhea), serious organ disorders (such as stroke and heart failure), a history of diagnosis of psychiatric disorders and corresponding treatments (such as antipsychotics, antidepressants and anticonvulsants), had used alcohol, antibiotics, probiotics or any other medications (oral or injectable) during the last three months.

This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University (No. S090, 2016) and carried out in accordance with the Declaration of Helsinki. All participants were aware of the risks and benefits of the study and signed informed consent forms. Thirty-seven HCs did not agree with the Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) scanning, but they all had completed the clinical assessment and fecal sample collection. And there were no statistical differences of characteristics between the HCs which agreed to brain scanning and those who did not (see Table S1 in Supplementary material).

2.2. Clinical assessment

Both HRs and UHRs completed the Scale of Prodromal Symptoms (SOPS) for screening of schizophrenic symptoms and were assessed with the Global Assessment of Function Scale, Modified Version (GAF-M) to evaluate their psychological, social, and occupational functionalities [24]. The SOPS is a 19-item scale, a part of the SIPS and includes four subscales for positive, negative, disorganized and general symptoms.

2.3. Fecal DNA extraction and sequencing

All samples were collected using Swube^{TM} dual swabs and stored at −80 °C for further processing. Each 0.2 g of raw sample was used for DNA extraction with QIAGEN QIAamp kit. We evaluated DNA concentration in each sample by fluorometry and

Table 1
General characteristics of the recruited subjects.

Variable	HR (n = 81)	UHR (n = 19)	HC (n = 69)	Statistic value (ChiSq or W)	*p*-value
Age, years (mean ± S.D.)	21.67 ± 5.75	20.47 ± 4.57	23.13 ± 3.89	4.56	0.103
Gender (male, female)	41 M, 40F	15 M, 4F	37 M, 32F	5.08	0.079
P score (mean ± S.D.) **	0.81 ± 1.48	11.47 ± 6.76	–	24.5	<0.001
N score (mean ± S.D.) **	1.8 ± 3.79	10.26 ± 5.13	–	120	<0.001
D score (mean ± S.D.) **	0.63 ± 1.23	4.89 ± 4.48	–	186	<0.001
G score (mean ± S.D.) **	0.95 ± 1.35	4.37 ± 3.52	–	300.5	<0.001
GAF score (mean ± S.D.) **	84.17 ± 6.45	58.89 ± 11.73	–	1453.5	<0.001

HR: high-risk patients; UHR: ultra-high risk patients; HC: health controls; ChiSq: Chi-square; M: male; F: female; P: positive symptom; N: negative symptom; D: disorganized symptom; G: general symptom; GAF: Global Assessment of Function Scale.
** The difference between the groups is significant with a *p*-value less than 0.01.