Epilepsy Research 147 (2018) 102–107

Contents lists available at ScienceDirect

Epilepsy Research

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

Altered composition of the gut microbiome in patients with drug-resistant epilepsy

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ARTICLE INFO

Keywords:
Drug-resistant epilepsy
Epilepsy
Microbiome
Microbiota
Ruminococcus

ABSTRACT

Objective: The relationship between the gut microbiota and the central nervous system has been gradually recognized while whether microbiome plays a role in the pathogenesis of drug-resistant epilepsy is still unknown. The aim of our work was to explore whether dysbiosis is involved in the mechanism of drug-resistant epilepsy.
Methods: Patients with epilepsy attending West China Hospital of Sichuan University were enrolled from March to May 2017. Patients were grouped into drug-resistant (n = 42) and drug-sensitive (n = 49) groups, another 65 healthy controls were from the same families of the patients. The fecal samples were collected and the microbiome composition was analyzed by high-throughout sequencing of the 16s ribosomal DNA.
Results: We found that the gut microbial community of drug-resistant epilepsy was significantly altered with an abnormal increased abundance of rare flora. While the gut microbiome composition of drug-sensitive epilepsy was similar with that of healthy controls. Specifically, patients with four seizures per year or fewer showed an increase of Bifidobacteria and Lactobacillus than those with more than four seizures per year.
Conclusions: Dysbiosis may be involved in the mechanism of drug-resistant epilepsy and restoring the gut microbial community may be a novel therapeutic method for drug-resistant epilepsy.

1. Introduction

Epilepsy is a chronic central nervous system disease affecting more than 70 million people worldwide. Nearly 30-40% of epilepsy patients are pharmacoresistant to two or more types of antiepileptic drugs, which is known as drug-resistant epilepsy and causes a great burden to their families and society (de Boer et al., 2008; Tang et al., 2017). Although new drugs have been developed, the treatment methods are still limited since the specific mechanisms remain largely unknown (Tang et al., 2017).

In recent years, a growing number of studies have found that the gut microbiome is closely connected with the central nervous system, and dysbiosis has been reported in many central nervous system (CNS) diseases such as Parkinson's disease (Scheperjans et al., 2015), multiple sclerosis (Mowry and Glenn, 2018) and Alzheimer disease (Zhan et al., 2016). The microbiome regulates the CNS through metabolites, neurotransmitters and inflammatory factors. The CNS, in turn, regulates the gut microbiome through the vagus nerve or hormone axis. The interaction between the gut microbiome and the CNS is called the gut-microbial-brain axis (Tremlett et al., 2017). However, whether the gut microbiome plays a role in the pathogenesis of drug-resistant epilepsy is still unknown. In this study, we analyzed the microbiome composition by high-throughput sequencing of the bacterial 16 s ribosomal DNA (rDNA) and found that dysbiosis might be involved in the pathogenesis of drug-resistant epilepsy.

2. Methods

2.1. Study participants and sample collection

The study was approved by the Ethics Committee of the West China Hospital of Sichuan University. A total of 91 patients (5 to 50 years) with epilepsy attending West China Hospital of Sichuan University were enrolled from March to May 2017. Forty-two of them were drug-resistant, and 49 of them were drug-sensitive. All patients met the 1981 ILAE criteria for epilepsy, and the drug-resistant epilepsy was defined according to the 2015 ILAE (Kwan et al., 2010). Sixty-five healthy controls were from the same families of the patients and had the same eating habits. Participants were excluded if they used antibiotics or probiotics within 3 months or had a known history of chronic disease.

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https://doi.org/10.1016/j.eplepsyres.2018.09.013
Received 21 May 2018; Received in revised form 7 September 2018; Accepted 22 September 2018
Available online 24 September 2018
0920-1211/

Clinical information including gender, age, seizure frequency, seizure types and antiepileptic drugs used were registered prospectively. Fecal samples were frozen immediately and transferred to a −80 °C freezer within 24 h.

2.2. Gene sequencing

Total DNA was extracted from the samples (100 mg, wet weight) using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA) following the recommended protocol of the manufacturer. A NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) and agarose gel electrophoresis was used for quality control.

The 16S rDNA gene amplification and sequencing was performed by the Realbio Genomics Institute (Shanghai, China). The primers F341 (5′-3′ ACTCCTACGGGRSGCAGCAG) and R806 (5′-3′GGACTACVVGGGTATCTAATC) were used for amplification of the V3-V4 regions of the bacterial 16S rDNA gene. PCR was performed on the GS FLX + system following the manufacturer's recommendation (Roche, Mannheim, Germany) (Ren et al., 2016). The cycling started with 3 min at 94 °C followed by 30 cycles of denaturation (94 °C for 3 min), annealing (57 °C for 45 s), extension (72 °C for 1 min) and a final extension at 72 °C for 2 min. Samples were then collected and quality-controlled by electrophoresis of a 1% agarose gel. The amplicons were then sequenced on the Illumina HiSeq PE250 platform.

2.3. Bioinformatics processing

Merged sequences were obtained by matching paired-end raw sequences obtained from the HiSeq platform, and raw data were then subjected to the quality control procedure using Quantitative Insights Into Microbial Ecology (QIIME) software (version 1.9.0) (Giloteaux et al., 2016). Briefly, the sequences with an average quality score less than 20, more than one ambiguous base, less than 220 bps or more than 500 bps were filtered out. Chimeric sequences were also filtered out from the dataset. All remaining sequences were then clustered to operational taxonomic units (OTUs) at 97% sequence similarity after singleton removal. Every OTU was represented by the reads with the most abundance and assigned taxonomically using the RDP classifier (Sun et al., 2017). The OTUs were rarefied, and 40,884 sequences per sample were used in subsequent analyses.

The within-sample diversity (i.e., α-diversity) was calculated by the following parameters: Chao1 index, PD index, Shannon index, and Simpson index. Distance matrices (i.e., β-diversity) were calculated by both the weighted and unweighted UniFrac distance metrics and visualized by a principal coordinate analysis (PCoA). UniFrac uses phylogenetic information to compare community differences between the samples. Weighted UniFrac takes the abundance of sequences into account, whereas unweighted UniFrac does not. The Wilcox.test R function was used for statistical comparisons of two groups, and the Kruskal.test R function was used for comparisons among more than two groups.

A linear discriminant effect size analysis (LEfSe) was used at different taxonomical levels to find the genera representing different groups. LEfSe combined both the statistical significance (Kruskal test and Wilcox test) and linear discriminate analysis and measured the magnitude of differentiation between groups. The threshold of the logarithmic linear discriminant analysis (LDA) score for discriminative features was 2.0.

A phylogenetic investigation of the communities by reconstruction of the unobserved states (PICRUSt) on Qiime was used to infer metagenomic information from the 16S rDNA sequences. Data were then entered into the HMP unified metabolic analysis network (HUMAnN) to find the significant differences in the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog (KO) abundances (Thompson et al., 2015).

Table 1
Characteristics of the study population.

	DR (n = 42)	EP (n = 49)	HC (n = 65)
Age (y, mean)	28.4 ± 12.4	25.1 ± 14.6	29.4 ± 13.8
Gender			
Female	23 (54.8%)	24 (49.0%)	33 (50.8%)
Male	19 (45.2%)	25 (51.0%)	32 (49.2%)
Disease duration (y, mean)[a]	11.5 ± 9.3	4.8 ± 5.2	
Seizure types (n, percentage)[a]			–
Generalized	8 (19.0%)	16 (32.7%)	–
Partial	4 (9.5%)	7 (14.3%)	–
Multiple forms	30 (71.4%)	27 (55.1%)	–
Frequency (seizures per year, (n, percentage)[a]			
0-4	8 (19.0%)	41 (83.7%)	–
5-12	17 (40.5%)	9 (18.4%)	–
13-52	14 (33.3%)	0	–
> 52	6 (14.3%)	1 (2.0%)	–
Medication (n, percentage)			
Oxcarbazepine	18 (42.9%)	18 (36.7%)	–
Levetiracetam	16 (38.1%)	17 (34.7%)	–
Valproate	12 (28.6%)	10 (23.8%)	–
Topiramate	15 (35.7%)	6 (12.2%)	–
Carbamazepine	7 (16.7%)	4 (8.2%)	–
Lamotrigine	5 (11.9%)	4 (8.2%)	–
Clonazepam	7 (16.7%)	0	–
Phenobarbital	4 (9.5%)	1 (2.0%)	–
Phenytoin	1 (2.3%)	0	–
Withdrawal > 3 years	0	4 (8.2%)	–

[a] $P < 0.05$. Data was reported as means ± SD For continuous variable, and percentage for non-parametric data.

3. Results

3.1. Study population characteristics

A total of 42 patients with drug-resistant epilepsy (DR), 49 patients with drug-sensitive epilepsy (DS) and 65 healthy controls (HC) were enrolled. To reduce the impact of diet on the gut flora, the healthy controls all came from the patients' families. The average age and sex ratio were similar among the three groups. However, the average disease duration, seizure frequency, and patients with multiple seizure types were significantly higher in the DR group. Oxcarbazepine was the most frequently used drug in both group, followed by Levetiracetam and Valproate (Table 1).

3.2. Subjects with drug-resistant epilepsy harbor an altered bacterial gut microbiota

After extraction from the fecal samples, DNA was amplified by PCR, and 16S rDNA sequences of the gut microbiome were obtained by high-throughput sequencing. An average of 59,794 ± 3191 clean reads were obtained per sample and clustered into OTUs under the similarity of 0.97. The α-diversity analyses showed that both community richness (measured by the Chao1 index and PD index) and evenness (measured by the Shannon index and Simpson index) were increased in the DR group than in the HC and DS groups (Fig. 1), and they were similar between the HC and DS groups. The following clinical parameters were also tested: age, gender, disease duration, and seizure frequency. The results showed that the α-diversity of the patients with 4 seizures or fewer per year were similar to that of HC while the patients with more than 4 seizures showed significantly higher α-diversity. Other clinical parameters appeared to have no effect on the α-diversity of the microbial community. The β-diversity analyses showed that the microbiome community of the samples in the DR group was different from that of the DS group (Fig. 1).

The overall gut microbiome composition was similar between the HC and DS groups at the phylum level with Bacteroidetes being the largest phylum (56.7 % in DS and 57.2% in HC) and Firmicutes being