The detailed supplement of method

Supplementary methods S1.

S1. Metagenomic analysis

S1.1 Experimental procedure

Total DNA was extracted from a participant's stool using a Stool DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. For library preparation, DNA samples were fragmented by sonication to a size of 350 bp, then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina (NEBNext® Ultra™ DNA Library Prep Kit) sequencing with further PCR amplification. PCR products were purified (AMPure XP system) and libraries were analyzed for size distribution by Agilent2100 Bioanalyzer and quantified using real-time PCR (Bio-Rad CFX96). For sequencing, the clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Novaseq 6000 sequencing platform and 150 bp paired-end reads were generated.

S1.2 Sequencing results pretreatment

Preprocessing the raw data obtained from the Illumina Novaseq 6000 sequencing platform was conducted using Readfq (V8, https://github.com/cjfields/readfq) to acquire the clean data for subsequent analysis. Considering the possibility of host pollution in samples, clean data underwent blast comparison with the host database, using Bowtie 2.2.4 software to filter the reads of host origin. (Bowtie2.2.4, http://bowtie-bio.sourceforge.net/bowtie2/index.shtml)

S1.3 Metagenome assembly

All the reads not used in the forward step of all samples were combined and then software SOAPdenovo (V2.04)/MEGAHIT (v1.0.4-beta) was used for mixed assembly; Scaftigs were obtained by breaking the mixed assembled Scaffolds from the N connection. Fragments shorter than 500 bp were filtered in all Scaftigs, generated from single or mixed assembly, for statistical analysis.

S1.4 Gene prediction and abundance analysis

Using Meta Gene Mark (V2.10, http://topaz.gatech.edu/GeneMark/) software, the Scaftigs (> 500 bp) assembled from both single and mixed assembly, the predicted open reading frame was filtered for length information shorter than 100 nucleotides [1,2] from the predicted results. For predicted open reading frames, CD-HIT software (V4.5.8, http://www.bioinformatics.org/cd-hit) [3] was adopted to remove redundancy and obtain the unique initial gene catalogue. Then, the clean data of each sample was mapped to the initial gene catalogue using Bowtie2.2.4[4,5]. Gene with reads < 2 in each sample were filtered to obtain the gene catalogue (Unigenes) eventually used for subsequently analysis. Finally, statistic the abundance information was determined based on the number of mapped reads and the length of the gene.

S1.5 Taxonomy and functional database annotation

DIAMOND software (V0.9.9, https://github.com/bbuchfink/diamond/)[6] was used to blast the Unigenes to sequences of Bacteria, Fungi, Archaea and viruses extracted from the NR database (Version:2018-01-02, https://www.ncbi.nlm.nih.gov/) of NCBI. For the finally aligned results of each sequence, as each sequence may have multiple aligned results, we selected those with an e value < the smallest e value *10 to take the Lowest Common Ancestor algorithm, which was applied to system classification of the MEGAN software [7] to confirm the species annotation information of sequences. A table containing the number of genes and the abundance information of each sample in each taxonomy hierarchy (kingdom, phylum, class, order, family, genus, species) were obtained based on the Lowest Common Ancestor annotation result and the gene abundance table. The abundance of a species in one sample was equal to the sum of the gene abundance annotated for the species; the gene number of a species in a sample equaled the number of genes whose abundance were nonzero.

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2. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59-65.

3. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics. 2012;28:3150-2.

4. Qin N, Yang F, Li A, Prifti E, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513:59-64.

5. Li J, Jia H, Cai X, Zhong H, et al. MetaHIT Consortium; Bork P, Wang J; MetaHIT Consortium. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 2014 ;32:834-41.

6. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods 2015;12:59-60.

7. Huson DH, Mitra S, Ruscheweyh HJ, et al. Integrative analysis of environmental sequences using MEGAN4. Genome Res 2011;21:1552-60.

Supplementary methods S2.

S2. High-throughput targeted metabolomics

S2.1 Metabolite Extractions

To extract metabolites from samples of serum, 400 μL of cold methanol/acetonitrile (1:1, v/v) extraction solvent was added to remove protein and extract the metabolites, then adequately vortexed. For absolute quantification of the metabolites, stock solutions of stable-isotope internal standards were simultaneously added to the extraction solvent. The mixture was collected into a new centrifuge tube, and centrifuged at 14,000 g for 20 min at 4°C, then the supernatant collected and dried in a vacuum centrifuge. For LC-MS analysis, the samples were re-dissolved in 100 μL acetonitrile/water (1:1, v/v) solvent and centrifuged at 14,000 g at 4°C for 20 min, then the supernatant was injected.

S2.2 LC-MS Analysis

Analyses were performed using an UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a QTRAP MS (6500, Sciex) in Shanghai Applied Protein Technology Co., Ltd.. The analytes were separated on HILIC (Waters UPLC BEH Amide column, 2.1 mm × 100 mm, 1.7 µm) and C18 columns (Waters UPLCBEH C18-2.1 × 100 mm, 1.7 µm). The sample was placed at 4°C during the whole analysis process. 6500 QTRAP (AB SCIEX) was performed in positive and negative switch mode. Multiple reaction monitoring was used for mass spectrometry quantitative data acquisition. The multiple reaction monitoring ion pairs are showed in the attached file. Pooled quality control (QC) samples were set in the sample queue to evaluate the stability and repeatability of the system.
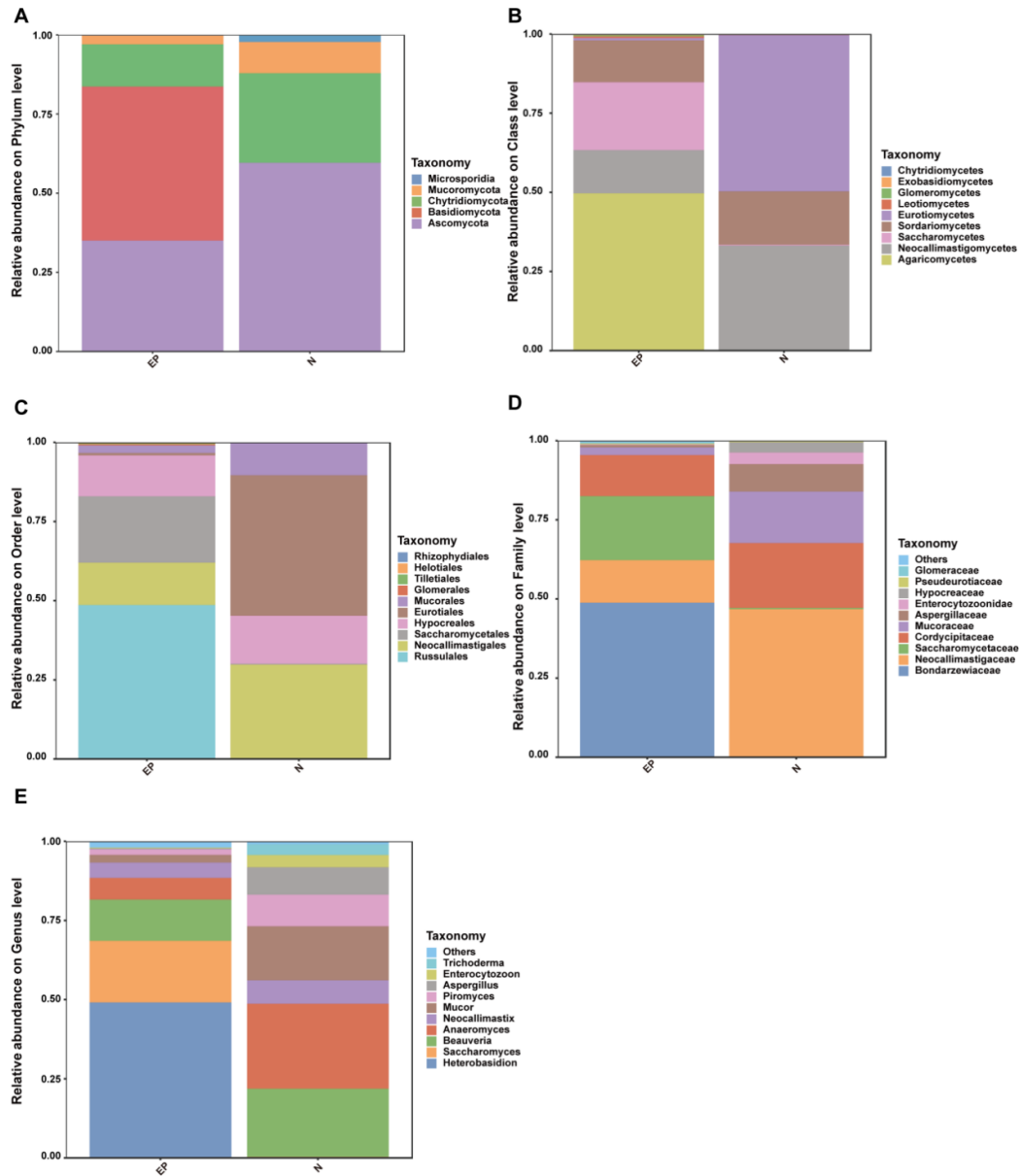
S2.3 Data process

MultiQuant or Analyst was used for quantitative data processing. The QCs were processed together with the biological samples. Metabolites in QCs with coefficient of variation less than 30% were denoted as reproducible measurements.

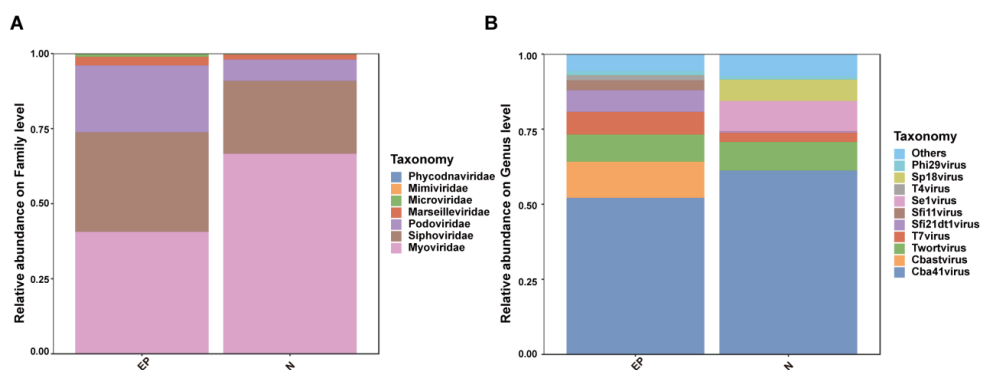**Supplementary Table S1.** The top 10 gut flora with the largest abundance in each group on phylum level.

Phylum name	Relative abundance in normal controls	Relative abundance in patients with epilepsy
Firmicutes	0.60630642	0.52199392
Actinobacteria	0.08658387	0.13697363
Bacteroidetes	0.16683237	0.18056470
Proteobacteria	0.03164099	0.04043603
Verrucomicrobia	0.00178457	0.00077334
Euryarchaeota	0.00064403	5.32E-05
Chlamydiae	0.00071584	0.00097614
Fusobacteria	7.36E-05	0.00017660
Candidatus Melainabacteria	0.00048153	0.00039289
Others	0.10417114	0.11674667

Supplementary Table S2. STAMP differential analysis of bacterial populations between the groups at the species level.

species	EP_Mean Rel(%)	N_Mean Rel(%)	Difference between means	P-value	95% lower CI	95% upper CI
Roseburia faecis	0.1777623	0.588362	-0.391588	0.001302	-0.764073	-0.141043
Roseburia intestinalis	0.106482	0.239969	-0.090185	0.001544	-0.131031	-0.049076
Roseburia sp. CAG:18_43_25	0.030389	0.134759	-0.088991	0.000523	-0.193601	-0.036792
Roseburia sp. CAG:18	0.026377	0.100742	-0.062562	0.001302	-0.135463	-0.024793
Butyrivibrio crossotus	0.007165	0.017402	-0.011650	2.04E-05	-0.015245	-0.006594
Lachnospiraceae bacterium TF01-11	0.005922	0.017114	-0.009638	0.000763	-0.015364	-0.004094
Roseburia sp. CAG:197_41_10	0.007075	0.011279	-0.007094	0.000121	-0.012541	-0.002653
[Bacteroides] pectinophilus	0.007176	0.012863	-0.006121	0.002149	-0.009466	-0.002640
Firmicutes bacterium CAG:95	0.009398	0.011148	-0.004788	0.002951	-0.008141	-0.001516
Roseburia sp. CAG:197	0.021185	0.007431	-0.004607	0.001824	-0.007188	-0.001951
Pseudobutyrivibrio ruminis	0.001843	0.006236	-0.002337	0.002149	-0.004296	-0.001015
Roseburia sp. CAG:100	0.003947	0.004679	-0.001874	0.004639	-0.002957	-0.000788
Roseburia sp. CAG:10041_57	0.004548	0.004052	-0.001827	0.005362	-0.003171	-0.000500
Providencia rettgeri	0.000832	0.000404	-0.000185	0.001756	-0.000441	-7.16E-05
Nocardia farcinica	0	0.000051	-8.51E-05	0.002089	-6.94E-05	0

Supplementary Figure S1. The abundance of eukaryotic communities in epilepsy patients at the phylum (A), class (B), order (C), family (D), and genus (E) levels.

Supplementary Figure S2. The abundance of viruses communities in epilepsy patients at the family (A), and genus (B)levels.

Supplementary Table S3. An overview of the metabolomic analysis outcome.

Epileptic patients vs Normal controls	179		amino acids and their derivatives accounted for 25.7%, lipids and their derivatives occupied 22.9%, and bile acids accounted for 17.9%
Serum differential metabolites			
Name	**Class**	**Foldchange**	**pvalue**
10E-Heptadecenoic acid	Fatty acids	1.466107729	0.030036481
10Z-Heptadecenoic acid	Fatty acids	1.455198774	0.048485219
11Z,14Z,17Z-Eicosatrienoic Acid	Fatty acids	1.385919482	0.025153048
11Z-Eicosenoic Acid	Fatty acids	1.66645794	0.008213937
2-Furoic acid	Furoic acid	1.520111901	0.027457927
7Z,10Z,13Z,16Z-Docosatetraenoic acid	Fatty acids	1.63224567	0.004419807
Asparagine	Amino acids	0.887036696	0.035906721
Beta-Alanine	Amino acids	0.852283414	0.041615514
Cystine	Amino acids	1.212246679	0.016297136
Elaidic Acid	Fatty acids	1.611798657	0.009907898
Gama-glutamylalanine	Amino acids	0.773775403	0.01422619
Glyceric acid	Carbohydrates	0.849017823	0.006330989
Kynurenine	Amino acids	0.815099109	0.003400228
Lignoceric Acid	Fatty acids	0.806846508	0.041914392
Methionine	Amino acids	0.886094457	0.043876598
Oleic acid	Fatty acids	1.588997149	0.01231258
Oxoadipic acid	Keto acids	1.837210554	0.008072284
Palmitoleic Acid	Fatty acids	1.75637843	0.032708483
Petroselinic acid	Fatty acids	1.579018116	0.010435385
Ribonic acid	Carbohydrates	0.767521672	0.002197188
Tryptophan	Amino acids	0.75719594	4.11096E-05