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Research Article

Cohort Profile: A Prospective Study of Gut Microbiota in Patients with Acute Ischemic Stroke

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Emerging evidence highlights the role in the gut microbiota (GM), integral parts of the gut-brain axis, and plays in developing various complications in patients with acute ischemic stroke (AIS). The link between the GM and disease can be actively utilized in the diagnosis of poststroke complications. AIS-GM cohort is a prospective study focusing on the link between gut microbial signature and adverse outcomes in patients with AIS. From September 2020 to July 2021, a total of 507 AIS patients were enrolled, their clinical baseline data and faeces samples during hospitalization were collected, and poststroke outcomes were evaluated by a variety of questionnaires. At present, 395 faeces samples of AIS patients completed were collected, analyzed the composition of microbiota, and tracked the prognosis and neuropsychiatric complications of AIS patients. After the patient was discharged, the out-of-hospital follow up was conducted on the 90th days, then repeated once a year, which included the collections of fecal and blood samples and measurements of poststroke outcomes. AIS-GM cohort could provide an opportunity to observe the dynamic changes of GM after stroke. AIS-GM cohort contributes to deep understanding of risk factors for AIS and the relationship between GM and AIS outcomes. AIS-GM cohort can be used as a new tool to assess the prognosis of stroke and further predict the development of disease.

1. Introduction

Acute ischemic stroke (AIS) is a paroxysmal and rapidly progressing neurological dysfunction, which occurs due to intracranial artery blockage or stenosis, leading to local tissue ischemia and hypoxic necrosis, even neurological impairment and long-term disability [1]. The clinical prognosis of AIS patients varies greatly, and its adverse outcomes mainly include death, disability, and recurrence of stroke [2]. The risk factors of adverse outcomes mainly include smoking, drinking, hypertension, diabetes, dyslipidemia, and atrial fibrillation, but these factors cannot fully reveal the occurrence of adverse AIS outcomes [3–5]. Until now, no specific risk factors could be used to identify high-risk patients with poor prognosis [6]. Therefore, it is necessary to determine new prognostic biomarkers of AIS, so as to reduce the incidence of adverse outcomes of AIS.

In recent years, with the progress in research on gut microbiota-gut-brain axis, gut microbiota (GM) plays an increasingly important role in the diagnosis and treatment of neuropsychiatric diseases [7, 8]. GM could influence neuropsychiatric disease, such as Alzheimer's disease, Parkinson's disease, depression, and AIS. The correlation between ischemic stroke and GM is widely concerned [9]. A large number of evidences showed the link between GM and AIS. This association might be anything from occurrence and development of AIS to adverse outcomes. Studies of stroke patients and mice models indicated that the altered composition and diversity of GM were common phenomenon [10–12]. Singh et al. reported that after stroke, mice

appeared by GM disturbance, with the reduced diversity of GM and excessive growth of *Bacteroides* [13]. Similarly, compared with the control group, the diversity of GM in patients with ischemic stroke was decreased significantly, along with the increased relative abundance of *Escherichia*, *Bacillus*, *Bifidobacterium*, and *Ruminococcus* as well as the decreased abundance of *Parabacteroides*, *Ekmann*, and *Proctor* [14]. Moreover, it was also reported that GM might participate in the pathogenesis and pathophysiology of ischemic stroke and affect the prognosis of ischemic stroke [15]. In our previous study, we revealed that GM of patients with ischemic stroke had significantly changed and explored that *Enterobacteriaceae* might be a candidate biomarker for early clinical prediction of poststroke cognitive impairment [16]. Additionally, the abundance of SCFAs producing bacteria in patients with poststroke cognitive impairments and depression was decreased [17]. Nevertheless, existing studies of the connection between GM and prognosis of stroke mainly focused on descriptive studies, cross-sectional and case-control studies [18]; it was necessary to conduct a cohort study exploring the causal relationship between altered GM and adverse outcomes of AIS.

AIS-GM cohort focused on the relation between gut microbial characteristics and poststroke outcomes. 507 AIS patients were enrolled, the clinical baseline data as well as the samples of blood and faeces were collected, and poststroke outcomes were evaluated by a variety of questionnaires. 16 s rRNA sequencing of GM after admission was used to analyze the composition of GM and to explore the relation between GM and prognosis of AIS patients. The out-of-hospital follow up was conducted on the 90^{th} days, then repeated once a year, which included collections of fecal samples and blood samples and measurements of psychiatric outcomes. This prospective cohort could provide an opportunity to observe the dynamic changes of GM after stroke. This cohort contributed to develop a novel tool for identifying adverse prognosis with AIS by specific GM.

2. Materials and Methods

2.1. Study Setting and Patients Involvement. The 588 participants with stroke in this AIS cohort were recruited between September 2020 to July 2021 at the Affiliated Hospital of Wenzhou Medical University, where most locals sought treatment, which meant a fair representation of the southern area of Zhejiang province in terms of age, socioeconomic status, and disease characteristics. The protocol of the study was reviewed and approved by The Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University (LCKY2020-207). Patients were free to refuse participation or withdraw from the study. In this cohort, peripheral blood samples, sociodemographic, and previous medical history data of each patient were collected and numbered at the time of registration; by the way, patients with special diets, such as vegetarianism, were excluded from the cohort.

The recruitment and retention process of AIS participants was shown in Figure 1. Diagnosis of AIS accorded with the latest definition of American Heart Association/American Stroke Association (patients with corresponding MRI/CT evidence of acute ischemia and no clinical or radiological indication of a noncerebrovascular etiology) [19]. 29 patients with transient ischemic attack, 21 patients with lacunar cerebral infarction, and 5 patients without MRI/CT evidence who did not meet the inclusion criteria were excluded. Potential participants were excluded if they suffered from systemic diseases (renal failure, respiratory failure, and circulatory failure), cirrhosis, systemic lupus erythematosus, malignant intestinal tumors, psychosis (schizophrenia or bipolar disease), and other neurological diseases (Alzheimer's disease, epilepsy, and intracranial space-occupying lesion). Furthermore, the potential participants conducted with gastrointestinal surgery, antibiotics, or probiotics (within three months before admission or within three months after admission) were also excluded on account of the possible changes in GM. Finally, the analysis was conducted in 507 patients with AIS (67.1 ± 12.7 y, 35.5% of female).

2.2. Data Collection and Measures

2.2.1. Collection of Clinical Data. Clinical physicians interviewed patients and reviewed the medical records to collect demographic data and health-related behaviors. Each patient was obtained a unique registration number corresponding to their file once they were diagnosed with AIS. After writing informed consent, all included participants were introduced to a collection of basic information, including age, sex, education, marriage status, current resident area, lifestyle (smoking and drinking), telephone numbers, and other contact information of family members for follow up, aiming to keep the dynamic observation of GM and disease; all computerized and paper records of which were reviewed. Moreover, we extracted a broad range of data, including vital signs, biochemical indicators of blood and urine, and imaging findings (see detailed information below). All collected data at baseline were summarized in Table 1 and were analyzed to account for random error and biological variation.

2.2.2. Anthropometric Measures. Basic signs of the human body were measured and recorded by professionals. Height and weight were collected by well-trained nurses through a calibrated wall fixed clinical stadiometer and weight scale. Waist circumference was obtained at the narrowest part of the torso by an anthropometric tape at the end of exhalation. The body mass index (BMI) was calculated based on the measurement results (obesity was classified as a BMI greater than $25\,kg/m^2$ according to Asian-specific criteria). Sitting blood pressure was measured at admission by well-trained nurses after a rest with 10 min.

2.2.3. Assessment of Medical History. The collection of medical history was consistent with corresponding standards. Hypertension was considered as the mean of three blood pressure measurements ≥140/90 or previously diagnosed by a physician or receiving treatment for hypertension at present. Type 2 diabetes mellitus was defined as the fasting glucose level of 126 mg/dL or greater or previously diagnosed as diabetes or currently being treated for diabetes. Dyslipidemia involved a combination of total cholesterol

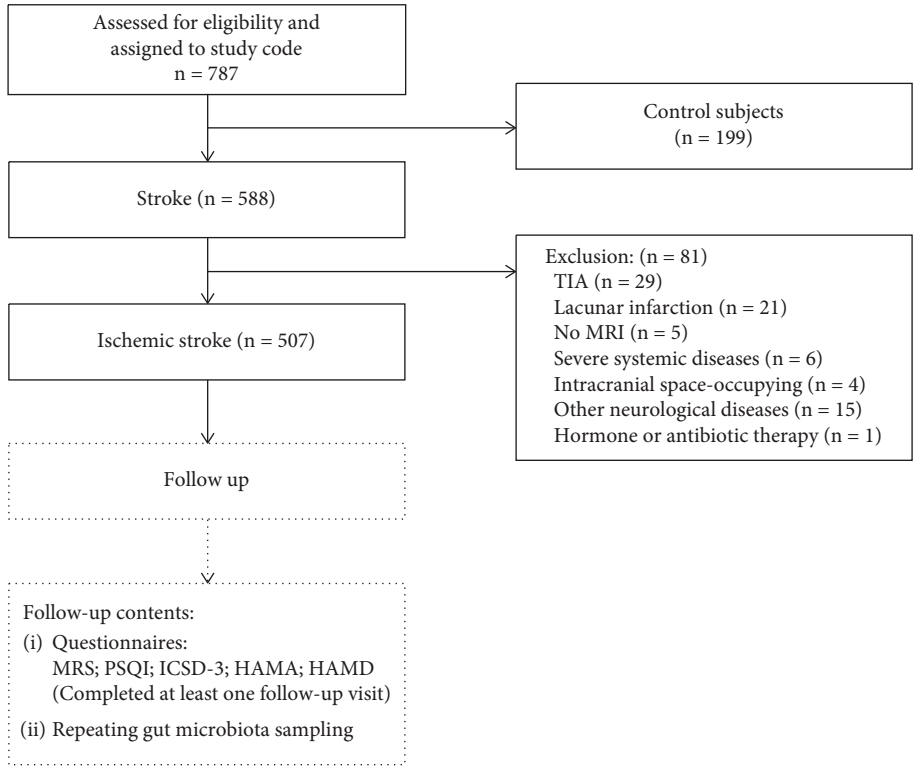

Figure 1: The schematic diagram of the inclusion/exclusion process. TIA indicates transient ischemic attack; mRS: modified Rankin's scale; PSQI: Pittsburgh's sleep quality index; ICSD-3: international classification of sleep disorders-third edition; HAMA: Hamilton's anxiety scale; HAMD: Hamilton's depression.

Table 1: Summary of variables collected for the Wenzhou ischemic stroke cohort study.

Classification	Contents	Method
General data	Name, gender, age, occupation, place of residence, contact information, education level, marital status, family history, etc.	Questionnaire/interview
Medical history	Past history and current medication	Questionnaire/interview
Health-related behaviors	Smoking and drinking history	Questionnaire/interview
Anthropometric measures	Height and weight	Examination
Blood pressure	Systolic blood pressure and diastolic blood pressure	Examination
Biochemical indicators		
Urine and common items	Proteins, ketones, blood, bilirubin, and nitrates	Examination
Blood and common items	CBC, total protein, albumin, FPG, triglyceride, total cholesterol, HDL-cholesterol, BUN, creatinine, uric acid, AST, ALT, hs-CRP, HbA1c, B12, folic acid, and Hcy	Examination
Blood and additional items	Lipid markers (LDL-cholesterol), hemostatic markers (prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimer), cardiac marker (troponin I), and thyroid function markers (FT3, FT4, TT3, TT4 and TSH)	Examination
Imaging items	Magnetic resonance imaging (MRI), CT angiography (CTA) or magnetic resonance angiography (MRA), cervical vessels ultrasonography and transcranial Doppler), heart rhythm (ECG and 24-Holter monitoring), and cardiac morphology (transthoracic echocardiogram).	Examination
Stool	PCR on 16 s rRNA gene sequence. PCR on 18 s rRNA gene sequence.	Laboratory analysis
Blood	GC-MS was used to detect the metabolites of gut microbiota	Laboratory analysis

CBC: complete blood count; FPG: fasting plasma glucose; HDL-cholesterol: high density lipoprotein cholesterol; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; hs-CRP: high sensitivity C-reactive protein; HbA1c: hemoglobin A1c, B12, vitamin B12; Hcy: homocysteine; LDL-cholesterol: low density lipoprotein cholesterol; FT3: free triiodothyronine; FT4: free tetraiodothyronine; TT3: total triiodothyronine; TT4: total thyroxine; TSH: thyroid-stimulating hormone; GC-MS: gas chromatography-mass spectrometry.

(TC) ≥ 200 mg/dL, triglycerides (TG) ≥ 200 mg/dL, low-density lipoprotein (LDL) ≥ 160 mg/dL, and high-density lipoprotein (HDL) ≤ 40 mg/dL for men and ≤50 mg/dL for women. Based on the Chinese guidelines on the prevention and treatment of dyslipidemia in adults (2016), hyperlipidemia was diagnosed according to the presence of TC ≥ 5.2 mmol/L, TG ≥ 1.70 mmol/L, a self-reported history of hyperlipidemia or a current lipid-lowering pharmacotherapy [20]. The definition of atrial fibrillation was considered as the appearance with the waveform of atrial fibrillation recorded on the electrocardiogram or 24-hour electrocardiogram during hospitalization or a self-reporting medical history or a current experience of atrial fibrillation-related treatment [21]. The previous experiences of suffering from cardiovascular diseases, such as myocardial infarction and stroke, were reported by patients themselves. Additionally, other factors concerned with behavior, including physical activity, smoking, and drinking, were divided into two categories (former: nonsmoking and nondrinking, quitting smoking and drinking, and second-hand smoking; current: continuing smoking and drinking at present).

2.2.4. Assessment of Blood and Urine Index. Blood and urine samples were collected to require the biochemical indicators, covering complete blood count (CBC), total protein, albumin, fasting plasma glucose (FPG), TG, TC, HDL-cholesterol, blood urea nitrogen (BUN), creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hs-CRP (high sensitivity C-reactive protein), hemoglobin A1c (HbA1c), vitamin B12, folic acid, homocysteine (Hcy), LDL-cholesterol (lipid markers), hemostatic markers (prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimer), Cardiac marker (troponin I), thyroid function markers (free triiodothyronine (FT3), free tetraiodothyronine (FT4), total triiodothyronine (TT3), total thyroxine (TT4), thyroid-stimulating hormone (TSH), and urine items, such as proteins, ketones, blood, bilirubin, and nitrates.

2.2.5. Assessment of Stroke Severity. The severity of stroke was assessed through National Institute of Health stroke scale (NIHSS) score on admission [22]. NIHSS score < 5 is defined as slight injury, and NIHSS score ≥ 5 is defined as moderate and severe injury. The higher NIHSS score, the more serious it is. The etiology of stroke was further divided into the following subtypes: large-artery atherosclerosis (LAA), cardioembolic (CE), small-vessel occlusion (SAO), and other etiology according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [23]. Besides, venous thrombosis, especially the lower extremity venous thrombosis, was ascertained and collated by ultrasound scans on admission. The clinical data on the history of whether employed with intravenous thrombolysis and the treatment during hospitalization were taken from the electronic medical records. Additionally, within 7 days after enrollment, total recruited participants underwent clinical examinations consisting of MRI, computed tomography angiography (CTA) or magnetic resonance angiography (MRA), cervical vessels ultrasonography, the transcranial Doppler, heart rhythm (ECG and the 24-Holter monitoring), and cardiac morphology (transthoracic echocardiogram). Then, the imaging evidences of each participant, including the location of ischemic stroke and cerebrovascular stenosis, were exhibited and recorded on CT and MRI for deeper exploration.

2.2.6. Questionnaires. The neurological recovery, sleep, and mental status of the patients were fully evaluated based on face-to-face interviews with patients or their close families by questionnaires, such as the modified Rankin scale (mRS), the Pittsburgh sleep quality index (PSQI), the international classification of sleep disorders (ICSD-3), Hamilton's anxiety scale (HAMA), and Hamilton's depression scale (HAMD). The process of performing questionnaires strictly followed the main diagnostic criteria and scoring criteria of the scale (Table 2). Moreover, we also conducted a questionnaire that assessed the dietary intake of patients over the previous month to exclude the impacts of diet on GM [24]. The diet questionnaire included the following elements: proportion of carbohydrate, fat, plant protein, animal protein, fruits and vegetables, type of staple food, taste preference, use of antibiotics or probiotics, consumption of alcohol, and exercise habits. The dietary questionnaire was described in detail in Table 2.

The estimation of poststroke disability distinguished six ordered grades of functional outcome and a death state based on mRS (0-1 implied the largest poststroke burden in the mental health and relationship domains; >3 indicated the greatest burden in independent living, mobility, and self-care domains) [25]. PSQI was a self-rated questionnaire on sleep quality and disturbances over a 1-month time interval, in which nineteen individual items generated seven "component" scores, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction, and the sum for these seven components yields one global score with a range of 0–21; higher scores indicated worse sleep quality [26]. ICSD-3 identified seven major categories that consisted of insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, parasomnias, and other sleep disorders [27]. In addition, anxiety symptoms were evaluated by HAMA consisting of 2 subscales (psychic anxiety and somatic anxiety), in which the symptoms were assessed using 5 grades, 0-4 referred from no symptom to extremely severe symptoms. HAMD scale applied for assessment of the depression symptoms included 7 subscales (anxiety/somatization, weight, cognitive impairment, diurnal variation, blockage, sleep disorders, and hopelessness), in which the symptoms underlined the divided 5 grades; 0-4 referred from no symptom to extremely severe symptoms.

2.3. Biological Samples

2.3.1. Stool Samples. The participants were instructed to place the excreted stool in the collection tube, flick the stool to the bottom of the tube, and avoid picking the part that was in contact with the bottom and edge of the

Table 2: Contents and scoring rules of cohort follow up.

Objective examination	Follow-up project	Major diagnostic sections
Neurologic disability measures	mRS	0—No symptoms. 1—No significant disability. Able to carry out all usual activities, despite some symptoms. 2—Slight disability. Able to look after own affairs without assistance but unable to carry out all previous activities. 3—Moderate disability. Requires some help but able to walk unassisted. 4—Moderately severe disability. Unable to attend to own bodily needs without assistance and unable to walk unassisted. 5—Severe disability. Requires constant nursing care and attention, bedridden, and incontinent. 6 - dead
Poststroke insomnia (PSI)	PSQI	(1) Subjective sleep quality (2) Sleep latency (3) Sleep duration (4) Habitual sleep efficiency (5) Sleep disturbances (6) Use of sleep medications (7) Daytime dysfunction
	ICSD-3	(1) Insomnia (2) Sleep-related breathing disorders (3) Central disorders of hypersomnolence (4) Circadian rhythm sleep-wake disorders (5) Parasomnias (6) Sleep-related movement disorders (7) Other sleep disorders
Posstroke depression (PSD)	HAMA	(1) Psychic anxiety (2) Somatic anxiety
	HAMD	7 subscales: anxiety/somatization, weight cognitive impairment, diurnal variation, blockage, sleep disorders, and hopelessness
Dietary habits	Diet questionnaire	(1) What is the proportion of grains, potatoes, beans, fish, meat, oil, fruits, and vegetables in your diet? (2) What kind of staple food do you eat? Rice or noodles? (3) What is your usual taste? Sweet or salty? Light or fried? (4) Have you used antibiotics or probiotics in the last month? (5) Do you exercise regularly? What kind and frequency of exercise?

mRS: modified Rankin's scale; PSQI: Pittsburgh's sleep quality index; ICSD-3: international classification of sleep disorders-third edition; HAMA: Hamilton's anxiety scale; HAMD: Hamilton's depression.

bedpan. The fresh specimens should be collected within 1-3 days of admission and at subsequent follow up, then temporarily frozen at -20°C before stored at -80°C without any additives.

2.3.2. Blood Samples. Each patient provided 5 mL blood under fasting conditions at enrollment. The blood samples were kept at room temperature for 60 min to complete coagulation and centrifuged at a speed of 1300 × g for 10 min at 20°C. After centrifugation, the serum was acquired and then immediately frozen at -80°C for further analyses.

2.3.3. Sample Storage and Data Management. A total of 395 fecal samples and 489 serum samples were collected in AIS-GM cohort according to standard operative procedures and stored at -80°C. Each stool sample was accompanied by a submission form, and each stool and serum sample was numbered and corresponds to the patient's medical record number.

2.3.4. Analysis of GM and Their Metabolites. The fecal samples were taken for 16 s rRNA sequencing as described previously [28]. Microbial DNA was extracted and purified using QIAamp DNA Mini Kit, and then PCR amplification was carried out in the V3-V4 hypervariable region of 16 s rRNA gene. The purified PCR products were sequenced by Illumina Miseq platform. The α diversity, such as Shannon's index, Simpson's index, and Chao1 and ACE index, and β diversity, such as principal coordinates analysis (PCoA), microbial compositions, and linear discriminant analysis effect size (LEfSe), were analyzed. The metabolites were analyzed by liquid chromatography tandem mass spectrometry (LC-MS) for quantitative determination of metabolites; the results of which were recorded in a data dependent manner on a QExactive. The EASY nLC-1000 liquid chromatography system (Thermo scientific, Odense, Denmark) was coupled to the mass spectrometer through a 2 cm C18 pre-column (300 μm inner diameter, 1.9 μm particle size) and a self-packed 15 cm C18 column (Pico Frit columns from New Objective) with a 75 μm inner diameter, packed with

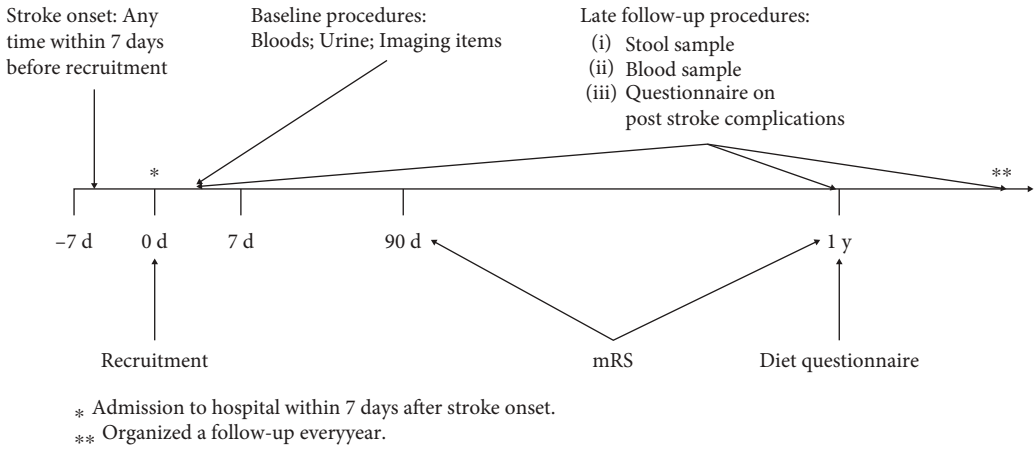

Figure 2: The schematic diagram of timeline of AIS-GM cohort profile. mRS: the modified Rankin scale.

(1.9 μm C18 Dr. Maisch Mat. No. r119.a). Metabolites were eluted with a mobile phase consisting of solvent A (0.1% formic acid) and B (80% acetonitrile in 0.1% formic acid).

2.4. Follow-Up Survey. As shown in Figure 2, follow up initiated the day after enrollment in the cohort and lasted until disenrollment from this cohort, which included out-of-hospital follow up. Within 7 days after the recruitment (in-hospital follow up), baseline procedures including bloods, urines, imaging items, collections of fecal samples, blood samples, and questionnaire were timely and effectively administrated. The out-of-hospital follow up was conducted in the form of face-to-face in the outpatient department or telephone on the 90th days, then repeated once a year. If the patient is not contacted at the first follow up, we would try to recontact them at different dates up to three times and suggested them to clinic for completing the follow up or filling the questionnaire by telephone. Elderly and frail patients could conduct proxy interviews with close family members to answer the questionnaires. The language barrier could be effectively resolved by a physician proficient in dialect who was arranged to contact the patients or their family members so as to maintain the follow-up rate. Meanwhile, a dietary questionnaire was added to later follow-up procedures one year after enrollment. By the time of December 15, 2021, 75.54% (383/507) of stroke patients completed baseline clinical assessment and one or more subsequent follow-up questionnaires.

3. Results

3.1. Baseline Characteristics. At enrollment, 507 patients with AIS treated in the department of neurology were identified as "AIS samples" (Table 3). The mean of age among all patients was 67.1 years (SD, 12.7); there were 64.5% of male patients, and most patients (82.4%) lived with their spouses. The distribution of participants in the four educational levels (115 illiterates, 196 primary school degrees, 129 junior middle school degrees, and 54 high school degrees and or above) showed that majority of them are less educated. At baseline, 22.1% of patients had a history of CVD, 73.4% of patients had hypertension, and 34.8% of suffered from diabetes. At present, fecal samples of 395 AIS patients were collected. The baseline characteristics of 395 AIS patients were as follows: mean of age was 66.9 years (SD, 12.2); there were 64.9% of male patients, and most patients (84.7%) lived with their spouses. The distribution of participants in the four educational levels (81 illiterates, 162 primary school degrees, 99 junior middle school degrees, and 44 high school degrees and or above) showed that majority of them are less

Table 3: Distribution of selected baseline characteristics in the AIS cohort.

Variables	Ischemic stroke samples	Patients with fecal samples
N	507	395
Sociodemographic factors		
Male	327 (64.5)	257 (64.9)
Age (years)	67.1 ± 12.7	66.9 ± 12.2
Marriage, married and living with a spouse	418 (82.4)	331 (84.7)
Education		
Illiterate	115 (23.3)	81 (21.0)
Elementary school	196 (39.7)	162 (42.0)
Middle school	129 (26.1)	99 (25.6)
High school and above	54 (10.9)	44 (11.4)
Health-related behaviors		
Smoking, current	180 (35.5)	139 (35.3)
Drinking, current	158 (31.2)	120 (30.5)
History of disease		
Hypertension	372 (73.4)	286 (72.6)
Diabetes mellitus	176(34.8)	142 (36.1)
CVD, history	112 (22.1)	83 (21.1)
Stroke severity, median (IQR)		
NIHSS	2 (1-4)	2 (1-4)
mRS	1 (0-2)	1 (0-2)

CVD: cardiovascular disease; NIHSS: National Institute of Health stroke scale; mRS: modified Rankin's scale.

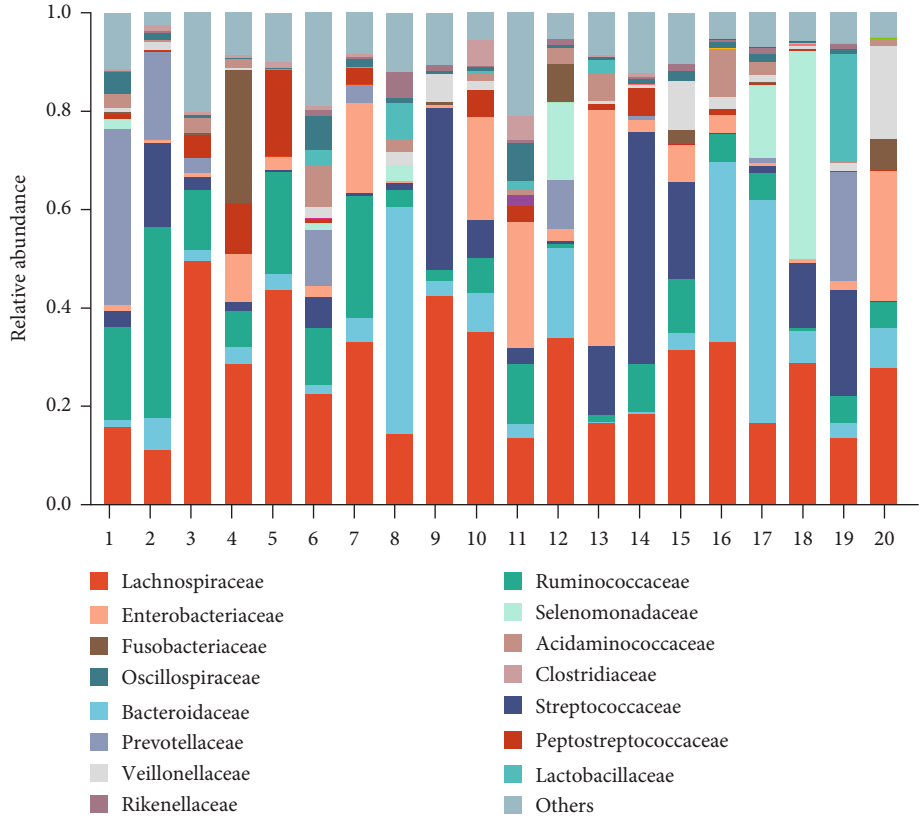

FIGURE 3: The composition of gut microbiota in AIS patients. *x*-axis, pilot samples of 20 AIS patients; *y*-axis, the relative abundance of gut microbe at family level.

educated. At baseline, 21.1% of patients had a history of CVD, 72.6% of patients had hypertension, and 36.1% of suffered from diabetes.

3.2. GM Analysis. As shown in Figure 3, 395 fecal samples from the participants were collected. We analyzed the composition of GM using 16 s RNA sequencing. 20 samples of AIS patients were selected to analyze the composition of GM. The family classification showed that the relative abundance of GM, including *Lachnospiraceae, Bacteroidaceae*, *Ruminococcaceae*, *Streptococcaceae*, *Enterobacteriaceae*, *Prevotellaceae*, *Selenomonadaceae*, *Peptostreptococcaceae*, *Fusobacteriaceae*, *Veillonellaceae*, *Acidaminococcaceae*, *Lactobacillaceae*, *Oscillospiraceae*, *Rikenellaceae*, and *Clostridiaceae*, accounted for more than 90% of the total microbiota. The most predominant three genera were *Lachnospiraceae, Bacteroidaceae*, and *Ruminococcaceae*. The composition and structure of GM in patients with AIS were different from those in healthy controls (Figure S1). This cohort will describe the relationship between the prognosis of AIS and the dynamic changes of GM.

4. Discussion

This cohort profile explored the dynamic changes and mechanisms of GM in prognosis of AIS patients via follow up. At present, 395 AIS patients have completed 16 s rRNA sequencing of GM after admission, analyzed the composition of microbiota, and tracked the prognosis and neuropsychiatric complications of AIS patients. The subsequent progress is to detect the concentration of GM metabolites in the fecal of poststroke patients by LC-MS, in order to investigate the mechanism of GM affecting stroke through metabolites. 90 days after cohort initiation, each subject was followed up through the scale and questionnaire to assess the complications of AIS patients. It is also planned to collect blood and stool samples of patients every year to expand the existing database. In addition, the database can be used to explore characteristic GM, which can be used in animal models to verify the influence of GM in the disease. This cohort can be used as a new tool to assess the prognosis of stroke and further predict the development of disease.

The advantage of prospective cohort is that it provides an unprecedented opportunity to observe the dynamic changes of GM and its metabolites after stroke. In addition, faeces of patients in acute stage, convalescent stage, and sequela stage of stroke were collected, respectively, and then the biological succession of GM was comprehensively analyzed according to the changes of patients' conditions. Importantly, AIS-GM cohort conducted direct and longitudinal medical assessments of prospectively AIS survivors based on physician assessments of all medical records, admission dates, discharge dates, and complete hospitalization records, which were considered accurate and reliable. Furthermore, AIS-GM cohort is open, the collection of patients is still continuing, and the findings are still being updated.

Limitations should be mentioned in this cohort profile. Firstly, as a single center study, this cohort profile may have problems with late effects and detection rates, and demography has not been extended to a wide range of regions in China. Secondly, this cohort profile lacks diet questionnaires. Although patients collected some important influencing factors, such as smoking, drinking, and blood pressure, and provided stool samples before treatment, other equally important factors, such as exercise and diet, were not recorded in time. Since the second follow up, we intend to use the diet questionnaire to partially compensate for the deficiencies in the previous baseline or the first follow up.

5. Conclusions

AIS-GM cohort profile highlighted the great potential of GM in the occurrence and development of AIS patients, which is helpful to deepen the understanding of AIS complications. In addition, this cohort profile will provide new ideas for further exploring biomarkers or intervention strategies of poststroke prognosis based on GM.

Data Availability

All the data relevant to this research are available in the body of the manuscript as supporting figure and tables. We do not have any ethical or legal consideration for us not to make our data publicly available.

Ethical Approval

The ethical approval obtained from The Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University (LCKY2020-207).

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

This work was carried out in collaboration among the authors. J.L. and J.S. contributed to the study design. Material preparation and data collection were performed by H.X., J.Z., Q.G. and S.Y. All authors analyzed the data. H.X. wrote the first draft of the manuscript. All authors discussed the results of the experiments and edited and approved the final version of the manuscript. Huijia Xie and Junmei Zhang contributed equally to this work.

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Supplementary Materials

Fecal samples from 20 AIS patients and 20 healthy subjects were analyzed by 16 s RNA sequencing. The diversity, community composition, and differential bacteria of GM were evaluated. Our results showed that the composition and structure of GM in patients with AIS were different from those in Con (Figure S1). Figure S1. The difference of gut microbiota between AIS patients and Control subjects. (A) The principal coordinate analysis (PCoA) study showed variations of gut microbiota composition between two groups, the significant P value was indicated, and each character represented a sample. (B) Venn diagram displayed the discrepancy and overlap of ASV between two groups. (C) The percent of different taxa at the Phylum level between two groups. (D) The percent of different taxa at the family level between two groups. (E) Linear discriminant analysis (LDA) effect size (LEfSe) analysis revealed significant bacterial differences in gut microbiota between the AIS patients (red) and Control subjects (green). *(Supplementary Materials)*

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