Research protocol

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Safety and effectiveness of prebiotics and probiotics in intervention of obesity in children with Prader-Willi syndrome

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1. Background

Prader-Willi syndrome (PWS) is a rare genetic disease with a wide range of clinical manifestations, including changes in body composition, excessive appetite and serious behavioral problems [1]. The combination of low energy consumption and high energy intake is considered to be the most common cause of hereditary obesity [2-4]. The clinical symptoms of PWS vary with age and present different nutritional stages. In infancy, it is characterized by weak sucking and feeding difficulties, and in early childhood, it gradually transits to lack of satiety, morbid obesity and related complications caused by gluttony, which are the most common causes of poor prognosis and death of PWS [5-8]. At present, there are no effective drugs to help control appetite, and obesity treatment drugs such as weight-loss drugs are not suitable for children, and the use of gastric volume reduction surgery for PWS is still controversial. Therefore, how to effectively control the appetite of children, avoid or reduce the occurrence and severity of obesity, and reduce the occurrence of related complications has become the most serious challenge for PWS treatment.

In recent years, more and more evidence shows that intestinal flora is closely related to the occurrence of obesity, and is considered as an important participant in the development of obesity and metabolic diseases [9,10] and mental health disorders including depression, anxiety and social behavior [11-13]. Probiotics, as a kind of microorganism that can improve the changes of intestinal micro-ecology, has been proved to improve biochemical indicators such as blood lipids in obese people. Several animal bifidobacteria subspecies and lactic acid bacteria strains have been proved to improve the risk factors of cardiac metabolism in adult obese patients by reducing central obesity and insulin resistance [14~16]. Specifically, the strain CECT 8145 (BPL1) was initially identified as an effective probiotics, which reduced fat deposition in the high-throughput screening of Cryptostrongylus elegans [17]. In this organism, BPL1 targets both energy homeostasis and tryptophan metabolism [17]. It is an important regulator of central nervous system processes, including satiety, anxiety and depression [13, 18]. Research on intestinal microbiota of PWS patients found that 17 children and adolescents with PWS were given low-calorie diet intervention in hospital, and the changes in intestinal microbiota directly contributed to improving obesity and metabolic health [19]. Olsen et al. studied the intestinal microbiota of 17 adult PWS subjects and the matched group of non-hereditary obesity, and concluded that intestinal bacteria play a positive role in insulin sensitivity [20]. The recently reported intestinal microbiota associated with obesity in PWS children found changes similar to those in simple obesity [21], while Peng et al.'s research suggested that there were some differences in intestinal bacterial and fungal communities in PWS children compared with the control group [22].

Since carrying out molecular genetic diagnosis of PWS, our hospital has successively carried out a national multi-center study on "early screening and intervention of PWS"; Set up PWS special clinic; Establish PWS Club of Pediatric Hospital Affiliated to Fudan University; Carry out special research on PWS MDT and special disease cohort; In 2018, it initiated the establishment of the "China Prad Willi syndrome clinical standardized diagnosis and treatment cooperation alliance", and took the lead in carrying out the national multi-center study of "Phase III clinical trial to evaluate the efficacy and safety of recombinant human growth hormone injection in the treatment of PWS" in China, and assisted the domestic growth hormone product to obtain the first approval of PWS treatment indications on June 30, 2022. So far, hundreds of cases have been accumulated, and long-term follow-up monitoring and multidisciplinary comprehensive management have been carried out, and rich clinical diagnosis and treatment, drug clinical trials and multi-center research experience have been accumulated.

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2. Research purpose

This study aims to explore the new method and potential mechanism of PWS treatment by observing the safety and effectiveness of prebiotics and probiotics in the intervention of obesity in children with PWS.

3. Research overview

3.1 Overall research and design

It is planned to carry out a 12-week randomized, single-blind, parallel controlled, and efficacy test single-center clinical intervention trial. The trial plan screened 60 PWS obese patients (3-10 years old) who met the enrollment criteria and were divided into three groups. At the time of enrollment, the subjects were randomly assigned to different groups according to the block randomization method.

- Group A: probiotics 2g × 2/day;
- Group B: probiotics 2g × 2/day+prebiotics 25g × 2/day;
- Group C: control group (food with equal energy and no functional ingredients)

The intervention products comply with the Food Safety Law of the People's Republic of China, the probiotic solid beverage of GB/T 29602 standard, and the food grade inulin extract prebiotics of GB/T 19883 standard.

3.2 Study population

PWS obese children aged 3-10 years.

3.2.1 Selection criteria

1)Pre-adolescent children with Prader Willi syndrome which were definitely diagnosed by gene testing.

2)Consistent with the diagnostic criteria for obesity.

3)Not participate in other research projects at present or three months before the research;

4)Agree to participate in the test and obtain the consent of their parents; voluntarily be the subjects and sign the informed consent form.

3.2.2 Exclusion criteria

1)Losing weight in ways other than the intervention measures of this project, such as taking weight loss drugs or known drugs that cause weight change;

2)Use antibiotics within 1 month before the study and lasted for 3 days or more;

3)Use probiotics within 1 month before the study and lasted for 3 days or more;

4)Complicated with liver and renal insufficiency (alanine aminotransferase and serum creatinine indexes exceed 2 times the upper limit of the normal value set by the hospital);

5)Have gastrointestinal diseases affecting food digestion and absorption (such as severe diarrhea, constipation, severe gastrointestinal inflammation, active gastrointestinal ulcer, acute cholecystitis, etc.); severe diarrhea refers to watery stool 3 or more times a day and lasts for 3 or more days. severe constipation refers to defecation 2 or less times a week with difficulty in defecation;

6)Surgery was performed within 1 year before the study (except for appendicitis and hernia surgery);

7)Have hepatitis B, active tuberculosis, AIDS and other infectious diseases;

8)Those who are suffering from mental illness and are taking psychotropic drugs such as antidepressants.

3.2.3 Withdrawal criteria

1)The tested children have serious violations of the study plan, such as taking weight loss drugs, and stopping the trial intervention plan on their own for a long time;
2)Other clinical events, such as fracture and trauma, sudden disease requiring surgery, cannot continue to be tested;
3)Subjects were absent for two consecutive times in three test visits;
4)If the subject and his caregiver withdraw their informed consent and request to withdraw from the study, the case record in this study is active termination, and all clinical data cannot be used for statistical analysis.

3.2.4 Test completion criteria

Subjects completed all protocols of the study.

3.2.5 Number of subjects and grouping method

Sixty eligible subjects were divided into three groups at a ratio of 1:1:1. At the time of enrollment, the subjects were randomly assigned to different groups according to the block randomization method. The randomization scheme was generated by the statisticians using SAS 9.4 statistical software.

3.3 Test drug

Group A: probiotics 2g × 2/day;
Group B: probiotics 2g × 2/day+prebiotics 25g × 2/day;
Group C: control group (food with equal energy and no functional ingredients)

Interventions are probiotic solid drinks in accordance with the Food Safety Law of the People's Republic of China and the GB/T 29602 standard, and prebiotics (strawberry dietary fiber jelly) in food grade inulin extract in accordance with the GB/T 19883 standard, which are provided free of charge by the research center with self-raised funds.

Table 1. Nutritional composition of probiotics

project	Every 100g	Nutrient reference value%
energy	1312 kJ	16%
protein	4.1 g	7%
Fat	0 g	0%
carbohydrate	58.5 g	20%
dietary fiber	30.1 g	120%
sodium	60 mg	3%

Table 2. Nutritional composition of prebiotics (strawberry dietary fiber jelly)

project	Every 100g	Nutrient reference value%
energy	448 kJ	6%
protein	0 g	0%
Fat	0 g	0%
carbohydrate	17.2 g	6%
dietary fiber	19.5 g	78%
sodium	93 mg	5%

3.4 Research steps and methods

① Research steps

- Intervention group (group A): from day 0: ① Sabijian ® Probiotic solid beverage 2g × 2/day oral; ② Accept the dietitian's diet and lifestyle recommendations. The dietitian collects the daily diet and exercise information of the subjects through the WeChat widget, and gives the corresponding personalized diet and exercise suggestions to the subjects according to the Diet Guide for Chinese School-age Children;
- Intervention group (group B): from day 0: ① probiotics solid beverage 2g × 2/day+prebiotic dietary fiber jelly 25g × 2/day oral; ② Accept the dietitian's diet and lifestyle recommendations. The dietitian collects the

daily diet and exercise information of the subjects through the WeChat widget, and gives the corresponding personalized diet and exercise suggestions to the subjects according to the Diet Guide for Chinese School-age Children;

- Control group (group C): healthy diet guidance was given only in the 0, 4 and 8 weeks (three times).

② Indicator detection

Use intervention food twice a day (30 minutes before meals). At the same time, they accept the daily diet content management and exercise prescription management of dietitians. The following tests were performed on the 0th, 4th and 12th week after enrollment:

- Main outcome measures: weight, height and BMI, BMI - SDS, waist circumference, body composition (body fat), and stool macrogene sequencing;
- Secondary indicators: ① fasting triglyceride, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) - cholesterol levels, fasting glucose, insulin and hemoglobin A1c (HbA1c) concentrations, insulin resistance index (HOMA-IR), blood pressure; ② Psychological behavior scale; ③ Clinical Trial Hyperappetite Questionnaire (HQ-CT); ④ Gastrointestinal symptoms (constipation, diarrhea, abdominal pain, excessive flatulence, bloody stool, nausea, dysphagia, anorexia, dyspepsia and acid reflux); ⑤ Stool frequency, stool form, etc.

The trial was observed for a total of 12 weeks, and there were 3 clinical follow-up test time points, which were weeks 0, 4 and 12. After the start of treatment, stage obesity assessment was conducted every 2 weeks in the first 12 weeks until the end of the study. In addition to the baseline, the time window of each visit is ± 3 days (based on the baseline).

4. Observation of adverse events

4.1 Definition of adverse events

4.1.1 Definition

Adverse events: adverse medical events occurred after patients or subjects involved in human trials received a drug (or product), but not necessarily related to treatment.

Serious adverse events: during the clinical trial, such events as hospitalization, prolonged hospitalization, disability, impact on work ability, life-threatening or death, and congenital malformation occurred.

4.1.2 Degree

Mild: the subject can tolerate, does not affect the treatment, does not need special treatment, and has no impact on the subject's rehabilitation.

Moderate: the subject is intolerable and needs special treatment, which has a direct impact on the rehabilitation of the subject.

Severe: endangering the life of the subject, causing death or disability, requiring immediate emergency treatment.

4.2 Recording and reporting of adverse events

This study will record the adverse events occurred during the study and any clinically relevant changes, and evaluate whether there is any correlation with the application of experimental drugs; And immediately take measures to correct and ensure the safety and interests of the subjects. The recorder shall sign and date it, and file it with the source file and submit it to the clinical research center (CRU) in the hospital.

Subjects may have clinical adverse events during treatment. Once adverse events (including important adverse events) occur, the occurrence time, clinical manifestation, treatment process and duration, outcome and relationship with the test drug (or test product) shall be recorded in detail on the case report form. In case of serious adverse events, fill in the form of serious adverse events and report to the sponsor, clinical medical ethics branch, relevant management departments and health administrative departments within 24 hours.

4.3 Risk prevention and treatment

The following risk minimization measures are taken in this study to ensure that the potential benefits of research participation exceed the relevant risks.

1. The application of prebiotics and probiotics in this study is based on the overlapping administration of the original PWS multidisciplinary comprehensive management. The study participants still maintain the original treatment plan during the study process, and the treatment risk is low.

2. Take the following measures to reduce potential risks.

(1) People younger than 3 years old were not included in this study. Compared with older children, liver and kidney functions of younger children are prone to damage, and the risk of adverse reactions is higher. Therefore, children under 3 years of age are not included in this study.

(2) The dosage selection of prebiotics and probiotics in this study is based on the product manual and previous studies, and no obvious adverse reactions

have been observed.

5. Statistical analysis

5.1 Sample size estimation

According to the main outcome index BMI, the sample size was estimated by using the inter-group parallel control design of measurement data. According to the BMI distribution of normal children and adolescents and previous clinical studies on PWS, the average BMI of PWS patients with obesity in Hefei is 22 kg/m2, and the standard deviation of BMI is 2.0 kg/m2 α= Under the assumption of 0.05 and 80% statistical efficiency, the sample size required to detect the 10% difference in the mean BMI before and after the intervention (BMI reduction of 2.0 kg/m2) is at least 16 subjects in each group. According to the 1:1:1 distribution of three groups of samples, 48 children were needed. In consideration of the factors of sample separation during the study, it is proposed to enroll 20 children in each group, with a total of 75 children recruited.

5.2 Statistics and analysis of research data

- All statistical tests are double-sided tests. A P value less than or equal to 0.05 will be considered statistically significant.
- The description of quantitative indicators will calculate the mean, standard deviation, median, minimum and maximum, and the classification indicators will describe the number of cases and percentage of each type.
- Enrollment and completion: summarize the number of enrollees and completion, and list the shedding cases. Describe the demographic characteristics (age, height, weight, vital signs, etc.), medical history, family history, etc. of the patient. The comparison of the three groups of general conditions will be analyzed by appropriate methods according to the type of indicators. The group t test or Wilcoxon rank sum test will be used for the comparison of quantitative data between groups, the chi-square test or accurate probability method will be used for classification data, and Wilcoxon rank sum test or CMH will be used for grade data- χ 2 Inspection.

6. Research on relevant ethical issues

6.1 Review by the Ethics Committee

This study will fully inform participants and their legal representatives of the risks and requirements of this study, and will provide participants and their legal representatives with any new information that may affect their continued participation in decision-making during the study. Only participants and legal

representatives who fully understand the risks, benefits and potential adverse events of this study and voluntarily provide consent will be included in this study.

The protocol, written informed consent and data directly related to the subjects were submitted to the Clinical Medical Ethics Branch, and the study was officially conducted after obtaining the written approval of the Clinical Medical Ethics Branch. Submit the annual research report to the Clinical Medical Ethics Branch every year.

Inform the Clinical Medical Ethics Branch in writing when the study is suspended and/or completed; Report all changes in the research work (such as the revision of the protocol and/or the number of informed consent) to the Ethics Committee in a timely manner, and implement these changes after obtaining the approval of the Clinical Medical Ethics Branch.

6.2 Informed consent

6.2.1 Procedures for obtaining informed consent

The researcher must provide the subject or his legal representative with an easy-to-understand informed consent form approved by the Clinical Medical Ethics Branch, and give the subject or his legal representative sufficient time to consider the study. The subject shall not be included in the group until the signed written informed consent form is obtained from the subject. During the participants' participation, all updated informed consent forms and written information will be provided to the subjects. The informed consent form shall be kept for future reference as an important document involving human trials.

7. Confidentiality measures

The results of the research through this project may be published in the magazine, but we will keep the information of the subject confidential according to the requirements of the law, and the personal information of the subject will not be disclosed unless required by the relevant laws. When necessary, the government administrative department, the Clinical Medical Ethics Branch and its relevant personnel can access the data of the subjects according to the regulations.

Informed consent

(Legal guardian version)

Clinical trial name: Safety and effectiveness of prebiotics and probiotics in intervention of obesity in children with Pradville syndrome

Main investigator: Lu Wei, Department of Endocrine Genetics and Metabolism, Pediatric Hospital Affiliated to Fudan University

Dear parent or legal guardian:

Your child will be invited to participate in a study. This informed consent form provides some information to help you and your child decide whether to participate in the study. Please read carefully. If you have any questions, please ask the researcher in charge of the study.

1. What is the research background and purpose of this study

Prader-Willi syndrome (PWS) is one of the main genetic causes of morbid obesity in children. The clinical symptoms change with age, presenting different nutritional stages, and gradually transiting to lack of satiety in early childhood, morbid obesity and related complications caused by overeating, which are the most common causes of poor prognosis and death of PWS. At present, there are no effective drugs to help control appetite, and obesity treatment drugs such as weight-loss drugs are not suitable for children, and the use of gastric volume reduction surgery for PWS is still controversial. Therefore, how to effectively control the appetite of children, avoid or reduce the occurrence and severity of obesity, and reduce the occurrence of related complications has become the most serious challenge for PWS treatment.

In recent years, more and more evidence has shown that intestinal microflora is closely related to the occurrence of obesity, and probiotics, as a kind of microorganism that can improve the changes of intestinal micro-ecology, has been proved to improve the blood lipids and other biochemical indicators of obese people. Our previous 16s rRNA sequencing results of fecal bacteria in PWS and normal control children suggest that the diversity of intestinal flora in PWS children is significantly reduced, the beneficial bacteria producing short chain fatty acids such as Faecalibacterium, Blautia, Prevotella, etc. are significantly decreased, and the opportunistic pathogenic bacteria such as Streptococcus, Escherichia-Shigella, Klebsiella, etc. are increased, suggesting that intestinal flora may play a role in the occurrence and development of obesity in PWS.

Therefore, the purpose of this study is to observe the safety and effectiveness of prebiotics and probiotics in the intervention of obesity in children with PWS, and explore new methods and potential mechanisms of PWS treatment.

2. How was the study conducted?

It will take about 12 weeks for your child to participate in and complete the study. The study will be carried out in a research center of the Pediatric Hospital affiliated to Fudan University. There will be 60 patients enrolled in the study, and the research center will recruit at least 75 patients.

- **Research content:**

Potential benefits of probiotics and prebiotics on body mass index, glucose and lipid metabolism and intestinal flora composition of PWS obese subjects. This study selected obese PWS patients as the study object, and compared probiotics, probiotics combined with probiotics (inulin) and placebo to explore the effects of probiotics combined with probiotics (inulin) on body mass index, glucose and lipid metabolism and intestinal flora composition of PWS patients with obesity.

- **Study design:**

This study is a 12-week randomized, single-blind, parallel controlled, and clinical intervention test with excellent effect. To evaluate the potential benefits of probiotics and prebiotics on body mass index, glycolipid metabolism and intestinal flora composition of PWS patients with obesity. Sixty eligible subjects were divided into three groups at a ratio of 1:1:1. At the time of enrollment, the subjects were randomly assigned to different groups according to the way of block randomization:

Intervention group (group A): from day 0: ① probiotics powder 2g × 2/day oral; ② Accept the dietitian's diet and lifestyle recommendations. The dietitian collects the daily diet and exercise information of the subjects through the WeChat widget, and gives the corresponding personalized diet and exercise suggestions to the subjects according to the Diet Guide for Chinese School-age Children;

Intervention group (group B): from day 0: ① probiotics powder 2g × 2/day+prebiotic dietary fiber jelly 25g × 2/day oral; ② Accept the dietitian's diet and lifestyle recommendations. The dietitian collects the daily diet and exercise information of the subjects through the WeChat widget, and gives the corresponding personalized diet and exercise suggestions to the subjects according to the Diet Guide for Chinese School-age Children;

Control group (group C): healthy diet guidance was given only in the 0, 4 and 8 weeks (three times).

Weight, height, blood pressure, BMI, BMI - SDS, waist circumference, body composition (body fat), and fecal macrogene sequencing were performed before treatment (0W) and at the 4th week (4Ws) and 12th week (12Ws) of treatment respectively; Glycolipid metabolism and other indicators: fasting triglyceride, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) - cholesterol levels, fasting glucose, insulin and hemoglobin A1c (HbA1c) concentrations, insulin resistance index (HOMA-IR).

The trial was observed for a total of 12 weeks, and there were 3 clinical follow-up test time points, which were weeks 0, 4 and 12. After the treatment, the stage obesity assessment was conducted every 2 weeks until the end of the study. In addition to the baseline, the time window of each visit is ± 3 days (based on the baseline).

- **Test termination criteria for subjects:**

In case of one of the following conditions, the test process should be stopped:

- ✧ Serious adverse events occur and have certain relationship with the test product;
- ✧ The tested children have serious violations of the study plan, such as taking weight loss drugs, and stopping the trial intervention plan on their own for a long time;
- ✧ Other clinical events, such as fracture and trauma, sudden disease requiring surgery, cannot continue to be tested;
- ✧ Subjects were absent for two consecutive times in three test visits;
- ✧ If the children and their caregivers withdraw their informed consent and request to withdraw from the study, the case record in this study is an active termination, and all clinical data cannot be used for statistical analysis.

3. Does my child have other treatment options?

This study is a superimposed treatment plan based on the original PWS multidisciplinary comprehensive management. No matter which group you are assigned to, the original treatment plan will continue. If the research results show that the treatment effect is not good, there are other clinical research programs, such as new long-acting glucagon-like peptide-1 (GLP-1) analogues or stomach volume reduction surgery, traditional Chinese medicine and so on, which can be considered. Please discuss these and other possible options with your doctor.

4. What does my child need to do in the study?

You need to provide true information about your medical history and current physical condition; Tell the research doctor about any discomfort of your child during this study; It is necessary to take your child to the clinic for follow-up and complete relevant examinations according to the scheduled time.

5. What are the risks and adverse reactions of my child participating in this study?

- Treatment risk: The application of prebiotics and probiotics, the intervention drug of the researcher, is the superimposed administration based on the original PWS multidisciplinary comprehensive management. Therefore, the study participants still maintained the original treatment plan during the study, and the treatment risk was low.
- Adverse reactions: Sabijian was used in the experiment ® Probiotic solid beverage and Sabijian ® Strawberry dietary fiber is a food-grade health care product that has been marketed. Generally, there will be no adverse reactions when consumed in the appropriate range, such as Sabijian ® Excessive strawberry dietary fiber may cause gastrointestinal symptoms such as diarrhea, abdominal distension and excessive intestinal exhaust. In the course of drug application, we will closely observe and monitor the situation of children. In case of any discomfort, new changes in the condition of the subject, or any unexpected situation in the study, whether related to drugs or not, the parents should contact the subject's primary doctor in time, and the doctor will make judgment and medical treatment. If any test-related damage occurs due to participation in the test, the subject

will receive timely treatment and corresponding compensation.

- In terms of blood sample collection: no additional blood sample is required for this study.

6. What are the benefits of this study for my children?

The application of prebiotics and probiotics in this study is based on the superimposed administration of the original PWS multidisciplinary comprehensive management. Therefore, no matter which group the study participants are assigned to, the original treatment plan will remain unchanged during the study, and will benefit from the treatment. If you are in the trial drug group, the trial drug may better improve your obesity and metabolic disorders, but we cannot guarantee this. In addition, the relevant information of this study will help to obtain important information about PWS obese children, and your participation will bring benefits to children suffering from the same pain in the future.

7. Will you be paid for participating in the research?

You will not receive any remuneration for participating in this experiment or this study. We will provide probiotics and prebiotic intervention drugs and intestinal flora detection related to this study for free.

8. What fees do I need to pay to participate in this study?

Participation in this research project will not increase your additional expenses. Your child will participate in this research, and all other relevant inspection items are routine outpatient follow-up inspection items except for the detection of fecal intestinal flora during the research process. You will not pay any additional fees for participating in this research project.

9. What if my child is injured during the study?

If your child is injured during the study or has adverse events during medication, please contact your research doctor, and your child will receive timely treatment; For any injury that has a causal relationship with the study or the trial drug, the sponsor will bear the medical expenses and give you corresponding economic compensation according to the relevant national laws and regulations.

10. Will I keep my child's information confidential?

During the study, we will collect your child's medical history information, laboratory and imaging examination results, and follow-up information. To ensure privacy, we will code some of your information, and your personal identifier (such as name, date of birth, address) will be replaced by a code (unique patient number), so that no one can determine your identity. All data are kept in the hands of the main researchers, and stored in the clinical research center for 3 years, and then destroyed. The biological samples collected during the study period are kept in the hands of the main researchers, in the clinical research center for 3 years, and then destroyed. Except for this study, you will

not use this information again in the future.

The results of the research through this project may be published in medical journals, but we will keep your research records confidential according to legal requirements. The personal information of the research subjects will be strictly confidential, and your personal information will not be disclosed unless required by relevant laws. If necessary, the government management department, the hospital ethics committee and other relevant researchers can access your data according to regulations.

11. Does my child have to participate in this study?

Whether to participate in the study depends entirely on your and your child's willingness. You and your child can refuse to participate in the study, or withdraw from the study at any time during the course of the study, which will not affect the relationship between you and the doctor, nor will it affect the loss of medical treatment or other benefits to your child.

If your child needs other treatment, or your child does not comply with the study plan, or has test-related injury or for any other reason, the study physician can terminate your child's participation in the study.

12. If I have questions, who should I contact?

If you have any questions or concerns about this study, you can consult the doctor in charge of this study: Lu Wei. The contact number is 021-64933281. If you have any questions about the right to participate in this study, please consult the Ethics Committee of the Pediatric Hospital affiliated to Fudan University, Tel: 021-64931221, Working hours: 8:00-11:30 a.m., 1:30-5:00 p.m. on weekdays.

Informed consent form (signature page)

I have read and understood the information in this informed consent. I have had the opportunity to ask questions and am satisfied with the answers to all questions. I have been given enough time and opportunity to ask about the details of the study and consider whether to participate in the study. I volunteered my child to participate in this study. Signing this informed consent does not mean that I will waive any of my legal rights.

I have been informed that I will receive a signed copy of this document.

Name of the tested child (in block letters):_____

Name of legal guardian (in block letters):_____

Signature of legal guardian:_____ Relationship with children:_____
Signature date: Contact number:_____

Statement of informed consent acquirer:

I confirm that I have explained the details of this study to the subject's guardian, including their rights, possible benefits and risks, and have given them a signed copy of the informed consent form.

Name of informed consent recipient_____
Signature of informed consent acquirer_____

Signature date_____

Contact number_____

Informed consent

(Written notice version for minors ≥ 8 years old)

You will be asked whether you are willing to assist in the research of a new type of treatment drug for obesity in PWS. Before you participate in the study, you need to discuss with your parents/guardians, and the doctor or nurse will explain the study to you and answer all your questions. If there are any problems, you can contact the research doctor at any time.

1. What is the research background and purpose of this study

PWS often develops into severe obesity due to uncontrollable appetite, lack of exercise ability and amount of exercise, and obesity related complications such as diabetes, metabolic syndrome, etc. slowly appear. If effective treatment cannot be carried out in time, bad consequences will occur. Probiotic solid beverage and prebiotic dietary fiber jelly are food-grade health products, which can help us reduce food intake, regulate the bacteria in the intestines, and help us lose weight. Our main purpose of this study is to observe the safety and effectiveness of prebiotics and probiotics on obesity in children with PWS, and to find new methods for PWS treatment.

2. What will I encounter if I participate in this study?

In the process of participating in this study, you need to come to the hospital with your father or mother or other family members to visit the doctor three times. During the outpatient visit, the doctor will ask you about the drugs you are taking, as well as your daily diet and exercise, and will carry out the same physical measurement and blood test as the previous regular follow-up, and at the same time, you need to cooperate to take some fresh stool. Finally, the doctor will call you for a follow-up.

3. Is there anything in the research that makes me feel uncomfortable, scared or uncomfortable?

During the course of the study, you may experience some side effects, which may cause you to suffer from loss of appetite, poor appetite and other discomfort.

You may also have other feelings. In the process of participating in the study, when you feel unwell or take any medicine, you must inform your parents or your life. You or your parents can call the doctor at any time.

4. Will it help me to participate in the research?

Participation in this study is expected to improve your obesity and metabolic disorder, but we cannot guarantee it. Your participation will certainly help children with the same disease as you.

5. Do I have to participate in this research?

You do not have to participate in this study. You can continue to use your

conventional medicine or ask your doctor to give other treatment drugs. After participating in this study, you can also withdraw from the study at any time if you change your mind. Whether you participate in this study or not, your doctor will give you the best medical care.

6、 If I have questions, who should I contact?

If you have any questions or concerns about this study, you can consult the doctor in charge of this study: Lu Wei. The contact number is 64933281. If you have any questions about the right to participate in this study, please consult the Ethics Committee of the Pediatric Hospital affiliated to Fudan University, Tel: 021-64931221, Working hours: 8:00-11:30 a.m., 1:30-5:00 p.m. on weekdays.

Informed consent form (signature page)

The doctor has explained this information to me in detail, and I have also asked the doctor about the words and expressions I don't understand. I understand that participation in this study is voluntary. After consideration, I am willing to participate in this study and cooperate with the doctor's diagnosis, treatment and follow-up. In case of any discomfort, I will notify my parents/guardian or doctor in time.

I understand that I can stop this study at any time, and the doctor will help me with other treatments.

Name of child subject in block letters:_____Date of birth:_____
Signature of child subject:_____ Date: _____

Doctor's statement

I confirm that I have explained the details of this trial to the patient, including their rights, possible benefits and risks, and have given them a signed copy of the informed consent form.

Signature of informed consent interview doctor (researcher):_____

Date of signature:_____ Tel.: _____

Informed consent

(<8 years old minor oral notice version)

Hello, children:

PWS often develops into severe obesity due to uncontrollable appetite, lack of exercise ability and amount of exercise, and obesity related complications such as diabetes, metabolic syndrome, etc. slowly appear. If effective treatment cannot be carried out in time, bad consequences will occur. Probiotic solid beverage and prebiotic dietary fiber jelly are food-grade health products, which can help us reduce food intake, regulate the bacteria in the intestines, and help us lose weight. Our main purpose of this study is to observe the safety and effectiveness of prebiotics and probiotics on obesity in children with PWS, and to find new methods for PWS treatment.

1. What do I need to do if I participate in the research?

If you try this new therapeutic drug, we will take care of you very carefully and carry out some tests on you. This is clinical research. You can ask your parents and doctors to help you decide whether to participate in this study. During your participation in this study, the doctor will ask you about the drugs you are taking, as well as your daily diet and exercise. You need to come to the hospital with your father or mother or other family members to visit the doctor three times, and the doctor will call you for a follow-up. If you want to participate in this study, we will also ask your father or mother if they agree.

2. Is there anything in the research that makes me feel uncomfortable, scared or uncomfortable?

We hope that therapeutic drugs can help you feel better, but sometimes therapeutic drugs may make you feel a little uncomfortable. So if you feel sick, you should tell your father or mother, or tell the doctor or nurse. We will take good care of you and make your uncomfortable feeling better soon. During the test, you may encounter the same situation as you used to see a doctor: (For example, the doctor or nurse will use a needle to draw blood from you (arm). It may hurt a little. Your arm may have red spots or bruises.)

3. Will it help me to participate in this research?

You may become better, such as weight control and obesity reduction, but we can't guarantee it. Your participation may help children with the same disease as you.

4. Is participation in this study voluntary?

No matter whether you choose to participate or not, no one will be angry. If you don't want to, you can not participate in this study. If you agree to participate in this study now and do not want to participate in it later, you can still stop participating in this study. Even if you don't want to participate in this study, your doctor will still take care of you.