Zhejiang University School of Stomatology Human Research Informed Consent Form

Dear Patient:

We sincerely invite you to participate in a clinical study titled "Oral Microbiome Research in Children with Autism Spectrum Disorder." This study is led by the Department of Conservative Dentistry, Affiliated Stomatological Hospital of Zhejiang University School of Medicine, the Department of Child Psychology, The Seventh People's Hospital of Hangzhou. The study plans to recruit 150 participants and has been reviewed and approved by the Ethics Committee of Affiliated Stomatological Hospital of Zhejiang University School of Medicine.

Before deciding whether to participate in this study, please carefully read the following information, which will help you understand the study, why it is being conducted, the procedures and duration of the study, and the potential benefits, risks, and inconveniences you may experience by participating.

Here is a detailed introduction about this research:

I. Research Overview

1. Research Background

Autism spectrum disorder (ASD) is a pervasive developmental disorder and has become the leading cause of mental disability among children in China. The onset of autism is closely related to genetic factors and environmental factors during early development. Recent studies have found that changes in the gut microbiome are associated with the pathogenesis of ASD.

Saliva immunoglobulin A (IgA) is the primary protective antibody in oral mucosal immunity, working alongside the innate immune system to inhibit the adhesion of microorganisms to mucosal and tooth surfaces, and promote the elimination of cariogenic microorganisms such as Streptococcus mutans. Given the altered oral health conditions in ASD patients, as well as the correlation between oral microbiome dysbiosis and mucosal immunity, saliva IgA levels, which are influenced by oral bacteria, may change in ASD participants.

The oral microbiome may have direct or indirect effects on the brain. Bacteria can enter the bloodstream through the mucosa or tooth roots, crossing the blood-brain barrier to reach the brain; oral-related bacteria can also promote increased levels of inflammatory factors in cerebrospinal fluid and affect the levels of neurotransmitters involved in learning and memory. There may be a connection between the oral microbiome and the brain in ASD children, suggesting a "microbiome-gut-brain axis."

2. Research Objectives

① To clarify the changes in IgA levels and oral microbiome abundance within ASD children of varying severity groups.

② To clarify the changes in IgA levels and oral microbial diversity between ASD children and normal children;

③ To determine the host immune response under oral microbial changes.

3. Research Content

Since oral health is affected by multiple factors, previous studies have observed that ASD patients have poorer oral health conditions. We propose the main scientific question of this project: What are the changes in the oral microbiome of autistic children? This study will

collect saliva and plaque from ASD children and healthy children, while also distributing oral hygiene questionnaires. Then, using RNA-seq technology, we will obtain the distribution of oral microbiota and compare the dominant microbial species in ASD children compared to normal children. The saliva IgA content will be measured using the Elisa method to verify the host immune response under oral microbial changes. Additionally, based on the severity of autism, we will analyze the correlation between the condition and the oral microbiome. This will provide experimental evidence for the screening of cariogenic microorganisms, the measurement of immunoglobulin A levels, the prediction of the relationship between oral microbiome and brain development, and the development of indicators for caries, saliva immunity, and active autism.

4. Sample Collection Methods and Sample Size

Children were not allowed to brush or rinse their mouths the night before and in the morning of sample collection. They needed to rinse their mouths gently with sterile distilled water half an hour before sampling. Saliva collection: Maintain saliva in the mouth for 1 minute, gently spit into the collection funnel, and use a 5mL sterile tube to collect 3mL of non-irritating saliva samples. Label and immediately store in dry ice buckets, then transfer to a -80℃ freezer for freezing and storage. Plaque collection: Use sterile cotton rolls to isolate the sampling site from saliva. Use sterilized cotton swabs to scrape supragingival plaque from the 11, 46 teeth on the labial/palatal and buccal/tongue sides of the subjects. Place the samples in 1mL Tris-EDTA (TE) buffer, label, and store in dry ice buckets, then transfer to a -80℃ freezer for freezing and storage.

This study aims to collect a total of 150 patients, including 50 normal children, 50 children with mild to moderate ASD, and 50 children with severe ASD. Each group will collect plaque, saliva, and questionnaires for detecting and analyzing microbial distribution and saliva IgA content.

5. Clinical Inclusion and Exclusion Criteria

Inclusion Criteria:

① Clear source: This project is supported by Hangzhou Seventh People's Hospital and The Stomatological Hospital Affiliated to Zhejiang University School of Medicine;

② Clear age, gender, and basic test completeness of all collected patients. Age 6-18 years, gender 不限;

③ The subject(s) or/and family members can understand the purpose of the trial, are willing to cooperate, voluntarily or/and with family members' consent to participate in the trial and sign the informed consent form.

Exclusion Criteria:

① Obvious brain structural abnormalities detected by magnetic resonance imaging;

② Severe sensory organ damage (blindness, hearing loss);

③ Organic gastrointestinal problems;

④ Taking antibiotics or immunosuppressive drugs within the past month;

⑤ Autism and other conditions clearly associated with genetics;

⑥ Oral mucosa with obvious abnormalities;

⑦ Wearing various orthodontic appliances and accessories.

II. Specific Procedures and Processes

If you agree to participate and meet the selection criteria, you will be included in this

study. This study employs a multicenter retrospective cohort design, and the specific research process is as follows:

The duration of this study is from the date of ethical approval to September 2025.

This project involves 2 centers: the Stomatological Hospital Affiliated to Zhejiang University School of Medicine and the Seventh People's Hospital of Hangzhou. The total recruitment target is 150 people, with our center recruiting 50 people.

III. What You Need to Do if You Participate in the Research

1. Expected Duration of Participation in the Trial

After you sign this Informed Consent Form, we will proceed with the research according to the established study protocol. Your expected duration of participation in the trial is 3 years.

2. Method of Grouping and Probability of Assignment to Each Group

According to the established research protocol, successfully enrolled participants will be assigned to 3 groups through the Autism Rating Scale method.

3. Procedures and obligations that participants need to follow

♦ We will collect, analyze, and observe your saliva IgA through saliva collection.

♦ We will collect, analyze, and observe your oral microbiota distribution through plaque collection.

IV. Potential Benefits of Participating in This Research

1. Experimental Content and Benefits/Risks Involved in the Study

♦ This study is non-interventional, aiming to achieve the research objective of studying the oral microecology of children with autism by collecting _dental plaque, saliva_ and analyzing _oral microbial distribution, saliva IgA_, without involving experimental content.

2. Benefits to Participants in the Research

This study may not directly benefit you. You may gain knowledge related to your health, receive feedback on relevant test results, and consult on related medical and health issues. Your participation may contribute to a better understanding of the causes, development, and impact of such diseases in the future, as well as aid in scientific diagnosis. The results of this study may help provide more appropriate preventive, diagnostic, and treatment interventions for you and patients with similar conditions in the future.

V. Potential adverse reactions, risks, and preventive measures of participating in this study

1. Potential adverse reactions and risks of participating in this study

♦ Since this study is _non-interventional_, it only involves the collection and analysis of your _plaque and saliva_. The risks you may face during the study will not be higher than the minimum risks you encounter in daily life or during routine physical or psychological examinations and tests.

♦This study is a __non-interventional__ study, which will collect _cases__ through questionnaires. The risks you may face during the study will not be higher than the minimum risks you encounter in daily life or during routine physical or psychological examinations, tests, or procedures. If any questions in the questionnaire make you feel uncomfortable, you have the right to refuse to answer.

♦Any treatment may cause unknown adverse reactions. Based on clinical experience, this study will not cause adverse reactions. This study only collects residual/waste tissue specimens and will not expose you to any risks beyond clinical routine procedures, but there is a

possibility of (local bleeding/perforation) during the collection of tissue specimens.

2. Risk prevention measures

♦This study only collects your medical record information, and there are no intervention behaviors involved, so no additional risk prevention measures are in place.

♦The risks you may encounter in this study will not be higher than the minimum risks you face in your daily life or during routine physical or psychological examinations/tests. Therefore, in addition to routine medical risk prevention measures, no additional risk prevention measures are set for this study.

3. Compensation and treatment available if the subject suffers research-related harm

♦The risks you may encounter in this study will not be higher than the minimum risks you face in your daily life or during routine physical or psychological examinations/tests, and no research-related harm will occur.

VI. Explanation of Costs

♦Participation in this study does not involve additional examinations or treatment items compared to routine clinical treatment, and therefore will not incur additional costs.

♦This study is observational, and only collects your _saliva, plaque_, without involving any clinical intervention measures, and therefore will not incur additional costs.

Seven、Compensation for participating in the study

♦This study is within the scope of routine treatment, and therefore you will not receive additional financial compensation.

♦This study is observational and involves only the collection of your __saliva, plaque__ without any clinical interventions, therefore you will not receive any additional financial compensation.

VIII. Alternative Options

♦There are no alternative options for this study at the moment;

♦If after careful consideration, you choose not to participate in this study, we will not use your _saliva, plaque_. Regardless of whether you participate in this study or not, your medical treatment and rights will not be affected.

IX Confidentiality of Your Personal Information

Your medical records (including research medical records and physicochemical test reports, etc.) will be stored in the hospital according to regulations. Personnel not involved in the research have no right to access your medical records without permission. Under conditions that do not violate confidentiality principles and relevant regulations, inspectors, auditors, ethics committee members, and drug regulatory inspection personnel may review your original medical records to verify the process and data of clinical trials. The public reports of the results of this study will not disclose your personal identity. We will make every effort within the allowed scope to protect the privacy of your personal medical records.

X Termination of Participation in the Research

You have the right to withdraw from the trial at any time and at any stage without discrimination or retaliation, and your medical treatment and rights will not be affected. If any new situation occurs that may affect your rights during the trial, we will promptly inform you, and you can decide whether to continue participating in this experiment. Additionally, your participation in this study will be terminated in the following cases:

1、Saliva and plaque samples cannot be purified;

2、Saliva and plaque samples are contaminated;

3、The patient has other diseases, such as genetic diseases or viral infections, which are not suitable for participating in this project research. The research physician believes it is necessary to terminate your participation in the study.

XI Ethics Committee

This study has been reported to the Medical Ethics Committee of the Stomatological Hospital Affiliated to Zhejiang University School of Medicine. After a comprehensive review by the committee, including the risk assessment of the subjects, it has been approved. During the research process, matters concerning ethics and rights can be contacted at the Medical Ethics Committee of the Stomatological Hospital Affiliated to Zhejiang University School of Medicine, Tel: 0571-87219287. (Workdays 8:00-12:00, 13:30-17:00)