项目来源与编号：

基于尿液五羟色胺等神经递质水平进行孤独症/自闭症患者筛查（非干预性）临床研究方案

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一、立项依据与研究背景

自闭症谱系障碍（Autistic Spectrum Disorder, ASD）是一种严重的神经发育性疾病[1]。在过去几十年中，自闭症的患病率急剧上升，现在美国 59 个儿童中就有 1 个患有 ASD[2]。根据有关残疾人的人口普查数据，中国的 ASD 患病率也再不断上升。先前的研究报道，大多数 ASD 儿童的血液中血清素（5-HT）含量升高[3 4]。最近的研究也表明，5-HT 是肠道神经系统（ENS）和中枢神经系统（CNS）发育的关键调节剂，并可能在 ASD 儿童中充当肠脑轴的纽带[3 5 6 7]。但是，如何在家里检查他们的 5-HT 水平和其他神经递质的改变，以帮助 ASD 的父母干预 ASD 的行为受损，是一个亟待解决的难题。

二、研究设计与方法

1. 研究目标

本研究基于合成生物学设计了一种全细胞生物传感器，用于 ASD 患儿尿液中的 5-HT 等神经递质的居家检查，以辅助 ASD 患儿的父母持续监测孩子的行为障碍。转化有改造质粒的工程细菌（大肠杆菌 BL21）能够检测尿液中 5-HT 的浓度并显示肉眼可见的颜色变化。

2. 研究内容

本研究为针对正常人群与自闭症患病人群的现况调查研究。针对正常人群（N = 15），本研究计划使用既往留存的尿液样本约 10 毫升，纳入标准为：

年龄 3-9 岁的正常儿童；

饮食正常；

针对 ASD 患病人群，本研究将在现场对参加研究的受试人（N = 15）采集约 15 毫升清洁中段尿液样本。本研究受试人群的纳入标准为：

年龄 3-9 岁的自闭症患者（CARS 评分＞30 分）；

饮食正常；

自愿参加本实验，家属签署知情同意书；（知情同意的全过程由刘星吟教授负责，南京市江宁区海之星自闭症儿童康复中心协助，由监护人签署相关知情同意书）

采集得到的尿液样本用于 5-HT 等神经递质的靶向质谱检测与生物传感器功能测试，后续计算筛检试验的灵敏度、特异度、阳性预测值、阴性预测值，不做其他用途。所有实验将在南京医科大学基础医学院病原生物学系刘星吟实验室（B511，B515）完成。

➢ **涉及生物样本采集的需写明：**

· 样本类型：尿液样本；
· 样本量：30 例；
· 样本来源：15 例（正常儿童）来自既往留存，15 例（ASD 患儿）计划采集；
· 剩余样本的处理与销毁：剩余样本将于南京医科大学基础医学院病原生物学系实验室内留存。

3. 拟解决的科学问题与预期成果

本研究拟通过尿液样本检验生物传感器效率，期望看到载有工程细菌的试纸可以对高 5-HT 自闭症患儿的尿液响应并变色，而对正常儿童的尿液无响应不变色。最终实现尿液 5-HT 等神经递质的居家检测，帮助 ASD 患儿的父母识别并早期干预患儿的社交行为障碍。

三、研究进度

2020 年 9 月 18 日-2020 年 9 月 20 日：完成样本收集；

2020 年 9 月 20 日-2020 年 10 月 31 日：完成样本中 5-HT 等神经递质水平的质谱测定，验证生物传感器功能。

四、参考文献

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(Non-Interventional) Clinical Research Plan of Project: "Screening of ASD Patients Based on Urinary Serotonin (or Other Neurotransmitters) Levels based on synthetic biology"

Undertaking Unit:

Department of Pathogen Biology,
School of Basic Medicine Science,
Nanjing Medical University

Project Leader: Xingyin Liu **Tel:** 86869397

Version Number: 1 **Date:** 9/11/2020

1. Project Background

Autistic Spectrum Disorder (ASD) is a severe neurodevelopmental disorder [1]. With its prevalence increasing dramatically over the past decades, ASD now affects 1 out of 59 children in the United States [2]. According to national census data on handicapped individuals, China also has an increased prevalence with ASD first on its list of top mental disabilities. Previous studies have reported that most ASD children displayed elevated Serotonin (5-HT) levels in the blood [3] [4]. Recent studies implicated that Serotonin is a critical modulator of enteric nervous system (ENS) and central nervous system (CNS) development and may function as a nexus for the gut-brain axis in ASD children[3] [5] [6] [7]. However, how to check their serotonin level and other altered neurotransmitters at home, so as to help ASD's parents intervene in the impaired behavior of ASD, is an urgent and challenging problem to be solved.

2. Project Design

2.1 Research Objectives

In this study, a whole-cell biosensor was designed based on synthetic biology for home inspection of 5-HT and other neurotransmitters in the urine of children with ASD to assist parents of children with ASD to continuously monitor their children's behavioral disorders. The engineered bacteria (*E. coli* BL 21) transformed with the modified plasmid can detect the concentration of 5-HT in the urine and show a visible color change.

2.2 Research Content

This study is a survey of the current situation of normal and autistic children. For the normal population (N = 15), this study plans to use approximately 10 milliliters of previously retained urine samples. The inclusion criteria are:

Normal children aged 3-9 years; Eating normally;

For the ASD population, t this study plans to collect about 15 milliliters of clean midsection

urine samples from the participants (N = 15) on site. The inclusion criteria are:

Autistic patients aged 3-9 years (CARS score> 30 points); Eating normally; Be voluntary to participate in this experiment, with an informed consent form signed by family members (Professor Xingyin Liu is responsible for the whole process of informed consent, assisted by Haizhixing Autistic Children Rehabilitation Center, Jiangning District, Nanjing, and the guardian will sign the relevant informed consent form).

The collected urine samples are used for the targeted mass spectrometry detection of neurotransmitters such as 5-HT and biosensor function testing, and the sensitivity, specificity, positive predictive value, and negative predictive value of the screening test are subsequently calculated. The samples will not be used for other purposes. All experiments will be completed in Prof. Xingyin Liu's Laboratory (B511 & B515) in the Department of Pathogen Biology, School of Basic Medical Sciences, Nanjing Medical University.

If it involves collection of biological samples, the following must be stated:

Sample type: Urine sample;

Sample size: N = 30;

Source of samples: 15 cases (normal children) are from the past, and 15 cases (children with ASD) are planned to be collected;

Processing and destruction of remaining samples: The remaining samples will be kept in the laboratory of the Department of Pathogen Biology, School of Basic Medicine Science, Nanjing Medical University.

2.3 Expected Results

This study intends to test the efficiency of the biosensor through urine samples. It is expected that the test paper loaded with engineered bacteria can respond to and change color in the urine of autistic children with high 5-HT level, but does not respond to the urine of normal children. Finally, we expect our engineered biosensor could serve as a home-based detection manner of neurotransmitters such as 5-HT in urine, helping parents of children with ASD to identify and intervene early in their social behavior disorders.

3. Research Schedule

September 18, 2020 to September 20, 2020: Complete sample collection;

September 20, 2020 to October 31, 2020: Complete the mass spectrometry determination of 5-HT and other neurotransmitters in samples and verify the function of the biosensor.

4. References

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