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Summary Protocol – Autism and Gastrointestinal Disorders – Updated 10/13/21

For the +ASD/+GI and +ASD/-GI groups, PI, Co-PI and research coordinator will assist in recruitment. When a patient has a regularly-scheduled medical appointment with The pediatric gastroenterologist, she will give the family a flyer with information about our study (included as a supporting document) and ask if they would like to be contacted with further information and to determine if inclusion criteria are met. The original IRB application includes the 'clinician flyer' and script for use as an introduction to the study, and the steps for how to recruit potential participants. This clinician flyer includes a box for families in which to write their phone number, so that our research team can contact them about the study. The flyer also contains a section that allows potential participating families to leave their phone number on the bottom half of the flyer, detach this half and deposit it in a locked box at the clinic reception desk, and take the top half home for general study information. Our research coordinator collects these phone numbers from the locked boxes and calls interested families to tell them more about the study and screen for eligibility. If the pediatric gastroenterologist determines the patient has GI dysfunction, specifically FC (which is found in >85% of children presenting with GI symptoms) then she will recommend best practices treatment or make a referral to another pediatric gastroenterologist should the family request this. If the family is not interested in joining the study, the pediatric gastroenterologist will still recommend the patient be evaluated by her practice, or another gastroenterologist. If the family does express interest in joining the study, a member of the research team will contact these families by phone, using Phone Script #1 (included as a supporting document), which includes eligibility screening by asking if participants meet inclusion criteria. Regardless if a family would like to participate in our study or not, if the pediatric gastroenterologist determines they should be evaluated and treated by a gastroenterologist and the family agrees, then she will make a make relevant arrangements for a follow-up appointment for initiation of best practices treatment or for the family to see another pediatricgastroenterologist.After contacting participants by phone, we will schedule an appointment for eligible study participants and primary caregivers to come to CHLA. Prior to the appointment, we will send an appointment reminder letter with the scheduled date and time, driving directions, and a list of important information to bring to the appointment (such as current medications and social security numbers, which are needed for participant compensation). Additionally, we will send the consent form by mail to be read and completed by caregivers at home. We do this to minimize the time required of families during their appointment at CHLA. Please note that we will complete the consent process in person when a family comes in for their appointment, we will confirm consent and assent, sign the consent documents, and return copies to participating families. Only after the consent process has been completed will we ask to access the completed questionnaires. For participants who will see the pediatric gastroenterologist, a clinical referral may be made by their primary care physician This referral to the pediatric gastroenterologist will be billed to the participating family or their insurance, as this study does not pay for the clinic appointment with the pediatric gastroenterologist. We make this fact clear to potential participating families during the consent process. Of the possible combinations, we will actively recruit two groups of participants (namely, +ASD/+FC, +ASD/-FC). We will work with participating families to complete all the items at the initial and subsequent visits at 3 months, 6 months and 12 months. These times were selected because pediatric gastroenterology follow-up for a child with a disorder typically follows these timepoints, and ASD symptom and associated behavioral changes in either group are expected to show changes after several months. The specific steps will not always occur in the order. For example, occasionally the pediatric gastroenterologist will see a participant before the clinical psychologist does; in all cases, consenting will occur first, however. A member of the research staff (who does not have an existing relationship with participants) will be responsible for all

interactions regarding consent. The consent and assent forms will be mailed home before the family arrives at CHLA, to allow them to be read at their leisure. Informed consent for the children will be obtained from the parent/legal guardian in writing before the child begins participation, and they will receive a copy of the form for their own records. Parents will be informed that consent is voluntary and can be withdrawn at any time without consequence. If, for any reason, the parent/legal guardian declines to sign the consent form, the child will not be allowed to participate in the study in any way. In most cases, consent forms will be mailed to screened participants and caregivers ahead of their appointment, allowing the consent form to be read at home. Consenting will be finalized in a room on the Saban first floor, where privacy is ensured. Once consent has been obtained from the parent/legal guardian, we will confirm with the child, in an age-appropriate manner, that he or she would like to participate. The assent form will be sent home in advance to be read at home, but we will also allow the child time to ask any questions or express dissent. Because some children with autism spectrum disorders have intellectual disability and/or lower language levels than their chronological age indicates, we will use the age 5-7 assent script (even if the individual is older than 5-7 years) for these individuals to verbally explain the study, and we will be very sensitive to dissenting behaviors from the child and continually check in with the caregiver for any sign that the child would like to not participate. The child will be asked if he/she would like to participate, and it will be explained that he/she may discontinue participation at any time with no penalty or consequence. Age-appropriate assent forms are included in this IRB application. If any child exhibits dissenting behaviors such as repeatedly saying “No” or “I don’t want to”, beginning to cry excessively for several minutes, hiding behind the parent/guardian, or refusing to interact with the research staff, the child will not be forced to co-operate. In this case, the parents will be consulted and given options including giving the child a break, discontinuing and rescheduling the session, or stopping participation in the study. If the child or parent chooses to discontinue participation in the study altogether, the family will then receive their compensation and leave with no negative consequences. During the consenting process, we will make it clear to participants that their medical care and services will not be affected if they do not complete any part of the study. Additionally, we make it clear that no genetic information will be added to their medical records, which makes the risk of future genetic discrimination extremely small. As explained in the primary caregiver consent form, a participant who agrees to be in this study also agrees to let the research team use theparticipant¿s protected health information (PHI). With this agreement, after a participant has been seen and evaluated by the pediatric gastroenterologist or the clinical psychologist or another clinician at CHLA for either their ASD or GI dysfunction (and the doctor has written a note into electronic medical records) our research team will access the participant’s medical record, to acquire the text of those notes. Within the medical record, our research team will only look at the dates and appointments relevant to their gastrointestinal problems or autism spectrum disorder, and then we will copy this information into our secure Redcap study database for analysis purposes. Only senior members of the research team, trained in proper procedures regarding PHI, will access participants¿ medical records. In addition to explaining that we will access PHI from the participants medical record, we also ask parents to complete a record release form. Additionally, on the consent form we offer participants the option to receive a one-page summary of the results and findings from the study (for general informational purposes only), two years after completion of the study (or at the time of publication of any results from the study). We offer this to families to keep them informed regarding any results that come out of their participating in our study. After consenting, if a participant has had an ASD-related assessment in the last 2 years at CHLA (which includes the ADOS, and assessments of cognitive functioning such as the Mullen assessment or a Stanford-Binet assessment), we ask the parent/guardian to sign a medical and behavioral record release form, to gain access to previous psychological assessments from providers at CHLA. This record release form, and the cover letter we use to contact providers to request the release of

records, are included with this application. This record release form is also used to gain access to participants¿ Star Panel medical records, as discussed above. For the +ASD/+FC group, participants will be evaluated by the pediatric gastroenterologist. The pediatric gastroenterologist will acquire a focused clinical history and perform a physical exam. This evaluation will occur in the pediatric GI clinic at CHLA. The pediatric gastroenterologist might also advise that a participant be evaluated further via endoscopic examination. If she advises this, it will be for medical reasons only. In the consent process, we will make it clear to families that endoscopic recommendation will only be for medical reasons and outside the limits of this study, and therefore not paid for by this study. For the two +ASD groups, participants will be evaluated by a licensed developmental psychologist, using a clinical evaluation/interview and/or the Autism Diagnostic Observation Schedule (ADOS-2; included with this application). This assessment will occur in a room on the 9th floor of DOT. The assessment is audio- and videotaped with consent of the parent/guardian. The ADOS is a standard assessment measure used in autism research and will be used in this study to confirm diagnoses of ASDs in participants. The results of the clinical evaluation/interview ADOS will be stored in our study database. Additionally, if a participant has been assessed for ASD in the last 2 year at CHLA, after consenting we ask parents/guardians to sign a release of medical records (included with this application as a supporting document, along with a cover letter we send to providers who are potentially releasing such information). This release requests providers to release to our study psychological assessments relevant to the participant's ASD diagnosis (such as the ADOS, and assessments of cognitive functioning). If the parent/guardian signs this release form, then our study team will contact the appropriate provider to acquire this information. In this case, participants will not be assessed again with the ADOS during the visit for this study, which will shorten the family's time commitment. Please note that this release request and cover letter are modeled after analogous documents used by another IRB-approved ASD genetics study at Vanderbilt. We do ask that parents/guardians bring copies of their child's previous assessments/reports to the research appointment, so that we may confirm when the previous assessment was performed (i.e., within the last year or not) and which study or provider to contact to request the release of records. Participants and caregivers will be asked to complete questionnaires (a subset are approved in the original IRB application and subsequent amendments), which in total will require approximately 1-1.5 hour per participant. Additional instruments are included in this renewal. We send these questionnaires (all in the option of English or Spanish) home in advance of the family's appointment, to be completed at their leisure, but we will also have time available as needed to answer questions about the questionnaires, during their appointment. One questionnaire will ask about general gastrointestinal history (including food allergies, medications taken, and nutrition history). Another questionnaire will ask about lower GI symptoms. Behavioral questionnaires will ask about social behaviors, emotional regulation, externalizing and internalizing behaviors and basic quality of life questions. Each questionnaire will take ~10-15 minutes to complete. We will provide either hard copies of the questionnaires or a laptop computer that caregivers can use to fill out these questionnaires through the REDCap Survey system. If caregivers prefer to not use a computer, we will also have paper copies of the questionnaires available. A member of the research team will be available during this time to answer any questions that caregivers or participants might have. We will also ask participants to donate a urine sample, for a total of 10 ml. These tubes will be processed and stored either in the Pediatric CRC or in the PI's 3rd floor laboratory in the The Saban Research Institute, which is one block from the Boone-Fetter and Peds-GI clinics. A research assistant working on this study will complete this sample processing and store frozen samples in a locked -80¿C freezer until biochemical analyses will be completed in bulk. Please note that these samples, stored with a coded identification number, will be stored in the PI's lab in the TSRI for further analysis. Participants also will be asked to donate saliva. We will ask for 3-5 ml of saliva, which will be stored and processed for DNA

extraction using a standard protocol in the PI's laboratory. If a subject is unable to spit to collect a saliva sample, we will use a cotton swab to wipe the inside of his/her cheek. There is no additional risk associated with this method of collection. Urine also will be collected at 3, 6 and 12 month visits. Saliva will not be collected after the first visit. At the end of the day at CHLA, all participants will be mailed a $50 gift card for compensation. Even if participants do not complete all questionnaires or do not donate a blood or saliva sample, they will still receive this compensation. Completion of the visits at 3 and 6 months will trigger mailed $25gift cards. The final visit completion will trigger mailing of a $50 gift card. Total compensation for completing the study for those with ASD only will be $50. Those with GID, with or without ASD, will be an additional $50. At the completion of the study, we will also mail participants an anonymous satisfaction survey to gain feedback on the study. The primary school teacher of an enrolled subject will also be invited to participate in the study. Teachers will be mailed consent forms (with attached introduction letter) and/or provided consent forms by the subject's parent. Teacher participation will involve completion of questionnaires on four occasions throughout the course of the study year. Teacher questionnaires will be administered around the same time subjects complete study visits. A return envelope will be provided for teachers to mail signed consent forms to the study team. Teachers will be given a $25 gift card for each completed questionnaire (4 total). Teacher questionnaires will be timed around the subject's study visits.

Statistical Methods

Sample size estimates were performed by our statistical consultant, Christianne Lane, PhD using G*Power (v. 3.1)61 with a = 0.05 and80% Power for the primary outcome SRS. The proposed sample size has sufficient sensitivity to detect small changes in primary and secondary outcomes acro
ss time effect size, even when stratified by group (f =.07 - .16, for intra-individual correlations 0.8 to 0.2, respectively), assuming 15%attrition across the year of follow-up. We also will be able to detect small increases in R2 in the entire sample (R2? = .04), and moderate increased within the ASD+/FC+ sample (R2? = .08). Using the SDs from our recent prospective study of the Vanderbilt cohort13 for ASD+/FC+ and ASD+/FC-groups, this should translate into detectible changes across time of ~ SRS scores of 3-5 points, and FC improvement effect of ~ 4.5 points. For F2-IsoP, the detectible change across time should be ~ 2.6-5.9, and FC improvement effect of ~3.0. Even with the complexity of these nested models, we will have sufficient power to detect the mediating effect of FC improvement on our behavioral and oxidative stress outcomes.