PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	How Stress, Discrimination, Acculturation, and the Gut Microbiome Affect Depression, Anxiety, and Sleep among Chinese and Korean Immigrants in the United States: A Cross-Sectional Pilot Study Protocol
AUTHORS	Kim, Sangmi; Zhang, Wenhui; Pak, Victoria; Aqua, Jasmine; Hertzberg, Vicki; Spahr, Chandler; Slavich, George; Bai, Jinbing

VERSION 1 – REVIEW

REVIEWER	Peters-Samuelson, Brandilyn Yeshiva University Albert Einstein College of Medicine
REVIEW RETURNED	09-Mar-2021

GENERAL COMMENTS	The study protocol “How Stress, Discrimination, Acculturation, and the Gut Microbiome Affect Depression, Anxiety, and Sleep among Chinese and Korean Immigrants in the United States: A Cross-Sectional Study Protocol” provides detailed procedures for a pilot study measuring sociodemographic factors and the gut microbiome in relation to mental health outcomes. The battery of surveys is comprehensive for the variables of interest. Some additional details of the microbiome analysis can be clarified, as described below. -add “pilot study” to the title and asbtract. The sample size is too small to generate conclusions so the pilot nature of the study should be emphasized more prominently. -the background might benefit from some additional explanation of the gut microbiome-brain axis, e.g. how is the microbiome hypothesized to effect the brain -exclusion criteria should include antibiotics in past 1 or 6 months, if feasible. -Clarify whether the DSF is created specifically for this study, or a validated survey like the others? Does it also assess medications taken or clinical mental health history (i.e. diagnosis of depression, on medications for depression/anxiety)? Would it be better to exclude such participants if the interest is unaddressed depression/anxiety? -Has the primescreen been validated for Asian Americans (Chinese or Korean)? Diet is very culture specific and some of the food items may not apply. Conversely, some important dietary intakes in Asians may not be captured by this questionnaire -Gut microbiome specimen collection – provide more detail about the contents of the sample collection kit and instructions. Do participants need to put the sample in the fridge because there is no preservation media in the sample tube? How will the sample be stable during mailing?

	-With the stool kit it may also be helpful to include a Bristol stool scale questionnaire, since this can be a confounder in microbiome analysis. -Will DADA2 be used to identify amplicon sequence variants (ASVs)? -Statistical analysis – be more specific regarding analysis of microbiome diversity (e.g. correlations will be used for alpha-diversity; PCoA for beta-diversity). Specify what measures will be used (e.g. richness, Shannon index, unifrac, JSD, etc). The authors may also use PERMANOVA (adonis function in R) to associate overall gut microbiome composition (beta-diversity) with participant characteristics. -" Associations between the gut microbiome and demographics and psychosocial factors will be analyzed using microbiomes' composition" – the method cited is referred to as ANCOM. ANCOM and LefSe are two different methods for detecting differentially abundant taxa, so it is not clear why ANCOM will be used to associated microbiome with predictors, and LefSe for associations with outcomes. The investigators may want to compare taxa associated with outcome for the two methods. -Page 20, line 15 – typo, the "greatest" mental health-related disease burden

REVIEWER	Park, Chorong Vanderbilt University
REVIEW RETURNED	24-Mar-2021

GENERAL COMMENTS	Asian Americans' psychological distress and its influence on health are time-sensitive and very important issues. However, the protocol paper has some points that need to be clarified or improved. 1. Please provide rationales why you only focus on the East Asian population (Korean + Chinese) rather than Asian American overall. South Asians (e.g. South Indians) or Southeast Asians (e.g. Filipinos) may have different acculturation experiences and discrimination experiences. If you wanted to look at the Asian population's overall experience, I would suggest including other Asian subgroups, such as South Indians, Bengalis, Filipinos, or Vietnamese. Otherwise, you might narrow down the scope, say that you are only focusing on East Asians, and provide rationales (why focusing on East Asians is important). 2. Inclusion criteria: You only include those who speak the Mandarin language. Those who only speak Cantonese would be excluded? Why you don't include Cantonese-speaking people? This would limit the generalizability of your findings. Also Mandarin is a speaking language, not a written language. Here, "can read and write English, Mandarin Chinese" should be changed to either "can speak English, Mandarin, or Korean" or "can read and write English, traditional and simplified Chinese, or Korean". Also you included 1st and 2nd generations. They would have very different acculturation and discrimination experiences. They may be different in terms of their diet and sleep, which may influence their gut microbiome. Since those two generations are different, the heterogeneity may influence your findings. The differences between the two generations should be addressed in statistical analysis and you need to justify why you want to include both generations. Also, the sample size is only 60 (30 Koreans, 30 Chinese) and if you also stratify them by generation, each cell would be only 15, maybe too small.

	3. Recruitment methods may cause some selection bias. The study protocol did not show what % of participants will be recruited from online vs. offline. The participants recruited from online communities or SNS would be younger and techier than those who recruited from offline communities (such as churches). it is necessary to compare two recruitment methods and if there are any differences in their demographics and the variables of interests. This needs to be addressed in the statistical analysis section. 4. Since this is the protocol paper, I expected more details on recruitment methods, settings, and the patient and public involvement section. Please provide more details on the settings (specifically for the offline recruitment sites, settings, what clinics, where they are located), and the advisory board in terms of the members' demographics, and how they can contribute to bridging you and the lay communities. For example, how frequently you will meet together, what agenda will be addressed, how their opinions will be adapted in the recruitment process, etc. 5. The numbers of items from all of the questionnaires are over 200. Doing this long survey online and delivered by email (without any helps from RAs) may cause many missing values. Need to consider paper-and-pencil methods or completing the surveys with RAs (while using Redcap on their Ipad or some other tablets) for at least those who are recruited from offline. Also despite the large efforts on completing self-reported surveys and collecting their stools, there is no explanation about participant compensation (how much and how you will deliver). 6. Lack of detailed information on the procedure. The authors mentioned that "when potential participants contact the research team directly or via referral, our research staff will contact them back and make an appointment to screen their eligibility and obtain consent to participate in the study". The appointment will be in-person appointment or will be done verbally over the phone? If it is an in-person appointment, the authors should provide details on the locations, settings, etc. 7. No discussion on limitation. The authors only mentioned cross-sectional study design as a limitation in strength and limitation (after abstract), despite several major limitations (e.g., small sample size, only focusing on Korean and Chinese Asian subgroups, only collecting surveys via email) 8. No dates of the study: the dates of the study should be included in the manuscript.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Brandilyn Peters-Samuelson, Yeshiva University Albert Einstein College of Medicine

Comments to the Author:

The study protocol “How Stress, Discrimination, Acculturation, and the Gut Microbiome Affect Depression, Anxiety, and Sleep among Chinese and Korean Immigrants in the United States: A Cross-Sectional Study Protocol” provides detailed procedures for a pilot study measuring sociodemographic factors and the gut microbiome in relation to mental health outcomes. The battery of surveys is comprehensive for the variables of interest. Some additional details of the microbiome analysis can be clarified, as described below.

Thank you very much for your positive assessment of this article and for your very helpful and constructive comments.

-add “pilot study” to the title and abstract. The sample size is too small to generate conclusions so the pilot nature of the study should be emphasized more prominently.

We have added ‘pilot study’ to both the title and abstract.

-the background might benefit from some additional explanation of the gut microbiome-brain axis, e.g. how is the microbiome hypothesized to effect the brain

Thank you for this suggestion. Additional explanation of the gut microbiome-brain axis has now been provided in the Introduction.

-exclusion criteria should include antibiotics in past 1 or 6 months, if feasible.

We collect the information on antibiotic use over the past month. As suggested, we now include this information in the exclusion criteria section. Please see ‘Study Design and Participants’ under Method. Because we began data collection before changing the screening survey, we will exclude participants who reported using antibiotics from statistical analysis.

-Clarify whether the DSF is created specifically for this study, or a validated survey like the others? Does it also assess medications taken or clinical mental health history (i.e. diagnosis of depression, on medications for depression/anxiety)? Would it be better to exclude such participants if the interest is unaddressed depression/anxiety?

The DSF is not a validated survey. However, most of the items used were derived from the NIH Common Data Elements and have been widely used, as well as in one of the author’s ongoing study.

Although the DSF does not specifically ask about individuals’ history of mental health conditions, it does ask whether a participant has ‘other diseases or conditions’ and has ever used mental health services. This information is now included under DSF.

Lastly, we did not exclude individuals with a history of depression/anxiety because the current pilot study is designed to explore relations between psychosocial risk factors, gut microbiome, and mental health outcomes, regardless of individuals’ diagnosis of depression or anxiety. However, as you pointed out, our long-term goal is to intervene with unaddressed depression and anxiety; therefore, our future work may exclude persons with a current or lifetime diagnosis of depression and/or anxiety.

-Has the primescreen been validated for Asian Americans (Chinese or Korean)? Diet is very culture specific and some of the food items may not apply. Conversely, some important dietary intakes in Asians may not be captured by this questionnaire

Thank you for this question and comment. To our knowledge, the PrimeScreen has been tested mostly among Caucasians and African Americans. Although our use of the PrimeScreen would be a good opportunity to test this measure among Chinese and Koreans, we acknowledge the measure’s limitation and have added this as a limitation in the Discussion section.

-Gut microbiome specimen collection – provide more detail about the contents of the sample collection kit and instructions. Do participants need to put the sample in the fridge because there is no preservation media in the sample tube? How will the sample be stable during mailing?

More specific information has now been added to the 'Gut Microbiome' section.

-With the stool kit it may also be helpful to include a Bristol stool scale questionnaire, since this can be a confounder in microbiome analysis.

Thank you very much for this suggestion. We will include the Bristol Stool Scale in our next study on this topic.

-Will DADA2 be used to identify amplicon sequence variants (ASVs)?

Yes, we have now added this point to the 'Statistical Analysis' section.

-Statistical analysis – be more specific regarding analysis of microbiome diversity (e.g. correlations will be used for alpha-diversity; PCoA for beta-diversity). Specify what measures will be used (e.g. richness, Shannon index, unifrac, JSD, etc). The authors may also use PERMANOVA (adonis function in R) to associate overall gut microbiome composition (beta-diversity) with participant characteristics.

Thank you for this suggestion. We have now added more specific measures and methods for analyzing microbiome diversity. Please see the 'Statistical Analysis' section.

-"Associations between the gut microbiome and demographics and psychosocial factors will be analyzed using microbiomes' composition" – the method cited is referred to as ANCOM. ANCOM and LefSe are two different methods for detecting differentially abundant taxa, so it is not clear why ANCOM will be used to associated microbiome with predictors, and LefSe for associations with outcomes. The investigators may want to compare taxa associated with outcome for the two methods.

Thank you for pointing that out. We deleted the sentence and reference. We have also clarified that we will use LefSe for characterizing the taxa differences between different levels of outcome variables. Please see the 'Statistical Analysis' section.

-Page 20, line 15 – typo, the "greatest" mental health-related disease burden
We have changed "greatest" to 'substantial.' Thank you.

Reviewer: 2
Dr. Chorong Park, Vanderbilt University
Comments to the Author:
Asian Americans' psychological distress and its influence on health are time-sensitive and very important issues. However, the protocol paper has some points that need to be clarified or improved.

1. Please provide rationales why you only focus on the East Asian population (Korean + Chinese) rather than Asian American overall. South Asians (e.g. South Indians) or Southeast Asians (e.g. Filipinos) may have different acculturation experiences and discrimination experiences. If you wanted

to look at the Asian population's overall experience, I would suggest including other Asian subgroups, such as South Indians, Bengalis, Filipinos, or Vietnamese. Otherwise, you might narrow down the scope, say that you are only focusing on East Asians, and provide rationales (why focusing on East Asians is important).

Thank you for this important question. This study focuses only on Chinese and Koreans for two reasons. First, and most substantively, Chinese and Koreans combined constitute the largest subgroup of Asians in the U.S. and therefore experience the greatest overall mental health-related disease burden. Second, and pragmatically, these groups are the sponsor's populations of interest. Specifically, Brazil, China, Ethiopia, India, and South Korea are designated by Emory University as priority countries to develop and implement country-based research initiatives for strategic engagement due to their higher concentration in the Greater Atlanta area. As you mentioned, Asian populations are heterogeneous with each subgroup's unique lived experience. Therefore, it seems appropriate to focus on Chinese and Koreans to limit the heterogeneity of the sample, especially given the limited sample size. To address this comment, we have now provided the rationale for focusing on Chinese and Koreans as our target populations in the 'Present Study' section of the Introduction.

2. Inclusion criteria: You only include those who speak the Mandarin language. Those who only speak Cantonese would be excluded? Why you don't include Cantonese-speaking people? This would limit the generalizability of your findings.

Also Mandarin is a speaking language, not a written language. Here, "can read and write English, Mandarin Chinese" should be changed to either "can speak English, Mandarin, or Korean" or "can read and write English, traditional and simplified Chinese, or Korean".

Also you included 1st and 2nd generations. They would have very different acculturation and discrimination experiences. They may be different in terms of their diet and sleep, which may influence their gut microbiome. Since those two generations are different, the heterogeneity may influence your findings. The differences between the two generations should be addressed in statistical analysis and you need to justify why you want to include both generations. Also, the sample size is only 60 (30 Koreans, 30 Chinese) and if you also stratify them by generation, each cell would be only 15, maybe too small.

Thank you for this detailed comment. First, Cantonese-speaking individuals are not excluded as Cantonese can also use Mandarin. Second, following your suggestion, we have changed the inclusion criterion to 'can read and write English, traditional and simplified Chinese, or Korean.' Please see 'Study Design and Participants' in the Method section. Third, we agree that risk factors and health outcomes can differ according to the immigrant's generation. Also, we acknowledge the limited sample size for the stratified analysis. Therefore, while it will not be possible to develop sub-groups based on this particular sample, our future larger study will examine these possible generational differences. We have now added this limitation (i.e., small sample size precluding the empirical test of the generational differences) as a limitation in the Discussion section.

3. Recruitment methods may cause some selection bias. The study protocol did not show what % of participants will be recruited from online vs. offline. The participants recruited from online communities or SNS would be younger and techier than those who recruited from offline communities (such as

churches). it is necessary to compare two recruitment methods and if there are any differences in their demographics and the variables of interests. This needs to be addressed in the statistical analysis section.

You are correct about the different demographics between those recruited online and offline. Because it has been several months since we recruited participants and we have not collected information on where they saw or heard about our research, we have now included this point as a limitation and will make sure to address this issue in our future research.

4. Since this is the protocol paper, I expected more details on recruitment methods, settings, and the patient and public involvement section. Please provide more details on the settings (specifically for the offline recruitment sites, settings, what clinics, where they are located), and the advisory board in terms of the members' demographics, and how they can contribute to bridging you and the lay communities. For example, how frequently you will meet together, what agenda will be addressed, how their opinions will be adapted in the recruitment process, etc.

Thank you for these suggestions. In 'Recruitment,' we have now included more specific information on the offline recruitment settings (e.g., community partners and locations). In addition, in 'Patient and Public Involvement,' we now describe the demographic characteristics of the advisory board members and the operation of the advisory board (e.g., how the members contribute, how frequently they meet, and meeting agenda).

5. The numbers of items from all of the questionnaires are over 200. Doing this long survey online and delivered by email (without any helps from RAs) may cause many missing values. Need to consider paper-and-pencil methods or completing the surveys with RAs (while using Redcap on their Ipad or some other tablets) for at least those who are recruited from offline. Also despite the large efforts on completing self-reported surveys and collecting their stools, there is no explanation about participant compensation (how much and how you will deliver).

Thank you for the comments. Because this study is being conducted amid the COVID-19 pandemic, we administer a non-contact online survey. Although we allow participants to opt-out of the questions they wish not to answer, our prior research using the current study's instruments showed little missing data. For example, most of the questionnaire items in the current study come from the Adult STRAIN due to their thorough and comprehensive assessment of individuals' lifetime stress. However, according to our prior research with Adult STRAIN, we rarely have missing data as it focuses on people's lives; participants were typically happy to answer the questions in the instrument. Moreover, after reviewing the current study's data collected so far, we found no missing data. Nevertheless, to ensure the data quality for statistical analyses, we will analyze the missing data, if any, to determine if they are missing at random or have any patterns that could induce bias. This is added to the 'Statistical Analysis' section. Besides, the information on participant compensation is added to the 'Data Collection' section.

6. Lack of detailed information on the procedure. The authors mentioned that "when potential participants contact the research team directly or via referral, our research staff will contact them back and make an appointment to screen their eligibility and obtain consent to participate in the study". The appointment will be in-person appointment or will be done verbally over the phone? If it is an in-person appointment, the authors should provide details on the locations, settings, etc.

Thank you for these suggestions. In the 'Data Collection' subsection, we have now provided clearer information on the consent process. In short, the research team will call potential participants and verbally obtain their consent. Due to the COVID-19 pandemic, there will be no in-person interactions with participants.

7. No discussion on limitation. The authors only mentioned cross-sectional study design as a limitation in strength and limitation (after abstract), despite several major limitations (e.g., small sample size, only focusing on Korean and Chinese Asian subgroups, only collecting surveys via email)

Limitations have now been added at the end of Discussion section.

8. No dates of the study: the dates of the study should be included in the manuscript.

Thank you. We have now provided the dates in the 'Present Study' section.

VERSION 2 – REVIEW

REVIEWER	Peters-Samuelson, Brandilyn Yeshiva University Albert Einstein College of Medicine
REVIEW RETURNED	12-May-2021

GENERAL COMMENTS	The authors have addressed all comments.