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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

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Abstract

Introduction: Although a considerable proportion of Asians in the United States experience depression, anxiety, and poor sleep, such health issues have been underestimated due to the model minority myth about Asians, the stigma attached to mental health, lower rates of seeking treatment, and a shortage of culturally tailored mental health services. Indeed, despite emerging evidence of the links between psychosocial risk factors, the gut microbiome, and depression, anxiety, and sleep quality, very few studies examined such relationships in Chinese and Korean immigrants in the U.S. The purpose of this study is to address this issue by (1) testing the usability and feasibility of the study's multilingual survey measures and biospecimen collection procedure among Chinese and Korean immigrants in the U.S., and (2) examining how stress, discrimination, acculturation, and the gut microbiome are associated with depression, anxiety, and sleep quality in this population.

Methods and Analysis: This is a cross-sectional study among 1st and 2nd generations of adult Chinese and Korean immigrants in the greater Atlanta area (Georgia, U.S.). We will collect (1) gut microbiome samples and (2) data on psychosocial risk factors, depression, anxiety, and sleep disturbance using a battery of online surveys with validated scales in English, Mandarin Chinese, and Korean. We aim to recruit 60 participants (30 Chinese and 30 Korean). We will profile participants' gut microbiome using 16S rRNA V3-V4 sequencing data, which will be analyzed by QIIME 2. Associations of the gut microbiome and psychosocial factors with depression, anxiety, and sleep disturbance will be analyzed using descriptive and inferential statistics, including linear regression.

Ethics and Dissemination: This study has been approved by the Institutional Review Board at XX University where the investigators are affiliated. Results will be made available to Chinese and Korean community members, the funder, and broad scientific societies and researchers.

Strengths and Limitations

- This study is the first to examine biological and psychosocial mechanisms underlying mental health and sleep quality among Chinese and Korean immigrants.
- The study will collect data among Asians in the U.S., who have been historically underrepresented in biomedical research.
- The study is timely, as the COVID-19 pandemic increased racial discrimination and stress among Asians in the U.S.
- The study uses a state-of-the-art measure of lifetime stress exposure (i.e., STRAIN) and several scales tailored to Asians.
- The cross-sectional design of the study will limit the testing of causal associations but set the stage for future longitudinal research.

Introduction

Asians are the fastest-growing racial group in the United States,[1] with Chinese (23%) and Koreans (9%) combined representing the largest subgroup of Asians. The increasing size of the Asian population nationwide calls for more attention to be paid to the unique health needs of this population, which has been historically underestimated and underrepresented, partly because of the "model minority myth" that characterizes Asians as being relatively successful with few problems.[2] However, Asian Americans experience many mental health problems including depression and anxiety in high proportions, making this topic an important public health priority, especially during the current COVID-19 pandemic.

Although depressive and anxiety disorders are the most common and debilitating psychiatric illnesses in the U.S. adult population,[3 4] the literature investigating these illnesses among Asians is limited.[5] This has occurred despite the fact that depression is the most frequently diagnosed mental disorder in Asian Americans. The pooled prevalence rate of depression ranges from 26.9% to 35.6%,[5] and the lifetime prevalence is estimated to be 9.1%.[6] Asian Americans, as compared to their White peers, tend to manifest more prevalent, persistent, and ongoing depressive symptoms.[5-7] This is a critical point, as depression is the leading cause of disability worldwide and can lead to more severe health consequences, including chronic physical health problems[8 9] and suicide. In fact, suicide is the leading cause of death for Asian Americans aged 15 to 24 years.[10] Additionally, anxiety disorders (panic disorder, agoraphobia without panic disorder, social phobia, generalized anxiety disorder, and post-traumatic stress disorder) are experienced among 10.2% of Asian Americans.[6] Moreover, due to the stigma attached to mental health conditions and the lack of culturally competent mental health services, Asian Americans are less likely than their White peers to ask for help and seek treatment,[4,8]

which contribute to the racial and ethnic disparities in mental health outcomes that are evident in the U.S. for Asian Americans.

Understanding depression and anxiety among Asian immigrants is complicated by the fact that their mental health is determined by multiple factors, including chronic stress exposure, racial discrimination, and level of acculturation.[5 7 11] It has been reported that the more Asian Americans are exposed to discrimination and acculturative life stress, the more likely they are to experience depression and anxiety.[12] Asian Americans experience racial discrimination on multiple levels (i.e., cultural, structural, interpersonal, and internalized).[13-15] Moreover, racial discrimination and aggression toward Asians has substantially increased during the COVID-19 pandemic. Relative to White, Black, and Hispanic peers, for example, Asian Americans are more likely to report that since the COVID-19 pandemic, people acted as if they were uncomfortable around them (39%), that they have been subjected to slurs or jokes (31%), and that they have feared someone might threaten or physically attack them (26%).[16] Moreover, 60% of Asian immigrants, including those with high educational attainment, experience acculturative stress associated with learning and fitting into a new culture, concerns about legal status, cultural conflicts, and language barriers.[17]

Given the complexity of the psychosocial determinants underlying depression and anxiety, it is challenging to identify Asian Americans at high risk of developing these mental health disorders, particularly given that they are more reluctant to disclose their mental health status to others.[5 17] Thus far, a few biomarkers have been used to predict depression, including cytokines and inflammatory markers, oxidative stress markers, endocrine markers, energy balance hormones, genetic/epigenetic factors, and structural and functional brain imaging.[18] Emerging evidence suggests that the gut microbiome also plays a critical role in human mental

health via the microbiome-gut-brain axis.[19] The gut microbiome is the collection of all genomes of the microbes in the human gastrointestinal tract.[20] The human gut hosts tens of trillions of microbes, representing 500 species on average.[21 22] Notably, it is heavily influenced by an individual's sociodemographic characteristics, changes in diet, lifestyle, stress, and geographic environment, all of which represent significant risk factors for depression and anxiety among Asian immigrants.[23 24] More specifically, migration from non-western nations to the U.S. is associated with a loss in the gut microbial diversity and function in a manner that may predispose Asian immigrants to high risk of metabolic diseases and mental disorders.[23] Therefore, subsequent changes in the gut microbiome (e.g., diversity and function) after migration provide a unique opportunity to study how living environment in the U.S. represents an external stimulus that affects immigrants' mental health in the context of stress, discrimination, and acculturation.[23 25 26]

Finally, when exploring the impact of psychosocial determinants and the gut microbiome on mental health, it is critical to address sleep quality.[27] Asian Americans are more likely to report short sleep duration than their White peers (33% vs 28%).[28] Sleep disturbance is one of the most prominent symptoms experienced by those with depression and anxiety disorder, and is incorporated into their diagnostic criteria and definitions.[29-31] Moreover, chronic stress is most frequently manifested as changes in sleep patterns or sleep disturbance.[32] Daily racial microaggressions have been associated with poorer sleep quality and shorter sleep duration the following day among Asian Americans.[33] Additionally, the gut microbiome has been associated with sleep disturbance and metabolic disorders.[34 35] Considered together, therefore, it is critical to examine psychosocial and biological pathways that might underlie depression, anxiety, and sleep

disturbance in Asian Americans in the context of migration and acculturation among Asian immigrants in the U.S.

Present Study

The goal of the present study is to study psychosocial and biological mechanisms of depression, anxiety, and sleep disturbance in order to help promote early prevention and personalized treatment for these conditions among Asian immigrants who commonly underutilize mental health services. The work is guided by conceptual framework presented in **Figure 1**. In conducting this research, we have two primary aims: (1) To test the usability and feasibility of the study's multilingual survey measures and biospecimen collection among Chinese and Korean immigrants in the U.S.; and (2) to collect pilot data for a larger study to examine the role of psychosocial determinants of health and the gut microbiome in depression, anxiety, and sleep disturbance among this population.

Methods

Study Design and Participants

An observational cross-sectional study design will be used. Our inclusion criteria for the sample population are: (1) aged 18 years or older; (2) self-identify as Chinese or Korean; (3) live in the greater Atlanta area in Georgia, U.S.; and (4) can read and write English, Mandarin Chinese, or Korean. Because this study aims to sample 1st and 2nd generation Chinese and Korean immigrants, we define 1st generation immigrants as those who are foreign-born living in the U.S., regardless of the duration and purpose of residence in the U.S., and we define 2nd generation immigrants as those who are US-born living in the U.S. The exclusion criteria include pregnant woman, as they undergo considerable psychosocial and biological changes during pregnancy that can affect their physical and mental health status. We will sample a total of 60

participants, including 30 Chinese and 30 Korean. As a pilot study's sample size, 24-40 is suggested to help obtain optimal sample size for both pilot and main trials.[36 37]

Recruitment

First, we will recruit potential participants through online and offline study announcements on social media (e.g., Twitter, Facebook), craigslist, ResearchMatch, Chinese and Korean online communities, websites, blogs, and flyers. Second, we will solicit help from gatekeepers in the community (e.g., churches, clinics, immigrant associations) who will refer interested individuals to the research team. Many studies have suggested collaboration with gatekeepers as one of the most effective way to reach out to Asian populations in the U.S.[38]

Patient and Public Involvement

We have established an advisory board comprised of not only academics with expertise in immigrant populations and mental health, but also community members from churches and clinics. The community members are Chinese or Korean themselves and serve Chinese or Koreans in the Greater Atlanta area. The goal of the advisory board is to demonstrate and improve the research team's engagement with and accessibility to the target population.

Data Collection

First, 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. To accomplish this, we have hired and trained culturally matched research staff who are fluent in English, Chinese, or Korean to perform the consent process in the participant's preferred language. Second, upon obtaining participants' informed consent and agreement to participate in the study, the research team will send an online survey link via email. Participants will administer the survey in their preferred language. During

the survey, participants will provide their name, mailing address, phone number, and email address. Participant names and mailing addresses will be used to ship gut microbiome data collection kits, which will include pictorial and written instructions in English, Mandarin Chinese, or Korean. Compensation for participating will be provided after completing the study.

Consistent with ethical guidelines, participants will be allowed to opt-out of parts of the data collection—either the online survey or specimen collection—and continue with other parts of the protocol as they wish. If a participant does opt out, they will be encouraged to provide a reason so we can better understand the situation. Their feedback on the usability of the study methods will help the research team modify and tailor the current data collection procedure further to Chinese and Korean immigrants for future research. If participants withdraw their consent, or if the research team learns that a participant does not meet the inclusion or exclusion criteria during the study, data collection will be stopped, and all collected biological material and data will be destroyed.

Self-reported Measures and their Translation

This study will use the battery of validated instruments described in **Table 1**. This battery will include the Demographics Short Form (DSF), Suinn-Lew Self Identity Acculturation Scale (SL-ASIA), Acculturative Stress Scale, Subtle and Blatant Racism Scale for Asian Americans (SABR-A^2), Stress and Adversity Inventory for Adults (Adult STRAIN), Pandemic Stress Index (PSI), PROMIS Short Form – Depression, PROMIS Short Form–Anxiety, Pittsburgh Sleep Quality Index (PSQI), and PrimeScreen, a brief dietary screening tool. All these instruments have already been validated and widely used in English.

For measures that have not yet been translated into Chinese and/or Korean, we contacted the instrument developers to obtain permission to use and translate them. We translated SABR-

A[2], Adult STRAIN, PSI, and PrimeScreen into Mandarin Chinese and Korean following the guideline of cultural translation and adaptation of instruments from the World Health Organization, which includes: forward translation, expert panel back translation, pre-testing and cognitive interviewing, and final version.[39] Our instrument translation team includes three research team members and one external member who were bilingual (fluent in English and Mandarin Chinese or Korean) with Ph.D. degrees in nursing or sociology and extensive experience with Asian immigrants, demography, mental health, and stress. Specifically, after one member translated all of the instruments into Chinese or Korean versions, another member translated them back into English versions. Then, both members compared the original English and back-translated English versions to evaluate the quality of the translation. Discrepancies in the translation and meanings were solved by consensus discussions between these two members to ensure conceptual equivalence across the translations. The steps taken as part of this multi-lingual survey development process is depicted in **Figure 2**.

DSF. The DSF is a 27-item questionnaire used to collect participants' general sociodemographic and health characteristics. The sociodemographic variables include age, gender, self-identified race, marital status, living arrangement, immigration, religious belief, and household income. Health-related variables include height, weight, lactose intolerance, use of antibiotics and probiotics, disease history, and the use of mental health services.

SL-ASIA. The original version[40] of the SL-ASIA is a 26-item questionnaire used to assess a person's level of acculturation, specifically historical background and cultural identity. We chose 5 items to measure participants' preference for food, music, custom, language proficiency, and the racial composition of close friends on a 5-point Likert scale. This adapted version has been used in other studies.[41] We will average the assigned values across the

questions into a total acculturation score. A higher total score indicates more Westernization or acculturation.

Acculturative Stress Scale. The Acculturative Stress Scale is a 36-item questionnaire used to measure acculturative stress on a 5-point Likert scale. Not counting the miscellaneous group, there are six subscales assessing perceived discrimination, homesickness, perceived hate, fear, stress due to change/culture shock, and guilt. In this study, an 8-item questionnaire from two domains of task-oriented stress (3 items) and emotion-oriented stress (5 items) will be adopted. Items for task-oriented stress include: "I feel nervous when communicating in English" and "I feel uncomfortable adjusting to new foods." Sample items for emotion-oriented stress include: "Homesickness bothers me" and "I feel sad living in unfamiliar surroundings." Acculturative stress in the adapted instrument will also be measured on a 5-point Likert scale from 0 (*strongly disagree*) to 4 (*strongly agree*). Individual scores will be summed to create a total score for each domain where a task-oriented stress score can range 0–12, and an emotion-oriented stress score can range 0–20. Higher scores indicate greater levels of acculturative stress. The adapted instrument has shown high internal consistency for both scales tested among Korean American elders (Cronbach's $\alpha = .73$ for task-oriented stress and .87 for emotion-oriented stress).[42]

SABR-A^2. The SABR-A is a 10-item questionnaire that asks about personal experience of subtle and blatant racism.[43] The subtle racism subscale (4 items) refers to instances of discrimination due implicitly to racial bias or stereotype (e.g., treated differently, overlooked). The blatant racism subscale (4 items) refers to instances of discrimination due explicitly to racial bias or stereotype (e.g., called names, commented about English proficiency). However, two out of ten items will not be included in these scales because according to the instrument's author, they were developed as exploratory items. Responses are measured on a 5-point Likert scale

from 1 (*almost never*) to 5 (*almost always*). All eight items will be averaged into a total racism score, and each set of the four items will be averaged into a subtle and blatant racism score, with higher scores indicating greater perceived racism. The internal consistency of the total, subtle, and blatant racism (sub)scales tested among self-identified Asian American undergraduate students was 0.84-0.88, 0.76-0.82, and 0.77-0.82, respectively.[43]

Adult STRAIN. The Adult STRAIN[44] measures a person's lifetime exposure to 55 different types of acute (e.g., deaths of relatives, job loss) and chronic stressors (e.g., ongoing health, work, relationship, and financial problems). Participants' responses will be used to calculate a standard set of 20 lifetime stress exposure scores, which are based on the type of stressors experienced, when they were experienced, their primary life domain, and their core social-psychological characteristic. More specifically, this summary score data will include the following computed variables: lifetime stressor count, lifetime stressor severity, early life (before age 18) stressor count, early life (before age 18) stressor severity, adulthood stressor count, adulthood stressor severity, lifetime count of acute life events, lifetime count of chronic difficulties, lifetime severity of acute life events, lifetime count of chronic difficulties, lifetime stressor count and severity by primary life domain (i.e., housing, education, work, treatment/health, marital/partner, reproduction, financial, legal/crime, other relationships, death, life-threatening situations, possessions), and lifetime stressor count and severity by core social-psychological characteristic (i.e., interpersonal loss, physical danger, humiliation, entrapment, role change/disruption). Higher score indicates greater life stress exposure across these categories. The STRAIN has been extensively validated in relation to a variety of mental and physical health outcomes,[45 46] and has excellent test-retest reliability over time for the main stress exposure outcomes (r-values ≥ 0.904).[47]

PSI. The PSI[48] is a 3-item measure of behavior changes and stress that individuals may have experienced during the COVID-19 pandemic. The questions are: "What are you doing/did you do during COVID-19 (coronavirus)?" with a checklist of items about behaviors, like social distancing; "How much is/did COVID-19 (coronavirus) impact your day-to-day life?"; and "Which of the following are you experiencing (or did you experience) during COVID-19 (coronavirus)?" with a checklist of items about emotional distress, substance use, sexual behavior, financial stress, stigma, and support.

PROMIS Short Form–Depression. The 28-item PROMIS Depression Item Bank assesses negative mood (e.g., sadness, guilt), negative views of self (e.g., self-criticism, worthlessness), negative social cognition (e.g., loneliness, interpersonal alienation), and decreased positive affect and engagement (e.g., loss of interest, meaning, and purpose).[49] Of these 28 items, 6 items have been selected to create the PROMIS Short Form–Depression, which has high reliability and precision that is comparable to the original 28-item scale.[49] The 6-item scale assesses depressive symptoms over the past 7 days and has response options ranging from 1 (*never*) to 5 (*always*). The raw scores will be transformed into T scores, with higher scores indicating more depressive symptoms.[49]

PROMIS Short Form–Anxiety. The PROMIS Anxiety Item Bank assesses self-reported fear, anxious misery, hyperarousal, and somatic symptoms related to arousal.[50] The PROMIS Short Form–Anxiety includes six items, whose reliability and precision are high and comparable to the full item bank.[50] The correlation of the adult full bank with the 6-item short form is between 0.90 and 0.95. The 6 items assess anxiety symptoms over the past 7 days and have response options ranging from 1 (*never*) to 5 (*always*). The raw scores will be transformed into T scores, with higher scores indicating more severe anxiety.[50]

PSQI. The PSQI is a 10-item scale containing 19 self-rated questions. It assesses sleep quality over a 1-month time interval. The instrument evaluates both objective (e.g., how often participants wake up during the night) and subjective aspects of sleep quality (e.g., how rested they typically feel after a night of sleep). These 19 questions are combined to form seven "component" scores, each of which has a range of 0-3 points, from 0 (*no difficulty*) to 3 (*severe difficulty*). Then, the seven component scores are summed to create a global PSQI score, ranging from 0-21, with higher scores indicating worse sleep quality. In primary insomnia patients, the overall PSQI global score correlation was .87 for test-retest reliability.[51] The total score of the Korean version of PSQI showed high internal consistency (Cronbach's $\alpha = 0.84$).[52]

PrimeScreen. The PrimeScreen is a 23-item dietary assessment questionnaire.[53] This self-reported measure evaluates the average frequency of consumption of specified foods and food groups, as well as 13 nutrients (e.g., vitamin and supplements) over the past 6 months.[53 54] Each item has five response categories: "less than once per week", "once per week", "2-4 times per week", "nearly daily or daily", or "twice or more per day". This measure has great reliability and validity for use in adults ages 19-65 years, including excellent reproducibility ($r = 0.70$) and comparability ($r = 0.61$) with the Semiquantitative Food Frequency Questionnaire (SFFQ) in foods and food groups, as well as excellent reproducibility ($r = 0.74$) and comparability ($r = 0.60$) with the SFFQ for nutrients.[53]

Gut Microbiome

To profile the gut microbiome, we will collect fecal specimens. The sample collection will be performed as indicated in the Human Microbiome Project protocol.[55] Specifically, we will coach participants to use the home-based specimen collection kits to obtain fecal samples. Fecal samples will be collected using pictorial instruction. All the instructions for the sample

collection will be prepared in English, Mandarin Chinese, and Korean. Upon completion of the specimen collection, participants will follow the packaging instructions (e.g., store in a refrigerator before shipping) and then ship their samples to the Nursing Biobehavioral Laboratory at XX University using pre-paid FedEx envelopes. All fecal samples will be stored at a -80℃ freezer until DNA extraction.

DNA Extraction and Sequencing of the Gut Microbiome

According to the Human Microbiome Project protocol, the microbial DNA will be extracted from fecal specimens using the PowerSoil isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). The 16S rRNA V3-V4 gene regions [56 57] will be extracted and sequenced. 16S rRNA amplicons will be generated using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, KK2600) and primers specific to 16S V3-V4 region of bacteria 341F (5'-CCTACGGGNGGCWGCAG-3')-805R (5'-GACTACHVGGGTATCTAATCC-3'). The PCR clean-up will be performed using AMPure XP beads (Beckman, A63880) and indices will be attached using the Nextera XT Index kit (Illumina, FC-131-1001). Final library pools will be quantitated via qPCR (Kapa Biosystems, catalog KK4824). The pooled library will be sequenced on an Illumina miSeq using miSeq v3 600 cycle chemistry (Illumina, catalog MS-102-3003) at a loading density of 8 pM with 20% PhiX, at PE300 reads. This process will be conducted at the Integrated Genomics Core at XX University. The microbial sequencing will lead to paired-end sequences for further analysis.

Statistical Analysis

Prior to analysis, all data will be reviewed for quality, distributions, and missing data bias. Mathematical transformations will be performed when necessary to normalize measures. Descriptive statistics (e.g., Mann-Whitney U test and Fisher's exact test because of the small

sample size) will be adopted to describe participants' characteristics as well as associations between the psychosocial and biological factors and the outcome variables (anxiety, depression, and sleep disturbance).

For the gut microbiome data, 16S rRNA sequences will be analyzed to obtain microbial diversity (α-diversity and β-diversity), taxonomic composition, and abundance analysis. QIIME 2 default parameters will be adopted to filter the sequences quality using DADA2.[58 59] Taxonomies will be assigned by the pre-trained classifier using Silva. Pearson or Spearman correlations will be used to determine associations among microbial diversity indices and the outcome variables. The principal coordinates analysis will also be used to visualize diversity patterns. Associations between the gut microbiome and demographics and psychosocial factors will be analyzed using microbiomes' composition.[60] Lastly, the linear discriminant analysis (LDA) effect size (LEfSe) [61] will be used to characterize the taxa differences between different levels of outcome variables: 1) Kruskal-Wallis sum-rank test will be adopted to detect features with significant differential abundance between the levels of outcome variables; 2) Wilcoxon rank-sum test will be adopted to further investigate significances of taxa through a set of pairwise tests among subclasses (e.g., psychosocial factors); and 3) LEfSe will use LDA to estimate the effect size of each differentially abundant feature. All analyses will be conducted using QIIME 2[62-64] and R 3.3.3. The statistical significance level will be set at $p < 0.05$.

Data Storage and Security

All of the survey data will be managed using REDCap,[65] which allows data errors, completeness, and validation checks to ensure maximum quality throughout. All fecal specimens will be stored in the Nursing Biobehavioral Laboratory at XX University. These specimens will only be used to address our research aims. All the survey data and specimens will be destroyed 3

years after the entire study is finished. The confidentiality of all data will be maintained within legal limits.

Discussion

Although numerous studies have examined risk processes associated with mental health and poor sleep in general, there is a distinct paucity of research on Asian immigrants in the U.S., despite the fact that this population is underserved and experiences the great mental health-related disease burden in America. To address this important issue, we will conduct the present study, which will be the first to examine biological and psychosocial mechanisms underlying depression, anxiety, and sleep symptoms among Chinese and Korean immigrants in the U.S. Considering that these populations are growing quickly, we expect that our findings will help advance our knowledge on racial and ethnic differences in mental health outcomes and the biopsychosocial pathways that underlie these effects.

Although these associations would be important to understand at any time, we believe these issues are particularly critical to study during the COVID-19 pandemic, given the increased rates of social conflict, discrimination, and, in some cases, injustice that have been experienced by Asians in the U.S during this time. Indeed, the impact of the COVID-19 pandemic on Asian immigrants has been extensive.[66] Public health measures designed to curb the spread of the virus, which have included lockdown, school and business closures, and travel restrictions, have had a tremendous impact on the stress levels and mental health of the general population.[67] Beyond this, though, Asians living in the U.S. have been stigmatized and victimized by media coverage perpetuating the naming of the COVID-19 virus as the 'Chinese Virus' or 'Kung Flu', which has in turn lead to racial discrimination and other social threats[66] that have been shown to strongly affect mental and physical health.[68] The cumulative social stress and threat experienced by Asian

immigrants, which include aggravated racial discrimination in addition to ongoing health, employment, and financial worries, will provide a unique opportunity to better understand how psychosocial factors and the microbiome affect mental health and sleep symptoms during a time of maximal importance and relevance.

In assessing Asian immigrants' cumulative life stress exposure, the adult STRAIN and PSI will help capture acute and chronic stressors of participants who have been going through the pandemic for an extended period of time. Importantly, some of the measures we have selected are tailored to Asian populations, which will enable us to collect more valid and reliable data that are reflective of Asians' lived experiences, including racial discrimination and acculturation. These culturally adapted measures will yield a unique and timely perspective on mental health and sleep outcomes in Asian immigrants. Looking forward, we expect this study to provide important data that can in turn be used to inform the development of a larger longitudinal study aimed at investigating causal relations between social and biological determinants of health, and mental health and sleep symptoms among Asian immigrants in the U.S.

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Author's contributions

SK prepared the first draft of this manuscript. All authors provided critical edits, critiqued the manuscript for intellectual content, and approved the final version for submission.

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Ethics approval

This study was approved by the Institutional Review Board at XX University (IRB ID: STUDY00000935).

Competing interest statement

None declared.

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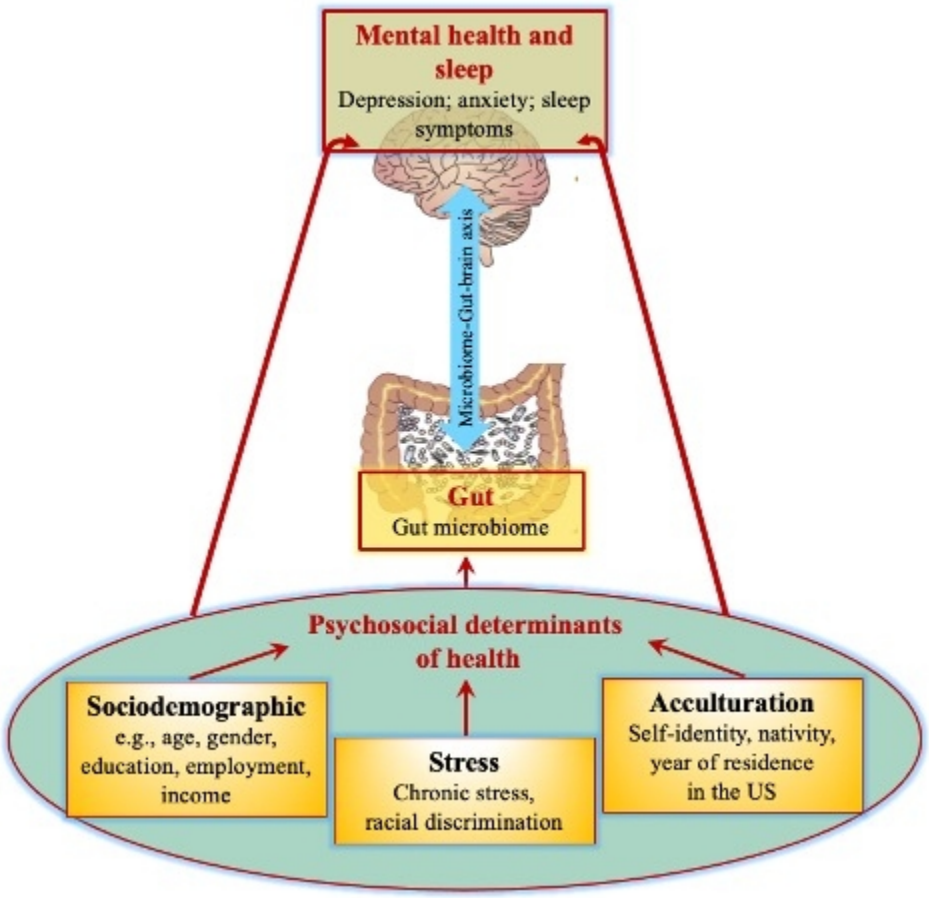

Figure 1

165x160mm (72 x 72 DPI)

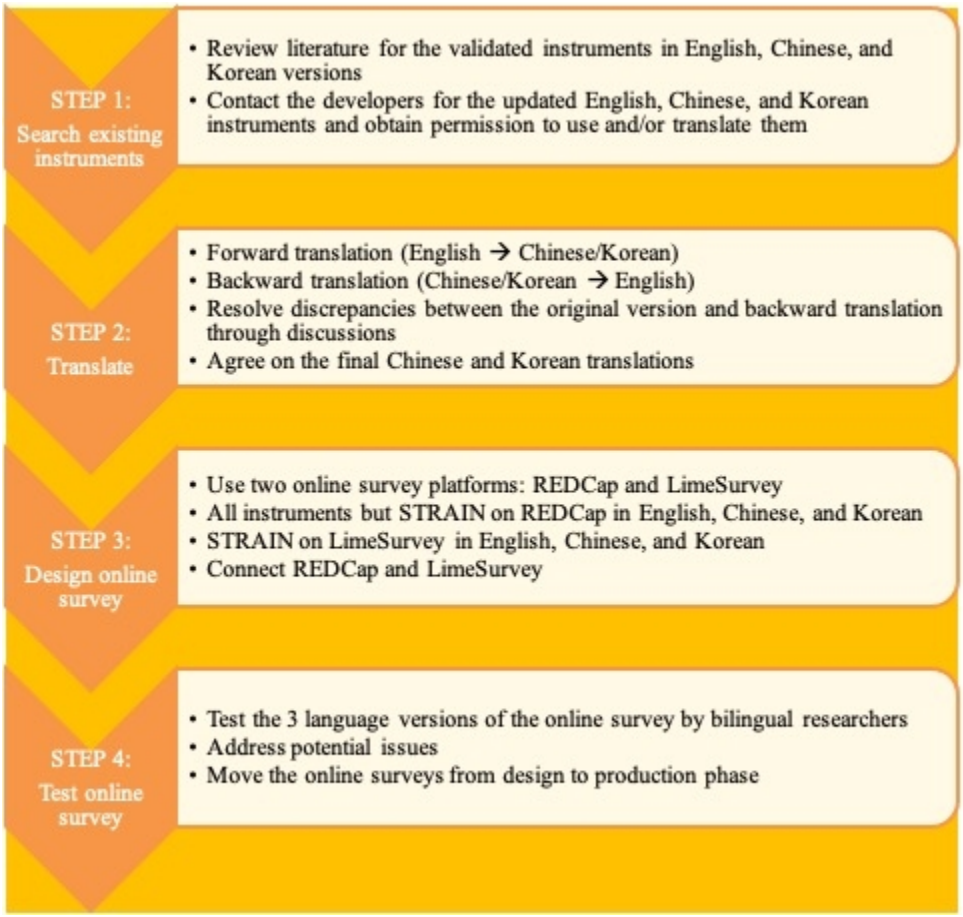

Figure 2

170x160mm (72 x 72 DPI)

Table 1. Study Measures

Variable	Measure	Instrument	Need for translation
Sociodemographic and clinical factors		Demographics Short Form (e.g., sociodemographic characteristics, health behaviors, medical history)	Y
Psychosocial factors	Acculturation	Suinn-Lew Self Identity Acculturation Scale	Y
		Demographic Short Form (e.g., foreign-born status, duration of US residence, age at immigration)	Y
	Stress	Stress and Adversity Inventory for Adults	Y
		Pandemic Stress Index	Y
		Acculturative Stress Scale	N
		Subtle and Blatant Racism Scale for Asian Americans	Y
	Diet	PrimeScreen Survey	Y
Biological factor	Gut microbiome	Fecal specimen	Y (instructions)
Mental health outcomes	Depression	PROMIS Short Form–Depression	N
	Anxiety	PROMIS Short Form–Anxiety	N
Sleep symptoms	Sleep quality	Pittsburgh Sleep Quality Index	N

Figure 1. Conceptual Framework

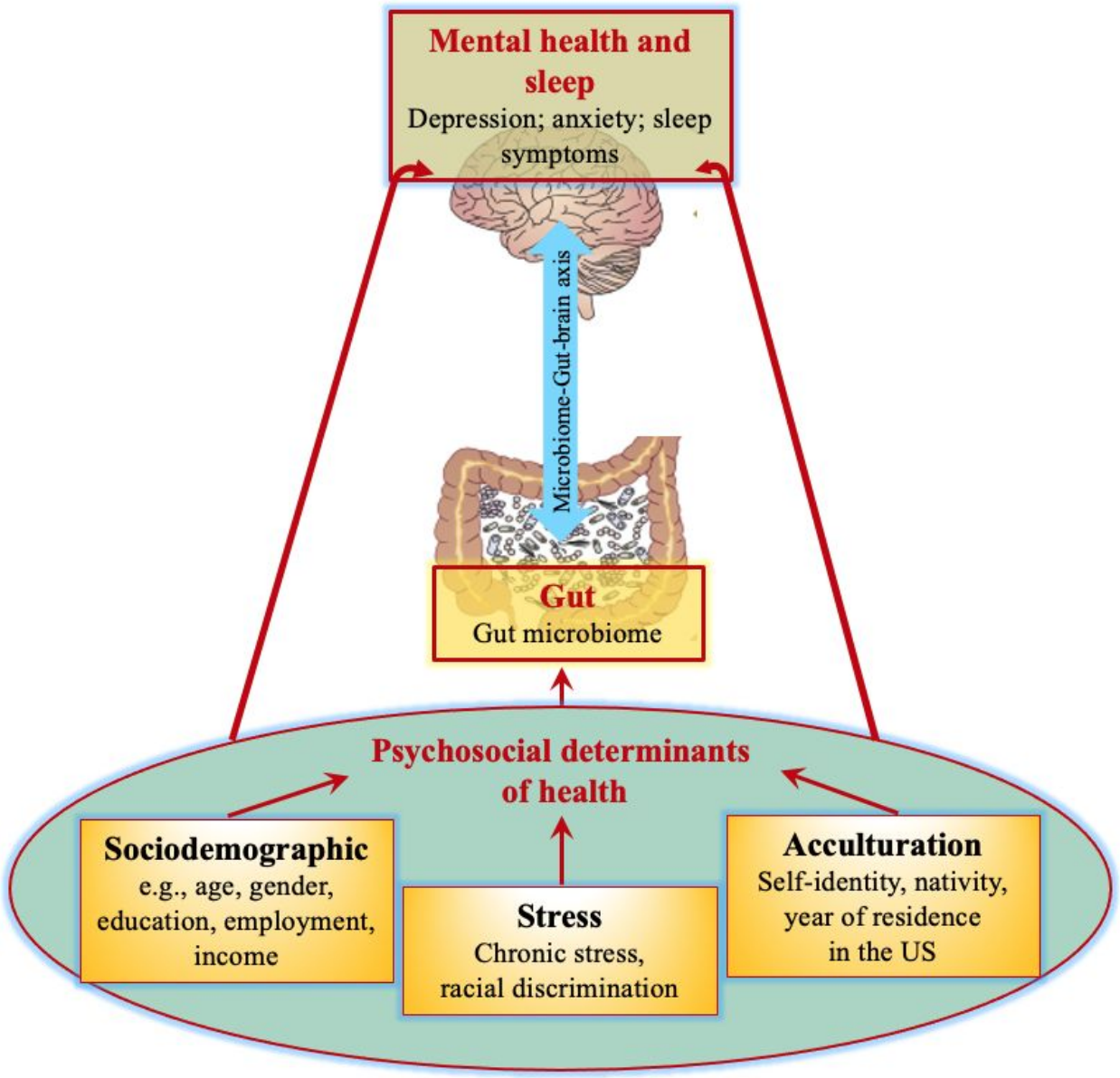

Figure 2. Multi-lingual Survey Development and Testing Process

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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

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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

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Abstract

Introduction: Although a considerable proportion of Asians in the United States experience depression, anxiety, and poor sleep, these health issues have been underestimated due to the model minority myth about Asians, the stigma associated with mental illness, lower rates of treatment seeking, and a shortage of culturally tailored mental health services. Indeed, despite emerging evidence of links between psychosocial risk factors, the gut microbiome, and depression, anxiety, and sleep quality, very few studies have examined how these factors are related in Chinese and Korean immigrants in the U.S. The purpose of this pilot study was to address this issue by (a) testing the usability and feasibility of the study's multilingual survey measures and biospecimen collection procedure among Chinese and Korean immigrants in the U.S., and (b) examining how stress, discrimination, acculturation, and the gut microbiome are associated with depression, anxiety, and sleep quality in this population.

Method and Analysis: This is a cross-sectional pilot study among 1st and 2nd generations of adult Chinese and Korean immigrants in the greater Atlanta area (Georgia, U.S.A.). We collected (a) gut microbiome samples and (b) data on psychosocial risk factors, depression, anxiety, and sleep disturbance using validated, online surveys in English, Chinese, and Korean. We aim to recruit 60 participants (30 Chinese, 30 Korean). We will profile participants' gut microbiome using 16S rRNA V3-V4 sequencing data, which will be analyzed by QIIME 2™. Associations of the gut microbiome and psychosocial factors with depression, anxiety, and sleep disturbance will be analyzed using descriptive and inferential statistics, including linear regression.

Ethics and Dissemination: This study has been approved by the Institutional Review Board at Emory University. Results will be made available to Chinese and Korean community members, the funder, and other researchers and the broader scientific community.

Strengths and Limitations

- This study is the first to examine psychosocial and biological mechanisms underlying mental health and sleep quality among Chinese and Korean immigrants.
- The study will collect data among Asians in the U.S., who have been historically underrepresented in biomedical research.
- The study is timely, as the COVID-19 pandemic has greatly increased stress and racial discrimination among Asians in the U.S.
- The study uses a state-of-the-art measure of lifetime stress exposure (i.e., the STRAIN) and several other culturally valid assessment instruments.
- The cross-sectional study design will limit the testing of directionality and causal associations but will help inform future longitudinal research.

Introduction

Asians are the fastest-growing racial group in the United States,[1] with Chinese (23%) and Koreans (9%) combined representing the largest subgroup of Asians. The increasing size of the Asian population nationwide calls for more attention to be paid to the unique health needs of this population, which has been historically underestimated and underrepresented, partly because of the "model minority myth" that characterizes Asians as being relatively successful with few problems.[2] However, Asian Americans experience many mental health problems including depression and anxiety in high proportions,[3 4] making this topic an important public health priority, especially during the current COVID-19 pandemic.

Although depressive and anxiety disorders are the most common and debilitating psychiatric illnesses in the U.S. adult population,[5 6] the literature investigating these illnesses among Asians is limited.[3] This has occurred despite the fact that depression is the most frequently diagnosed mental disorder in Asian Americans. The pooled prevalence rate of depression ranges from 26.9% to 35.6%,[3] and the lifetime prevalence is estimated to be 9.1%.[4] Asian Americans, as compared to their White peers, tend to manifest more prevalent, persistent, and recurrent depressive symptoms.[3 4 7] This is a critical point, as depression is the leading cause of disability worldwide and can lead to other severe health problems, including chronic physical health conditions[8 9] and suicide. In fact, suicide is the leading cause of death for Asian Americans aged 15 to 24 years.[10] Additionally, anxiety disorders—including panic disorder, agoraphobia without panic disorder, social phobia, generalized anxiety disorder, and post-traumatic stress disorder—are experienced by 10.2% of Asian Americans.[4] Moreover, due to the stigma attached to mental illness and the lack of culturally competent mental health services, Asian Americans are less likely than their White peers to ask for help and seek treatment,[4,8]

which further contribute to the racial and ethnic disparities in mental health outcomes that are evident for Asian Americans living in the U.S.

Understanding depression and anxiety among Asian immigrants is complicated by the fact that their mental health is determined by several factors, including chronic stress exposure, racial discrimination, and level of acculturation.[3 7 11] It has been reported that the more Asian Americans are exposed to discrimination and acculturative life stress, the more likely they are to experience depression and anxiety.[12] Additionally, Asian Americans experience racial discrimination on multiple levels (i.e., cultural, structural, interpersonal, and internalized).[13-15] Moreover, racial discrimination and aggression toward Asians has substantially increased during the COVID-19 pandemic. Relative to White, Black, and Hispanic peers, for example, Asian Americans are more likely to report that since the COVID-19 pandemic, people acted as if they were uncomfortable around them (39%), that they have been subjected to slurs or jokes (31%), and that they have feared someone might threaten or physically attack them (26%).[16] Finally, 60% of Asian immigrants, including those with high educational attainment, experience acculturative stress associated with learning and fitting into a new culture, concerns about legal status, cultural conflicts, and language barriers.[17]

Given the complexity of the psychosocial determinants underlying depression and anxiety, it is challenging to identify Asian Americans at high risk of developing these psychiatric disorders, particularly given that they are more reluctant to disclose their mental health status to others.[3 17] Thus far, a few biomarkers have been used to predict depression, including cytokines and inflammatory markers, oxidative stress markers, endocrine markers, energy balance hormones, genetic/epigenetic factors, and structural and functional brain imaging.[18] Emerging evidence suggests that the gut microbiome also plays an important role in human mental health

via the microbiome-gut-brain axis, a bidirectional network that enables the gut microbiome to affect the brain and mental health through immune, neural, and hormonal pathways.[19] The gut microbiome is the collection of all genomes of the microbes in the human gastrointestinal tract.[20] The human gut hosts tens of trillions of microbes, representing 500 species on average.[21 22] Notably, it is heavily influenced by an individual's sociodemographic characteristics, changes in diet, lifestyle, stress, and geographic environment, all of which represent significant risk factors for depression and anxiety among Asian immigrants.[23 24] More specifically, migration from non-western nations to the U.S. is associated with a loss in the gut microbial diversity and function in a manner that may predispose Asian immigrants to high risk of metabolic diseases and mental disorders.[23] Therefore, subsequent changes in both the diversity and function of the gut microbiome after migration provide a unique opportunity to study how living environment in the U.S. represents an external stimulus that affects immigrants' mental health in the context of stress, discrimination, and acculturation.[23 25 26]

Finally, when exploring the impact of psychosocial determinants and the gut microbiome on mental health, it is important to address sleep quality.[27] Asian Americans are more likely to report short sleep duration than their White peers (33% vs 28%).[28] Sleep disturbance is one of the most prominent symptoms experienced by those with depression and anxiety disorder, and is incorporated into the diagnostic criteria and definitions of these disorders.[29-31] Moreover, chronic stress frequently manifests as increases in sleep disturbance and/or changes in sleep patterns.[32] Daily racial microaggressions have been associated with poorer sleep quality and shorter sleep duration the following day among Asian Americans.[33] Additionally, the gut microbiome has been associated with sleep disturbance and metabolic disorders.[34 35] Considered together, therefore, it is critical to examine psychosocial and biological pathways that might underlie depression, anxiety,

and sleep disturbance in Asian Americans in the context of migration and acculturation among Asian immigrants in the U.S.

Present Study

The goal of the present study (06/01/2020 – 05/31/2021) is to study psychosocial and biological mechanisms of depression, anxiety, and sleep disturbance to help inform early prevention and personalized treatment strategies for these conditions for Asian immigrants who commonly underutilize mental health services. Of many Asian subgroups, we chose to focus on Chinese and Korean as the target populations for two main reasons: (a) together, they represent the largest subgroup of Asian Americans in the U.S., as well as in the Greater Atlanta area where the study is located; and (b) as a result, they experience the greatest proportion of disease burden associated with depressive and anxiety disorders among Asian Americans. This work is guided by the conceptual framework presented in **Figure 1**. In conducting this research, we have two primary aims: (a) test the usability and feasibility of the study's multilingual survey measures and biospecimen collection procedures among Chinese and Korean immigrants living in the U.S.; and (b) collect pilot data for a subsequent larger study to examine the roles that psychosocial factors and the gut microbiome play in depression, anxiety, and sleep disturbance in this population.

Method

Study Design and Participants

An observational, cross-sectional study design will be used. The inclusion criteria for the sample population are: (1) aged 18 years or older; (2) self-identify as Chinese or Korean; (3) live in the greater Atlanta area in Georgia, U.S.; and (4) can read and write English, traditional and simplified Chinese, or Korean. Because this study aims to sample 1st and 2nd generation

Chinese and Korean immigrants, we define 1st generation immigrants as those who are foreign-born living in the U.S., regardless of the duration and purpose of residence in the U.S., and we define 2nd generation immigrants as those who are U.S.-born living in the U.S. The exclusion criteria include having used antibiotics during the past month and being a pregnant woman, as they undergo considerable psychosocial and biological changes during pregnancy that can affect their physical and mental health status. We will sample a total of 60 participants, including 30 Chinese and 30 Korean. This is based on data showing that a sample size of 24-40 is optimal as a pilot study for helping inform subsequent research.[36 37]

Recruitment

First, we will use both online and offline recruitment strategies. The former will involve posting study advertisements on social media (e.g., Twitter, Facebook), craigslist, ResearchMatch, and Chinese and Korean online communities (e.g., online Chinese Church Group via WeChat, Georgia Tech Korean Student Association), websites, and blogs. The latter recruitment strategy will involve working with community partners, including a Korean church in Atlanta, an Emory Clinic in Atlanta, and a local clinic in Johns Creek, Georgia. They will introduce our study to their Chinese or Korean congregation, patients, or members. This recruitment strategy is consistent with prior work showing that collaborating with gatekeepers is one of the most effective ways to reach and conduct high-quality research with Asian populations in the U.S.[38]

Patient and Public Involvement

We have established an advisory board comprised of not only academics with expertise in immigrant populations and mental health, but also community members from churches and clinics. The demographic characteristics of the advisory board members are: (1) professor (male,

Caucasian, expertise: mental health), (2) professor (female, Caucasian, expertise: international social demography), (3) pastor (male, Korean), (4) professor/clinician #1 (male, Caucasian, medical doctor), and (5) clinical instructor/clinician #2 (female, Chinese, nurse practitioner). The community members are Chinese or Korean themselves, or serve Chinese or Koreans in the Greater Atlanta area. The goal of the advisory board is to demonstrate and improve the research team's engagement with and accessibility to the target population. The board members in academia will share their knowledge and experience of working with racial/ethnic minority populations, and those in communities will provide the study's information and refer interested individuals to the research team. The board will convene as a group 1-2 times a year via conference calls, although the research team can contact individual board members for consultation as needed. The agenda of the advisory board meetings will include (but not be limited to) recruitment strategies to reach out to Chinese and Korean communities, motivational strategies, how each board member can help connect the communities to the research team, and the board's expectations after their service (e.g., authorship in papers, sharing the study findings with the community members they serve).

Data Collection

First, when potential participants contact the research team directly or via referral, the research staff will email them back to make an appointment. Then, on the scheduled date and time, we will call them to screen their eligibility and obtain their verbal consent to participate in the study. To accomplish this, we have hired and trained culturally matched research staff members who are fluent in English, Chinese, or Korean to perform the consent process in the participant's preferred language. Due to the COVID-19 pandemic, there will not be any in-person interactions with participants. Second, upon obtaining participants' informed consent and

agreement to participate in the study, the research team will send an online survey link via email. Participants will administer the survey in their preferred language. During the survey, participants will provide their name, mailing address, phone number, and email address. Participants' names and mailing addresses will be used to ship the gut microbiome data collection kits, which will include pictorial and written instructions in English, Chinese, or Korean. Compensation for participating will be provided after completing the study. The compensation will be prorated: participants who complete both the online survey and specimen collection will receive a $30 e-gift card, whereas those completing only the online survey will receive a $10 e-gift card. E-gift cards will be emailed to the email addresses provided by the participants.

Consistent with ethical guidelines, participants will be allowed to opt-out of any parts of the data collection that they wish (e.g., specific online survey questions, specimen collection) and continue with other parts of the study protocol as they wish. If a participant does opt out, they will be encouraged to provide a reason so we can better understand the situation. Their feedback on the usability of the study methods will help the research team modify and tailor the current data collection procedure further to Chinese and Korean immigrants for future research. If participants withdraw their consent, or if the research team learns that a participant does not meet the inclusion or exclusion criteria during the study, data collection will be stopped, and all collected biological material and data will be destroyed.

Self-report Measures and their Translation

This study will use the battery of validated instruments described in **Table 1**. The battery will include the Demographics Short Form (DSF), Suinn-Lew Self Identity Acculturation Scale (SL-ASIA), Acculturative Stress Scale, Subtle and Blatant Racism Scale for Asian Americans

(SABR-A^2), Stress and Adversity Inventory for Adults (Adult STRAIN), Pandemic Stress Index (PSI), PROMIS Short Form – Depression, PROMIS Short Form–Anxiety, Pittsburgh Sleep Quality Index (PSQI), and PrimeScreen, a brief dietary screening tool. All these instruments have already been validated and are widely used in English.

Table 1. Study Measures

Variable	Measure	Instrument	Need for translation
Sociodemographic and clinical factors		Demographics Short Form (e.g., sociodemographic characteristics, health behaviors, medical history)	Y
Psychosocial factors	Acculturation	Suinn-Lew Self Identity Acculturation Scale	Y
		Demographic Short Form (e.g., foreign-born status, duration of US residence, age at immigration)	Y
	Stress	Stress and Adversity Inventory for Adults	Y
		Pandemic Stress Index	Y
		Acculturative Stress Scale	N
		Subtle and Blatant Racism Scale for Asian Americans	Y
	Diet	PrimeScreen Survey	Y
Biological factor	Gut microbiome	Fecal specimen	Y (instructions)
Mental health outcomes	Depression	PROMIS Short Form–Depression	N
	Anxiety	PROMIS Short Form–Anxiety	N
Sleep symptoms	Sleep quality	Pittsburgh Sleep Quality Index	N

For measures that have not yet been translated into Chinese and/or Korean, we contacted the instrument developers to obtain permission to use and translate them. We translated SABR-A^2, Adult STRAIN, PSI, and PrimeScreen into Chinese and Korean following the guideline of cultural translation and adaptation of instruments from the World Health Organization, which involves: forward translation, expert panel back translation, pre-testing and cognitive interviewing, and final version.[39] Our instrument translation team included three research team members and one external member who were bilingual (fluent in English and Simplified or Traditional Chinese or Korean) with Ph.D. degrees in nursing or sociology and extensive

experience with Asian immigrants, demography, mental health, and stress. Specifically, after one member translated all of the instruments into Chinese or Korean versions, another member translated them back into English. Then, both members compared the original English and back-translated English versions to evaluate the quality of the translation. Discrepancies in the translation and meanings were solved by consensus discussions between these two members to ensure conceptual equivalence across the translations. The steps taken as part of this multi-lingual survey development process is depicted in **Figure 2**.

DSF. The DSF is a 27-item questionnaire used to collect participants' general sociodemographic and health characteristics. Most of the items were derived from the National Institutes of Health (NIH) Common Data Elements.[40] The questionnaire has been used in an ongoing study sponsored by the NIH (1K99NR017897-01, PI: Bai). The sociodemographic variables include age, gender, self-identified race, marital status, living arrangement, immigration, religious belief, education, and household income. Health-related variables include height, weight, lactose intolerance, use of antibiotics and probiotics, disease history, and the use of mental health services.

SL-ASIA. The original version[41] of the SL-ASIA is a 26-item questionnaire used to assess a person's level of acculturation, specifically historical background and cultural identity. We chose 5 items to measure participants' preference for food, music, custom, language proficiency, and the racial composition of close friends on a 5-point Likert scale. This adapted version has been used in other studies.[42] We will average the assigned values across the questions into a total acculturation score. A higher total score indicates more Westernization or acculturation.

Acculturative Stress Scale. The Acculturative Stress Scale is a 36-item questionnaire used to measure acculturative stress on a 5-point Likert scale. Not counting the miscellaneous group, there are six subscales assessing perceived discrimination, homesickness, perceived hate, fear, stress due to change/culture shock, and guilt. In this study, an 8-item questionnaire from two domains of task-oriented stress (3 items) and emotion-oriented stress (5 items) will be adopted. Items for task-oriented stress include: "I feel nervous when communicating in English" and "I feel uncomfortable adjusting to new foods." Sample items for emotion-oriented stress include: "Homesickness bothers me" and "I feel sad living in unfamiliar surroundings." Acculturative stress in the adapted instrument will also be measured on a 5-point Likert scale from 0 (*strongly disagree*) to 4 (*strongly agree*). Individual scores will be summed to create a total score for each domain where a task-oriented stress score can range 0–12, and an emotion-oriented stress score can range 0–20. Higher scores indicate greater levels of acculturative stress. The adapted instrument has shown high internal consistency for both scales tested among Korean American older adults (Cronbach's $\alpha = .73$ for task-oriented stress and .87 for emotion-oriented stress).[43]

SABR-A^2. The SABR-A is a 10-item questionnaire that asks about personal experience of subtle and blatant racism.[44] The subtle racism subscale (4 items) refers to instances of discrimination due implicitly to racial bias or stereotype (e.g., treated differently, overlooked). The blatant racism subscale (4 items) refers to instances of discrimination due explicitly to racial bias or stereotype (e.g., called names, commented about English proficiency). However, two out of ten items were not included in this study because according to the instrument's author, they were developed as exploratory items. Responses are measured on a 5-point Likert scale from 1 (*almost never*) to 5 (*almost always*). All eight items will be averaged into a total racism score, and each set of the four items will be averaged into a subtle and blatant racism score, with higher

scores indicating greater perceived racism. The internal consistency of the total, subtle, and blatant racism (sub)scales tested among self-identified Asian American undergraduate students was 0.84-0.88, 0.76-0.82, and 0.77-0.82, respectively.[44]

Adult STRAIN. The Adult STRAIN[45] measures a person's lifetime exposure to 55 different types of acute (e.g., deaths of relatives, job loss) and chronic stressors (e.g., persistent health, work, relationship, financial problems) (see https://www.strainsetup.com). Participants' responses will be used to calculate a standard set of 20 lifetime stress exposure scores, which are based on the type of stressors experienced, when they were experienced, their primary life domain, and their core social-psychological characteristic. More specifically, this summary score data will include the following computed variables: lifetime stressor count, lifetime stressor severity, early life (before age 18) stressor count, early life (before age 18) stressor severity, adulthood stressor count, adulthood stressor severity, lifetime count of acute life events, lifetime count of chronic difficulties, lifetime severity of acute life events, lifetime count of chronic difficulties, lifetime stressor count and severity by primary life domain (i.e., housing, education, work, treatment/health, marital/partner, reproduction, financial, legal/crime, other relationships, death, life-threatening situations, possessions), and lifetime stressor count and severity by core social-psychological characteristic (i.e., interpersonal loss, physical danger, humiliation, entrapment, role change/disruption). Higher scores indicate greater life stress exposure across these categories. The STRAIN has been extensively validated in relation to a variety of cognitive, mental, and physical health outcomes,[46-50] and has excellent test-retest reliability over time for the main stress exposure outcomes (r-values ≥ 0.904).[45]

PSI. The PSI[51] is a 3-item measure of behavior changes and stress that individuals may have experienced during the COVID-19 pandemic. The questions are: "What are you doing/did

you do during COVID-19 (coronavirus)?" with a checklist of items about behaviors, like social distancing; "How much is/did COVID-19 (coronavirus) impact your day-to-day life?"; and "Which of the following are you experiencing (or did you experience) during COVID-19 (coronavirus)?" with a checklist of items about emotional distress, substance use, sexual behavior, financial stress, stigma, and support.

PROMIS Short Form–Depression. The 28-item PROMIS Depression Item Bank assesses negative mood (e.g., sadness, guilt), negative views of the self (e.g., self-criticism, worthlessness), negative social cognition (e.g., loneliness, interpersonal alienation), and decreased positive affect and engagement (e.g., loss of interest, meaning, and purpose).[52] Of these 28 items, 6 have been selected to create the PROMIS Short Form–Depression, which has high reliability and precision that is comparable to the original 28-item scale.[52] The 6-item scale assesses depressive symptoms over the past 7 days and has response options ranging from 1 (*never*) to 5 (*always*). The raw scores will be transformed into *T* scores, with higher scores indicating more depressive symptoms.[52]

PROMIS Short Form–Anxiety. The PROMIS Anxiety Item Bank assesses self-reported fear, anxious misery, hyperarousal, and somatic symptoms related to arousal.[53] The PROMIS Short Form–Anxiety includes six items, which have reliability and precision estimates that are high and comparable to the full item bank.[53] The correlation of the adult full bank with the 6-item short form is between 0.90 and 0.95. The 6 items assess anxiety symptoms over the past 7 days and have response options ranging from 1 (*never*) to 5 (*always*). The raw scores will be transformed into *T* scores, with higher scores indicating more severe anxiety.[53]

PSQI. The PSQI is a 10-item scale including 19 self-rated questions. It assesses sleep quality over a one-month time interval. The instrument evaluates both objective (e.g., how often

participants wake up during the night) and subjective aspects of sleep quality (e.g., how rested they typically feel after a night of sleep). These 19 questions are combined to form seven "component" scores, each of which has a range of 0-3 points, from 0 (*no difficulty*) to 3 (*severe difficulty*). Then, the seven component scores are summed to create a global PSQI score, ranging from 0-21, with higher scores indicating worse sleep quality. In primary insomnia patients, the overall PSQI global score exhibited an excellent test-retest reliability of .87.[54] The total score of the Korean version of PSQI showed high internal consistency (Cronbach's $\alpha = 0.84$).[55]

PrimeScreen. The PrimeScreen is a 23-item dietary assessment questionnaire.[56] This self-reported measure evaluates the average frequency of consumption of specified foods and food groups, as well as 13 nutrients (e.g., vitamin and supplements) over the past six months.[56 57] Each item has five response categories: "less than once per week", "once per week", "2-4 times per week", "nearly daily or daily", or "twice or more per day". This measure has great reliability and validity for use in adults aged 19-65 years, including excellent reproducibility ($r = 0.70$) and comparability with the Semiquantitative Food Frequency Questionnaire (SFFQ) in foods and food groups ($r = 0.61$), as well as excellent reproducibility ($r = 0.74$) and comparability ($r = 0.60$) with the SFFQ for nutrients.[56]

Gut Microbiome

To profile the gut microbiome, we will collect fecal specimens using the sample collection procedure used in the Human Microbiome Project protocol.[58] Specifically, we will coach participants to use the home-based specimen collection kits to obtain fecal samples. The kits will include one pair of gloves, one toilet basin, and one biohazard bag with four small stool collection tubes (Fisher Scientific Co. LLC., Pittsburgh, PA, USA). Fecal samples will be collected using pictorial instruction. Specifically, after voiding stool into the toilet basin, the

participant will use the spoon in the cap of the stool collection tube to collect stool and then cap the tube. This stool specimen collection process is repeated two more times with the same voided stool specimen for a total of three tubes (one for gut microbiome analysis, one for quality control, and one for backup).

All the instructions for the sample collection will be prepared in English, Chinese, and Korean. Upon completion of the specimen collection, participants will follow the packaging instructions (e.g., store in a refrigerator for 24 hours before shipping). The samples will be put in the biohazard bag and then into a padded, labeled freezer bag with an ice pack. Participants will ship the samples to the Nursing Biobehavioral Laboratory at Emory University using pre-paid FedEx shipping, which takes approximately 24 hours to arrive at the lab. All fecal samples will be stored at a -80℃ freezer until DNA extraction.

DNA Extraction and Sequencing of the Gut Microbiome

According to the Human Microbiome Project protocol, the microbial DNA will be extracted from fecal specimens using the PowerSoil isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). The 16S rRNA V3-V4 gene regions [59 60] will be extracted and sequenced. 16S rRNA amplicons will be generated using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, KK2600) and primers specific to 16S V3-V4 region of bacteria 341F (5'-CCTACGGGNGGCWGCAG-3')-805R (5'-GACTACHVGGGTATCTAATCC-3'). The PCR clean-up will be performed using AMPure XP beads (Beckman, A63880) and indices will be attached using the Nextera XT Index kit (Illumina, FC-131-1001). Final library pools will be quantitated via qPCR (Kapa Biosystems, catalog KK4824). The pooled library will be sequenced on an Illumina miSeq using miSeq v3 600 cycle chemistry (Illumina, catalog MS-102-3003) at a loading density of 8 pM with 20% PhiX, at PE300 reads. This process will be conducted at the

Integrated Genomics Core at Emory University. The microbial sequencing will lead to paired-end sequences for further analysis.

Statistical Analysis

Prior to analysis, all data will be reviewed for quality, distributions, and missing data bias (e.g., missing at random). Mathematical transformations will be performed when necessary to normalize scores. Descriptive statistics (e.g., Mann-Whitney U test and Fisher's exact test because of the limited sample size) will be adopted to describe participants' characteristics, as well as associations between the psychosocial and biological factors and the outcome variables (i.e., depression, anxiety, and sleep disturbance).

For the gut microbiome data, 16S rRNA sequences will be analyzed to obtain microbial diversity (i.e., α-diversity and β-diversity), taxonomic composition, and abundance analysis. QIIME 2™ default parameters will be used to identify amplicon sequence variants (ASVs) and filter the sequences quality using DADA2.[61 62] Taxonomies will be assigned by the pre-trained classifier using Silva. Differences between the microbiomes across samples will be characterized by α-diversity metrics (Shannon, Chao-1, Faith's phylogenetic diversity, and Pielou's evenness) and β-diversity distances (Bray-Curtis distance, unweighted and weighted UniFrac distance). Pearson or Spearman correlations will be used to determine associations among microbial diversity indices (α-diversity and β-diversity) and the outcome variables. The principal coordinates analysis (PCoA) will also be used to visualize diversity patterns. The linear discriminant analysis (LDA) effect size (LEfSe)[63] will be used to characterize the taxa differences between different levels of outcome variables: (a) Kruskal-Wallis sum-rank test will be adopted to detect features with significant differential abundance between the levels of outcome variables; (b) Wilcoxon rank-sum test will be adopted to further investigate

significances of taxa through a set of pairwise tests among subclasses (e.g., psychosocial factors); and (c) LEfSe will estimate the effect size of each differentially abundant feature. All analyses will be conducted using QIIME 2™ [64-66] and R 3.3.3. The statistical significance level will be set at $p < 0.05$.

Data Storage and Security

All of the survey data will be managed using REDCap,[67] which evaluates data errors, completeness, and validation checks to ensure maximum data quality. All fecal specimens will be stored in the Nursing Biobehavioral Laboratory at Emory University. These specimens will only be used to address our research aims. All the survey data and specimens will be destroyed three years after the entire study is finished. The confidentiality of all data will be maintained within legal limits.

Discussion

Although numerous studies have examined risk processes associated with mental health and poor sleep, there is a distinct paucity of research on Asian immigrants in the U.S., despite the fact that this population is underserved and experiences substantial mental health-related disease burden in America. To address this important issue, we will conduct the present study, which will be the first to examine psychosocial and biological mechanisms underlying depression, anxiety, and sleep symptoms among Chinese and Korean immigrants in the U.S. Considering that these populations are growing quickly, we expect that the findings will help advance our knowledge on racial and ethnic differences in mental health outcomes and the biopsychosocial pathways that underlie these effects.

Although these associations would be important to understand at any time, we believe these issues are particularly critical to study during the COVID-19 pandemic, given the increased

rates of social conflict, discrimination, and, in some cases, injustice that have been experienced by Asians in the U.S during this time. Indeed, the impact of the COVID-19 pandemic on Asian immigrants has been extensive.[68] Public health measures designed to curb the spread of the virus, which have included lockdown, school and business closures, and travel restrictions, have had a tremendous impact on the stress levels and mental health of the general population.[69 70] Beyond this, though, Asians living in the U.S. have been stigmatized and victimized by media coverage perpetuating the naming of the COVID-19 virus as the 'Chinese Virus' or 'Kung Flu', which has in turn lead to racial discrimination and other social threats[68] that have been shown to strongly affect mental and physical health.[71] The cumulative social stress and threat experienced by Asian immigrants, which include aggravated racial discrimination in addition to ongoing health, employment, and financial worries, will provide a unique opportunity to better understand how psychosocial factors and the microbiome affect mental health and sleep symptoms during a time of maximal importance and relevance.

In assessing Asian immigrants' cumulative life stress exposure, the Adult STRAIN and PSI will help assess acute and chronic stressors of participants who have been going through the pandemic for an extended period of time. Importantly, some of the measures we have selected are tailored to Asian populations, which will enable us to collect more valid and reliable data that are reflective of Asians' lived experiences, including racial discrimination and acculturation. These culturally adapted measures will yield a unique and timely perspective on mental health and sleep outcomes in Asian immigrants.

This study has some limitations. They include a limited sample size and cross-sectional study design. The small sample size limits power and data analysis options at the more granular level (e.g., stratified analysis by immigrant generation). Also, the sample limits generalizability

to other Asian subgroups due to studying only Chinese and Koreans. In terms of the measures, although diet is culture-specific, the PrimeScreen has not been extensively validated among Chinese or Korean populations. Despite some dietary intake not captured by the PrimeScreen, we expect its impact on the study findings to be minimal, as diet will be treated as a control variable in analyses. Lastly, the demographic characteristics may differ between those recruited online and offline. Considering many of the recruitment strategies use online platforms, the participants could be skewed toward a younger population with a shorter duration of U.S. residence, resulting in limited variation for these data. Therefore, a future study should collect information on how participants were recruited (online vs. offline) and consider this in statistical analyses. Nevertheless, looking forward, we expect this study to provide important preliminary data that can in turn be used to inform the development of a larger longitudinal study aimed at investigating associations between psychosocial and biological determinants of health, and mental health and sleep symptoms among Asian immigrants in the U.S.

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Author's contributions

The study's concept and design were conceived by SK, WZ, VP, VSH, and JB. GMS produced a secure online STRAIN portal, monitored stress data collection, and implemented and managed the stress data collection protocol. SK, WZ, and JB translated the survey instruments and other study materials into Chinese or Korean. SK, WZ, JKA, JB, and CMS developed the online survey and managed the online survey platforms. SK, WZ, VP, JKA, and JB were involved in participant recruitment. SK, WZ, JKA, and JB collected data and consented participants. SK, WZ, JKA, and JB will analyze the data, and VSH and GMS will guide and supervise data analysis. SK prepared the first draft of this manuscript. All authors provided critical edits, critiqued the manuscript for intellectual content, and read and approved the final version for submission.

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Ethics approval

This study was approved by the Institutional Review Board at Emory University (IRB ID: STUDY00000935).

Competing interest statement

None declared.

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Figure legends

Figure 1. Conceptual Framework

Figure 2. Multi-lingual Survey Development and Testing Process

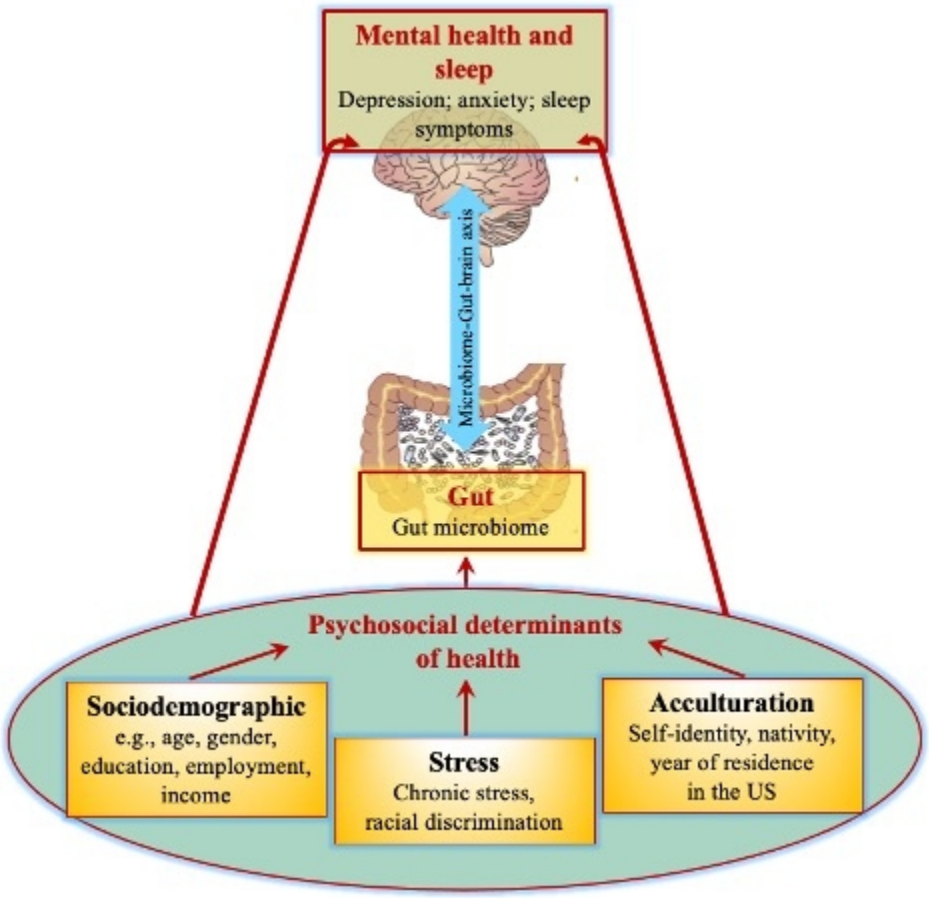

Figure 1

165x160mm (72 x 72 DPI)

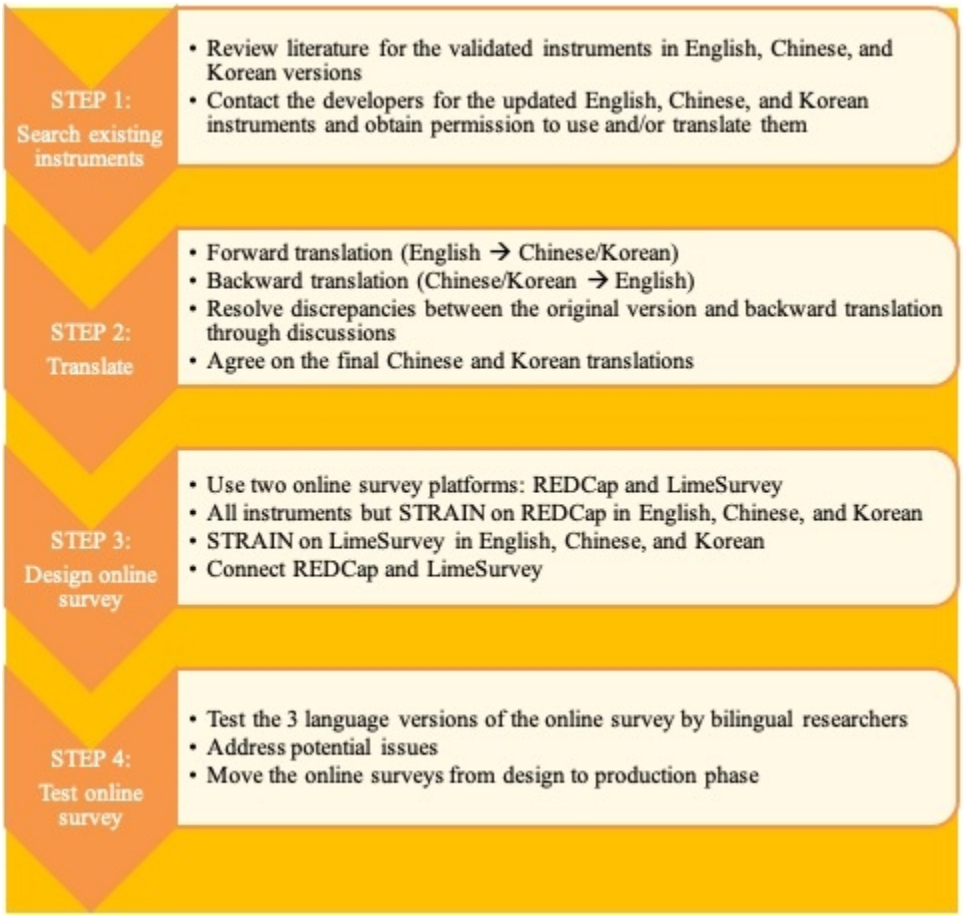

Figure 2

170x160mm (72 x 72 DPI)