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The Adolescent Stress Experiences over Time Study (ASETS):

A Prospective Longitudinal Study of Sexual Minority Adolescents in the United States

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Abstract

Introduction: Sexual minority adolescents (SMA) report higher rates of anxiety, self-harm, depression and suicide than heterosexual peers. These disparities appear to persist into adulthood and may worsen for certain subgroups, yet the mechanisms that drive these concerns remain poorly understood. Minority stress theory, the predominant model for understanding these disparities, posits that poorer outcomes are due to the stress of living in a violently homophobic and discriminatory culture. Although numerous studies report associations between minority stress and behavioral health in adolescence, no study has comprehensively examined how minority stress may change throughout the course of adolescence, nor how stress trajectories may predict differences in health during this critical developmental period.

Methods and analysis: Between May 15, 2018 and April 1, 2019, we recruited a U.S. national sample of diverse SMA (N = 2,559) age 14-17 through social media and respondent-driven sampling strategies. A subset of participants (N = 1,076) enrolled in the longitudinal component and will be followed each six months until July 1, 2022. Primary outcomes include symptoms of depression, anxiety, and PTSD; suicidality and self-harm; and substance use. The key predictor is minority stress, operationalized as the Sexual Minority Adolescent Stress Inventory. We will use parallel cohort-sequential latent growth curve models to test study hypotheses within a developmental framework.

Ethics and dissemination: All participants provided assent to participate, and longitudinal participants provided informed consent at the first follow-up survey after reaching age 18. All study procedures were reviewed and approved by the institutional review board at the authors' home institution, including a waiver of parental permission given the potential for harm due to unintentional "outing" to a parent during the consent process. The final anonymous data set will

be available upon request, and research findings will be disseminated through academic channels and products tailored for the lay community.

Strengths and Limitations of This Study

Strengths:

- This study leverages a newly developed, valid and psychometrically sound measure of minority stress in a large, diverse national sample of adolescents.
- The longitudinal cohort design permits the first examination of change in minority stress experiences over time.
- The cohort sequential modeling approach also supports the first examination of how minority stress influences health across adolescence.

Limitations:

- All outcome measures are self-reported and may be subject to recall and responses biases; no confirmatory behavioral data will be collected.
- Generalizability of study findings may be constrained by study eligibility criteria, strict data quality procedures, and recruitment methods.

The Adolescent Stress Experiences over Time Study (ASETS): A Prospective Longitudinal Study of Sexual Minority Adolescents in the United States

Introduction

Sexual minority (e.g., lesbian, gay, bisexual, pansexual) adolescents (SMA) experience significant behavioral health disparities compared to their heterosexual peers. In particular, SMA experience higher rates of internalizing psychopathology including depression, anxiety and self-harm[1-3] and externalizing behaviors such as substance use,[4-6] suicide attempt and completion[(7, 8)] Longitudinal studies suggest that these disparities persist into young adulthood and may even worsen. For example, data from a national study of adolescents (Add Health) showed that average longitudinal trajectories for substance use among SMA are disparate from heterosexual youth beginning in early adolescence and increase as youth transition into young adulthood.[9] When examining individual trajectories of suicidality, all sexual minority groups (lesbian, gay, bisexual, mostly heterosexual) reported higher rates of suicidality across all four waves than their heterosexual peers, from mid-adolescence to early adulthood.[10]

There are also behavioral health disparities among SMA by demographic subgroup. For example, sexual minority girls are more likely to report both considering and attempting suicide than sexual minority boys,[11, 12] and bisexual youth show larger substance use disparities than other sexual minority groups.[9] SMA living in rural areas also experience different behavioral health outcomes than their urban counterparts due to confidentiality concerns, values, and limited access to cities with more extensive peer networks[13] and a more comprehensive social support system.[14] While there are likely to be subgroup differences among racial and ethnic lines as well, scholars have noted a relative absence of racial and ethnic diversity in sexual minority research,[15-17] and even in large meta-analytic studies, the lack of racial and ethnic diversity in

sampling is noted as a significant limitation.[12, 18] Understanding the experience of these youth is increasingly relevant, as recent national survey data suggest that racial and ethnic minority youth are more likely than white segments of the U.S. population to identify as SMA.[19]

The primary theoretical framework for understanding the disparities found among sexual minorities is the minority stress theory (MST),[20-22] which has been endorsed by the Centers for Disease Control and Prevention,[23] the National Academy of Medicine,[24] and Healthy People 2030.[25] MST suggests that discrimination, violence, and victimization due to a pervasive homophobic culture are the primary sources of stress and most probable driving mechanisms of mental health disparities among sexual minorities, including SMA.[21, 26-28] Numerous cross-sectional studies have attributed poor behavioral health outcomes among adolescents to minority stressors, such as negative disclosure experiences with family and peers,[1, 27-29] becoming homeless upon disclosure,[30] in-school victimization (bullying) by students and faculty members,[31, 32] and experiences of violence.[12, 33, 34] However, no study has ever comprehensively examined this relationship longitudinally. Despite recognition that stigmatizing experiences can disrupt adolescent development and contribute to negative outcomes,[35] the gap between theoretically predicted relationships and empirical evidence to support them is largely due to four key concerns:

(1) Studies of minority stress during adolescence have been fraught with *poor psychometric measurement*.[13, 36] A review of psychometric measurements assessing discrimination against sexual minorities found that across 162 articles, nearly all had suboptimal psychometric properties.[37] Few studies have used empirically validated measures, and most measures had been developed using small investigator-led samples or adapted from measures with adults in other minority populations.[18] Previously available general stress

measures, even those validated for use with adolescents, do not allow us to differentiate between common developmental stressors and those associated with minority stress.

(2) There is an absence of studies examining minority stress and behavioral health in adolescents *over time*. Only six studies (with four unique samples) have examined the relationship between minority stress and subsequent behavioral health outcomes, and each has several major limitations: (a) lack of a well-constructed comprehensive measure of minority stress for adolescents; (b) reliance on small regional samples; and (c) lack of repeated-measures analyses and trajectory modeling to assess patterns of change in minority stress during this critical developmental time period.[22, 38-40] Although the field has generally assumed minority stress is the most probable cause of persisting behavioral health concerns among SMA, no study has examined this directly.

(3) Although some *subgroup differences* in behavioral health outcomes have been documented, their determinants are not well understood. As previously described, differential outcomes are noted in sexual minority samples by race, ethnicity, gender, and geography, and authors have called for increased attention to subgroup analyses in future research with SMA.[10, 12, 18, 41] These experiences have been explored in only a handful of studies, primarily with adult samples.[42-44] Although the assumption is that minority stress also drives these disparities, no studies have systematically explored subgroup differences in minority stress over time.

(4) The presence or absence of *protective factors* may add to the confusion. Some studies suggest avoidance strategies[45] or emotionally focused cognitive restructuring[46] may be helpful; others have recommended finding accepting friends, having supportive parents or family members, identifying supportive adults at school, and relying on SMA community resources (e.g., gay–straight alliances, SMA community centers) as methods for coping with

minority stress.[14] However, not all subgroups of SMA may have these opportunities. For example, youth who live in rural areas may have less access to affirming resources[13] and be more likely to live in areas with less protective school policies.[47] Thus, the relationships between minority stress, demographics, protective factors and outcomes remain poorly understood.

The current study is the first to address these four major gaps in the extant literature. We can now measure minority stress in adolescents with a psychometrically sound instrument, the Sexual Minority Adolescent Stress Inventory (SMASI), which was developed and validated by the research team in prior work funded by the National Institutes of Health (NIH).[48-50] Using this measure, we will conduct a systematic investigation of minority stressors and behavioral health over time in a large, diverse national sample. With repeated measures of minority stress and a modeling approach (cohort sequential latent growth curve modeling – see *Data Analysis*) that considers change across age rather than time, we can answer questions not previously addressed, such as whether minority stress increases over time as young teenagers develop throughout adolescence; when do minority stressors peak; whether there are demographic differences in the frequency, severity and pattern of minority stressors; and whether changes in minority stressors over time predict corresponding changes in health outcomes over time. Furthermore, we can test whether trajectories of minority stress are inversely associated with protective factors over time and if they too differ by demographic subgroup.

Thus, the present study will serve as one of the first longitudinal studies conducted with this vulnerable population. We address critical methodological design factors necessary to conduct high-quality longitudinal research with SMA, including: (a) a safe and effective recruitment approach, with built-in mechanisms to protect SMA from being "outed" via study

participation, which could increase risk of victimization (e.g., kicked out of home); (b) repeated measures over time of important psychosocial predictors and outcomes; (c) recruitment of participants at ages 14–17, because they are a particularly neglected subpopulation in SMA studies;[51] and (d) a mechanism for recruiting "hidden youth" who have not disclosed their sexual orientation to others, including their parents, resulting in a lack of both scientific and clinical understanding about them. Upon completion, the study will provide critical information needed to inform the nature and timing of intervention efforts for this high-need, underserved, and difficult-to-reach population of youth.

Methods and Analysis

Sample Selection

Population Definition. Recent studies suggest that upwards of 10% of youth do not identify as exclusively heterosexual.[10] Throughout this protocol, we use the term *sexual minority adolescents*, or SMA, to refer to adolescent individuals who endorse same-sex attraction or identity. Attraction includes romantic or sexual feelings, whereas identity describes how youth label themselves (e.g., lesbian, gay, bisexual).[52] These are consistent with constructs commonly used to operationalize sexual orientation.[53, 54] We recognize *adolescents* generally as youth aged 13 to 20, a common international convention.[55] However, we restricted recruitment in this study to youth aged 14 to 17, as we have in our preliminary work, given literature suggesting youth commonly begin to define their sexual identity during these years.[56]

*Study Eligibility***.** Youth were eligible to participate in the study if they were at least 14 and no more than 17 years old; were cisgender male or female (i.e., reported a current gender identity consistent with their sex assigned at birth); resided in the United States, as determined by ZIP code; identified as not 100% heterosexual using *Add Health* guidelines (i.e., identified as

mostly heterosexual, bisexual, gay, lesbian, or unsure);[57] and were willing and able to provide assent to participate.

Stratification Variables. To ensure geographic diversity, ZIP code was recoded into two additional variables: region and urbanicity. Region (West, Southwest, Midwest, Northeast, and Southeast; see Table 1) was based on the state associated with the participant's reported ZIP code. Urbanicity (rural or urban) was determined based on the Rural Urban Commuting Area (RUCA)[58] codes. Specifically, "urban" was defined as a ZIP code corresponding to RUCA codes of 1.0, 1.1, 2.0, 2.1, 4.1, 5.1, 7.1, 8.1, and 10.1. "Rural" was defined as all other valid RUCA codes. When a ZIP code was associated with a RUCA 3.1 score, that score was used; for ZIPs that were not assigned a RUCA 3.1 score due to changes in the classification system between RUCA versions 2 and 3, the RUCA 2.0 score was used.

Table 1. Assignment of U.S. states to regions.

U.S. Region	**U.S. States**		
West	Alaska	Idaho	Utah
	California	Montana	Washington
	Colorado	Nevada	Wyoming
	Hawaii	Oregon	
Southwest	Arizona	Oklahoma	Texas
	New Mexico		
Midwest	Illinois	Michigan	North Dakota
	Indiana	Minnesota	Ohio
	Iowa	Missouri	South Dakota
	Kansas	Nebraska	Wisconsin
Northeast	Connecticut	Massachusetts	Pennsylvania
	Delaware	New Hampshire	Rhode Island
	Maine	New Jersey	Vermont
	Maryland	New York	
Southeast	Alabama	Kentucky	South Carolina
	Arkansas	Louisiana	Tennessee
	Florida	Mississippi	Virgina
	Georgia	North Carolina	West Virgina

Participant Recruitment

Targeted Advertising. Initial participants were recruited through advertising on Facebook/Instagram (which now share a single advertising platform) and YouTube. Advertisements varied slightly by platform, but all included language asking youth to "Share Your Voice" and described basic details of the research study and incentives that participants could earn. Advertising was stratified by gender, geographic region, and urbanicity. This resulted in 20 target cohorts, as each of the five regions encompassed four unique groups: rural males, rural females, urban males, and urban females. We used two different sets of advertising images: one featuring females (for the female groups) and one featuring males (for the male groups). To reach each of these groups, general specifications included age (14-17 years), gender (women or men), and location. Facebook/Instagram allows bulk uploading of up to 2,500 ZIP codes per ad. A total of 44 targeted ads were required to reach all possible combinations of region, urbanicity, and gender, as some combinations included nearly 10,000 eligible ZIP codes.

Facebook/Instagram also allows advertisers to target audiences based on interests. Using keywords enumerated by members of the research team, we identified specific interest terms by gender. Male-targeted interests included Gay-friendly; Gay, Lesbian, Bisexual, Transgender, Straight Alliance; Homosexuality; LGBT community; LGBT symbols; and Pansexuality. Female-targeted interests included all of the male-targeted interest keywords plus Lesbian Connection and Lesbian Romance.

Simultaneously, the research team identified YouTube channels for review using keywords including LGBTQ, gay, coming out, transition, and trans. Channels were reviewed for visibility, reach, and engagement of each channel, operationalized as the number of subscribers per channel and number of video views for each channel's three most-viewed videos. We

initially identified 47 possible YouTube channels that had high visibility and engagement among LGBTQ+ adolescents, using a combination of keyword searches (e.g., LGBTQ, gay, coming out, transition) and subscriber and video view counts; after reviewing this list, we advertised to 23 channels that were verified YouTube accounts, able to accept advertisements, and agreed up on by the study team as being relevant to sexual minority adolescents. Using the Google advertising system, we placed advertisements for the ASETS study directly on the pages of those channels.

Respondent-Driven Sampling. Respondent-Driven Sampling (RDS) is a type of chain-referral sampling that allows for identified members of a hidden group, called "seeds," to recruit other group members from their personal networks.[59] Participants who completed their survey and were initially deemed eligible for retention were asked if they might be interested in referring friends who they thought might be eligible to participate. Participants who confirmed their interest in referring friends to the study were provided with an email that contained three unique survey links as well as two different language prompts to encourage peers to participate. In return for successfully recruiting an eligible participant who completed the survey, the recruiter participant (seed) was paid $10 per referral for up to three eligible participants.

Baseline Study Procedures

Initial Eligibility Screening. Advertisement clicks and referral links all directed youth to a screening page in Qualtrics that asked a series of demographic questions to determine their eligibility based on age in years, gender, ZIP code, and sexual attraction. Ineligible participants were thanked for their interest in the study and then re-routed to a separate Qualtrics survey where they could optionally provide contact information (email and/or phone number) to be included in outreach for future studies. Eligible participants were shown the IRB-approved study

assent text and asked to confirm assent in order to proceed with the main survey, implemented in Qualtrics (see *Measures*).

Post-Survey Data Collection. After completing the survey, the participant was re-routed to a separate Qualtrics survey for payment in order to keep their personally identifiable information separate from their main study data. This payment survey asked the participant for their private email address at which to receive an electronic gift card. Participants were also asked if they knew other sexual minority youth, and if so, whether they would consider referring any of those youth into the study, to aid RDS recruitment. Finally, participants were asked whether they would be interested in participating in the longitudinal study and given fields to provide up to five different contact methods if so. Contact options included email, phone numbers for call/text, and usernames for Facebook, Twitter, Snapchat, and any other social media accounts that allow for personal messaging. Participants were able to rank their provided methods of contact in order of preference.

Final Eligibility Determination. Each business day a study team member downloaded any new surveys from Qualtrics. Variables were created to represent region, urbanicity, response declinations (total number of "Decline to answer" responses across the entire survey); survey duration, and attention validation (number of attention-control questions the respondent answered correctly).[60] Participants who failed to complete the entirety of the survey–that is, they exited the survey prior to completing and being routed to the payment survey–were excluded and could not be paid due to lack of contact information. Participants determined to have engaged in any type of fraudulent activity were also immediately excluded from both study eligibility and pay. "Fraudulent activity" included providing information or response patterns, either within the main survey data or on the payment and contact information survey, that

confirmed duplicate response by a previous participant, or screening out of a first survey attempt (i.e., determined to be ineligible) and immediately re-accessing the survey with false responses in an attempt to access the full survey.[61-64] Fraudulent participants were identified by duplicate IP address, duplicate email and/or contact information, similar patterned responses throughout survey (including open-ended responses with identical or unique wording), and/or survey time stamps. Fraudulent participation was not compensated even if sufficient contact information was provided.

Participants who completed the survey but provided very low quality data, defined as either an unrealistically short survey completion time (≤10 minutes), a low attention-control score (≤1 out of 4 correct responses), or very high (≥35) "Decline to answer" response count, were compensated for their participation but were excluded from the baseline dataset and not invited to participate in the longitudinal study or refer peers via RDS. Participants who had survey duration times of 10 – 15 minutes, attention-control scores of 2, and moderately high (≥25) responses of "Decline to answer" were compensated for participation and further evaluated for inclusion on a case-by-case basis. By applying all of the above-described procedures prior to longitudinal recruitment, we ensured that only participants who provided valid and trustworthy data would be enrolled in the longitudinal study.

Incentive Compensation. All baseline participants who were eligible for compensation, whether or not their data were retained for analysis, were sent a $15 Amazon gift card to the private email address they provided in the payment survey. Participants whose data were retained for analyses were assigned a unique four-digit participant identifier at this time.

All participants were recruited into the study and completed their baseline surveys (N = 2,559) between May 15, 2018 and April 1, 2019. Figure 1 illustrates the number of individuals retained and excluded at each step of the baseline recruitment and data collection process.

Longitudinal Study Procedures

Longitudinal Enrollment. Approximately one week from the date of a participant's baseline survey completion, participants who expressed interest in longitudinal participation were entered into a master tracking log file. This artificial delay helped ensure that we could detect participants willing to engage in fraudulent behavior, including participants who were trying to take the baseline survey multiple times in an attempt to receive multiple payments, prior to inviting them to be part of the longitudinal study. Participants who reached this longitudinal recruitment stage were contacted by a research assistant in real-time via the participant's preferred contact method, using a study-specific username or account shared by the research team. Participants were first reminded that they recently completed an online survey.

In an effort to protect their privacy and ensure that we were speaking with the right individual, we asked them to please tell us what that survey was about. Participants who provided the correct information (e.g., "LGBT youth") were asked if they were interested in learning more about the longitudinal study. Participants who expressed interest were given information about the study outlining their involvement, including a written information sheet. Those who agreed to participate were then asked to confirm or update their contact information, and the research assistant verified the participant could receive emails from the study team that did not end up in their spam/junk folders. Participants were reminded that the study team's next contact with them would be through an automated monthly check-in survey every month (see *Monthly Check-In Surveys*) and that they would receive an email in approximately six months for

their next full survey. Finally, they were provided with all methods of contact to reach the study team and were encouraged to reach out in the event they had questions, concerns, or comments.

RDS Referrals. Longitudinally enrolled participants were given the option to refer peers into the baseline survey for an additional incentive, i.e., RDS referrals. Participants who stated they may know others who might be interested were provided three custom Qualtrics referral links, which contained an embedded RDS code that both identified the new participant as an RDS referral and allowed the study team to link the new survey to the referring participant for a referral payment. All referred participants went through the same validation, eligibility, and payment process as those who entered the study through direct outreach methods. Additionally, the participant who referred them was provided with a $10 Amazon gift card as a referral incentive. Referrers were not paid referral incentives for distribution of survey links to youth who were ineligible for participation or those whose surveys were excluded from retention due to low data quality. Participants who attempted to refer themselves were easily identified by the quality assurance protocol previously described; in the case of self-referrals, the participant was immediately excluded from both the baseline and longitudinal study due to their demonstrated willingness to defraud the study team.

Monthly Check-In Surveys. Because the study was conducted entirely online, having up-to-date contact information for all participants was of critical importance. Additionally, with six months in between full surveys, it was important to have more regular contact with participants in order to maintain rapport and interest in the study. Thus, a brief check-in survey, consisting of one item asking whether any of the participant's contact information had changed within the last 30 days, was automatically emailed to each longitudinal participant near the first day of every month. If a participant indicated that their contact information had changed, they were then

prompted to provide any new or updated contact information. If a participant failed to respond to the automated check-in survey by the 15th of each month, a research assistant would manually reach out to them once through each of the participant's preferred contact methods. Each check-in survey was accompanied by a raffle where all respondents to the check-in survey within the calendar month were entered into a random drawing to receive a $100 Amazon gift card, regardless of whether their contact information had changed.

Longitudinal Follow-Up Surveys. A unique link to each Qualtrics follow-up survey was created by the study team for each participant. This link, provided to the participant once they became eligible to complete the survey, contained embedded information about the date on which they completed their previous survey along with their assigned unique participant identifier. This allowed information about prior participation dates to be pre-populated in survey items requesting retrospective information in an effort to aid in recall.

At the start of every week, all participants whose follow-up survey date fell within that week (i.e., a multiple of 6 months after their baseline survey date) were sent an automated survey link in an email from the Qualtrics platform. Automated messages were sent on Monday, Wednesday, and Sunday of the first week of eligibility. Participants who failed to complete their follow-up survey by the third automated attempt would begin to receive manual messages from research assistants containing their unique embedded survey link. Manual attempts to contact the participant were first sent via the participant's most recently indicated preferred contact method. After three months, their survey window would close and participants would not be able to complete that wave of data collection to ensure any two consecutive waves of data collection would reflect a minimum time difference of three months apart. Follow-up data collection began

on August 15, 2018, and will conclude on July 1, 2022 when the three-month window of the 36-month survey closes.

Longitudinal Eligibility and Payment Determination. When a participant completed a follow-up survey, the participant was re-routed to the separate Qualtrics survey page for payment using the same procedures as at baseline. Each business day a study team member would access the main survey through Qualtrics and download the previous day's surveys. Variables were again created in each follow-up dataset including survey duration, attention control score, and count of "decline to answer" responses. Participants who failed to complete the entire survey or who had very low quality data (as defined above), were encouraged to retake the survey with an explanation provided as to why they were asked to retake the survey. This outreach process continued for five total manual attempts alternating every other day. This process was replicated at all subsequent survey waves. The longitudinal enrollment and retention diagram is shown in Figure 2; to date, five participants have withdrawn from continued follow-up, bringing the longitudinal sample to N=1,071.

Measures

Beginning with baseline data collection and at each follow-up survey, the following measures were collected:

Outcomes. We focus analysis on understanding behavioral health, consistent with terminology from the Substance Abuse and Mental Health Services Administration,[37] to describe an integrated approach focused on the promotion of emotional health and prevention of mental illness, alcohol and other drug use, and associated outcomes (e.g., suicide). Behavioral health outcomes included: *depressive symptoms*, measured with the CESD-4;[65] *anxiety,* assessed with the Generalized Anxiety Disorder 7-item measure (GAD-7);[66] symptoms of *Post-*

Traumatic Stress Disorder (PTSD),[67] using the Abbreviated PTSD Checklist-Civilian (PCL-C); and five questions from the Youth Risk Behavior Survey (YRBS) to assess *suicidality and self-injury*.[68] All responses were recoded to binary indicators of suicidal ideation, plan, attempt, attempt resulting in injury, and self-injurious behavior. Finally, YRBS items were also used to assess lifetime and past-30-day *substance use,*[68] including binary indicators of whether a participant had used alcohol, tobacco, marijuana, prescription pain relievers, prescription tranquilizers, and prescription stimulants.

Key Predictor: Minority Stress. The focal measure in this study is the Sexual Minority Adolescent Stress Inventory[48-50] (SMASI; whole-scale omega (ω)=.97), which relies on 54 main items to measure 10 domains of minority stress: social marginalization (8 items, ω=.93), family rejection (11 items, ω=.94), internalized homonegativity (7 items, ω=.94), identity management (3 items, ω=.77), homonegative climate (4 items, ω=.88), intersectionality (3 items, ω=.82), negative disclosure experiences (5 items, ω=.77), religion (5 items, ω=.93), negative expectancies (3 items, ω=.81), and homonegative communication (5 items, ω=.77). An optional subscale assessed stress experiences at work among youth with any work history (10 items, ω=.96). At baseline, youth were asked if they have had each experience "ever" and "in the past 30 days;" the stem language changes to "since [they] last took the survey" and "in the past 30 days" at subsequent time points.

Demographic Covariates. Variables used for eligibility screening included *age* (in years). *Sex assigned at birth* (male or female) was used in concert with *gender identity* (response options included: male; female; trans male/trans man; trans female/trans woman; genderqueer; gender nonconforming; nonbinary; gender identity not listed here) to determine cisgender status. For eligibility screening that depended on survey programming logic, *sexual minority identity* was

determined with the recommended item from Add Health guidelines:[57] "If you had to pick one of the following options, please choose the description that best fits how you think about yourself," with all response options other than "100% heterosexual (straight)" qualifying for eligibility. ZIP code was assessed to verify *U.S. residence* and subsequently recoded for stratification into *urbanicity* and *region* as previously described.

Additional demographic variables included open-ended *sexual identity,* assessed by asking "What would you say is your sexual orientation or identity?" and a text box for an open-ended responses that could be recoded for later analyses. *Race and ethnicity* was a forced-choice item with response options of Native American/American Indian/Alaska Native; Asian/Pacific Islander; Black or African American; White/Caucasian; Latino/Hispanic; Multi-racial (with a text box to specify); and Race/ethnicity not listed here (with a text box to specify). Gender expression was captured with one item asking, "A person's appearance, style, dress, or the way they walk or talk may affect how people describe them. How do you think other people would describe you?" Response options were on a 7-point Likert-type scale from "Very feminine" to "Very masculine." *School enrollment* was captured with a binary indicator of whether participants are currently enrolled in school, and *educational attainment* measured the highest grade already completed (less than 7th grade; 7th grade; 8th grade; 9th grade; 10th grade; 11th grade; High school graduate or GED; Some college; Trade school certification or Associate's (AA) degree; Bachelor's (BA/BS) degree or higher). Additionally, participants were asked whether they were eligible for free or reduced price lunch at the school they most recently attended as a proxy for *socioeconomic status* (response options: "Yes," "No," "I don't know"). *Work status* was assessed with one item asking whether participants are currently working, with response options of "Yes, full-time"; "Yes, part-time"; "No, but I have previously had a job"; or

"No, and I have not previously had a job". In follow-up surveys, participants were asked whether they had worked since the last time they took the survey ("Yes" or "No"). *Living situation* was assessed by asking participants with whom do they currently live. Response options included: two parents; mother only; father only; grandparents or other relatives; foster parents; group home; alone or with roommates in own apartment or home; boyfriend/girlfriend/romantic partner/spouse; with friends or couch-surfing; homeless or on the street; and other (with a text box to specify). Participants were asked whether they had become involved in the *foster care system* ("Yes", "No", or "Unsure"). Participants were asked about their *primary language* spoken a) at home and b) with their friends; response options included English; Spanish; or another language (with text box to specify). Participants were asked to indicate their personal and family religion from a comprehensive list.[69] Although not used in the eligibility process, sexual attraction to men, women, and gender nonconforming people was also assessed with Likert-type response options ranging from "Not at all" to "Extremely."

Theoretical Covariates and Protective Factors. Participants completed the Perceived Stress Scale (PSS), a measure of *general stress,*[70, 71] to ensure we can control for other common adolescent stress experiences unrelated to minority stress. *Social support*[72] from friends, family, and a significant other was measured with the Multidimensional Scale of Perceived Social Support. *Disclosure* questions were asked to understand who, if anyone, knows the participant is LGBTQ (baseline) or who, if anyone, the participant has disclosed their sexual orientation to for the first time since the last survey (follow-up surveys). Options included: mother; father; siblings; other adult relatives; peers/supervisor(s) at work; members of the participant's religious community; teacher(s); peers at school, childhood friends; heterosexual friends; LGBTQ friends; and girlfriend/boyfriend/partner. Response options were "Yes," "No," or "Not Applicable" (if

they did not have the listed person in their life). A series of binary items assessed the presence of supportive resources such as *gay-straight alliances (GSA)* and *mentorship*, as follows: Three items captured whether the person currently had regular access to a GSA; whether their current (or most recently attended) school has a group or club specific to the LGBTQ+ community; and whether they participated in meetings or activities sponsored by an LGBTQ+ club at their school (or most recently attended school). Participants were also asked whether there is an adult 25 years or older, currently in their life, who they consider to be a mentor. Those who responded "yes" were asked whether this person is part of their immediate family; if they responded yes, they received an additional question asking, "Other than an immediate family member (or the person who raised you), is there an adult 25 years or older who you consider to be your mentor?" These items were recoded into a binary item reflecting the presence or absence of a non-family mentor age 25 or older. Finally, adolescent *coping strategies* were captured with the Coping Strategies Inventory-Short Form,[73] which includes 16 items on four subscales (problem-focused engagement; problem-focused disengagement; emotion-focused engagement; and emotion-focused disengagement). An additional 20 items assessed *LGBT-specific coping strategies*. The first ten questions asked about potentially recurring events, such as "I spent time with the LGBTQ community" and "I tried to gain new knowledge about the LGBTQ community." Response options for these statements included "Never," "Sometimes," "Often," and "Regularly." The second ten questions asked participants for a binary response indicating if they had ever had the positive experience, such as "I went to an LGBTQ pride event" and "I've been in a romantic relationship."

Additional Measures. Although not the primary focus of the study, additional measures were added at later waves of data collection to probe emerging findings in the literature on SMA

behavioral health. These included a more thorough investigation into the experiences of homeless and precariously housed youth; intersectionality of SGM status with cultural identity; experiences with body dysmorphia; sexual behavior, intimate partner violence, and nonconsensual distribution of explicit images ("revenge porn"); healthcare access and utilization; and specific forms of marijuana or nicotine used in the past 30 days.

Data Analysis Plan

We propose using cohort sequential latent growth curve modeling (LGCM)[74] to describe how minority stress and its associated behavioral health outcomes change over time among SMA. Compared with traditional longitudinal analysis methods such as comparing pre- and post-test scores or change by data collection wave, the cohort sequential LGCM approach examines individual change over age and is more appropriate for modeling developmental processes, coinciding with theoretical paradigms that are often person-centered longitudinal pathways.[75, 76]

Several preliminary steps and decisions will be made prior to longitudinal model estimation and will vary depending on the type of analysis (e.g., ordinary least squares vs. logistic regression). Multicollinearity and influential cases will be assessed.[77] Distributional properties of all continuous and categorical variables will be evaluated, and we will apply appropriate transformation or robust estimation procedures to correct for non- normally distributed variables.[123] Attrition analyses will be conducted to understand missingness. Missing data will be handled in all growth models using full information maximum likelihood estimators in Mplus assuming data are missing completely at random or missing at random.[78] Multiple imputation[79] methods will also be used when appropriate. Depending on the analysis and the hypothesis being tested, demographic and some substantive variables will be included as covariates to increase the specificity of the effects; e.g., geographic region, ethnicity, or general

stress. Proper functional forms of trajectories will be identified prior to estimating full unconditional LGCMs.[80] Structural equation models will be evaluated using commonly accepted fit indices (e.g., chi-square, CFI, IFI, TLI, and RMSEA) and modification indices (e.g., LaGrange multipliers).[81] Standard guidelines for small (.2), medium (.5), and large (.8) effect sizes[82] will be adopted. Confirmatory factor analysis will be used to assess measurement invariance of the SMASI and all outcome measures over time.

General approach to hypothesis testing. LGCMs will be estimated for the SMASI total score, the SMASI subscales, and each of the behavioral health outcomes. The use of a cohort sequential LGCM will allow for the modeling of change in each outcome trajectory as adolescents age during the course of our study by plotting latent means across age to understand developmental trends among participants. These are useful in examining within-person change across time and between-person variability.[80] Furthermore, LGCMs provide group-level statistics, including the average amount of change over time (i.e., slope), the average starting point (i.e., intercept), and the relationship between the two.[83, 84] One important advantage of LGCM is the implementation and comparison of appropriate functions to best fit the trend of the data. It is likely that several patterns of growth during the course of adolescence may emerge – that is, two or more separate slopes may be modeled within the same trajectory to demonstrate divergence in trends.[83, 85] These separate but related pieces incorporate the piecewise function, which allows for several linear slopes to be modeled within the same construct (e.g., minority stress) and can provide information about differences in construct level (i.e., intercept) and growth velocity (i.e., slope) at varying stages of adolescence.

Due to the hypothesized relationships between minority stress and the behavioral health outcomes (depression, suicidality, problem behaviors, and substance use), we propose to analyze

these trajectories simultaneously via the use of parallel cohort sequential LGCM models.[85] By modeling several growth processes (e.g., minority stress and depression) at once, we can evaluate the relationship of slopes and intercepts both within and across measures to understand their interrelated effects over time. This approach will allow us to test the following working hypotheses (WH):

WH1. There will be differences in minority stress across adolescent development. A cohort sequential LGCM[86] for the total SMASI score and each of its 10 subscales will be estimated to describe individual and group-level trajectories of minority stress among all SMA during the course of the study period. This will establish the best-fitting LGCM and allow the selection and implementation of the most appropriate piecewise function. We will test piecewise models with one (i.e., linear across development) to four growth trajectories to determine the number of different stages of adolescence that best represent the data. The best-fitting model for each of the outcomes will be evaluated according to fit statistics (e.g., AIC, BIC, RMSEA, CFI/TLI) and by examining differences in chi- square statistics of nested models.

Once the number of stages is chosen, the intercepts and slopes can be evaluated for each LGCM. Each model will capture the hypothesized differences in growth by estimating intercept means (defined as the starting point of the growth stage) and slope means (change over time) for each of the growth processes and the correlations among and between them. We hypothesize that the intercepts of all growth functions will show statistically significant residual variance, indicating that adolescents vary significantly in their minority stress levels at the initial stage of each growth process. We further hypothesize that the slopes of all of the growth trajectories will show significant residual variance, indicating that youth experience varied rates of increase or decrease of minority stress over time. Such findings would demonstrate that trajectories of

minority stress across adolescence differ among individual youth.

WH2.1. Trajectories of minority stress and behavioral health outcomes, including depression, suicidality, behavioral problems, and substance use, will be associated over time (i.e., considered parallel processes). We will estimate cohort sequential piecewise LGCMs for each of the behavioral health outcomes to measure growth over time. Separate models describing depression; suicidality; internalizing, externalizing, and total problem behaviors; and five substance use outcomes (alcohol, tobacco, marijuana, prescription drugs, and hard drugs) will be estimated. A similar process to the analysis for WH1 will be applied to building these models. Subsequently, the LGCMs of both the SMASI total score and behavioral health outcome will be combined in a parallel LGCM to evaluate the relationship between the two variables over time. Significant coefficients corresponding to the regression paths between the SMASI and health outcomes would provide strong evidence that minority stress affects behavioral health outcomes over time.

WH2.2. Reporting higher levels of minority stress in early adolescence will be associated with poorer behavioral health outcomes in later adolescence. Within the parallel LGCM framework, we will regress the intercepts and slopes of all behavioral health outcomes onto the intercept(s) of the SMASI to determine whether and how levels of minority stress predict later health outcomes during adolescence. Specifically, we hypothesize that: (a) The intercept of the SMASI latent variable (or in the case of a multiple trajectory piecewise model, the intercept of the first trajectory) will be positively and significantly associated with the intercept(s) of the behavioral health outcome, indicating that higher levels of minority stress in early adolescence result in worse health at each stage of development. (b) The first intercept of the SMASI will be significantly, positively associated with all outcome slopes, such that high levels of minority

stress in early adolescence will result in a steeper increase in behavioral health problems in all growth stages; and (c) the slope(s) of the SMASI will differentially predict the rate of change in later growth stages of behavioral health, such that a steeper increase or decrease in minority stress throughout adolescence will predict corresponding increases or decreases in behavioral health.

WH3.1. There will be significant differences in outcome trajectories by demographic subgroup (race and ethnicity, gender, sexual identity, urbanicity). Building on the previous analyses, we will use four demographic stratification variables (race and ethnicity, gender, sexual identity, and urbanicity) to explore whether there are subgroup differences (e.g., male vs. female; gay vs. lesbian vs. bisexual) in trajectories of minority stress and behavioral health outcomes across adolescence. For example, prior literature suggests that girls are more likely to experience suicidality in adolescence than boys[12, 15] and bisexuals are more likely to engage in substance use than other sexual minority groups.[16] We hypothesize that we will see significant group differences in our data that confirm these findings. In a series of analysis using the multiple group function in Mplus, we will evaluate the structural invariance of each of our final parallel LGCMs across the subgroups comprising each of our stratification variables. The intercept and slope coefficients for each growth process will first be estimated freely across groups; the loadings will then be constrained to be equal across groups. If there is no decrement in fit (i.e., CFI $\Delta<.01$ or nonsignificant chi-square difference test), we will conclude the model has structural invariance and thus there are no differences in either minority stress or behavioral health outcome process across demographic subgroups. If significant decrements in fit emerge (e.g., when constraining across gender in the suicidality model), we will systematically free parameters to determine which intercept(s) or slope(s) differ by group and in which direction.

Because no longitudinal study of this nature has been conducted, there is no evidence to support *a priori* hypotheses about minority stress differences by subgroup. Commensurate with the extant literature, however, we expect to find subgroup differences for each of our behavioral health outcomes. Therefore, we hypothesize that the parallel LGCMs will not demonstrate structural invariance.

WH3.2: Trajectories of minority stress will be inversely associated with protective factors over time and will differ by demographic subgroup. Using the approach described under WH2.1, we will first estimate the LGCM for protective factors (either simultaneously, i.e. with a latent variable, or as separate measures depending on results of preliminary analyses). Next, we will model the trajectories of the protective factor(s) and the SMASI total score simultaneously to estimate a parallel LGCM; minority stress growth parameters will be regressed on protective factors. Finally, as in WH3.1, we will examine the protective factor/minority stress parallel process model for differences by demographic subgroup using the multiple group function in Mplus and examining constrained and unconstrained models. We hypothesize that protective factors will show an overall inverse trajectory to minority stress; i.e., greater intercepts and slopes of protective factors will be associated with lower intercepts and slopes of minority stress, and vice-versa. We further hypothesize that the parallel LGCMs of protective factors and minority stress will not demonstrate structural invariance – that is, there will be subgroup differences in the growth processes, owing to hypothesized sociodemographic differences in protective factors (e.g., greater accessibility of social support systems in urban compared to rural environments).

Sample Size Calculation

For LGCM analyses, statistical power depends on sample size, degrees of freedom (the number of known minus free parameters), variable distributions, amount of missing data, measure reliabilities, and strength of the relationships among variables. Based on the code provided by Preacher and Coffman (2006),[87] we used the hypothesis-testing framework for Root-Mean-Square Error of Approximation (RMSEA) as a vehicle to estimate the power for LGCM in our study. For the first, simplest models to be implemented (i.e., one intercept, one linear slope), we expect 34 degrees of freedom; this value will decrease with each additional trajectory estimated in the piecewise models (e.g., a 4- slope trajectory will have 7 degrees of freedom). With α=0.05, null hypothesis RMSEA of 0.05 and alternative hypothesis RMSEA of 0.08, degrees of freedom ranging from 7 to 56, and nominal statistical power of 0.80, a longitudinal sample size of up to 1,075 may be needed to achieve adequate power for all analyses depending on the exact size of the model. The range of statistical power for all proposed models provided by a sample of this size is between .80 to .99, depending on the closeness of the null and alternative hypotheses.

Patient and Public Involvement

Youth advisors were first involved in 2013 during an initial qualitative study funded by the Zumberge Foundation. That study provided the original basis for closed-ended items that eventually evolved into the SMASI measure. The current study design is a direct result of interviews conducted with SMA between 2013-2015, a small study of minority stress conducted between 2014-2016, and a set of focus groups conducted in 2016-2017 to understand stress and health patterns among the population. Youth were not directly involved in the choice of outcome measures; however, youth at several LGBTQ+ drop-in centers were involved in the development of study protocols (e.g., advertisements used) and helped provide guidance on recruitment and

retention methods. Some study participants were also directly involved in recruitment via their choice to refer other youth through RDS procedures. We are in the process of forming a youth advisory board that will assist with choosing the methods and developing plans for dissemination of study results to participants and linked communities.

Ethics and Dissemination

A comprehensive informed assent document was provided to eligible youth immediately upon screening into the study, and indeed, assent to participate was required in order to begin survey data collection. All study participants were willing and able to provide assent at the baseline survey. Because SMA constitute a vulnerable group whose parents may not be aware of their sexual minority status, we were granted a waiver of parental consent. At the beginning of each follow-up survey, participants who had reached age 18 since completing the previous survey were consented using adult protocols for informed consent. All study procedures for both baseline and longitudinal follow-up activities were reviewed and approved by the institutional review board (IRB) at the authors' home institution. Because the study is purely observational with no researcher-controlled intervention, there is no external data safety monitoring board for the study. However, a member of the research team reviews study data immediately upon downloading the new data files each business day, and any open-ended statements or data that could potentially suggest participant safety concerns are immediately brought to the attention of the study investigators, who are considered mandated reporters in the State of California. Statements are reviewed and assessed for information concerning abuse or neglect of a child; abuse or neglect of an elder; or threat that the participant will harm themselves or someone else. A IRB-approved standard operating procedure is in place in the event of a positive disclosure;

however, to date, no participant has disclosed any imminent safety concerns, and no other adverse events have been reported.

To enhance protection for study data, we obtained an NIH Certificate of Confidentiality The final dataset will include self-reported demographic and behavioral health data, as described above, from surveys completed by the research participants. All identifying data will be destroyed at the end of the study after analysis. The final anonymous data set will be made available to other qualified members of the scientific community upon request per policies of the NIH and the IRB at the authors' institution. We are committed to participating in the sharing and building of research knowledge, and will adhere to the NIH Policy on Sharing of Unique Research Resources including the Guidelines for Recipients of NIH Grants and Contracts. Requests for research resources that are generated as part of this project (e.g., qualitative outcomes, the stress measurement instrument) will be distributed in a timely manner.

Finally, the purpose of the current research is to examine pathways that may predict differing behavioral health outcomes in sexual minority adolescents. To that end, the overarching purpose is to share our developed resources with the community. As the research team completes analyses and arrives at empirical results, we have contracted with a creative graphics firm to develop infographics that cleanly summarize research findings with terminology suitable for the lay public. In addition to presenting our work in peer-reviewed manuscripts and scientific meetings, we are pursuing opportunities to share our findings with the broader community, including hosting the infographics and other study materials and derivatives on the website of the University of Southern California Center for LGBTQ+ Health Equity.[88]

Figure Captions

Figure 1. CONSORT flow diagram for enrollment into baseline study phase (final *N*=2,559).

Figure 2. CONSORT flow diagram for enrollment and retention in longitudinal study phase (current *N*=1,071).

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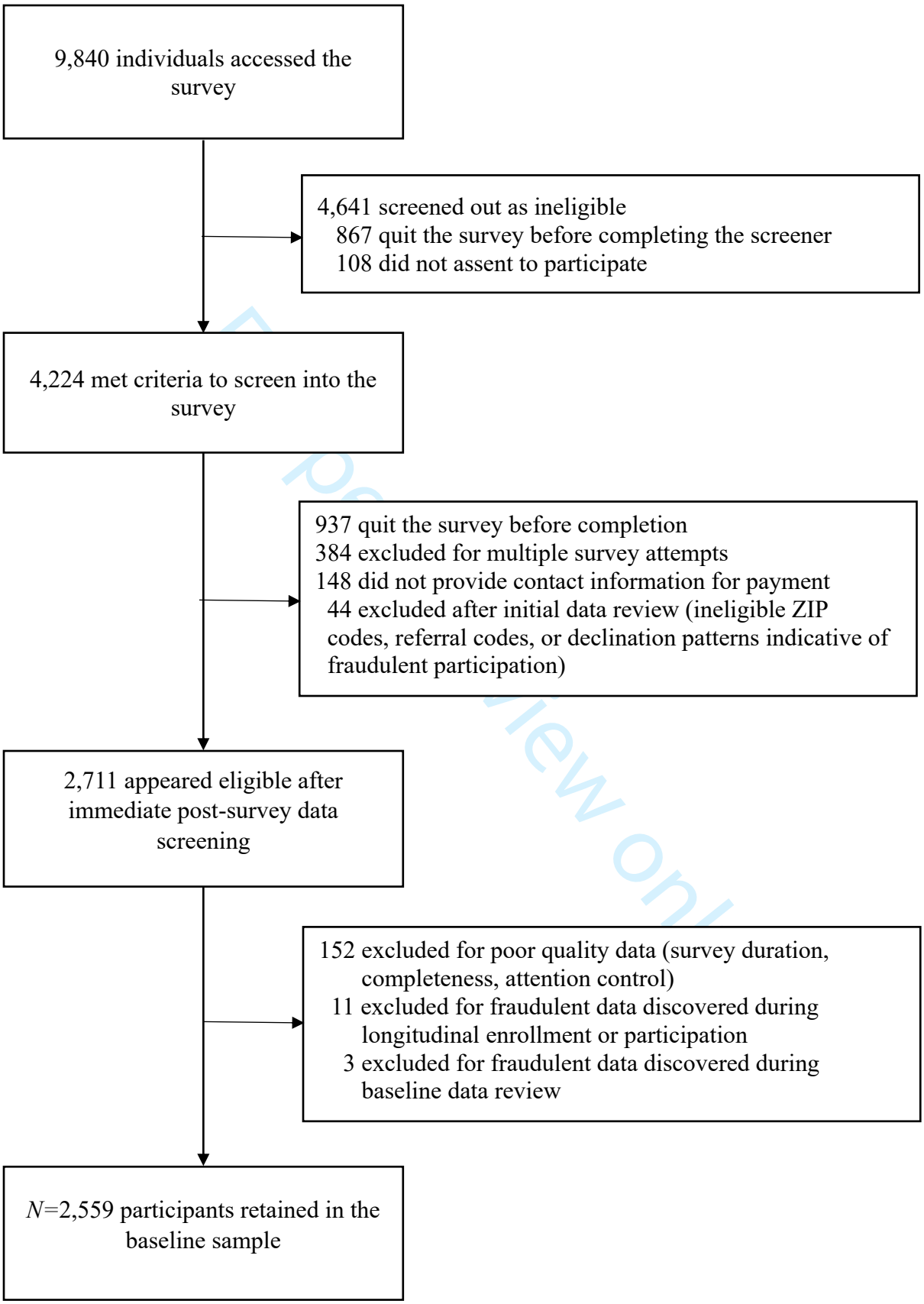

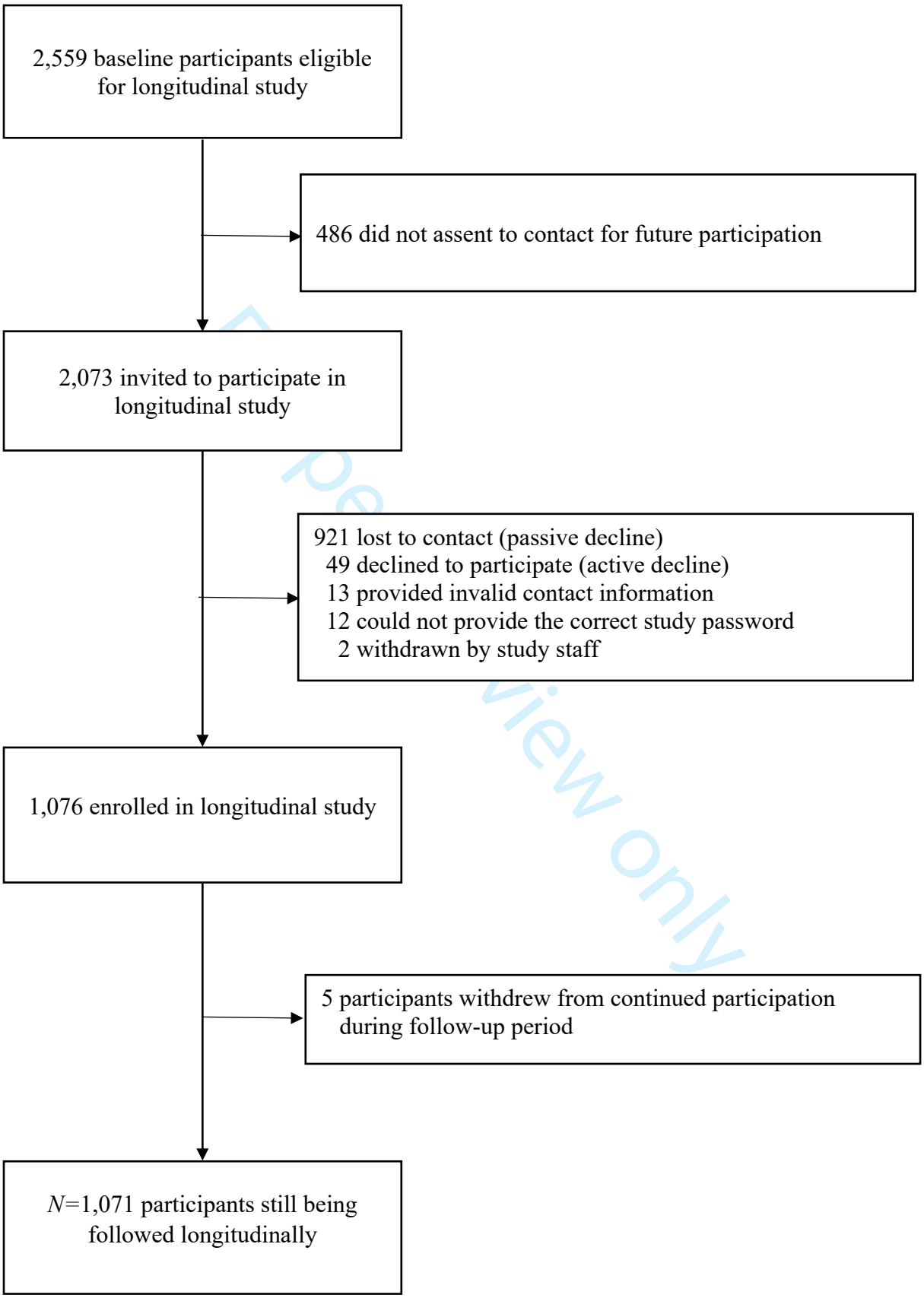

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The Adolescent Stress Experiences over Time Study (ASETS) Protocol: Design and Methods of A Prospective Longitudinal Study of Sexual Minority Adolescents in the United States

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The Adolescent Stress Experiences over Time Study (ASETS) Protocol: Design and Methods of a Prospective Longitudinal Study of Sexual Minority Adolescents in the United States

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Abstract

Introduction: Sexual minority adolescents (SMA) report higher rates of anxiety, self-harm, depression and suicide than heterosexual peers. These disparities appear to persist into adulthood and may worsen for certain subgroups, yet the mechanisms that drive these concerns remain poorly understood. Minority stress theory, the predominant model for understanding these disparities, posits that poorer outcomes are due to the stress of living in a violently homophobic and discriminatory culture. Although numerous studies report associations between minority stress and behavioral health in adolescence, no study has comprehensively examined how minority stress may change throughout the course of adolescence, nor how stress trajectories may predict health outcomes during this critical developmental period.

Methods and analysis: Between May 15, 2018 and April 1, 2019, we recruited a U.S. national sample of diverse SMA ($N = 2,558$) age 14-17 through social media and respondent-driven sampling strategies. A subset of participants ($N = 1,076$) enrolled in the longitudinal component and will be followed each six months until July 1, 2022. Primary outcomes include symptoms of depression, anxiety, and PTSD; suicidality and self-harm; and substance use. The key predictor is minority stress, operationalized as the Sexual Minority Adolescent Stress Inventory. We will use parallel cohort-sequential latent growth curve models to test study hypotheses within a developmental framework.

Ethics and dissemination: All participants provided assent to participate, and longitudinal participants provided informed consent at the first follow-up survey after reaching age 18. All study procedures were reviewed and approved by the University of Southern California Social-Behavioral Institutional Review Board, including a waiver of parental permission given the potential for harm due to unintentional "outing" to a parent during the consent process. The final

anonymous data set will be available upon request, and research findings will be disseminated through academic channels and products tailored for the lay community.

Strengths and Limitations of This Study

- This study leverages a newly developed, valid and psychometrically sound measure of minority stress in a large, diverse national sample of adolescents.
- The longitudinal cohort design permits the first examination of change in minority stress experiences over time among adolescents.
- The cohort sequential modeling approach also supports the first examination of how minority stress influences health across adolescence.
- All outcome measures are self-reported and may be subject to recall and responses biases; no confirmatory behavioral data will be collected.
- Generalizability of study findings may be constrained by study eligibility criteria, strict data quality procedures, and recruitment methods.

The Adolescent Stress Experiences over Time Study (ASETS) Protocol: Design and Methods of a Prospective Longitudinal Study of Sexual Minority Adolescents in the United States

Introduction

Sexual minority (e.g., lesbian, gay, bisexual, pansexual) adolescents (SMA) experience significant behavioral health disparities compared to their heterosexual peers. In particular, SMA experience higher rates of internalizing psychopathology including depression, anxiety and self-harm[1-7] and externalizing behaviors such as substance use[8-11] and suicide attempt and completion.[(12-14)] Longitudinal studies suggest that these disparities persist into young adulthood and may even worsen. For example, data from a national study of adolescents (*Add Health*) showed that average longitudinal trajectories for substance use among SMA are disparate from heterosexual youth beginning in early adolescence and increase as youth transition into young adulthood.[15] When examining individual trajectories of suicidality, all sexual minority groups (lesbian, gay, bisexual, mostly heterosexual) reported higher rates of suicidality across all four waves than their heterosexual peers, from mid-adolescence to early adulthood,[16] a phenomenon also found in a recent systematic review by Gilbey et al.[17]

There are also behavioral health disparities among SMA by demographic subgroup. For example, sexual minority girls are more likely to report both considering and attempting suicide than sexual minority boys,[18, 19] and bisexual youth show larger substance use disparities than other sexual minority groups.[15] SMA living in rural areas also experience different behavioral health outcomes than their urban counterparts due to confidentiality concerns, values, and limited access to cities with more extensive peer networks[20] and a more comprehensive social support system.[21] As there are likely to be subgroup differences among racial and ethnic lines as well,

scholars have called for attention to racial and ethnic diversity in sexual minority research generally.[22-24] Even in large meta-analytic studies that include adolescents and/or young adults, the lack of racial and ethnic diversity in sampling is noted as a significant limitation that often precludes subgroup comparisons.[19, 25] Recent studies of Black[26] and Latinx[27] adolescents do suggest that intersectional differences may exist, and understanding their experience is increasingly relevant: although population estimates specific to adolescents are lacking, national survey data suggest that racial and ethnic minority youth are more likely than white segments of the U.S. population to identify as SMA.[28]

The primary theoretical framework for understanding the disparities found among sexual minorities is the minority stress theory (MST),[29-31] which has been endorsed by the Centers for Disease Control and Prevention,[32] the National Academy of Medicine,[33] and Healthy People 2030.[34] MST suggests that discrimination, violence, and victimization due to a pervasive homophobic culture are the primary sources of stress and most probable driving mechanisms of mental health disparities among sexual minorities, including SMA.[30, 35-38] Numerous cross-sectional studies have attributed poor behavioral health outcomes among adolescents to minority stressors, such as negative disclosure experiences with family and peers,[1, 36, 37, 39] becoming homeless upon disclosure,[40] in-school victimization (bullying) by students and faculty members,[41-43] and experiences of violence.[19, 44-46] However, no study has ever comprehensively examined the relationship between minority stress and health outcomes longitudinally among adolescents. Despite recognition that stigmatizing experiences can disrupt adolescent development and contribute to negative outcomes,[47] the gap between theoretically predicted relationships and empirical evidence to support them is largely due to four key concerns:

(1) Studies of minority stress during adolescence have been fraught with *poor psychometric measurement*.[20, 48] A review of psychometric measurements assessing discrimination against sexual minorities found that across 162 articles, nearly all had suboptimal psychometric properties.[49] Few studies have used empirically validated measures, and most measures had been developed using small investigator-led samples or adapted from measures with adults in other minority populations.[25] Previously available general stress measures, even those validated for use with adolescents, do not allow us to differentiate between common developmental stressors and those associated with minority stress.

(2) There is an absence of studies examining minority stress and behavioral health in adolescents *over time*. Only six studies (with four unique samples) have examined the relationship between minority stress and subsequent behavioral health outcomes, and each has several major limitations: (a) lack of a well-constructed comprehensive measure of minority stress for adolescents; (b) reliance on small regional samples; and (c) lack of repeated-measures analyses and trajectory modeling to assess patterns of change in minority stress during this critical developmental time period.[31, 50-52] Although the field has generally assumed minority stress is the most probable cause of persisting behavioral health concerns among SMA, no study has examined this directly. As a related concern, no studies have provided evidence that SMA can be effectively retained over time outside of general population studies. Although not a primary outcome of the current study, establishing the feasibility of population retention is a critical step for future prospective research.

(3) Although some *subgroup differences* in behavioral health outcomes have been documented among adolescents, their determinants are not well understood. As previously described, differential outcomes are noted in sexual minority samples by race, ethnicity, gender, and

geography, and authors have called for increased attention to subgroup analyses in future research.[16, 19, 25, 53] These experiences have been explored in primarily young adult and adult samples.[54-56] Although some evidence is emerging to support the assumption is that minority stress also drives these disparities,[43, 57-61] few studies have systematically explored subgroup differences in minority stress over time, particularly among youth. A large study that is well-powered to examine differences among multiple demographic groups – that is, able to model more than simple binary comparisons – remains needed.

(4) The presence or absence of *protective factors* may add to the confusion. Some studies suggest avoidance strategies[62] or emotionally focused cognitive restructuring[63] may be helpful; others have recommended finding accepting friends, having supportive parents or family members, identifying supportive adults at school, and relying on SMA community resources (e.g., gay–straight alliances, SMA community centers) as methods for coping with minority stress.[21] However, not all subgroups of SMA may have these opportunities. For example, youth who live in rural areas may have less access to affirming resources[20] and be more likely to live in areas with less protective school policies.[64] Thus, the relationships between minority stress, demographics, protective factors and outcomes remain poorly understood.

The current study is the first to address these four major gaps in the extant literature. We can now measure minority stress in adolescents with a psychometrically sound instrument, the Sexual Minority Adolescent Stress Inventory (SMASI), which was developed and validated by the research team in prior work funded by the National Institutes of Health (NIH).[65-67] Using this measure, we will conduct a systematic investigation of minority stressors and behavioral health over time in a large, diverse national sample. With repeated measures of minority stress

and a modeling approach (cohort sequential latent growth curve modeling – see *Data Analysis*) that considers change across age rather than time, we can answer questions not previously addressed, such as whether minority stress increases over time as young teenagers develop throughout adolescence; when do minority stressors peak; whether there are demographic differences in the frequency, severity and pattern of minority stressors; and whether changes in minority stressors over time predict corresponding changes in health outcomes over time. Furthermore, we can test whether trajectories of minority stress are inversely associated with protective factors over time and if they too differ by demographic subgroup.

Thus, the present study will serve as one of the first longitudinal studies conducted with this vulnerable population. We address critical methodological design factors necessary to conduct high-quality longitudinal research with SMA, including: (a) a safe and effective recruitment approach, with built-in mechanisms to protect SMA from being "outed" via study participation, which could increase risk of victimization (e.g., kicked out of home); (b) repeated measures over time of important psychosocial predictors and outcomes; (c) recruitment of participants at ages 14–17, because they are a particularly neglected subpopulation in SMA studies;[68] and (d) respondent-driven sampling methods to recruit youth who may have not disclosed their sexual orientation to others, including their parents, resulting in a lack of both scientific and clinical understanding about them. Upon completion, the study will provide critical information needed to inform the nature and timing of intervention efforts for this high-need, underserved, and difficult-to-reach population of youth.

Methods and Analysis

Sample Selection

Population Definition. Recent studies suggest that upwards of 15% of youth do not identify as exclusively heterosexual.[69, 70] Throughout this protocol, we use the term *sexual minority adolescents*, or SMA, to refer to adolescent individuals who endorse same-sex attraction or identity. Attraction includes romantic or sexual feelings, whereas identity describes how youth label themselves (e.g., lesbian, gay, bisexual).[71] These are consistent with constructs commonly used to operationalize sexual orientation.[72, 73] We recognize *adolescents* generally as youth aged 13 to 20, a common international convention.[74] However, we restricted recruitment in this study to youth aged 14 to 17, as we have in our preliminary work, given literature suggesting youth commonly begin to define their sexual identity during these years.[75] We also required participants to be cisgender, i.e., to express a gender identity congruent with their sex assigned at birth, at the time of recruitment. At the time this study was open to enrollment, the SMASI had only been validated with cisgender adolescents. As the primary purpose of this study was the longitudinal validation of the SMASI instrument, we chose to mirror the inclusion criteria on which the SMASI was initially developed and validated in order to reduce error variance when assessing minority stress attributable to sexual identity, given the present inability to differentiate between sexual and gender minority stress among adolescents who are both sexual and gender minorities. Although transgender and nonbinary youth were excluded from enrolling in the baseline sample, maintaining a cisgender identity over time was not a requirement for continuation in the longitudinal portion of the study; indeed, we expect changes in gender identity over time, and will assess those in later waves (see *Measures)*.[76] A separate NIH-funded study (R21HD082813-01A1) is now underway to examine gender minority stress among transgender and nonbinary youth and parse out sexual versus gender minority stress experiences.

*Study Eligibility***.** Youth were eligible to participate in the study if they were at least 14 and no more than 17 years old; were cisgender male or female (i.e., reported a current gender identity consistent with their sex assigned at birth); resided in the United States, as determined by ZIP code; identified as not 100% heterosexual using *Add Health* guidelines (i.e., identified as mostly heterosexual, bisexual, gay, lesbian, or unsure);[77] and were willing and able to provide assent to participate.

Stratification Variables. To ensure geographic diversity, ZIP code was recoded into two additional variables: region and urbanicity. Region (West, Southwest, Midwest, Northeast, and Southeast; Table 1) was based on the state associated with the participant's reported ZIP code.

Table 1. Assignment of U.S. states to regions.

U.S. Region	U.S. States		
West	Alaska	Idaho	Utah
	California	Montana	Washington
	Colorado	Nevada	Wyoming
	Hawaii	Oregon	
Southwest	Arizona	Oklahoma	Texas
	New Mexico		
Midwest	Illinois	Michigan	North Dakota
	Indiana	Minnesota	Ohio
	Iowa	Missouri	South Dakota
	Kansas	Nebraska	Wisconsin
Northeast	Connecticut	Massachusetts	Pennsylvania
	Delaware	New Hampshire	Rhode Island
	Maine	New Jersey	Vermont
	Maryland	New York	
Southeast	Alabama	Kentucky	South Carolina
	Arkansas	Louisiana	Tennessee
	Florida	Mississippi	Virginia
	Georgia	North Carolina	West Virginia

Urbanicity (rural or urban) was determined based on the Rural Urban Commuting Area (RUCA)[78] codes. Specifically, "urban" was defined as a ZIP code corresponding to RUCA

codes of 1.0, 1.1, 2.0, 2.1, 4.1, 5.1, 7.1, 8.1, and 10.1. "Rural" was defined as all other valid RUCA codes. When a ZIP code was associated with a RUCA 3.1 score, that score was used; for ZIPs that were not assigned a RUCA 3.1 score due to changes in the classification system between RUCA versions 2 and 3, the RUCA 2.0 score was used.

Participant Recruitment

Targeted Advertising. Initial participants were recruited through advertising on Facebook/Instagram (which now share a single advertising platform) and YouTube. Advertisements varied slightly by platform, but all included language asking youth to "Share Your Voice" and described basic details of the research study and incentives that participants could earn. Advertising was stratified by gender, geographic region, and urbanicity. This resulted in 20 target cohorts, as each of the five regions encompassed four unique groups: rural males, rural females, urban males, and urban females. We used two different sets of advertising images: one featuring females (for the female groups) and one featuring males (for the male groups). To reach each of these groups, general specifications included age (14-17 years), gender (women or men), and location. Facebook/Instagram allows bulk uploading of up to 2,500 ZIP codes per ad. A total of 44 targeted ads were required to reach all possible combinations of region, urbanicity, and gender, as some combinations included nearly 10,000 eligible ZIP codes.

Facebook/Instagram also allows advertisers to target audiences based on interests. Using keywords enumerated by members of the research team, we identified specific interest terms by gender. Male-targeted interests included Gay-friendly; Gay, Lesbian, Bisexual, Transgender, Straight Alliance; Homosexuality; LGBT community; LGBT symbols; and Pansexuality. Female-targeted interests included all of the male-targeted interest keywords plus Lesbian Connection and Lesbian Romance.

Simultaneously, the research team identified YouTube channels for review using keywords including LGBTQ, gay, coming out, transition, and trans. Channels were reviewed for visibility, reach, and engagement of each channel, operationalized as the number of subscribers per channel and number of video views for each channel's three most-viewed videos. We initially identified 47 possible YouTube channels that had high visibility and engagement among LGBTQ+ adolescents, using a combination of keyword searches (e.g., LGBTQ, gay, coming out, transition) and subscriber and video view counts; after reviewing this list, we advertised to 23 channels that were verified YouTube accounts, able to accept advertisements, and agreed up on by the study team as being relevant to sexual minority adolescents. Using the Google advertising system, we placed advertisements for the ASETS study directly on the pages of those channels.

Respondent-Driven Sampling. Respondent-Driven Sampling (RDS) is a type of chain-referral sampling that allows for identified members of a hidden group, called "seeds," to recruit other group members from their personal networks.[79] Participants who completed their survey and were initially deemed eligible for retention were asked if they might be interested in referring friends who they thought might be eligible to participate. Participants who confirmed their interest in referring friends to the study were provided with an email that contained three unique survey links as well as two different language prompts to encourage peers to participate. In return for successfully recruiting an eligible participant who completed the survey, the recruiter participant (seed) was paid $10 per referral for up to three eligible participants.

Baseline Study Procedures

Initial Eligibility Screening. Advertisement clicks and referral links all directed youth to a screening page in Qualtrics that asked a series of demographic questions to determine their eligibility based on age in years, gender, ZIP code, and sexual attraction. Ineligible participants

were thanked for their interest in the study and then re-routed to a separate Qualtrics survey where they could optionally provide contact information (email and/or phone number) to be included in outreach for future studies. Eligible participants were shown the Institutional Review Board (IRB)-approved study assent text and asked to confirm assent in order to proceed with the main survey, implemented in Qualtrics (see *Measures*).

Post-Survey Data Collection. After completing the survey, the participant was re-routed to a separate Qualtrics survey for payment in order to keep their personally identifiable information separate from their main study data. This payment survey asked the participant for their private email address at which to receive an electronic gift card. Participants were also asked if they knew other sexual minority youth, and if so, whether they would consider referring any of those youth into the study, to aid RDS recruitment. Finally, participants were asked whether they would be interested in participating in the longitudinal study and given fields to provide up to five different contact methods if so. Contact options included email, phone numbers for call/text, and usernames for Facebook, Twitter, Snapchat, and any other social media accounts that allow for personal messaging. Participants were able to rank their provided methods of contact in order of preference. This step in the process was critical to establishing retention for the longitudinal study.

Final Eligibility Determination. Each business day a study team member downloaded any new surveys from Qualtrics. Variables were created to represent region, urbanicity, response declinations (total number of "Decline to answer" responses across the entire survey); survey duration, and attention validation (number of attention-control questions the respondent answered correctly).[80] Participants who failed to complete the entirety of the survey–that is, they exited the survey prior to completing and being routed to the payment survey–were

excluded and could not be paid due to lack of contact information. Participants determined to have engaged in any type of fraudulent activity were also immediately excluded from both study eligibility and pay. "Fraudulent activity" included providing information or response patterns, either within the main survey data or on the payment and contact information survey, that confirmed duplicate response by a previous participant, or screening out of a first survey attempt (i.e., determined to be ineligible) and immediately re-accessing the survey with false responses in an attempt to access the full survey.[81-84] Fraudulent participants were identified by duplicate IP address, duplicate email and/or contact information, similar patterned responses throughout survey (including open-ended responses with identical or unique wording), and/or survey time stamps. Fraudulent participation was not compensated even if sufficient contact information was provided.

Participants who completed the survey but provided very low quality data, defined as either an unrealistically short survey completion time (≤10 minutes), a low attention-control score (≤1 out of 4 correct responses), or very high (≥35) "Decline to answer" response count, were compensated for their participation but were excluded from the baseline dataset and not invited to participate in the longitudinal study or refer peers via RDS. Participants who had survey duration times of 10 – 15 minutes, attention-control scores of 2, and moderately high (≥25) responses of "Decline to answer" were compensated for participation and further evaluated for inclusion on a case-by-case basis. By applying all of the above-described procedures prior to longitudinal recruitment, we ensured that only participants who provided valid and trustworthy data would be enrolled in the longitudinal study.

Incentive Compensation. All baseline participants who were eligible for compensation, whether or not their data were retained for analysis, were sent a $15 Amazon gift card to the

private email address they provided in the payment survey. Participants whose data were retained for analyses were assigned a unique four-digit participant identifier at this time.

All participants were recruited into the study and completed their baseline surveys (N = 2,558) between May 15, 2018 and April 1, 2019. Figure 1 illustrates the number of individuals retained and excluded at each step of the baseline recruitment and data collection process.

Longitudinal Study Procedures

Longitudinal Enrollment. Approximately one week from the date of a participant's baseline survey completion, participants who expressed interest in longitudinal participation were entered into a master tracking log file. This artificial delay helped ensure that we could detect participants willing to engage in fraudulent behavior, including participants who were trying to take the baseline survey multiple times in an attempt to receive multiple payments, prior to inviting them to be part of the longitudinal study. Participants who reached this longitudinal recruitment stage were contacted by a research assistant in real-time via the participant's preferred contact method, using a study-specific username or account shared by the research team. Participants were first reminded that they recently completed an online survey.

In an effort to protect their privacy and ensure that we were speaking with the right individual, we asked them to please tell us what that survey was about. Participants who provided the correct information (e.g., "LGBT youth") were asked if they were interested in learning more about the longitudinal study. Participants who expressed interest were given information about the study outlining their involvement, including a written information sheet. Those who agreed to participate were then asked to confirm or update their contact information, and the research assistant verified the participant could receive emails from the study team that did not end up in their spam/junk folders. Participants were reminded that the study team's next

contact with them would be through an automated monthly check-in survey every month (see *Monthly Check-In Surveys*) and that they would receive an email in approximately six months for their next full survey. Finally, they were provided with all methods of contact to reach the study team and were encouraged to reach out in the event they had questions, concerns, or comments.

RDS Referrals. Longitudinally enrolled participants were given the option to refer peers into the baseline survey for an additional incentive, i.e., RDS referrals. Participants who stated they may know others who might be interested were provided three custom Qualtrics referral links, which contained an embedded RDS code that both identified the new participant as an RDS referral and allowed the study team to link the new survey to the referring participant for a referral payment. All referred participants went through the same validation, eligibility, and payment process as those who entered the study through direct outreach methods. Additionally, the participant who referred them was provided with a $10 Amazon gift card as a referral incentive. Referrers were not paid referral incentives for distribution of survey links to youth who were ineligible for participation or those whose surveys were excluded from retention due to low data quality. Participants who attempted to refer themselves were easily identified by the quality assurance protocol previously described; in the case of self-referrals, the participant was immediately excluded from both the baseline and longitudinal study due to their demonstrated willingness to defraud the study team.

Monthly Check-In Surveys. Because the study was conducted entirely online, having up-to-date contact information for all participants was of critical importance. Additionally, with six months in between full surveys, it was important to have more regular contact with participants in order to maintain rapport and interest in the study. Thus, a brief check-in survey, consisting of one item asking whether any of the participant's contact information had changed within the last

30 days, was automatically emailed to each longitudinal participant near the first day of every month. If a participant indicated that their contact information had changed, they were then prompted to provide any new or updated contact information. If a participant failed to respond to the automated check-in survey by the 15th of each month, a research assistant would manually reach out to them once through each of the participant's preferred contact methods. Each check-in survey was accompanied by a raffle where all respondents to the check-in survey within the calendar month were entered into a random drawing to receive a $100 Amazon gift card, regardless of whether their contact information had changed.

Longitudinal Follow-Up Surveys. A unique link to each Qualtrics follow-up survey was created by the study team for each participant. This link, provided to the participant once they became eligible to complete the survey, contained embedded information about the date on which they completed their previous survey along with their assigned unique participant identifier. This allowed information about prior participation dates to be pre-populated in survey items requesting retrospective information in an effort to aid in recall.

At the start of every week, all participants whose follow-up survey date fell within that week (i.e., a multiple of 6 months after their baseline survey date) were sent an automated survey link in an email from the Qualtrics platform. Automated messages were sent on Monday, Wednesday, and Sunday of the first week of eligibility. Participants who failed to complete their follow-up survey by the third automated attempt would begin to receive manual messages from research assistants containing their unique embedded survey link. Manual attempts to contact the participant were first sent via the participant's most recently indicated preferred contact method. After three months, their survey window would close and participants would not be able to complete that wave of data collection to ensure any two consecutive waves of data collection

would reflect a minimum time difference of three months apart. Follow-up data collection began on August 15, 2018, and will conclude on July 1, 2022 when the three-month window of the 36-month survey closes.

Longitudinal Eligibility and Payment Determination. When a participant completed a follow-up survey, the participant was re-routed to the separate Qualtrics survey page for payment using the same procedures as at baseline. Each business day a study team member would access the main survey through Qualtrics and download the previous day's surveys. Variables were again created in each follow-up dataset including survey duration, attention control score, and count of "decline to answer" responses. Participants who failed to complete the entire survey or who had very low quality data (as defined above), were encouraged to retake the survey with an explanation provided as to why they were asked to retake the survey. This outreach process continued for five total manual attempts alternating every other day. This process was replicated at all subsequent survey waves. The longitudinal enrollment and retention diagram is shown in Figure 2; to date, six participants have withdrawn from continued follow-up, bringing the longitudinal sample to N=1,070.

Measures

Beginning with baseline data collection, the following measures were collected:

Outcomes. We focus analysis on understanding behavioral health, consistent with terminology from the Substance Abuse and Mental Health Services Administration,[49] to describe an integrated approach focused on the promotion of emotional health and prevention of mental illness, alcohol and other drug use, and associated outcomes (e.g., suicide). Behavioral health outcomes included: *depressive symptoms*, measured with the CESD-4;[85] *anxiety,* assessed with the Generalized Anxiety Disorder 7-item measure (GAD-7);[86] symptoms of *Post-*

Traumatic Stress Disorder (PTSD),[87] using the Abbreviated PTSD Checklist-Civilian (PCL-C); and five questions from the Youth Risk Behavior Survey (YRBS) to assess *suicidality and self-injury*.[88] All responses were recoded to binary indicators of suicidal ideation, plan, attempt, attempt resulting in injury, and self-injurious behavior. Finally, YRBS items were also used to assess lifetime and past-30-day *substance use,*[88] including binary indicators of whether a participant had used alcohol, tobacco, marijuana, prescription pain relievers, prescription tranquilizers, and prescription stimulants.

Key Predictor: Minority Stress. The focal measure in this study is the Sexual Minority Adolescent Stress Inventory[65-67] (SMASI; whole-scale omega (ω)=.97), which relies on 54 main items to measure 10 domains of minority stress: social marginalization (8 items, ω=.93), family rejection (11 items, ω=.94), internalized homonegativity (7 items, ω=.94), identity management (3 items, ω=.77), homonegative climate (4 items, ω=.88), intersectionality (3 items, ω=.82), negative disclosure experiences (5 items, ω=.77), religion (5 items, ω=.93), negative expectancies (3 items, ω=.81), and homonegative communication (5 items, ω=.77). An optional subscale assessed stress experiences at work among youth with any work history (10 items, ω=.96). At baseline, youth were asked if they have had each experience "ever" and "in the past 30 days;" the stem language changes to "since [they] last took the survey" and "in the past 30 days" at subsequent time points.

Demographic Covariates. Variables used for eligibility screening included *age* (in years). *Sex assigned at birth* (male or female) was used in concert with *gender identity* (response options included: male; female; trans male/trans man; trans female/trans woman; genderqueer; gender nonconforming; nonbinary; gender identity not listed here) to determine cisgender status. For eligibility screening that depended on survey programming logic, *sexual minority identity* was

determined with the recommended item from Add Health guidelines:[77] "If you had to pick one of the following options, please choose the description that best fits how you think about yourself," with all response options other than "100% heterosexual (straight)" qualifying for eligibility. ZIP code was assessed to verify *U.S. residence* and subsequently recoded for stratification into *urbanicity* and *region* as previously described.

Additional demographic variables included open-ended *sexual identity,* assessed by asking "What would you say is your sexual orientation or identity?" and a text box for an open-ended responses that could be recoded for later analyses. *Race and ethnicity* was a forced-choice item with response options of Native American/American Indian/Alaska Native; Asian/Pacific Islander; Black or African American; White/Caucasian; Latino/Hispanic; Multi-racial (with a text box to specify); and Race/ethnicity not listed here (with a text box to specify). Gender expression was captured with one item asking, "A person's appearance, style, dress, or the way they walk or talk may affect how people describe them. How do you think other people would describe you?" Response options were on a 7-point Likert-type scale from "Very feminine" to "Very masculine." *School enrollment* was captured with a binary indicator of whether participants are currently enrolled in school, and *educational attainment* measured the highest grade already completed (less than 7th grade; 7th grade; 8th grade; 9th grade; 10th grade; 11th grade; High school graduate or GED; Some college; Trade school certification or Associate's (AA) degree; Bachelor's (BA/BS) degree or higher). Additionally, participants were asked whether they were eligible for free or reduced-price lunch at the school they most recently attended as a proxy for *socioeconomic status* (response options: "Yes," "No," "I don't know"). *Work status* was assessed with one item asking whether participants are currently working, with response options of "Yes, full-time"; "Yes, part-time"; "No, but I have previously had a job"; or

"No, and I have not previously had a job". In follow-up surveys, participants were asked whether they had worked since the last time they took the survey ("Yes" or "No"). *Living situation* was assessed by asking participants with whom do they currently live. Response options included: two parents; mother only; father only; grandparents or other relatives; foster parents; group home; alone or with roommates in own apartment or home; boyfriend/girlfriend/romantic partner/spouse; with friends or couch-surfing; homeless or on the street; and other (with a text box to specify). Participants were asked whether they had become involved in the *foster care system* ("Yes", "No", or "Unsure"). Participants were asked about their *primary language* spoken a) at home and b) with their friends; response options included English; Spanish; or another language (with text box to specify). Participants were asked to indicate their personal and family religion from a comprehensive list.[89] Although not used in the eligibility process, sexual attraction to men, women, and gender nonconforming people was also assessed with Likert-type response options ranging from "Not at all" to "Extremely."

Theoretical Covariates and Protective Factors. Participants completed the Perceived Stress Scale (PSS), a measure of *general stress,*[90, 91] to ensure we can control for other common adolescent stress experiences unrelated to minority stress. *Social support*[92] from friends, family, and a significant other was measured with the Multidimensional Scale of Perceived Social Support. *Disclosure* questions were asked to understand categories of individuals who may know the participant is LGBTQ (baseline) or to whom, if anyone, the participant has disclosed their sexual orientation to for the first time since the last survey (follow-up surveys). Options included: mother; father; siblings; other adult relatives; peers/supervisor(s) at work; members of the participant's religious community; teacher(s); peers at school, childhood friends; heterosexual friends; LGBTQ friends; and girlfriend/boyfriend/partner. Response options were

"Yes" (selected if they had disclosed to one or more of the people in the stated category), "No" (selected if they had not disclosed to anyone in that category), or "Not Applicable" (if they did not have the listed person in their life).

A series of binary items assessed the presence of supportive resources such as *gay-straight alliances (GSA)* and *mentorship*, as follows: Three items captured whether the person currently had regular access to a GSA; whether their current (or most recently attended) school has a group or club specific to the LGBTQ+ community; and whether they participated in meetings or activities sponsored by an LGBTQ+ club at their school (or most recently attended school). Participants were also asked whether there is an adult 25 years or older, currently in their life, who they consider to be a mentor. Those who responded "yes" were asked whether this person is part of their immediate family; if they responded yes, they received an additional question asking, "Other than an immediate family member (or the person who raised you), is there an adult 25 years or older who you consider to be your mentor?" These items were recoded into a binary item reflecting the presence or absence of a non-family mentor age 25 or older.

Finally, adolescent *coping strategies* were captured with the Coping Strategies Inventory-Short Form,[93] which includes 16 items on four subscales (problem-focused engagement; problem-focused disengagement; emotion-focused engagement; and emotion-focused disengagement). An additional 20 items assessed *LGBT-specific coping strategies*. The first ten questions asked about potentially recurring events, such as "I spent time with the LGBTQ community" and "I tried to gain new knowledge about the LGBTQ community." Response options for these statements included "Never," "Sometimes," "Often," and "Regularly." The second ten questions asked participants for a binary response indicating if they had ever had the

positive experience, such as "I went to an LGBTQ pride event" and "I've been in a romantic relationship."

With the exception of sex assigned at birth and race/ethnicity, which were only captured at baseline, all of the above measures were also collected at each follow-up time point. This includes explicitly inquiring about sexual identity and gender identity at each wave, given the fluidity of these identities during adolescence.

Additional Measures. Although not the primary focus of the study, additional measures were added at later waves of data collection to probe emerging findings in the literature on SMA behavioral health. These included a more thorough investigation into the experiences of homeless and precariously housed youth; intersectionality of SGM status with cultural identity; experiences with body dysmorphia; sexual behavior, intimate partner violence, and nonconsensual distribution of explicit images ("revenge porn"); healthcare access and utilization; specific forms of marijuana or nicotine used in the past 30 days; and lifetime and past 30-day use of cocaine and methamphetamine.

Data Analysis Plan

We propose using cohort sequential latent growth curve modeling (LGCM)[94] to describe how minority stress and its associated behavioral health outcomes change over time among SMA. Compared with traditional longitudinal analysis methods such as comparing pre- and post-test scores or change by data collection wave, the cohort sequential LGCM approach examines individual change over age and is more appropriate for modeling developmental processes, coinciding with theoretical paradigms that are often person-centered longitudinal pathways.[95, 96]

Several preliminary steps and decisions will be made prior to longitudinal model estimation and will vary depending on the type of analysis (e.g., ordinary least squares vs.

logistic regression). Multicollinearity and influential cases will be assessed.[97] Distributional properties of all continuous and categorical variables will be evaluated, and we will apply appropriate transformation or robust estimation procedures to correct for non-normally distributed variables (e.g., specifying the WLSMV estimator for binary indicators).[98, 99] Attrition analyses will be conducted to understand missingness. Missing data will be handled in all growth models using full information maximum likelihood estimators in Mplus assuming data are missing completely at random or missing at random.[100] Multiple imputation[101] methods will also be used when appropriate. Depending on the analysis and the hypothesis being tested, demographic and some substantive variables will be included as covariates to increase the specificity of the effects; e.g., geographic region, race/ethnicity, or general stress. Prior to estimating full unconditional LGCMs, we will identify the best-fitting functional forms of trajectories (i.e., linear, quadratic, cubic, and/or piecewise) for each variable.[102] Structural equation models will be evaluated using commonly accepted fit indices (e.g., chi-square, CFI, IFI, TLI, and RMSEA) and modification indices (e.g., LaGrange multipliers).[103] Standard guidelines for small (.2), medium (.5), and large (.8) effect sizes[104] will be adopted. Confirmatory factor analysis will be used to assess measurement invariance of the SMASI and all outcome measures over time.

General approach to hypothesis testing. LGCMs will be estimated for the SMASI total score, the SMASI subscales, and each of the behavioral health outcomes. The use of a cohort sequential LGCM will allow for the modeling of change in each outcome trajectory as adolescents age during the course of our study by plotting latent means across age to understand developmental trends among participants. These are useful in examining within-person change across time and between-person variability.[102] Furthermore, LGCMs provide group-level

statistics, including the average amount of change over time (i.e., slope), the average starting point (i.e., intercept), and the relationship between the two.[105, 106] One important advantage of LGCM is the implementation and comparison of appropriate functions to best fit the trend of the data. It is likely that several patterns of growth during the course of adolescence may emerge – that is, two or more separate slopes may be modeled within the same trajectory to demonstrate divergence in trends.[105, 107] These separate but related pieces incorporate the piecewise function, which allows for several linear slopes to be modeled within the same construct (e.g., minority stress) and can provide information about differences in construct level (i.e., intercept) and growth velocity (i.e., slope) at various points throughout adolescence.

Due to the hypothesized relationships between minority stress and the behavioral health outcomes (depressive symptoms, anxiety, PTSD symptoms, suicidality, and substance use), we propose to analyze these trajectories simultaneously via the use of parallel cohort sequential LGCM models.[107] By modeling two growth processes (e.g., minority stress and depressive symptoms) at once, we can evaluate the relationship of slopes and intercepts both within and across measures to understand their interrelated effects over time. This approach will allow us to test the following working hypotheses (WH):

WH1. There will be differences in minority stress across adolescent development. A cohort sequential LGCM[108] for the total SMASI score and each of its 11 subscales will be estimated to describe individual and group-level trajectories of minority stress among all SMA during the course of the study period. This will establish the best-fitting LGCM and allow the selection and implementation of the most appropriate piecewise function. We will test piecewise models with one (i.e., linear across development) to four growth trajectories to determine which function best represents the data. This will also allow us to understand how and when changes

may occur throughout adolescence. The best-fitting model for each of the outcomes will be evaluated according to fit statistics (e.g., AIC, BIC, RMSEA, CFI/TLI) and by examining differences in chi-square statistics of nested models.

Once the functional form is chosen, the intercepts and slopes can be evaluated for each LGCM. Each model will capture the hypothesized differences in growth by estimating intercept means (defined as the starting point of the growth period) and slope means (change over time) for each of the growth processes and the correlations among and between them. We hypothesize that the intercepts of all growth functions will show statistically significant residual variance, indicating that adolescents vary significantly in their minority stress levels at the initial point of each growth process. We further hypothesize that the slopes of all of the growth trajectories will show significant residual variance, indicating that youth experience varied rates of increase or decrease of minority stress over time. Such findings would demonstrate that trajectories of minority stress across adolescence differ among individual youth.

WH2.1. Trajectories of minority stress and behavioral health outcomes will be associated over time (i.e., considered parallel processes). We will estimate cohort sequential LGCMs for each of the behavioral health outcomes to measure growth over time. Separate models describing depressive symptoms, anxiety, PTSD symptoms, suicidal ideation and attempt, self-injury, and four substance use outcomes (alcohol, tobacco, marijuana, and prescription drugs) will be estimated. A similar process to the analysis for WH1 will be applied to building these models. Subsequently, the LGCMs of the SMASI total score with each behavioral health outcome will be combined in a parallel LGCM to evaluate the relationship between the two variables over time. Regression coefficients reflecting influence of minority stress on each outcome (i.e., regression of health outcome slopes and intercepts onto SMASI slopes and intercepts) will be estimated.

Significant coefficients corresponding to the regression paths from the SMASI to health outcomes would provide strong evidence that minority stress affects behavioral health outcomes over time.

WH2.2. Reporting higher levels of minority stress in early adolescence will be associated with poorer behavioral health outcomes in later adolescence. Within the parallel LGCM framework, we will regress the intercepts and slopes of all behavioral health outcomes onto the intercept(s) of the SMASI to determine whether and how levels of minority stress predict later health outcomes during adolescence. Specifically, we hypothesize that: (a) The intercept of the SMASI latent variable (or in the case of a multiple trajectory piecewise model, the intercept of the first trajectory) will be positively and significantly associated with the intercept(s) of the behavioral health outcome, indicating that higher levels of minority stress in early adolescence result in worse health at each unique phase of development; (b) The first intercept of the SMASI will be significantly, positively associated with all outcome slopes, such that high levels of minority stress in early adolescence will result in a steeper increase in behavioral health problems in all growth periods; and (c) the slope(s) of the SMASI will differentially predict the rate of change in later health outcome growth periods, such that a steeper increase or decrease in minority stress throughout adolescence will predict corresponding increases or decreases in behavioral health.

WH3.1. There will be significant differences in outcome trajectories by demographic subgroup. Building on the previous analyses, we will use four demographic stratification variables (race and ethnicity, gender, sexual identity, and urbanicity) to explore whether there are subgroup differences (e.g., male vs. female; gay vs. lesbian vs. bisexual/pansexual vs. queer) in trajectories of minority stress and behavioral health outcomes across adolescence. For example,

prior literature suggests that girls are more likely to experience suicidality in adolescence than boys[18,19] and bisexual youth are more likely to engage in substance use than other sexual minority groups of the same age.[15] We hypothesize that we will see significant group differences in our data that confirm these findings. In a series of analyses using the multiple group function in Mplus, with up to four identity groups modeled within a single analysis, we will evaluate the structural invariance of each of our final parallel LGCMs across the subgroups comprising each of our stratification variables. The intercept and slope coefficients for each growth process will first be estimated freely across groups; the loadings will then be constrained to be equal across groups. If there is no decrement in fit (i.e., CFI $\Delta<.01$ or nonsignificant chi-square difference test), we will conclude the model has structural invariance and thus there are no differences in either the minority stress or the behavioral health outcome process across demographic subgroups. If significant decrements in fit emerge (e.g., when constraining across gender in the suicidality models), we will systematically free parameters to determine which intercept(s) or slope(s) differ by group and in which direction. Because no longitudinal study of this nature has been conducted, there is no evidence to support *a priori* hypotheses about minority stress differences by subgroup. Commensurate with the extant literature, however, we expect to find subgroup differences for each of our behavioral health outcomes. Therefore, we hypothesize that the parallel LGCMs will not demonstrate structural invariance.

WH3.2: Trajectories of minority stress will be inversely associated with protective factors over time and will differ by demographic subgroup. Using the approach described under WH2.1, we will first estimate the LGCM for protective factors (either simultaneously, i.e. with a latent "protective factors" variable, or as separate manifest measures depending on results of preliminary analyses). Next, we will model the trajectories of the protective factor(s) and the

SMASI total score simultaneously to estimate a parallel LGCM; minority stress growth parameters will be regressed on protective factors. Finally, as in WH3.1, we will examine the protective factor/minority stress parallel process model for differences by demographic subgroup using the multiple group function in Mplus and examining constrained and unconstrained models. We hypothesize that protective factors will show an overall inverse trajectory to minority stress; that is, greater intercepts and slopes of protective factors will be associated with lower intercepts and slopes of minority stress, and vice-versa. We further hypothesize that the parallel LGCMs of protective factors and minority stress will not demonstrate structural invariance – that is, there will be subgroup differences in the growth processes, owing to hypothesized sociodemographic differences in protective factors (e.g., greater accessibility of social support systems in urban compared to rural environments).

Sample Size Calculation

For LGCM analyses, statistical power depends on sample size, degrees of freedom (the number of known minus free parameters), variable distributions, amount of missing data, measure reliabilities, and strength of the relationships among variables. Based on the code provided by Preacher and Coffman (2006),[109] we used the hypothesis-testing framework for Root-Mean-Square Error of Approximation (RMSEA) as a vehicle to estimate the power for LGCM in our study. For the first, simplest models to be implemented (i.e., one intercept, one linear slope), we expect 34 degrees of freedom; this value will decrease with each additional trajectory estimated in the piecewise models (e.g., a 4-slope trajectory will have 7 degrees of freedom). With α=0.05, null hypothesis RMSEA of 0.05 and alternative hypothesis RMSEA of 0.08, degrees of freedom ranging from 7 to 56, and nominal statistical power of 0.80, a longitudinal sample size of up to 1,075 may be needed to achieve adequate power for all

analyses depending on the exact size of the model. For WH3.1 and WH3.2, which examine differences in minority stress and outcome trajectories by subgroups, statistical power depends on both group size and total sample size, as we cannot assume identical fit of the initial model in all groups.[110] Using the trajectory forms developed for the previous hypotheses, we can support simultaneous trajectory comparisons with at least 190 participants per group; with a planned sample of N=1,075, we would potentially be adequately powered to examine up to five demographic subgroups simultaneously. However, given the low likelihood of perfectly even recruitment across all demographic strata, it may be more feasible to limit models to four groups to ensure adequate group size without overly condensing across meaningful categories. With these approaches in mind, the range of statistical power for all proposed models provided by a sample of this size is between .80 to .99, depending on the closeness of the null and alternative hypotheses.

Patient and Public Involvement

Youth advisors were first involved in 2013 during an initial qualitative study funded by the Zumberge Foundation. That study provided the original basis for closed-ended items that eventually evolved into the SMASI measure. The current study design is a direct result of interviews conducted with SMA between 2013-2015, a small study of minority stress conducted between 2014-2016, and a set of focus groups conducted in 2016-2017 to understand stress and health patterns among the population. Youth were not directly involved in the choice of outcome measures; however, youth at several LGBTQ+ drop-in centers were involved in the development of study protocols (e.g., advertisements used) and helped provide guidance on recruitment and retention methods. Some study participants were also directly involved in recruitment via their choice to refer other youth through RDS procedures. We are in the process of forming a youth

advisory board that will assist with choosing the methods and developing plans for dissemination of study results to participants and linked communities.

Ethics and Dissemination

A comprehensive informed assent document was provided to eligible youth immediately upon screening into the study, and indeed, assent to participate was required in order to begin survey data collection. All study participants were willing and able to provide assent at the baseline survey. Because SMA constitute a vulnerable group whose parents may not be aware of their sexual minority status, we were granted a waiver of parental consent. At the beginning of each follow-up survey, participants who had reached age 18 since completing the previous survey were consented using adult protocols for informed consent. All study procedures for both baseline and longitudinal follow-up activities were reviewed and approved by the Social-Behavioral IRB at the University of Southern California. Because the study is purely observational with no researcher-controlled intervention, there is no external data safety monitoring board for the study. However, a member of the research team reviews study data immediately upon downloading the new data files each business day, and any open-ended statements or data that could potentially suggest participant safety concerns are immediately brought to the attention of the study investigators, who are considered mandated reporters in the State of California. Statements are reviewed and assessed for information concerning abuse or neglect of a child; abuse or neglect of an elder; or threat that the participant will harm themselves or someone else. An IRB-approved standard operating procedure is in place in the event of a positive disclosure; however, to date, no participant has disclosed any imminent safety concerns, and no other adverse events have been reported. The protocol included providing referrals to support resources for all participants, and following up with specific additional resources for

those who screened at risk for suicidality (e.g., both general and LGBTQ-specific crisis services).

To enhance protection for study data, we obtained an NIH Certificate of Confidentiality The final dataset will include self-reported demographic and behavioral health data, as described above, from surveys completed by the research participants. All identifying data will be destroyed at the end of the study after analysis. The final anonymous data set will be made available to other qualified members of the scientific community upon request per policies of the NIH and the University of Southern California IRB. We are committed to participating in the sharing and building of research knowledge, and will adhere to the NIH Policy on Sharing of Unique Research Resources including the Guidelines for Recipients of NIH Grants and Contracts. Requests for research resources that are generated as part of this project (e.g., qualitative outcomes, the stress measurement instrument) will be distributed in a timely manner.

Finally, the purpose of the current research is to examine pathways that may predict differing behavioral health outcomes in sexual minority adolescents. To that end, the overarching purpose is to share our developed resources with the community. As the research team completes analyses and arrives at empirical results, we have contracted with a creative graphics firm to develop infographics that cleanly summarize research findings with terminology suitable for the lay public. In addition to presenting our work in peer-reviewed manuscripts and scientific meetings, we are pursuing opportunities to share our findings with the broader community, including hosting the infographics and other study materials and derivatives on the website of the University of Southern California Center for LGBTQ+ Health Equity.[111]

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Figure 1. CONSORT flow diagram for enrollment into baseline study phase (final N=2,558).

Figure 2. CONSORT flow diagram for enrollment and retention in longitudinal study phase (current N=1,070).

Figure 1. CONSORT flow diagram for enrollment into baseline study phase (final *N*=2,558).

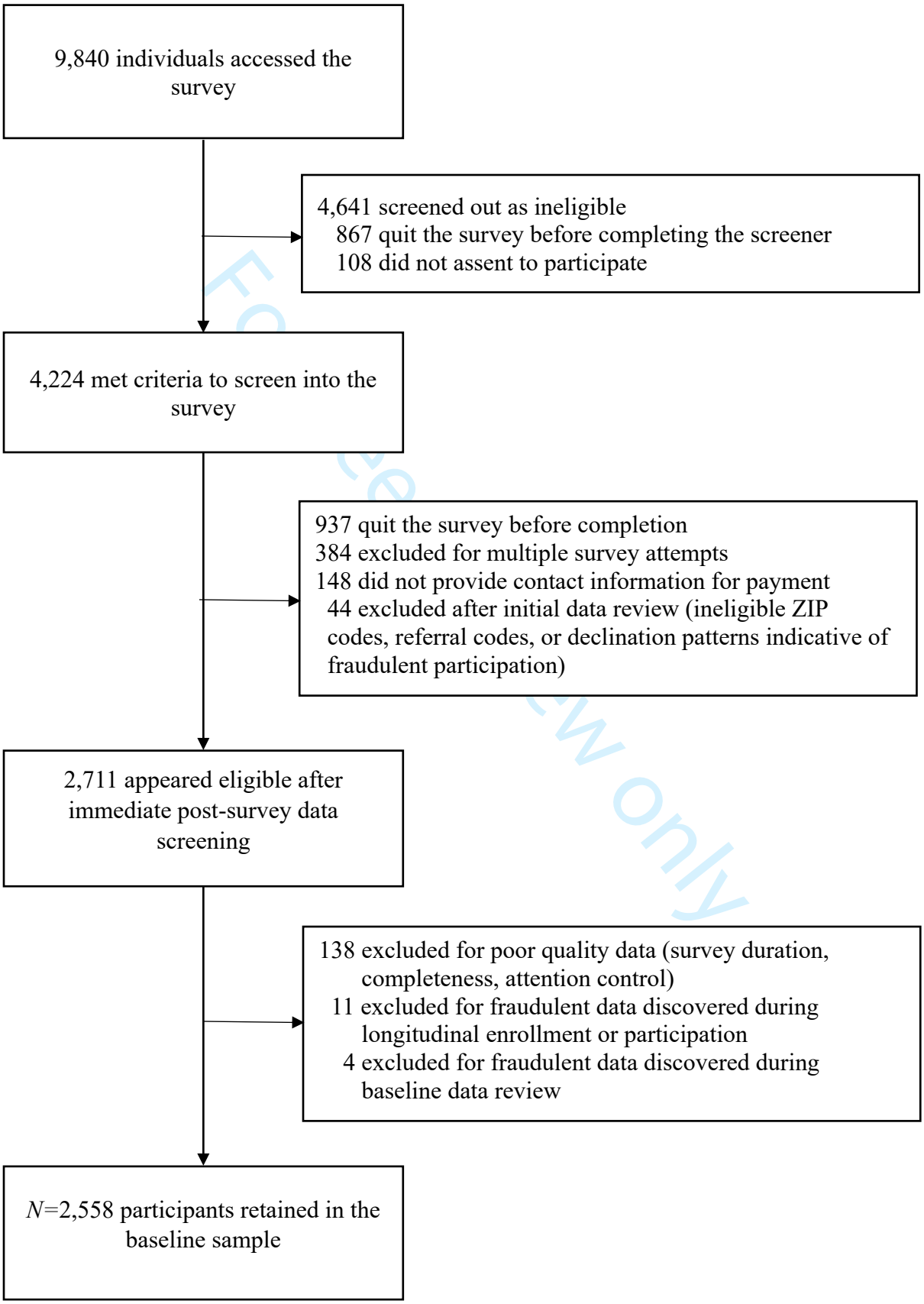
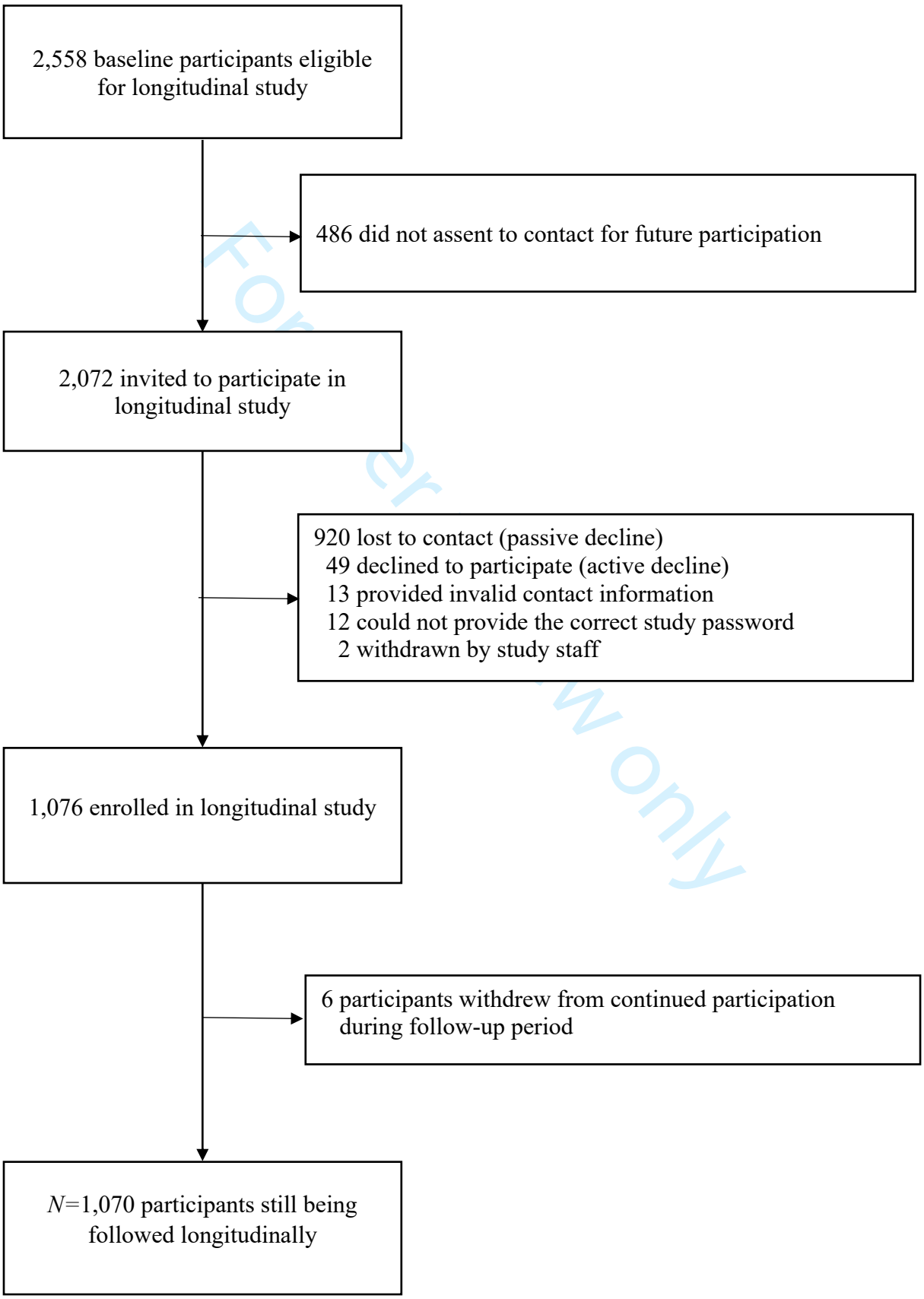


Figure 2. CONSORT flow diagram for enrollment and retention in longitudinal study phase (current *N*=1,070).



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Manuscript Location
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	page 1 lines 1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	page 2 lines 11-18
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	pages 5-9
Objectives	3	State specific objectives, including any prespecified hypotheses	page 9 lines 3-8; specific hypotheses on page 26 line 17, page 27 lines 14-15, page 28 lines 4-5 and 19-20, page 29 lines 19-20
Methods			
Study design	4	Present key elements of study design early in the paper	page 9 lines 10-17
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	page 11 lines 7-11; page 16 lines 3-5; page 19 lines 1-3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Eligibility: page 11 lines 1-6 Sources: pages 12-13 and page 17 lines 5-18 Follow-up: page 14 lines 3-17; page 16 line 7-page 17 line 4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	pages 19-24
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	pages 19-24
Bias	9	Describe any efforts to address potential sources of bias	page 10 lines 17-22; page 11 lines 7-11; page 13 lines 11-13
Study size	10	Explain how the study size was arrived at	page 30 line 13 - page 31 line 12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	page 24 line 16 – page 26 line 15 Groupings: page 28 lines 20-23
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	pages 24-30

		(*b*) Describe any methods used to examine subgroups and interactions	page 28 line 20 – page 30 line 11
		(*c*) Explain how missing data were addressed	page 25 lines 5-8
		(*d*) If applicable, explain how loss to follow-up was addressed	page 25 lines 5, 7-8 (loss to follow-up not yet established as data collection is still ongoing)
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	page 16 lines 3-4; page 19 lines 14-15; Figures 1 and 2
		(b) Give reasons for non-participation at each stage	Figures 1 and 2
		(c) Consider use of a flow diagram	Included (Figures 1 and 2)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	N/A
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	N/A
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/A
Generalisability	21	Discuss the generalisability (external validity) of the study results	N/A
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original	Page 1 lines 22-25

study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.