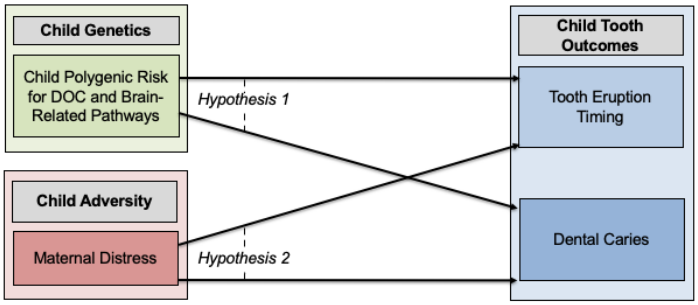
RESEARCH PLAN

SIGNIFICANCE and INNOVATION

A1. Dental caries (or tooth decay) are a common and burdensome chronic health condition, especially for children. According to former Surgeon General, David Satcher, cavities and other types of oral health problems represent a "silent epidemic" that is ranging in America (pg. 88; [54]). Although largely preventable, dental caries have been the most common chronic disease globally for over a decade[55,56]. Worldwide each year, an estimated 7.3 billion new cases of caries occur in permanent teeth and 1.8 billion in deciduous (baby) teeth[55]. In the United States, 23% of children aged 2-5 and 52% of children aged 6-8 have one or more caries[57]. If left untreated, dental caries can cause intense chronic pain, mouth infection, and even lead to hospitalization or death[58,59]. Untreated caries can also result in major impacts to children and their families, including sleep disruption[60], decreased school performance[61,62], lower school attendance[61,63], and missed work for parents[60]. Given these findings, there is an urgent need to identify factors that contribute to caries risk and can lead to the identification of strategies to prevent, as early as possible, the onset of dental caries in children.

Figure 1. The proposed K18 research study



A2. Genetic factors may, in part, predispose children to develop caries in their primary and permanent teeth and influence their age of tooth emergence. High sugar consumption, poor oral hygiene, lack of fluoride, and low socioeconomic status are four well-known risk factors for caries. However, even among people with these risk factors, there is variability in caries onset and progression, suggesting that other factors are likely at play. Because caries have been shown to cluster in families, experts have speculated that dental caries may have a heritable component. To that end, twin and adoption studies have shown that between 20% to 65% of the individual differences in susceptibility to caries may be explained by genetics[64]. To identify specific genetic risks, two types of genetic studies have been conducted: (1) candidate gene studies, which are hypothesis-driven studies that examine genes thought to shape risk; and (2) genome-wide association studies (GWAS), which are hypothesis-free studies that examine genetic variants across the genome. Results from both types of studies have accumulated to show that caries do have a genetic basis[65-67]. There are now multiple commonly-occurring genetic variants (or single nucleotide polymorphisms; SNPs) identified that influence the occurrence of dental caries, whether in primary or permanent teeth. These genetic variants map to several dozen genes involved in tooth mineral formation (*TFIP11*, *AMBN*, *AMELX*)[68], tooth and craniofacial development (*DLX4*)[69], taste perception (*TAS2R38*, *TAS2R3*, *TAS2R4*, *TASR25*)[69], or have lesser-known functions (*ALLC; C5orf66*)[70,71]. Notably, the effect size of these variants can be moderately large (odds ratios of up to 1.7, per each SNP). Genetic studies have also investigated between-person differences in the timing of normal tooth eruption (meaning, the movement of a tooth from its growth site in the alveolar bone of the jaw to its functional site in the oral cavity), as children who have earlier eruption times may be more prone to developing caries. This work has identified about a dozen genes that predict age at tooth eruption[72,73].

A3. Though relatively less well-studied, children's experiences of early life adversity may also influence primary tooth eruption timing and caries risk. Childhood adversity is the broad term used to describe a wide range of events or circumstances that pose a serious threat to a child's physical or psychological well-being[5]. Maternal distress is a major subtype of childhood adversity that encapsulates acute or ongoing stressors, traumas, and emotional or behavioral problems, including depression, in the mother. Studies have shown maternal distress can predict child psychopathology[74]. A handful of studies have also examined the role of maternal distress on children's oral health [75-84]. By and large, these studies report detecting a positive association between global indexes of parental stress (e.g., dysfunctional interactions in the parent-child relationship; child difficulties) and childhood caries [78-84]. In contrast, for more specific stressors, including reports of maternal depressive symptoms, results have been more mixed: while one study reported the incidence of childhood caries was higher among parents with greater depressive symptoms (n=235)[84], two studies found no association (n=538[85] and n=617[83]). Further, we and others have shown that early life adversity may accelerate biological aging[15]. Thus, maternal distress may also predict early age at tooth eruption. In the only empirical study we found on this topic of accelerated aging, researchers discovered and replicated a finding that exposure to childhood adversity, including mental illness in the household, associated

with earlier eruption of first molars[86]. Teeth that emerge earlier (versus later) in development may be more susceptible to dental caries, owing to longer exposure to maternal distress or other social-environmental risk factors. Given that maternal depression and parenting stress are common in the population, these findings related to the effects of maternal distress could affect between 5 to 43% of children growing-up in the US[87-89].

A4. Despite promising evidence of a link between genetic factors and maternal distress on risk for caries and early age at tooth emergence, at least two unanswered research questions impede progress in the field, which the proposed K18 research will address.

A4.1. What other genes shape tooth development and caries formation? From twin and adoption studies, genetics is thought to explain between 20-65% of the susceptibility to caries. Yet, only a fraction of that liability has been explained through genome-wide association studies (GWAS). Thus, how can we bridge that gap to identify the "missing heritability"[90,91]? One strategy widely pursued in the genetics of complex traits has been to increase study sample size, assuming that new genetic variants can be identified from larger studies. A complementary and perhaps more cost-efficient strategy may be to also leverage data from existing studies to conduct "cross-disorder analyses,"[92] meaning studies that interrogate how genetic risk for one outcome (e.g., a psychiatric disorder) associates with risk for a second outcome (e.g., a DOC phenotype). The hypothesis behind such studies is that multiple adverse outcomes may result from a given risk factor. In developmental psychology, this concept of "one to many" is known as multifinality[93]; in genetics, it is referred to as pleiotropy[94]. To the best of our knowledge, no cross-disorder studies have been conducted to investigate the potential shared genetic links between either caries formation or tooth emergence and brain-based disorders.

Yet, nascent evidence suggests there may be a "tooth-brain axis", or biological link between tooth formation and brain development[30]. Receptors for neuropeptides, including serotonin and melatonin, are expressed by ameloblasts (the cells that form enamel) and potentially modulate enamel formation[95,96]. Other markers specific to glial cells (the most abundant cell type in the central nervous system) are also expressed in dental pulp[97]. Like enamel, brain structures are derived in ontogeny from ectodermal tissue[98], supporting observations that developmental defects in enamel are disproportionately common among people with cerebral palsy, Down Syndrome, and other brain-based congenital conditions[98,99]. Thus, enamel formation appears to not only track ameloblast function, but it may also be susceptible to processes affecting early brain development[100,101]. Further, genes regulating brain development (e.g., axonogenesis; neurogenesis) and cytokine secretion, a key pathway in brain-mediated stress response circuitry[102], were found to be a top pathway explaining dental caries in at least one study[103]. Together, these findings have led some to suggest "it is possible that the timetable of key neurodevelopmental events is imprinted in an individual's teeth"[104]. If a tooth-brain axis exists, then studies of genetic risk for brain attributes could provide an efficient way to identify new genes linked to DOC attributes.

A4.2. When does maternal distress influence caries risk and tooth eruption timing? From a systematic review of the literature, we identified only one longitudinal study on the effects of maternal distress on oral health. In this very large study of infants (n=790,758), researchers found that risk of hospitalization due to caries was increased by about double among the offspring of women who experienced maternal stress-related or anxiety disorders (hazard risk=1.7) and depression (hazard risk=1.8) before or during pregnancy[105]. Without longitudinal data, it is difficult to discern if and how the relationship between maternal distress and both caries formation and early tooth emergence may vary across child development. Life course theory[106-109] suggests timing features may be important in shaping oral health. For instance, there may be a *sensitive period* [106,110-113] when the effect of maternal distress varies as a function of when in development the adversity occurred. There may also be *accumulation* effects[114-116], in which the effect of adversity increases with the number of occasions exposed, regardless of timing. Finally, there may also be *recency* effects[117], in which the effect of adversity is stronger for more proximal events. With prospective and repeated measures data obtained from a longitudinal study, scientists could study such timing dimensions. Because the prevalence of maternal distress varies over time, such timing differences may exist and if known, could allow scientists to identify new opportunities for preventing caries, especially among children who have an earlier age at tooth emergence.

A5. To begin to investigate these research questions, I will analyze data from one of the Center for Oral Health Research in Appalachia's (COHRA) datasets, called COHRA2. As described in **B1**, COHRA2 is a prospective, longitudinal birth-cohort study led by Dr. Mary Marazita (Mentor) and others. COHRA2 contains genome-wide genetic (GWAS) data, repeated measures of maternal distress, as well as measures of tooth eruption and dental caries. I will receive guidance to analyze these COHRA2 data from Dr. Marazita and an interdisciplinary team of advisors, who were carefully assembled to complement one another and together, provide the necessary ingredients to help me accomplish my long-term career goals (see **Biosketches** and

Future Goals and Objectives). In **Aim 1**, I will derive polygenic risk scores (PRS), which is a bioinformatics strategy[118,119] to characterize a person's genetic liability to a given health outcome. PRS are calculated from GWAS data by summarizing genetic risk across hundreds of thousands of SNPs. As described further in **B4**, I will derive multiple sets of PRSs, using GWAS studies of brain-related phenotypes. My goal in Aim 1 will be to evaluate the extent of correlation among these PRSs in COHRA2 and determine the degree to which the PRS of brain-related markers associate with my two dental outcomes (dental caries and age at tooth emergence), even after controlling PRS values for my two outcomes and other covariates. In **Aim 2**, I will investigate the role of repeated measures of maternal distress on age at first tooth emergence and number of caries, using a novel statistical technique called the structured life-course modeling approach (SLCMA[120,121], pronounced "slick-mah"). My team has applied the SLCMA to multiple types of data[8,12,14-16,122]. As noted in **B4**, the SLCMA enables researchers to test different life course theoretical models[106-108] to determine which model explains the most amount of variability in a given outcome. Both of these aims address the **limited rigor in prior studies**: As noted in A4, no longitudinal studies of childhood adversity have been conducted in relation to primary tooth emergence or dental caries formation. Further, only three studies to my knowledge have examined genetic risk scores in relation to a DOC phenotype[123-125]; none, to my knowledge, have focused on poly-genic risk scores.

A6. The proposed research questions, design, and methodology have significant scientific and technical merit. This work aligns with NIDCR's Notice of Special Interest (NOSI) to "integrate and analyze available genetic…phenotypic…and environmental…data to identify novel association of genetic variants with DOC traits" and "develop robust bioinformatic and computational methods" (NOT-DE-20-006).

A7. The Significance of the Proposed Research Project. Successful completion of these aims will change the way DOC researchers address questions about early life adversity by providing a new paradigm that brings greater specificity to how to conceptualize, characterize, and measure the timing of adversity. It will prompt genetic researchers studying DOC phenotypes to consider interrogating brain-based pathways. When the aims are achieved, this study will increase understanding of genetic correlates of the "tooth-brain axis." It will also determine when exposure to maternal distress, a common type of childhood adversity exposure, is most harmful in increasing caries risk and early age at tooth emergence. The proposed work thus has the potential to transform our understanding of the molecular mechanisms underlying risk for these DOC phenotypes. The potential significance of the proposed research is therefore exceptional in that these results could exert a sustained influence on the field by identifying the *windows of vulnerability* when adversity is most harmful and by suggesting the *windows of opportunity* when enriching exposures may be most beneficial. These results would change the standard for how studies of "early" life adversity are conducted by DOC researchers.

A8. The Potential Impact. This K18 study aligns with several priorities in the NIDCR Strategic Plan (2021-2026), including but not limited to: Strategic Priority 1 (Integrate Oral and General Health) to identify "unique and shared taxonomies among the oral cavity, gut, and the brain" and Strategic Priority 2 (Precision Dental Medicine) to identify the "precise multi-omic elements and mechanisms that contribute to DOC disease".

APPROACH

B1. Methods for the Center for Oral Health Research in Appalachia's (COHRA2) dataset

B1.1. Study Overview, Sample, and Procedures. COHRA2 is a prospective, longitudinal birth-cohort examining the interplay of genetic, microbiological, environmental, and behavioral factors leading to early-life oral health disparities[126,127]. COHRA2 was developed in response to findings from the COHRA1 study, which revealed a high prevalence of dental caries in young children from families living in the Northern Appalachia region. COHRA2 includes 1,172 European-ancestry adult mother-child pairs who were recruited and enrolled prenatally, beginning in November 2011 for West Virginia-based mothers and January 2012 for Pennsylvania-based mothers and ending recruitment in 2017. Mothers were recruited through 42 partnering health and dental care offices, clinics, hospitals, and community centers, as well as other community sampling methods (e.g., radio and television ads, WIC offices, Head Start) across the state of West Virginia (n=555) and through one hospital and its associated clinics and offices in southwest Pennsylvania (n=617). Data have been collected on mothers and offspring through phone interviews, home visits, and in-person assessments. Phone interviews were conducted by a professional and HIPAA-compliant research survey service. 47 data collection periods have occurred there was ~10% attrition between each study visit. By age 5, 554 mother/child pairs were still in the longitudinal study out of the original 1,172 enrolled, representing 47% retention at that point, comparable to many similar studies worldwide[1]. There is slight variation in assessment measures and timepoints between the two sites, with the Penn site adding measures and measurement periods to the common protocol (See **Table 1**). These data can be combined, however, because the majority of timepoints

are the same and we propose using the common timepoints for the overall analyses. We can also repeat analyses separately for the two sites to assess the congruence of results.

B1.2 Measures of Dental Caries and Tooth Eruption. *Dental caries* were assessed from age 2 months to 10 years during yearly intra-oral exams performed by a trained/licensed dentist or hygienist. Providers used the Decayed, Missing, or Filled Tooth Surfaces (DMFS) Index to evaluate the status of each primary tooth (e.g., decayed, healthy, restored) and surface, as part of the *PhenX Toolkit Dental Caries Experience Prevalence Protocol*. I will analyze the number of DMFS at age 5, the age before primary tooth exfoliation. *Tooth eruption* was assessed through the DMFS and phone interviews conducted every 6 months from 2 months of age to 10 years. Interviewers asked mothers if their child had any new teeth, how old the child was when their first tooth came in, and how many teeth the child has now. I will analyze the age (in months) at first tooth emergence.

B1.3. Genetic Data. Salivary DNA was collected from children at age 2 months and thereafter, if needed, using Oragene Discover kits (OGR-500, DNA Genotek). 1001 children were genotyped using an Illumina Infinium beadchip platform at the Center for Inherited Disease Research at Johns Hopkins. SNPs and subjects not meeting quality control standards were removed. SNPs not directly genotyped were imputed using the Trans-Omics for Precision Medicine (TOPMed) Imputation server (EAGLE for phasing and Minimac for imputation).

B1.4. Measures of Maternal Distress. On up to 23 different measurement occasions (see **Table 1**), mothers reported their experiences of stressors and mental health symptoms commonly examined in studies of maternal distress[74-84]. These domains were measured using single items or well-known, psychometrically-sound, and standardized measures. With these data, we can characterize the effect of the developmental timing, accumulation, and recency of maternal distress on children's dental caries and age at tooth eruption.

(1) Global Stress was measured using the 10-item Cohen's *Perceived Stress Scales* (CPSS)[128,129], one of the most widely-used instruments to capture perceptions of stress. The CPSS assesses the degree to which mothers appraise situations in their life as stressful, focusing on how unpredictable, uncontrollable, and overloaded mothers found their lives in the past month. Response options range from 0=never to 4=very often. Sample items include: "In the last month, how often have you been upset because of something that happened unexpectedly?" and "In the last month, how often have you felt confident about your ability to handle your personal problems?." Items are summed to derived total scores, which can range from 0 to 40. Prior studies have shown the CPSS demonstrates excellent psychometric properties[128].

(2) Parenting Stress was measured using an 8-item adapted version of the *Parenting Stress Index Short-Form* (PSI-SF), which captures stress, exhaustion, and burdens in their role as a parent[130,131]. Mothers indicated how often they experienced certain feelings concerning their parenting responsibilities and emotions towards their child. Response options range from 1=never to 5=almost always. Sample items include "How often would you say that your child gets on your nerves?", and "How often do you find that being a mother is much more work than pleasure?". The PSI-SF had high internal consistency reliability (α=0.72) in COHRA2.

(3) Depressive Symptoms were assessed using the *Center for Epidemiological Studies of Depression Scale* (CES-D), a 20-item self-report measure of current depressive symptoms[132]. CES-D items capture core symptoms of depression in the past week: anhedonia, depressed mood, and behavioral symptoms (e.g., felt depressed; sleep was restless; enjoyed life; had crying spells; felt sad; felt people disliked you). Dozens of studies report the CES-D has strong psychometrics, including in samples of women and mothers[133,134].

Table 1: Scheduled Assessment of Childhood Adversity and Dental Markers in COHRA2 by Age (prenatal, weeks, and years)

	Pre-natal	Weeks			Years																		
		8-10	26	~1st eruption	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10
Tooth Eruption*																							
Dental Caries				a																			
CPSS*																							
PSI-SF*																							
CES-D*																							

* = Phone Interview, a = The Pennsylvania site completed an additional in-person visit, including a dental assessment, approximatley 1-month after mothers reported their child's first tooth eruption via phone interview.

B1.5. Measures of Covariates. In all analyses, we will adjust for potential child and mother factors that could confound the associations we detect between PRS, maternal distress, child caries formation, and tooth eruption timing[126,135-138]. COHRA2 has a comprehensive set of factors measured prenatally, which we can examine in our analyses, while ensuring temporality in the associations we will study. Notably, many of these factors, including characteristics of the primary caregiver, breast and bottle feeding, diet, and oral hygiene, are

those prior studies[105] were unable to include in their analysis on the effects of maternal distress. Child-specific variables are birth measures from Pennsylvania-recruited children only (approximately 52% of total sample), including a microbial community sample, birth term, and antibiotic use. However, most children have genetic data, which will allow us to control for ancestry informative markers, to control for genetic differences in ancestry. Mother-specific variables include prenatal oral health variables (e.g., microbial community samples, gum inflammation, enamel hypoplasia, microbial community, salivary pH level), medication and illness history, dental anxiety, and dental health history. Lastly, we will also adjust for the environmental measures of mother-child Medicaid status, demographics (e.g., age, income), and fluoride levels from a home water sample. To avoid model overfitting and outcome misspecification across aims, given the ratio of the number of potential covariates to the total sample size, we will use propensity score approaches[139] as a data reduction strategy. Propensity score approaches accommodate binary and continuous exposure variables and in some instances can be more efficient in predicting outcome variation[140].

B2. We use multiple strategies to ensure a robust and unbiased approach. (1) Our measures in B1.2 are based on the PhenX Toolkit. PhenX offers researchers detailed data collection protocols vetted by experts to ensure its measures are well-established, reproducible, reliable, and from major reference studies[141]. (2) As noted in B1.5, we will ensure our results are not explained by other factors by controlling for covariates. (3) As noted in B4, use of PRS values and the SLCMA approach are unbiased. (4) To further minimize bias, we will report all results (positive and negative) for both aims in scholarly articles, including sufficient detail so these results can be reproduced. (5) We will account for the modest level of missing data on COHRA2 study variables using multiple imputation[142,143]. (6) Finally we can attempt replication of findings from COHRA2 in a second independent sample, called the Avon Longitudinal Study of Parents and Children (ALSPAC; n=~1400 with dental assessment)[144]. ALSPAC is a birth cohort that also has genetic and maternal distress measures in addition to measures of our dental phenotypes (i.e., a parent-reported measure of first tooth eruption age; a clinical examination of caries experiences at ages 3, 4, and 5[70,145-147]. Dr. Dunn has worked for more than a decade with ALSPAC data[11,14,148,149]. These replication analyses would be likely started and completed after the K18, as time would unlikely permit for it to be fully done during the one-year K18 period.

B3. Plans to address relevant biological variables. Where sample size allows, we will stratify analyses by sex or test for statistical interactions by sex. Prior work shows genetic susceptibility to dental caries[64] and the effects of maternal distress, including depression[150-152], may differ between males and females, underscoring the biological and social relevance of sex. If we lack the power to stratify the sample or test for interactions, we can use these data to obtain estimates of effect, which could inform a larger study to examine sex differences.

B4. Data Analysis.

Aim 1: Using polygenic risk scores (PRS) derived from large-scale analyses of brain structures and disorders, investigate the association between these PRSs on tooth emergence and caries. In Aim 1, I will derive PRSs in COHRA2 using standard PRS protocols[153,154] and the PRSice software[155]. PRS are calculated within a dataset by summing the number of trait-associated alleles across genetic loci, after weighting each loci (or SNP) based on the empirical effect estimate derived from an external GWAS. Three external GWASs datasets will be used to construct 28 PRSs values of brain-related phenotypes: (1) GWASs of 11 psychiatric disorders (i.e., ADHD, Alzheimer's disease, autism, bipolar disorder, eating disorders, major depressive disorder, obsessive-compulsive disorder/Tourette syndrome, PTSD, schizophrenia, substance use disorders, and all other anxiety disorder [156-166]); these data will be downloaded from the Psychiatric Genomics Consortium, in which I am involved: (2) GWASs of volumetric measures from 4 brain regions (subcortical, intracranial, hippocampal volume, subcortical) and a GWAS of cortical surface area and thickness[167-170]; these data will come from the Enhancing Neuro Imaging Genetics through Meta Analysis (ENIGMA) consortium; and (3) 11 GWASs of neurological disorders (e.g., epilepsy, ischemic stroke, migraine, multiple sclerosis, Parkinsons, etc) downloaded from the Brainstorm consortium[171]. To test **Hypothesis 1**, I will first examine the correlation between the 26 PRSs (11 + 6 + 11) to identify those exceeding r=0.80; for any pairs of PRS above this level, I will select one PRS from the pair, at random, to analyze. I expect to include 20 PRSs. I will then investigate in two separate regression analyses (one for each outcome) the association between the brain-based PRSs and caries and age at primary tooth emergence. I will examine these associations before and after adjusting for covariates, which will allow me to determine the extent to which children with high genetic risk for brain-related phenotypes also have a higher likelihood of dental caries and earlier age at tooth eruption. I will also control for PRS values for primary caries risk and age at first tooth emergence, using data from the largest GWAS of these outcomes[70,72,73]. While imperfect (given their use of self-report/non-clinically assessed outcome measures), it may reduce bias and allow for detection of new and non-overlapping genetic signal.

Aim 2: Using a novel analytic technique called the structured life course modeling approach (SLCMA) that my team has been implementing with longitudinal data, assess how the *timing* of children's exposure to maternal distress (e.g., global and parenting stress; depressive symptoms; other stressors) associates with primary tooth eruption timing and number of dental caries. In Aim 2, we will use the SLCMA to investigate the extent to which the timing of children's exposure to maternal distress predicts their age at tooth emergence and risk for caries. The major advantage of the SLCMA relative to other statistical approaches (e.g., standard regression; latent variable modeling) is that it provides an unbiased way to compare multiple competing life course models simultaneously and identify the most parsimonious explanation for the observed outcome variation[16,172,173]. In this two-stage approach, the best-fitting life course models identified in the first stage (as determined through r^2 values) are carried forward to a second stage, where measures of effect (regression beta coefficients) are estimated in a multivariate regression framework. Sensitive period life course models will be encoded as the presence vs. absence of maternal distress exposure at each measurement timepoint (we expect to collapse data into 4 or fewer time periods, to reduce multiple testing and because the SLCMA cannot operate in the presence of strongly-correlated exposures ($r>0.9$). To rule-out alternative explanations for sensitive period effects, we will also test accumulation and recency models. The accumulation model will be encoded as the total number of time periods the child was exposed to maternal distress. The recency model will be encoded as a weighted sum of the number of exposures to maternal distress, with later exposures weighted higher. As we have shown, SLCMA automatically adjusts for the number of life course models tested in the first stage, thus there are no concerns about the multiple testing of different life course models. To adjust for confounding, each encoded exposure variable and outcome will be regressed on the covariates and SLCMA applied to the residuals. To determine the extent to which exposure to maternal distress exerts time-dependent effects on our two outcome variables, we will run 6 total SLCMA models (one for each of the 3 types of maternal distress markers and for the 2 outcomes). Our prior studies have shown that testing each exposure on its own is important, as each type of adversity can have differential health effects[8,14]. However, prior to analysis, we will examine bivariate correlations to determine if collapsing by type of maternal distress is warranted. Consistent with **Hypothesis 2**, we expect to detect the sensitive period model, specifically during pregnancy, as the best fitting model to our outcomes for the global stress and depressive symptoms measure; we expect to detect sensitive period effects of parenting stress at age 2.5.

B5. Power Calculations. Even under conservative conditions, all analyses are powered to ≥80%. Aim 1: With a sample size of 1001, we have 80% power for each PRS tested to detect ≥2.2% of the outcome variation, controlling for 5 covariates (i.e., propensity score values) and a two-sided Bonferroni-corrected alpha of 0.0025 (0.05/20 PRSs) to adjust for multiple testing. This level of variation is on par (or less than) the PRS contribution to many complex traits[174-176]. Aim 2: With a sample size of 1250, the SLCMA has 90% power identify the best-fitting life course hypothesis from the 6 tested (4 for sensitive periods; 1 for accumulation; 1 for recency), if the effect present in the underlying population is at least r^2=8% in size; this effect size is comparable to our prior findings yielding r^2=7-8% estimates[177].

B6. Potential Problems, Alternative Strategies, and Benchmarks. Despite a sufficient degree of between-child variability in each measure, and my use of a large sample, I may be unable to find support for these hypotheses. If my approach does not work, I will report null results, as negative findings are important in their own right. I can also investigate the role of maternal distress on gene sets (meaning groups of genes in defined biological pathways). I can also explore more proximal markers, including oral health behaviors in the mother. Even if after these attempts there is no signal to detect, I will still achieve my learning objectives, due to having a better understanding of dental outcomes and processes. See mentor statement for **timeline for success**.

B7. Relevance of this research plan to my research career objectives. The research plan is appropriate to my stage of research development. It provides a vehicle for me to develop my research skills as described in the career enhancement plan. The proposed research project is a novel extension of my research, as I have never before examined factors contributing to tooth emergence or dental caries. The research plan will help me develop a rigorous research program in DOC research by providing me with foundational knowledge in three areas (concepts in tooth development and dental hard tissues; traditional measures to record tooth growth; and state-of-the-art approaches to measure tooth growth), which will position me to become a leader of team science focused on the effects of genetic and social-environmental adversities on DOC-related markers. This K18 study will also provide pilot data for a future R01 study to investigate gene-environment interplay (GxE), or how the effects of childhood adversity on DOC markers might be worsened (or lessened) among children with certain genetic backgrounds. Such work aligns with my commitment to reduce the burden of disease resulting from childhood adversity exposure and other forms of social vulnerability, including in high-risk populations.