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Study protocol to quantify the genetic architecture of sonographic cervical length and its relationship to spontaneous preterm birth

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ABSTRACT

Introduction A short cervix (cervical length <25 mm) in the midtrimester (18–24 weeks) of pregnancy is a powerful predictor of spontaneous preterm delivery. Although the biological mechanisms of cervical change during pregnancy have been the subject of extensive investigation, little is known about whether genes influence the length of the cervix, or the extent to which genetic factors contribute to premature cervical shortening. Defining the genetic architecture of cervical length is foundational to understanding the aetiology of a short cervix and its contribution to an increased risk of spontaneous preterm delivery.

Methods/analysis The proposed study is designed to characterise the genetic architecture of cervical length and its genetic relationship to gestational age at delivery in a large cohort of Black/African American women, who are at an increased risk of developing a short cervix and delivering preterm. Repeated measurements of cervical length will be modelled as a longitudinal growth curve, with parameters estimating the initial length of the cervix at the beginning of pregnancy, and its rate of change over time. Genome-wide complex trait analysis methods will be used to estimate the heritability of cervical length growth parameters and their bivariate genetic correlation with gestational age at delivery. Polygenic risk profiling will assess maternal genetic risk for developing a short cervix and subsequently delivering preterm and evaluate the role of cervical length in mediating the relationship between maternal genetic variation and gestational age at delivery.

Ethics/dissemination The proposed analyses will be conducted using deidentified data from participants in an IRB-approved study of longitudinal cervical length who provided blood samples and written informed consent for their use in future genetic research. These analyses are preregistered with the Center for Open Science using the AsPredicted format and the results and genomic summary statistics will be published in a peer-reviewed journal.

Strengths and limitations of this study

- This study will be the first to characterise the genetic architecture of cervical length and its longitudinal change during pregnancy.
- This study will be the first to estimate the bivariate genetic correlation between cervical length and gestational age at delivery.
- While the study cohort is not large enough to identify individual genetic variants associated with cervical length, it is well powered to analyse aggregate genome-wide summary statistics in order to estimate trait heritability and bivariate genetic correlations, and to develop a polygenic risk score to identify women with the highest risk of developing a short cervix and delivering preterm.
- The study cohort predominately comprises women who self-identify as Black/African American; although the findings of this study may not be generalisable to women from other populations or ancestry groups, they could improve screening and clinical care for a population of women who are disproportionally affected by health disparities in preterm birth and perinatal outcomes.

BACKGROUND AND INTRODUCTION

A short cervix (cervical length <25 mm) in the midtrimester (18–24 weeks) of pregnancy is a powerful predictor of maternal risk for delivering preterm (<37 weeks),[1–29] and the only biomarker for spontaneous preterm birth that can be coupled with an effective clinical intervention.[30–47] Preterm birth and prematurity-related conditions are the leading causes of perinatal morbidity and mortality worldwide[48–51] and in the USA,[52 53] where there is a pronounced and persistent racial disparity in the incidence of preterm birth and its associated health outcomes.[54–59] Although the rate of medically indicated preterm births is on the rise,[48 51 58 60] most preterm births are idiopathic, and occur spontaneously.[51 60] Thus, prevention of spontaneous preterm birth remains a major public health priority.[56 58 61 62] A better understanding

of the primary pathogenic mechanisms contributing to spontaneous preterm birth is needed to develop effective strategies for reducing the morbidity and mortality associated with prematurity.

Despite extensive evidence of a genetic contribution to gestational age at birth,[63–83] there has been little success identifying specific genetic variants that influence the timing of labour and delivery.[84] The difficulty in gene discovery may be due, in part, to the syndromic nature of spontaneous preterm birth.[85 86] Multiple mechanistic pathways in both the mother and the fetus—each influenced by unique genes and environmental risk factors—are thought to contribute to the premature onset of labour.[49 58 60 87] For this reason, statistical genetic methods may be more successful at identifying genes associated with individual risk factors, rather than the final common outcome of spontaneous preterm birth. A genetic study of cervical length could prove highly informative, given that the length of the cervix is an easily measured, quantitative trait that is highly correlated with risk for spontaneous preterm delivery.[88]

Biomechanical properties of the cervix

The uterine cervix has two opposing functions during pregnancy: first, it must remain firmly closed to prevent intrauterine infection, spontaneous abortion, or preterm delivery; and second, at the onset of labour, it must open to allow successful parturition.[89 90] These changes are reflected in the histology,[91–94] biochemistry[94–97] and biomechanical properties[98–100] of the cervix. While the uterine corpus is predominantly composed of smooth muscle (ie, myometrium), the uterine cervix is fundamentally a connective tissue.[89 90] Smooth muscle cells constitute approximately 10% of the cervix stroma, with the remainder comprising collagen and elastin fibres, interspersed with cervical fibroblasts.[89 90] The structural integrity of the cervical stroma is essential for carrying a pregnancy to term, and relies on the strength and organisation of the fibrous network structure, rather than the contractile strength of smooth muscle.[98–100] Remodelling of this collagen-rich, connective tissue is a complex process which begins early in pregnancy, and culminates with softening, effacement and dilation of the cervix at parturition.[91–97]

The length of the cervix is defined as the distance between the external os and the functional internal os,[88] and can be easily measured by transvaginal ultrasonography over the course of a pregnancy.[101–103] Estimates for the mean length of the cervix in the midtrimester vary between 35 and 45 mm, depending on the population,[1 2 9–11 88 104–109] with cervical lengths shorter than 25 mm before 24 weeks meeting the clinical definition of a short cervix.[2 88] Typically, the cervix progressively shortens with increasing gestational age,[2] decreasing between 0.1–0.3 mm per week after 15 weeks of gestation.[8 110–112]

Relationship between cervical length and spontaneous preterm birth

A short cervix in the midtrimester is associated with a sixfold increase in the risk of preterm delivery.[2] The shorter the cervix, and the earlier in pregnancy the shortening occurs, the higher the risk for spontaneous preterm delivery.[1–29] In women with a cervical length <25 mm, every additional 1 mm of cervical shortening is associated with a 3% increase in the odds of spontaneous preterm delivery.[113 114] The rate of change in cervical length is also significantly associated with an increased risk of preterm delivery,[2 115–118] independent of the initial measurement.

Mean cervical length in the midtrimester is significantly shorter—and the incidence of a short cervix is more than twice as high—among Black women living in North America and Europe, compared with women from other racial and ethnic backgrounds.[104 106–109 119] Cervical shortening begins at an earlier gestational age, and occurs more rapidly, among Black/African American mothers,[120 121] and midtrimester cervical length is more predictive of risk for spontaneous preterm delivery for Black/African American women, compared with white/Caucasian American women.[108 122] While structural and social risk factors are strongly associated with an increased risk of premature cervical shortening and spontaneous preterm delivery among Black/African American women,[119–122] the observed variation in mean midtrimester cervical length among women from different continental ancestry groups raises the question of whether population-level differences in the frequency of risk alleles may also be contributing to the risk of premature cervical shortening and subsequent risk for spontaneous preterm delivery.[71 123]

A biometrical genetic approach to the study of cervical length

Sonographic measurements of cervical length in the midtrimester and gestational age at delivery are two quantitative phenotypic traits that can be approximated by a normal distribution.[2 9–11 114] These sampling distributions are characterised by mean and variance statistics, which provide an estimate for interindividual differences within each trait in the population. The relative contributions of genetic and environmental factors influencing population-level variation in these traits can be estimated using biometric modelling techniques in a genetically informative cohort. The concept of *heritability* describes the proportion of the total phenotypic variation in a trait that can be attributed to genetic variation among individuals in the population.[124–126]

The broad-sense heritability of spontaneous preterm birth is estimated between 25% and 40%,[65 66 68 70 76 78 80–82] and can be separated into fetal and maternal components explaining 11%–35% and 13%–20% of the phenotypic variance, respectively.[78 80 81] Heritability estimates vary significantly by population, although variation in gestational age at birth among ancestry groups can be primarily attributed to sociodemographic and environmental factors.[127] Although the maternal and fetal genetic

contributions to spontaneous preterm delivery are well described in the literature, there is no estimate for the heritability of cervical length, and very little is known about how genes influence the length and rate of change of the cervix during pregnancy.[69 72 84 128–130]

Classical twin and family studies are the most common methods for estimating trait heritability through comparison of genotypic and phenotypic similarities between pairs of family members, stratified by their genetic relatedness.[124–126 131] For instance, observing an increased phenotypic correlation among monozygotic twin pairs (who are genetically identical) compared with dizygotic twin pairs (who share, on average, 50% of their genetic material) would indicate the contribution of genetic factors to trait variance. A recent meta-analysis of 17 804 traits from 2748 twin studies published in the last 50 years estimates an average heritability of 49% across all categories of complex human traits.[131] The average heritability of traits specifically related to female reproduction is estimated at 45%, with 144 of the 164 studied traits consistent with a simple and parsimonious model in which all trait resemblance between twins can be attributed to additive genetic influences.[131]

No large twin or family cohorts to date have collected data on cervical length, and the lack of a heritability estimate discourages large scale genetic studies aiming to identify the contribution of individual genes to cervical length and its rate of change during pregnancy.[124–126] A solution exists in modern statistical methods for estimating heritability using genome-wide association data from large, population-based cohorts of unrelated individuals.[132–134] Genome-wide complex trait analysis (GCTA) can be used to estimate the proportion of phenotypic variation in a population that is attributable to common genetic variants, in the form of single nucleotide polymorphisms (SNPs) at millions of positions across the genome.[132–134] SNP-based heritability estimation is based on the same fundamental concept as twin and family methods; that is, the correlation between shared genotypes and shared phenotypes. If the degree of genetic similarity between pairs of individuals is positively correlated with the degree of phenotypic similarity between individuals, this suggests that genetic variation contributes to phenotypic variation in the trait.[132–134] While twin and family-based methods use theoretically derived estimates of genetic relatedness based on known pedigrees, SNP-based methods estimate the degree of genetic similarity between individuals empirically from the observed genotypic SNP data.[132–134] The estimated coefficient of genetic similarity between two individuals—that is, the mean number of shared alleles for all genotyped SNPs, weighted by the frequency of each allele in the population—is represented in a genetic relationship matrix (GRM), which contains a single value for each pair of individuals in the cohort. Instead of testing for an association between the phenotype and the genotype at each SNP independently, the GRM is used to estimate the phenotypic variance explained by genetic variation across all genotyped SNPs simultaneously.[132–134] Although SNP-based heritability estimates are often lower than those reported by classical twin studies due to methodological limitations, they can be used to approximate the lower bound of genetic contributions to phenotypic variance and help contextualise the results of genome-wide association studies.[124–126]

A simple extension of these methods can be used to estimate the coheritability, or genetic covariance, between two traits.[135] Just as phenotypic variance can be partitioned into genetic and environmental components to estimate heritability, phenotypic covariance between two traits can also be decomposed into its constituent genetic and environmental components.[136 137] A strong genetic correlation between cervical length and gestational age at delivery would suggest that some of the same genes influence the expression of both traits, and that assessing the genetic risk for one trait would allow estimation of the genetic risk for the second trait via cross-trait polygenic analyses.[138–140]

Although a genetic correlation between cervical length and gestational age at delivery would suggest an underlying genetic aetiology shared between the two traits, it would not reveal any information about the causal mechanisms that lead to the observed correlation. Indeed, cross-sectional association-based analysis methods have limited power to unravel the mechanistic pathways that underly complex diseases. However, because genetic variants are fixed at conception, and therefore not subject to the question of reverse causation, they can be used to test the direction of causality in an observed association between an intermediate phenotype or modifiable risk factor, such as cervical length, and a clinically relevant outcome, such as spontaneous preterm delivery. A mediation model can be constructed within the structural equation modelling (SEM) framework to model the relationship between predictor and outcome variables, both directly and an indirectly, mediated by a third, intermediary variable.[141–144] SEM can be used to test whether the same genetic factors influence both traits independently (ie, horizontal pleiotropy), or if unique genetic factors influence cervical length, which then mediates the risk for spontaneous preterm delivery through a short or rapidly shortening cervix during pregnancy. Understanding if, and how, maternal genetic risk for spontaneous preterm delivery is mediated by cervical change during pregnancy may improve the predictive value of midtrimester cervical length for use in universal screening programmes, and inform clinical interventions for women at high risk for spontaneous preterm delivery associated with a short cervix.

METHODS AND PROPOSED ANALYSIS

Objective

The primary aim of this study is to characterise the genetic architecture of cervical length and its bivariate genetic correlation with gestational age at delivery. The hypotheses are as follows: (1) maternal genetic variation contributes directly to variance in cervical length and its rate of change during pregnancy; (2) there is a bivariate genetic correlation between cervical length/change and gestational age at delivery; and (3) cervical length/change causally mediates a portion of the maternal genetic contribution to gestational age at delivery.

Study start and end dates

The proposed study will use phenotypic data and biological specimens collected from women enrolled under the protocol entitled *Biological Markers of Disease in the Prediction of Preterm Delivery, Preeclampsia and Intra-Uterine Growth Restriction: A Longitudinal Study (NCT00340899*) between November 2005 and Novermber 2016. Research activities specific to this project began in August 2017 (research design and planning). DNA samples isolated from banked biological specimens provided by the 5000 selected participants were sent to a commercial laboratory for genotyping via low-pass whole genome sequencing in May 2021. Genotyping is projected to be completed by February 2022. Statistical analysis following the proposed protocol will begin once genotyping is complete.

Study participants

This study involves women enrolled in a longitudinal study of cervical length at the Center for Advanced Obstetrical Care and Research (CAOCR) at Hutzel Women's Hospital in Detroit, Michigan. The center in affiliated with Wayne State University and the Detroit Medical Center, and is an integral part of the Perinatal Research Branch of The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (National Institutes of Health, U.S. Department of Health and Human Services). This research was approved by the Institutional Review Boards of Wayne State University (WSU IRB#110605MP2F) and NICHD/NIH/DHHS (OH97-CH-N067). All study participants were enrolled between 2005 and 2017 and provided written informed consent before the collection of demographic or clinical information, images, or biological samples. From a set of 8226 pregnancies with serial cervical length measurements available, 5971 pregnancies were selected on the basis of the following criteria: a singleton pregnancy, at least 2 cervical length measurements performed between 8 and 40 weeks of gestation, availability of a blood sample and consent to its use in future genetic research, and availability of relevant demographic and clinical characteristics (weight, height, age, parity, etc). Women with a medically induced preterm delivery or a termination during study participation were excluded from the current analysis. Additional exclusion criteria include a history of cervical trauma or any serious medical conditions (such as severe chronic hypertension or renal insufficiency, congestive heart disease, chronic respiratory insufficiency, etc). Biological samples from 5000 participants meeting these criteria were selected for genotyping. The study cohort of 5000 women comprises women who self-identify as Black/African (4640 women, 93%), Caucasian/white (139 women, 3%), or Biracial/other racial identity (220 women, 4%).

Patient and public involvement

Patient/public input was not consulted regarding the design, conduct, reporting or dissemination plans of our research.

Data collection and outcome measures

Demographic characteristics, relevant medical history and pregnancy outcome data were obtained for each participant via medical record abstraction. Maternal peripheral blood samples were collected from each participant during their enrolment period in the original study. DNA extracted from buffy coat isolations has been sent to a commercial laboratory for low-pass whole genome sequencing (sequencing depth 1×).

Cervical length is measured in millimetres (mm) using a transvaginal 12–3 MHz ultrasound endocavitary probe (SuperSonic Imagine).[102] Serial cervical length measurements were obtained between 8 and 40 weeks of gestation when patients were seen for prenatal care visits in the CAOCR clinic. Cervical stiffness was also assessed in some women via cervical elastography, by measuring the percentage of displacement or deformation of cervical tissue during manual application of oscillatory pressure. The primary outcome measure, gestational age at delivery, is measured from the first day of a woman's last menstrual period and confirmed by ultrasound.

Statistical methods and approach

Relationships among repeated measures of cervical length during pregnancy will be modelled as a longitudinal growth curve, with parameters describing the initial length of the cervix and its rate of change over time. The intercept parameter (intercept term (INT)) will represent the best estimate for the baseline measurement of cervical length in early pregnancy, while the slope parameter (SLP) will represent the linear rate of change in cervical length during pregnancy. Higher order parametric terms can also be incorporated to model non-linear growth. Individual growth trajectories and parameters will be estimated for each participant in the cohort, and associations between INT, SLP and the outcome variable will be tested to determine if the starting value or rate of change of cervical length varies significantly with respect to gestational age at delivery.

Quality control for genomic data will conform to the current best practices for the platform used for genotyping. Each individual will be empirically assigned to an ancestry group using genome-wide molecular variation and an external reference panel of known ancestry. If a small number of outliers are identified, they can be excluded from analysis. Otherwise, analyses can be performed within empirically assigned ancestry groups and then meta-analysed. This approach has the advantage of minimising genomic inflation and sample loss due to exclusion of low frequency, unknown or admixed ancestry groups. The open-source, whole-genome association analysis toolset PLINK will be used to test for association between genetic variants (eg, SNPs) and individual estimates of cervical length growth parameters as quantitative traits,[145] for the purpose of constructing a polygenic risk score (PRS) for a short cervix. The PRS method will be used to aggregate the effects of many moderately associated SNPs across the genome for multiple significance thresholds (eg, $p<0.00001$, $p<0.0001$, $p<0.001$, etc)

and the phenotypic variance accounted for by SNPs within each threshold will be calculated.[146] The PRS approach will allow further characterisation of the genetic architecture of cervical length by estimating the total number of genetic loci influencing phenotypic variance and the effect sizes and frequency of risk alleles in the population. Furthermore, a PRS can be used to infer genetic overlap between two traits, such as cervical length and gestational age at delivery, and to predict associated phenotypes based on a genetic profile.[139 140 147 148]

The genomic-relatedness-based restricted maximum-likelihood (GREML) method from the GCTA framework will be used to estimate the SNP-based heritabilities of cervical length growth parameters and gestational age at delivery within the sample, and calculate the genetic correlation between these phenotypes.[132–134 136] The SEM framework will be used to test for mediation of the relationship between maternal genetic variation and gestational age at delivery. A mediation model will assess the effects of common genetic variants (SNPs and/or PRS) on gestational age at birth, both directly and indirectly, mediated through cervical length growth parameters (INT and SLP). The standard criteria of a p value less than 0.05 will be used to draw inferences. A false discovery rate correction will be applied based on iterations of the model used.

Power calculation

The estimation of statistical power was performed for the questions outlined in the study objectives for a sample size of 5000 participants. While individual SNP association tests will be performed, this study lacks the statistical power to identify the contribution of a single SNP while controlling for multiple tests at the genome-wide level (ie, family-wise error rate of 5×10^{-8}). The summary statistics from individual SNP association tests will be used to estimate the aggregate GCTA and PRS statistical summaries as previously described. Statistical power for GCTA methods followed the approach as described by the GREML power calculator.[149] We estimate 80% power to detect a cervical length heritability of 0.16 or greater using the GCTA method (as previously mentioned, the average heritability female reproductive traits is around 0.45, based on 164 estimates from twin studies[131]). For the bivariate GCTA approach, we estimate 80% power to detect a genetic correlation between cervical length and gestational age at delivery of 0.36 or greater, and 80% power to estimate a mediating role of cervical length on the relationship between the PRS and gestational age at delivery for a wide range of scenarios in which the proportion of this direct effect that is mediated ranges from 10% to 100%.

DISCUSSION

This study is designed to decipher the genetic architecture of cervical length and its genetic relationship to spontaneous preterm birth in a large cohort of Black/African American women with longitudinal cervical length measurements across pregnancy. Characterising the number, effect size and population frequency of genetic variants influencing cervical changes during pregnancy is essential to inform a mechanistic understanding of cervical shortening and its contribution to maternal liability for preterm birth.

This project has the potential to identify genetic factors contributing to the increased incidence of spontaneous preterm birth and the higher relative risk for preterm delivery associated with a short cervix in Black/African American women. The development of a PRS for assessing maternal genetic liability to cervical shortening and subsequent risk for spontaneous preterm birth could aid in clinical risk assessment for women of African ancestry, and help identify high risk women who could benefit from early intervention to prevent preterm delivery.[150] A PRS would be particularly useful for primigravida, who have no medical history of pregnancy to inform their risk for spontaneous preterm delivery, and who may not receive cervical length screening as standard of care in the absence of other known risk factors. Rapid assessment of a patient's genetic risk for developing a short cervix and delivering preterm could help identify additional patients who would benefit from effective clinical interventions, such as vaginal administration of progesterone for the prevention of spontaneous preterm birth.

Cervical length screening by transvaginal ultrasound is the best available technique for predicting and preventing preterm birth when paired with the administration of vaginal progesterone in patients with a short cervix. A simple extension of the methods described in this study design could be used to test for genetic moderation of the relationship between vaginal progesterone treatment and cervical length change during pregnancy. A pharmacogenomic study of the women in the cohort who were treated with vaginal progesterone due to a pregnancy complicated by a short cervix could be conducted to identify genetic alleles that modify the responsiveness of progesterone treatment in women with a short cervix and determine whether response to progesterone is informed by the developed PRS or individual genetic variants (SNPs).

Limitations

Although the study cohort is not large enough to identify individual genetic variants associated with cervical length change or gestational duration while controlling for multiple tests at the genome-wide level, it is well powered to analyse aggregate genome-wide summary statistics in order to estimate trait heritability and bivariate genetic correlations, and to develop a PRS to identify women with the highest risk of developing a short cervix and delivering preterm.

The study cohort predominately comprises women who self-identify as Black/African American; although the findings of this study may not be generalisable to women from other populations or ancestry groups, they could improve

screening and clinical care for a population of women who are disproportionally affected by health disparities in preterm birth and perinatal outcomes. While heritability estimates and alleles/allele frequencies vary from population to population, we do not expect the underlying mechanistic relationships between genes, cervical change and gestational duration to differ substantially between our study population and other global populations. Thus, we hope that a better understanding of how genes contribute to cervical change during pregnancy and risk for preterm delivery can inform perinatal care for women from all populations across the globe.

The proposed study does not address the influence of fetal genes, which are predicted to play a role in the timing of birth. Furthermore, there may be overlapping fetal and maternal genes associated with cervical length and spontaneous preterm birth, given that matrix metabolism is implicated in both cervical ripening and changes in the fetal membranes preceding parturition.[84] Fetal genetic variants may also promote preterm premature rupture of membranes,[84 151–153] which often occurs in the setting of a prematurely shortened cervix.[154–156] The proposed cohort has a rich biobank, including fetal blood samples, that will allow follow-up studies of the fetal genetic contributions to cervical length changes during pregnancy.

Although this study will examine maternal genetic contributions to the correlation between cervical length and gestational age at delivery, we do not discuss all of the environmental and sociodemographic factors that may also contribute.[157] Additionally, population differences in vaginal microbiome states, which are associated with cervical inflammation,[158–161] may also contribute to the association between cervical length and gestational age at birth.[162 163] Cervicovaginal samples collected from the cohort will allow evaluation of the vaginal microbiome and its relationship to cervical length and the presence of proinflammatory cytokines.

ETHICS APPROVAL

The Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)/National Institutes of Health/U.S. Department of Health and Human Services (Detroit, MI, USA) approved the study. Participants were enrolled under the protocols Biological Markers of Disease in the Prediction of Preterm Delivery, Preeclampsia and Intra-Uterine Growth Restriction: A Longitudinal Study (WSU IRB#110605MP2F and NICHD/NIH# OH97-CH-N067). All participants provided written informed consent for the collection of cervical length data and blood samples for future genetic research studies.

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Contributors Study designed by TPY, JFS, SSH and RR. The initial manuscript written by HMW and TPY, JFS, RR, SSH, and ALT provided editing and review. Input on technical issues and methodological approaches was provided by SJL, BTW, ALT, NG-L and C-DH. All authors have read and approved the final manuscript.

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