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# BMJ Open

## Cohort profile: Understanding Pregnancy Signals and Infant Development (UPSIDE), a pregnancy cohort study on prenatal exposure mechanisms for child health

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4 **Cohort profile: Understanding Pregnancy Signals and Infant Development**  
5 **(UPSIDE), a pregnancy cohort study on prenatal exposure mechanisms for child health**  
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**ABSTRACT**

**Purpose:** Extensive research suggests that maternal prenatal stress and anxiety are reliably related to perinatal and child health outcomes – which may persist into adulthood. However, basic questions remain regarding mechanisms involved. To better understand these mechanisms, we developed the Understanding Pregnancy Signals and Infant Development (UPSIDE) cohort study, which has several distinguishing features, including repeated assessments across trimesters, analysis of multiple biological pathways of interest, and incorporation of placental structure and function as mediators of child health outcomes.

**Participants:** Healthy pregnant women were recruited at <14 weeks gestation. Study visits occurred in each trimester and included extensive psychological, socio-demographic, health behavior, and biospecimen collection. Placenta and cord blood were collected at birth. Children visits (ongoing) occur at birth and 1, 6, 12, 24, 36, and 48 months of age and use standardized anthropometric, clinical, behavioral, biological, and neuroimaging methods.

**Findings to date:** We recruited 326 pregnancies; 294 (90%) were retained through birth. Success rates for prenatal biospecimen collection were high across all trimesters (96-99% for

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3 blood, 94-97% for urine, 96-99% for saliva, 96% of placentas, 88% for cord blood, and 93% for  
4 buccal swab). Ninety-four percent of eligible babies (n=277) participated in a birth exam;  
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6 postnatal visits are ongoing.  
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11 **Future plans:** The current phase of the study will follow children through age 48 months to  
12 examine child neurodevelopment and physical development. In addition, the cohort participates  
13 in the National Institutes of Health's Environmental influences on Child Health Outcomes  
14 (ECHO) program, a national study of 50,000 families examining early environmental influences  
15 on perinatal outcomes, neurodevelopment, obesity, and airway disease. Future research will  
16 leverage the rich repository of biological samples and clinical data to expand research on the  
17 mechanisms of child health outcomes in relation to environmental chemical exposures,  
18 genetics, and the microbiome.  
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### 30 **Strengths and Limitations:**

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32 • The UPSIDE cohort features intensive, serial biospecimen and questionnaire collection  
33 from the first trimester of pregnancy through age 4 that will allow us to test hypotheses  
34 regarding the pathways by which psychosocial stress impacts children's development  
35 during critical and sensitive periods.  
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- 38 • Our comprehensive assessment of the placenta, including both morphometric and  
39 molecular markers, provides novel data on the role that this under-studied organ plays  
40 as a mediator of the association between maternal exposures and child outcomes.  
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- 43 • The study is underpowered to assess clinical outcomes as by design, we recruited a  
44 medically low-risk cohort.  
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## INTRODUCTION:

For several decades, epidemiological studies have provided robust evidence of an association between maternal prenatal stress or anxiety and child health outcomes<sup>1-6</sup>. The large and growing collection of studies that has emerged from both high-income and low/middle-income countries, suggests that prenatal maternal anxiety or stress is a plausible illustration of a “developmental programming effect” on child health outcomes<sup>7-12</sup>. The developmental programming model proposes that *in utero* exposures instigate an adaptive response in the fetus/child that is carried forward in development and has persisting effects on behavior and biology<sup>13-19</sup>. Central to this hypothesis is the concept that early exposures have a privileged – or different – effect on biological systems than those occurring later in development. The resulting clinical and public health implications of this model are substantial because they suggest that the timing of intervention may be as important as its content. The aim of this paper is to introduce a new cohort, Understanding Pregnancy Signals and Infant Development (UPSIDE), which includes several measurement and design advantages to advance our understanding of maternal prenatal psychosocial stress and child health outcomes.

A key exposure variable in this large collection of studies may be broadly interpreted as prenatal maternal psychological distress. Assessment of maternal psychological distress can derive from many sources, including clinical interviews as well as maternal self-report inventories of anxiety, depressive symptoms, trauma, major life-event stressors, and “pregnancy-specific” worry. The persistence of reported impacts of maternal psychological distress on child outcomes, despite variation in exposure measurement, suggests that the association is robust<sup>20-25</sup>. The corollary – how maternal experience of or exposure to distress creates an exposure variable for the fetus – is far less clear and likely involves multiple, interdependent biological pathways. That is, if stress is indeed a causal factor, it may operate via neuroendocrine, immune, autonomic, or other

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3 physiological mechanisms<sup>26-29</sup>. Initial research on the mediating mechanisms, based on strong  
4 evidence from experimental animal studies, targeted the hypothalamic-pituitary-adrenal (HPA)  
5 axis<sup>30</sup>. The biological case for its involvement in the stress response (most typically in the form  
6 of cortisol, the downstream product of HPA activation) is certain, its transplacental transfer is  
7 well-established, and the application to placental mechanisms is evident (placental enzyme 11-  
8  $\beta$ -HSD2)<sup>31 32</sup>. Nonetheless, that biological model is too limited. Human studies have not  
9 provided consistent evidence that prenatal maternal anxiety or stress impacts child development  
10 through HPA-related mechanisms<sup>30 33-37</sup>. Moreover, several lines of research raise alternative  
11 mechanisms. One of the most important of these is maternal inflammation, represented by  
12 research on the Maternal Immune Activation (MIA) model. Research findings show that  
13 circulating proinflammatory markers in pregnancy predict an increased risk of significant  
14 neurodevelopmental problems in the child<sup>38-43</sup>. Other studies indicate that prenatal sex steroids  
15 may also be a plausible predictor of child development<sup>44</sup> and may be confounded with stress  
16 and stress physiology<sup>45-48</sup>. Accordingly, a first major methodological and conceptual strength of  
17 this study is the assessment of biomarkers relevant to alternative pathways (e.g. cytokine  
18 profiles, steroidogenic activity) across pregnancy and in multiple biological sample types (e.g.  
19 maternal blood, cord blood, placenta).

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41 A second key feature of the UPSIDE study is its focus on and intensive assessment of the  
42 maternal-fetal-placenta unit. Despite the placenta's critical role in transmitting maternal signals  
43 to the developing fetus, direct measurement of the placenta has been notably absent from the  
44 vast majority of studies on prenatal stress and child development<sup>49 50</sup>. There are both practical  
45 and scientific reasons for the limited research that integrates interrogation of the placenta in  
46 studies of prenatal exposures and child outcomes. The practical matter concerns sample  
47 collection and processing, particularly the 24/7 coverage that this requires if spontaneous  
48 deliveries are included. Scientifically, there is variability among studies in which placental



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3 markers are assessed; placenta weight, gene expression, and epigenetics have all received  
4 some attention, almost always in separate reports. In this cohort study, we expand direct,  
5 comprehensive measurement of the placenta to advance the field in several important ways.  
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7 First, it is increasingly clear that the placenta contributes to maternal perinatal health <sup>51-53</sup>, with  
8 clear implications for neonatal and, by extension, child health. It is also becoming more widely  
9 appreciated that prenatal exposures (including maternal anxiety or stress but also  
10 environmental exposures) may alter placenta structure and function <sup>54-56</sup>. Finally, there is now a  
11 growing evidence base linking placenta measures to child outcomes such as obesity and  
12 neurodevelopment <sup>57-59</sup>. What has been missing from this field are prospective pregnancy cohort  
13 studies that track mother-child dyads from early gestation through early childhood, while also  
14 collecting detailed placenta data. To that end, the UPSIDE study includes extensive  
15 measurement of placenta structure and function from imaging, histology, and  
16 immunohistochemistry, genetics, and pathology reports.  
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32 In addition to these conceptual advances, UPSIDE includes several key design elements that  
33 will inform our study of the multiple physiological pathways by which maternal psychological  
34 stress may impact child development. These include: (1) serial maternal questionnaire and  
35 biomarker data across all trimesters to examine critical and sensitive windows of gestation; (2)  
36 pediatric visits at seven time points from birth to 4 years of age to assess neurodevelopment as  
37 well as growth, reproductive development, and HPA axis activity; (3) consideration of potentially  
38 important covariates and confounders that are sometimes overlooked in studies of child  
39 development (e.g. maternal and child diet, physical activity, sleep).  
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## 51 **COHORT DESCRIPTION:**

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3 **Study setting and recruitment:** From December 2015–April 2019, women were recruited  
4 during their first trimester of pregnancy from outpatient obstetric clinics affiliated with the  
5 University of Rochester. Eligibility criteria included: <14 weeks gestation, age 18 or older,  
6 singleton pregnancy, no known substance abuse problems or a history of psychotic illness, and  
7 ability to communicate in English. Women with major endocrine disorders (such as polycystic  
8 ovary syndrome), high-risk pregnancies, or significant obstetric problems were excluded. Infants  
9 born prior to 37 weeks gestation were not included in postnatal study phases. Women who were  
10 recruited and delivered and then had a subsequent pregnancy during this time period were also  
11 invited to participate for the second pregnancy, towards the goal of examining intra-individual  
12 differences in prenatal maternal and placental biology as a future research direction. The study  
13 protocol was approved by the University of Rochester School of Medicine and Dentistry (URMC)  
14 Internal Review Board and Rutgers University. All participants provided informed consent.  
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30 **Patient and public involvement:** No patient involvement.  
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35 **Overview of UPSIDE study activities:** Prenatal participation in UPSIDE consisted of face-to-  
36 face visits in each trimester, including biospecimen collection and questionnaires. At birth, the  
37 placenta and cord blood were collected, and the infant underwent a neonatal physical exam  
38 prior to hospital discharge. Additional postnatal visits occur when children are 1, 6, 12, 24, 36,  
39 and 48 months of age and biospecimen collection as well as observational and performance-  
40 based assessments of the child; parents complete questionnaires on child and family health and  
41 exposures. In general, data collection for UPSIDE followed several key principles: (1) repeated  
42 measures over time; (2) complementary biospecimen and questionnaire data collection; (3)  
43 ability to test multiple/competing hypotheses. Study activities and biospecimen collections are  
44 summarized in Tables 3 and 4, respectively, and described in greater detail below.  
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3 **Maternal constructs and measures:** At baseline, participants provided sociodemographic  
4 information; time-sensitive data (e.g. employment, marital status) were updated at each study  
5 visit. Additional measures relevant to psychosocial stress, biological pathways of interest, and  
6 child neurodevelopment were collected during pregnancy as described below. To assess  
7 possible timing effects of exposures as well as changes in maternal mental health across  
8 pregnancy, assessments were repeated in each trimester and, when applicable, at postnatal  
9 visits.  
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19 **Maternal psychosocial measures.** Anxiety was measured using the Penn State Worry  
20 Questionnaire (PSWQ)<sup>60</sup>. This 16-item self-report instrument targets symptoms of worry (e.g.,  
21 “my worries overwhelm me”) and has been successfully used with this population in our  
22 previous work<sup>61</sup> and others’<sup>62</sup>. Anxiety related specifically to pregnancy was measured using a  
23 7-item scale (adapted from Huizink et al.) that assesses specific worry about the health of the  
24 developing fetus and the birth process<sup>3</sup>. We included alternative measures of maternal mental  
25 health to examine the extent to which detected effects are specific to prenatal maternal anxiety.  
26 The 10-item Edinburgh Postnatal Depression Scale (EPDS) was administered in each trimester  
27 to assess symptoms of depression<sup>63</sup>. Domestic abuse and violence were assessed using a 5-  
28 item screener inquiring about events occurring in the past year and lifetime based on previously  
29 used tools<sup>64 65</sup>. To facilitate comparison with other study populations and assess global stress,  
30 the 14-item Perceived Stress Scale (PSS) was administered at all visits (prenatal and  
31 postnatal)<sup>66</sup>. In the 3<sup>rd</sup> trimester, mothers reported stressful life events occurring across  
32 pregnancy on a 26-item scale derived from that used in previous studies<sup>20 67</sup>. To assess  
33 potentially traumatic experiences occurring in childhood (age 0-17) that may impact adult health  
34 and well-being, the 10-item Adverse Childhood Experiences (ACEs) scale was administered in  
35 the third trimester<sup>68</sup>. Maternal discrimination was assessed using the Experiences of  
36 Discrimination (EOD) scale which includes Response to Unfair Treatment, Discrimination, and  
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3 Everyday Discrimination scales based on race and ethnicity only<sup>69</sup>. Maternal aggression was  
4 measured through the 29-item Buss Perry Aggression Scale which includes subscales for  
5 Anger, Hostility, Physical Aggression, and Verbal Aggression<sup>70</sup>. To evaluate neighborhood  
6 stressors, participants completed the City Stress Inventory, an 18-item scale focusing on  
7 neighborhood disorder and exposure to violence<sup>71</sup>. Social support was measured using a  
8 modified 30-item version of the Interpersonal Support Evaluation List (ISEL)<sup>72</sup>. Finally, we  
9 administered the Couples Satisfaction Index, a 4-item measure of relationship satisfaction<sup>73</sup>.

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19 **Maternal health behaviors and other covariates.** To complement the extensive maternal  
20 psychosocial assessments described above, mothers completed additional measures related to  
21 psychosocial stress and/or child neurodevelopment. **Physical Activity.** The Pregnancy Physical  
22 Activity Questionnaire (PPAQ), which assesses household/caregiving, occupational,  
23 sports/exercise, sedentary, light, moderate, and vigorous activities, was administered in order to  
24 estimate average weekly energy expenditure<sup>74</sup>. **Sleep.** The Pittsburgh Sleep Quality Index  
25 (PSQI), a 19-item questionnaire, assesses sleep disturbances and sleep quality over a 1-month  
26 time period<sup>75</sup>. **Diet.** Two 24-hour dietary recalls were collected during mid- to late pregnancy to  
27 assess nutrient intake. Participants were interviewed by a trained nutrition coordinator using the  
28 Automated Multiple-Pass Method developed by the United States Department of Agriculture<sup>76</sup>.  
29 The resulting data are analyzed using the Nutrition Data System for Research software (NDSR,  
30 2017 version, University of Minnesota Nutrition Coordinating Center, Minneapolis, MN)<sup>77</sup>. The  
31 National Cancer Institute method is then applied to estimate usual food and nutrient intake<sup>78</sup>.  
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47 **Cognition.** To assess maternal cognition, participants completed a mental rotation task, the 24-  
48 item Vandenberg-Kuse test<sup>79</sup>, during the third trimester of pregnancy. Additionally, mothers  
49 complete an abbreviated Verbal Comprehension subtest of the Wechsler Adult Intelligence  
50 Scale IV (WAIS-IV)<sup>80</sup>, at one time point during the postnatal period.  
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3 **Prenatal maternal biospecimen collection and analysis.** UPSIDE collects extensive  
4 maternal biospecimens as described below and in Table 4. In addition to the specific analyses  
5 described, for all sample types, additional aliquots are banked for future research. **Blood.** In  
6 each trimester, a 40mL blood sample was collected and processed to provide aliquots of serum,  
7 plasma, cells, and whole blood (3<sup>rd</sup> trimester only) for a variety of analyses. Ongoing analysis of  
8 these samples includes (1) sex steroid hormones (estrone, estradiol, estriol, testosterone, and  
9 free testosterone) using liquid chromatography with tandem mass spectrometry (LC-MS/MS)<sup>81</sup>;  
10 (2) placental corticotropin releasing hormone using radioimmunoassay<sup>82</sup>; (3) immune and  
11 related markers [e.g. high sensitivity cytokines, C-reactive protein (CRP), TGF-beta, angiogenic  
12 markers, and Mullerian inhibiting factor (MIF)]. Maternal blood was collected by labor and  
13 delivery nursing staff upon admission for delivery for additional assessment of immune markers.  
14 **Saliva.** Participants were trained to collect diurnal saliva for cortisol measurement using the  
15 standard passive drool procedures developed by the MacArthur Research Network on  
16 Socioeconomic Status and Health<sup>83</sup>. Samples (approximately 1 mL) were collected at home at  
17 five pre-determined points across the day (at wake-up, 45 minutes after wake-up, 2.5 hours  
18 after wake-up, 8 hours after wake-up, 12 hours after wake-up) on a single day in each trimester  
19 (for a total of 5 samples per trimester or 15 samples across the pregnancy). An additional  
20 passive drool saliva sample was collected by mothers at face-to-face visits and will be used to  
21 assess the oral microbiome. **Urine.** At each prenatal visit, a urine sample was collected, after  
22 which the specific gravity (dilution) and temperature of the sample were measured using a  
23 handheld refractometer (National Instrument Company, Inc., USA). Five mL are frozen for future  
24 use. **Buccal swab.** In the third trimester, a buccal cell sample for DNA analysis was collected by  
25 swabbing the inside of the participant's cheek (MAWI iSWAB, 250 series) after which samples  
26 were stored according to manufacturer guidelines. **Vaginal swab.** In the third trimester, when a  
27 vaginal swab is taken by the provider to test for the presence of Streptococcus B as part of

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3 standard obstetric care, an additional swab was collected for future analysis of the vaginal  
4 microbiome.  
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9 **Birth biospecimen collection and analysis.** Samples were collected at the time of delivery  
10 (usually within 1 hour) and banked at -80°C. Analyses including hormone and immune assays,  
11 environmental chemical assessments, and genetics are ongoing.  
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16 **Cord bloods.** Cord bloods were collected in two venues: (1) mixed cord bloods (up to 25mL)  
17 from the delivery room by delivery staff; and (2) fetal arterial and venous bloods (up to 30mL  
18 each) drawn from the placental vasculature by trained coordinators immediately following  
19 delivery. The bloods collected from the cord, umbilical vein, and umbilical artery were placed  
20 into additive free tubes, K2-EDTA tubes, and sodium heparin tubes, depending on the volume  
21 collected. Peripheral blood mononuclear cells (PBMCs) extracted using Ficoll-Paque and red  
22 blood cells (RBCs) reserved from processing cord bloods were stored in liquid nitrogen.  
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35 **Placental tissue collection.** Fresh core villous tissue was collected by a trained coordinator  
36 using a flap technique to leave the maternal decidua surface intact. For RNA analysis, two  
37 50mg tissue sections were washed in phosphate buffered saline, placed in cryovials, and flash  
38 frozen in liquid nitrogen. About 30g of additional placental tissue was extracted using the same  
39 technique and frozen unwashed in liquid nitrogen for other types of analyses.  
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48 **Placental pathology.** All placentae underwent a detailed pathology examination that included  
49 standard gross and histologic protocols as well as novel assessment of placental  
50 vascularization patterns on the chorionic plate. Using a standard digital camera with polarizing  
51 filters, a trained coordinator took a series of 2D photographs of the fresh tissue prior to core  
52 villous specimen sampling. From the 2D photographs, fetal vascular data will be extracted,  
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3 arterial and venous surface vascular networks mapped, and virtual slices created. Chorionic  
4 surface vasculature branching will be further analyzed using computer extraction techniques,  
5 yielding continuous measures including number of branch generations, number of branches off  
6 base of cord, number of branch points in network, and mean distance from end of artery to end  
7 of nearest vein.  
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15 Additional photos were taken after removal of the placental specimens to indicate locations of  
16 the collections. Weight was collected after removal of the cord and membranes using a  
17 standard scale, and cord length was measured (to the nearest 0.1cm) using a tape measure.  
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19 Cord twists were counted to measure the twist index. Biopsies of the cord were taken at the  
20 insertion site and 10cm from the insertion site, then placed in cassettes. A section of membrane  
21 was cut into a square and rolled, then placed in a cassette. Placentae were reviewed by a  
22 pathologist to assess for histology, anomalies, and infections. All tissues were placed into  
23 formalin for fixation for at least 72 hours. After fixation, the placentae were sectioned and  
24 images were obtained of each section. Biopsies of tissue from four quadrants, plus any  
25 additional abnormal tissues that were noted, were collected into cassettes for further analysis.  
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27 The remaining tissues were retained for future assessments.  
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41 **3D placental imaging.** Three dimensional digital scans of all placentae were collected to  
42 assess placental morphology. The custom-built scanner contains two webcams mounted on a  
43 bar providing binocular view, and a turntable on which the placenta is placed. The scanner took  
44 8 images of the top and bottom of the placenta and the software assembled these resulting 16  
45 images into a 3-D shape. Morphometric measures to be obtained from 3D images include  
46 estimated volume, surface area, thickness, shape, and symmetric difference.  
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3 **Prenatal and Birth Record Abstraction.** Clinical data was abstracted from the UPMC eRecord  
4 system. Prenatal record abstraction included medical, surgical, gynecological, and reproductive  
5 history, prenatal visit records, ultrasound measurements, and clinical lab values. Delivery chart  
6 abstraction included admission date and time, gestational age on admission, labor onset and  
7 duration, rupture of membranes, highest intrapartum temperature, group B streptococcus status,  
8 maternal white blood cells, delivery date and time, fetal position and mode of delivery,  
9 complications, medications, and maternal morbidity. In addition, relevant data from newborn  
10 nursery records were abstracted including birth weight and length, head circumference, APGAR  
11 scores, admission unit for baby, first recorded temperature, first recorded blood glucose, Kaiser  
12 sepsis risk score, cord arterial pH, neonatal resuscitation, and neonatal complications.  
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26 **Child study activities.** Child visits occur at birth as well as 1, 6, 12, 24, 36, and 48 months of  
27 age. Consistent with the study emphasis on longitudinal measures over time, many  
28 assessments are repeated at multiple time points, as age appropriate. At present, all birth and 1  
29 month visits have been completed, whereas 6, 12, 24, and 36 month visits are ongoing, and 48  
30 month visits will start in late 2020. Here we provide an overview of the child data collected  
31 across all of these timepoints by domain of interest.  
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41 **Neurodevelopment.** UPSIDE study children participate in an extensive battery of  
42 neurodevelopmental assessments (Table 3). Some of these assessments (e.g. Bayley Scales of  
43 Infant Development-III) have been widely used in hundreds of pediatric studies and provide  
44 global measures of development, whereas others are more targeted, selected based on  
45 previous work linking them to prenatal stress, inflammation, or sex steroid exposure. All  
46 assessments are based on direct observation (as opposed to maternal report), unless otherwise  
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3 Cognition and language. The Bayley Scales of Infant Development-III (BSID-III), a widely used  
4 tool for assessing mental and motor development in infants and toddlers, is administered at 6,  
5 12, and 24 months<sup>84</sup>. Of primary interest are the Cognitive scaled-score and the Language  
6 scaled-score, including both receptive and expressive communication subtests which assess  
7 memory, sensorimotor development, preverbal behaviors and communication and vocabulary  
8 development. At 24 months, complementary data on early vocabulary are obtained through the  
9 MacArthur-Bates Communicative Development Inventory: Words and Gestures which asks  
10 parents to mark on a checklist which phrases, words, and sound effects their child understands,  
11 says, or signs <sup>85</sup>. Executive Function is assessed at 24 and 36 months using age-appropriate  
12 standardized tasks that evaluate the child's working memory ("Spin the Pots"), impulse control  
13 ("Snack Delay"), and inhibitory control ("Reverse Categorization")<sup>86-88</sup>. At 36 and 48 months we  
14 administer additional tasks from the NIH ToolBox Early Childhood Cognition Battery including:  
15 Flanker Inhibitory Control and Attention Test (executive function and attention), Dimensional  
16 Change Card Sort Test (cognitive flexibility), Picture Sequence Memory (episodic memory), and  
17 Picture Vocabulary Test (language development)<sup>89-92</sup>. Finally, at age 4 years, the Wechsler  
18 Preschool and Primary Scale of Intelligence (WPPSI-IV) is administered to facilitate calculation  
19 of verbal, performance and full scale IQ <sup>93</sup>.

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40 Temperament and behavior. The Laboratory Temperament Assessment Battery (Lab-TAB) is  
41 an observational tool for examining multiple dimensions of infant temperament <sup>94</sup>. We administer  
42 Lab-TAB fear tasks involving the presentation of an unpredictable mechanical toy (6, 12, and 24  
43 months) and a remote-controlled spider (12 and 24 months). In the Anger/Frustration task (6  
44 and 12 months), the infant is allowed to engage with a novel toy and is subsequently interrupted  
45 by gentle arm restraint. At 6, 12, and 24 months, mothers complete the Infant Behavior  
46 Questionnaire – revised (IBQ-R), a 191-item questionnaire on infant temperament that assesses  
47 14 different dimensions (Approach, Vocal Reactivity, High Intensity Pleasure, Smile and  
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3 Laughter, Activity Level, Perceptual Sensitivity, Sadness, Distress to Limitations, Fear, Falling  
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5 Reactivity, Low Intensity Pleasure, Cuddliness, Duration of Orienting, Soothability, Social Fear,  
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7 Attentional Shifting)<sup>95</sup>. At 12 months we also administer the Strange Situation Procedure, widely  
8  
9 used to assess parent-infant attachment relationships<sup>96 97</sup>.

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13 Sexually dimorphic neurodevelopment. We administer a series of specialized tasks that, in  
14  
15 previous research, have demonstrated sex differences even in early infancy. At 1 and 6 months,  
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17 infants engage in a task assessing preferences for faces<sup>98</sup>. At 6, 12, and 24 months, children  
18  
19 complete a series of computer-based tasks assessing preferences for sex-stereotypical toys  
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21 (e.g. doll versus toy truck) and social stimuli<sup>99 100</sup>. A computerized mental rotation task  
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23 developed for use in infants is administered at 6, 12, 24, and 36 months<sup>101</sup>. Finally at 12 and 24  
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25 months, the child engages in an independent play task designed to assess preferences for  
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27 stereotypically female, male, and gender neutral toys<sup>102</sup>.

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32 Eating behaviors. Eating behaviors are assessed using multiple questionnaires and  
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34 observational tasks at the child's 36-month visit. The Children's Eating Behavior Questionnaire,  
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36 Preschool Adapted Liking Survey, and Comprehensive Feeding Practices Questionnaire are  
37  
38 completed by the parent to indicate the child's eating behaviors and parental influences, such as  
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40 food responsiveness, emotional overeating, food preference, food restriction, and family food  
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42 environment<sup>103-105</sup>. Food reinforcement is an observational task used to examine the reinforcing  
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44 value of food in children, which indicates motivation to eat<sup>106</sup>. A food ranking task was  
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46 developed to evaluate the child's ability to indicate his/her food preference by ranking pictures of  
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48 food items. To complement the eating behavior tasks and provide information on the child's  
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50 usual dietary intake, at the 36-month visit, the parent completes 24-hour dietary recalls for the  
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52 child (similar to the one completed by mothers during pregnancy).

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3 Neuroimaging. At 1 month of age, magnetic resonance imaging (MRI) is collected on a Siemens  
4 Prisma with a 32 channel head coil using a standard protocol that assesses anatomical scans,  
5 Diffusion Tensor Imaging, and Resting State Functional Connectivity. MRI is conducted while  
6 the infant is in a natural sleep. An second MRI will be performed at age 4. When the child is 24  
7 and 36 months, a electroencephalogram (EEG) assessment is conducted. In the EEG, select  
8 stimuli are presented and the brain's measured response, known as event-related potentials  
9 (ERP), is recorded. Our stimulus is an auditory event-related potential, called mismatch  
10 negativity (MMN). In the MMN assessment, repetitive sounds are interrupted by an occasional  
11 odd sound, that differs in frequency and duration.  
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24 **Anthropometry.** At each visit, child anthropometric measurements are collected by trained  
25 research coordinators. In general, measurements are collected in duplicate at each time point,  
26 with a third measurement obtained when the first two differ by more than a pre-specified  
27 amount. Weight, length, and head circumference are measured at every postnatal visit using  
28 standard protocols<sup>107</sup>. Weight (to the nearest 0.01kg) is measured using a Seca Infant Scale  
29 (Model #334). Length (to the nearest 0.1cm) is measured using a Seca Infantometer  
30 (Model#416), tape measure, or wall-mounted stadiometer (depending on age). Head  
31 circumference is measured by placing a tape measure just above eyebrows and wrapping it  
32 around the widest part of the head. Skinfold thicknesses (suprailiac, subscapular, and tricep)  
33 are obtained to the nearest 0.1 mm using calibrated Holtain calipers<sup>107 108</sup>. From birth to 24  
34 months, anogenital distance, a marker of prenatal androgen exposure, is measured to the  
35 nearest 0.1 mm using dial Vernier calipers<sup>109</sup>. A second purported measure of prenatal  
36 androgen exposure, second to fourth digit ratio (2D:4D) of the child's right hand is measured to  
37 the nearest 0.1 mm at 36 and 48 months also using dial Vernier calipers<sup>110</sup>. Finally, at 36 and 48  
38 months, waist circumference is measured to the nearest 0.1 cm by wrapping a tape measure  
39 around the body at the level of the umbilicus<sup>107</sup>.  
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5 **Child biospecimen collection.** Postnatal biospecimen collection occurs at each child visit  
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7 (Table 4). Analysis of immune and HPA axis markers is ongoing, whereas additional analysis of  
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9 banked biospecimens is pending. **Rectal swab.** At birth as well as 1, 6, 12, and 24 months, a  
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11 sterile swab applicator is dipped into sterile phosphate buffered saline, inserted into the child's  
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13 anal orifice up to the floxed portion of the swab, and stored in a conical tube for future  
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15 microbiome analyses<sup>111</sup>. **Buccal swab.** At birth, 12, 24, and 48 months, a buccal specimen is  
16  
17 collected for genetic analysis at least 60 minutes after the last recorded feeding. Both cheeks  
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19 are swabbed using a Mawi iSWAB collection kit (250 series) and the vial is stored according to  
20  
21 manufacturer guidelines. **Saliva.** At 6, 12, 24, and 48 months, saliva is collected to measure  
22  
23 cortisol using Salimetrics SalivaBio swabs. To assess cortisol response to a stressor (blood  
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25 draw at 6 months, strange situation task at 12 months, physical exam at 24 months) a series of  
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27 swabs are collected: at the start of the visit (6 and 24 months only), pre-stressor, 15 minutes  
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29 post-stressor, and 30 minutes post-stressor. At 24 and 48 months, an additional saliva swab is  
30  
31 collected for assessment of the oral microbiome. **Stool.** At 1 and 6 months, a stool sample is  
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33 collected at study visits using standard protocols (or by parents using an at-home collection kit if  
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35 sample collection at the visit is not possible). **Urine.** Urine is collected at the 1, 6, and 12 month  
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37 visits using an Earth's Best chlorine free diaper equipped with either a urine collection bag  
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39 and/or sterile cotton balls. **Blood.** At 6, 12, and 48 months, approximately 10mL of blood is  
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41 collected by a pediatric nurse (4 mL in a K2-EDTA tube and 3 mL in each of 2 sodium heparin  
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43 tubes at 6 and 12 months, 8mL into a sodium heparin tube, 2mL into a K2-EDTA tube, and 3mL  
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45 into a tube without additives at 48 months). **Nails.** At 12, 24, and 36 months, fingernails and  
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47 toenails are collected at study visits (or by parents using an at-home collection kit). **Breastmilk.**  
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49 Mothers who are breastfeeding at 1 and 6 months provide a breastmilk sample (up to 45mL).  
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51 Mothers collect the sample during the visit (when possible) using a new, sterile Harmony  
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3 Medela manual breast pump. When that is not possible, mothers bring in a breastmilk sample  
4 that was collected at home.  
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### 8 9 **FINDINGS TO DATE:**

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11 In total, 312 women were recruited into the study, of whom 14 enrolled for more than one  
12 pregnancy, resulting in a total of 326 study pregnancies (Table 1; Figure 1). Of these, 294  
13 mothers gave birth to an infant in the study. The 32 women who signed informed consent but  
14 did not give birth to a study infant included 3 screen failures, 5 pregnancy losses, 8 who became  
15 ineligible during pregnancy, 2 who were lost to follow up, and 14 who chose to withdraw. Here  
16 we report on the mothers who gave birth to an infant in the study.  
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26 On average, women were  $28.9 \pm 4.7$  years old at recruitment and the majority are White (61.2%)  
27 and Black (25.5%), with 9.9% reporting Hispanic ethnicity. The participants are  
28 socioeconomically diverse with a 34.2% having a high school degree or less, while 25.7% had  
29 obtained a post-college degree. At the time of recruitment 74.9% were employed and 60.3%  
30 were married or living as married. Self-reported alcohol use and smoking during pregnancy  
31 were both relatively uncommon (3.4% and 7.8%, respectively).  
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41 The cohort was mildly psychosocially risk enriched and overall symptoms were stable across  
42 trimesters with ICC's for the various psychosocial scales ranging from 0.68-0.80 (Table 2).  
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44 There was a small increase in average EPDS scores across pregnancy, however the proportion  
45 of women scoring  $\geq 13$  (the most commonly used cutoff indicating possible depression) was  
46 stable across pregnancy (9.5-10.6% across trimesters). Anxiety (as assessed by the PSWQ)  
47 was similarly stable. At baseline, 36%, 44%, and 14% of women were classified as having low,  
48 moderate, and high levels of worry, and these proportions remained consistent across  
49 pregnancy. Women typically worried less about their fetuses after the first trimester, with slight  
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3 increases in worry about delivery over time. In the third trimester, 67.9% of women reported at  
4 least one ACE, with 16.7% reporting four or more. Similarly, the average number of stressful life  
5 events during pregnancy was relatively low ( $2.46 \pm 2.80$ ), however there was great inter-  
6 individual variation such that some women reported up to 17 events. By design, this was a low-  
7 medical risk cohort at recruitment therefore relatively few participants had major pregnancy  
8 complications including hypertensive disorders of pregnancy (n=22, 7.5%) and gestational  
9 diabetes (n=6; 2.0%). The average gestational age at birth was 39.5 weeks and only 14 (4.7%)  
10 babies were born preterm.  
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22 Success rates for biospecimen collection were consistently high across trimesters (blood: 95-  
23 99%; urine 94-97%; saliva 96-100%). In addition, 272 mothers (93%) provided a buccal swab  
24 for DNA analysis and 258 mothers (75%) provided a vaginal swab. At birth, the placenta and  
25 cord blood were obtained in 96% and 88% of women, respectively. Finally, 277 infants (94%)  
26 participated in an exam at birth; of these 271 (98%) provided a buccal swab and a rectal swab  
27 (for microbial analysis) was obtained on 261 (94%). One-month visits were recently completed  
28 with additional postnatal visits at 6, 12, 24, 36, and 48 months underway.  
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### 39 **STRENGTHS AND LIMITATIONS:**

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41 There are several notable strengths of the UPSIDE study. The first is our extensive, rigorous  
42 biospecimen collection starting in the first trimester and continuing throughout pregnancy. This  
43 is made possible by focusing recruitment and prenatal visits primarily at a small set of obstetric  
44 clinics located in close proximity to the labs where processing and storage occurs. Similarly,  
45 birth biospecimen collection (placenta and cord blood) is a strength and based on our  
46 experience, a large “SWAT” team is needed for around the clock collection and processing of  
47 placentae within 3 hours after birth (ideally within 1 hour). The need for a 24/7 dedicated on call  
48 team is further illustrated by the fact that the majority of study births occurred outside of  
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3 business hours (Figure 2). Similarly, we implemented multiple mechanisms to identify when  
4 study participants were admitted for delivery. The successful strategies discussed above  
5 allowed UPSIDE to acquire 96% of placentae. Our extensive placental assessments and  
6 biorepository will provide exceptionally rich data on both morphology and molecular biology,  
7 allowing us to address novel questions about the placenta in relation to maternal exposures and  
8 child outcomes.  
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18 Another strength of the study is our intensive longitudinal follow-up of mother-child dyads with a  
19 focus on repeated measures to assess intra-individual changes over time. In the prenatal  
20 period, for example, our extensive phenotyping of mothers occurs at three time points, allowing  
21 us to look at trimester-specific impacts and to potentially differentiate between different domains  
22 of psychosocial stress (e.g. anxiety, depression, stressful life events). During the postnatal  
23 period, we again adopt this model, with seven visits occurring from birth to age 4. This intensive  
24 visit schedule allows us to assess domains of neurodevelopment (as age-appropriate) over time  
25 and facilitates serial collection of relevant biospecimens that may yield mechanistic insights. Our  
26 use of standard measures of development and cognition (such as the WPPSI and BSID-III),  
27 moreover, is complemented by tools developed to examine more specific aspects of  
28 development that are plausibly linked to our pathways of interest (e.g. social preferences and  
29 sex-typical play behavior in relation to sex steroids).  
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45 At the same time, there are several limitations of note. The deliberate recruitment of low-medical  
46 risk pregnancies means that we are underpowered to test hypotheses regarding pregnancy  
47 complications or outcomes. Similarly, our relatively small sample size and overall healthy  
48 population precludes examining pediatric clinical outcomes such as birth defects, autism  
49 spectrum disorders or developmental delays; however, our ability to look at continuous  
50 measures of development will yield insights into neurodevelopmental variation within the typical  
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3 spectrum. Finally, given evidence that preterm infants develop along a very different trajectory  
4 than term infants, preterm infants were not included in postnatal follow-up, so we cannot look at  
5 outcomes in this special group.  
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### 10 11 **COLLABORATION:**

12 Interested investigators may contact the PIs (EB, TO'C) in writing regarding potential  
13 collaborations involving data and/or biospecimens from the UPSIDE study. Potential  
14 collaborators will be asked to write a concept proposal for their proposed analysis which will be  
15 reviewed by the UPSIDE Executive Committee. After concept proposal approval, collaborators  
16 will submit analysis plans and proof of IRB approval to the Executive Committee prior to  
17 receiving data/samples. Requests for collaborations will be considered on an ongoing basis;  
18 however, in general, external collaborations will be started once the primary study aims have  
19 been addressed.  
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### 31 32 **FUTURE DIRECTIONS:**

33 The rich biospecimen collection and intensive phenotyping of the UPSIDE cohort lends itself to  
34 a wide range of ancillary studies and future directions. At present, ancillary studies to examine  
35 environmental chemicals (synthetic chemicals and metals) and air pollution in relation to  
36 placental function, perinatal outcomes, and infant development are in progress. Also underway  
37 is a complementary study of participating mothers looking at trajectories of cardiometabolic  
38 health from early pregnancy through three years postpartum. Additional studies are planned to  
39 examine the microbiome (vaginal, oral, gut, and breastmilk) in relation to various endpoints  
40 including pediatric oral health and neurodevelopment. As part of the ECHO consortium, data  
41 and biospecimens from our cohort are harmonized with up to 50,000 other participating mother-  
42 child dyads from cohort studies around the U.S. with the goal of providing novel insights into  
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3 factors that shape multiple facets of children's health (including pre-, peri-, and postnatal  
4 outcomes, upper and lower airway health, obesity, neurodevelopment, and positive health).  
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9 **Author affiliations.** All authors (with the exception of CS) are affiliated with the University of  
10 Rochester School of Medicine and Dentistry. The senior author (EB) also holds an appointment  
11 at Rutgers School of Public Health. CS's primary affiliation is Placental Analytics.  
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16  
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55 **Competing interests.** None declared.  
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5 **Patient consent for publication.** Not required.  
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9 **Ethics approval.** This study was approved by the University of Rochester School of Medicine  
10 and Dentistry Institutional Review Board (#58456) and the Rutgers University Institutional  
11 Review Board (PRO20160001514).  
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18 **Provenance and peer review.** Not commissioned; externally peer reviewed.  
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22 **Data availability statement.** Once complete cohort data has been collected and cleaned, it will  
23 be available pending approved concept proposal, analysis plan, and documentation of IRB  
24 approval.  
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**Table 1. Baseline characteristics of mother-child dyads participating in UPSIDE (n=294<sup>1</sup>).**

	Mean (SD)	Min-Max	n (%)
<b>Maternal characteristics<sup>2</sup></b>			
<b>Continuous/ordinal</b>			
Age (years)	28.9 (4.7)	18-41	
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.2 (6.9)	16.98-49.77	
Poverty to income ratio	3.8 (3.9)	0.04-44.6	
Household size (persons)	3.2 (1.5)	1-11	
Sleep (Pittsburgh Sleep Index)	6.3 (3.5)	0-17	
<b>Categorical</b>			
Race			
White			180 (61.2)
Black			75 (25.5)
Asian			11 (3.7)
Mixed Race			8 (2.7)
Other <sup>3</sup>			20 (6.8)
Hispanic			29 (9.9)
Education			
<High school			8 (2.9)
High school			85 (31.3)
Some college			39 (14.3)
College degree			70 (25.7)
Post-college degree			70 (25.7)
Employed			215 (74.9)
Married/living as married			173 (60.3)
Medicaid status			110 (42.1)
Nulliparous			82 (31.3)
Smoking during pregnancy (any)			23 (7.8)
Alcohol use during pregnancy (any)			10 (3.4)
<b>Paternal characteristics<sup>2</sup></b>			
Age (years)	30.9 (5.6)	18 – 57	
Race			
White			170 (59.9)
Black			79 (27.8)
Asian			5 (1.8)
Other <sup>3</sup>			30 (10.6)
<b>Infant characteristics</b>			
Female			152 (51.7)
Gestational age at birth (weeks)	39.5 (1.6)	27.7-42.7	
Birth weight (g)	3352.8 (495.0)	2195-4654	
Birth length (cm)	51.1 (3.1)	40-60	

1 n's for individual variables may differ slightly due to missing data

2 At time of enrollment, with the exception of Sleep which was assessed in the second trimester.

3 "Other" includes American Indian/Alaska Native and individuals self-reporting as "other".

**Table 2. Psychosocial assessments repeated across pregnancy.**

Scale	Trimester 1	Trimester 2	Trimester 3	ICC
	mean±SD; (range)	mean±SD; (range)	mean±SD; (range)	
Depressive Symptoms (EPDS)	5.56 ± 4.73 (0-21)	5.94 ± 4.93 (0-23)	5.95 ± 5.13 (0-29)	.73
Anxiety Symptoms (PSWQ)	44.41 ± 13.36 (16-77)	44.09 ± 13.72 (17-80)	44.31 ± 13.69 (16-80)	.80
Pregnancy Specific Anxiety				
Worries about the baby	7.97 ± 4.3 (4-20)	7.24 ± 3.77 (4-20)	7.18 ± 3.99 (4-20)	.68
Worries about delivery	6.96 ± 3.64 (3-15)	6.65 ± 3.46 (3-15)	7.19 ± 3.47 (3-15)	.72
Perceived Stress (PSS)	19.9 ± 8.11 (2-42)	20.03 ± 8.1 (0-49)	19.83 ± 8.03 (2-39)	.77

Abbreviations: EPDS: Edinburgh Postnatal Depression Scale; ICC: Interclass correlation coefficient; PSS: Perceived Stress; PSWQ: Penn State Worry Questionnaire

**Table 3. Summary of UPSIDE child assessments.**

	Age at assessment (months)						
	birth	1	6	12	24	36	48
<b>Anthropometric measures</b>							
Height/weight	x	x	x	x	x	x	x
Head circumference	x	x	x	x	x	x	x
Skinfold thickness	x	x	x	x	x	x	x
Anogenital distance	x	x	x	x	x		
Waist circumference						x	x
Digit ratio						x	x
<b>Neurodevelopmental measures (by domain)</b>							
<i>Temperament</i>							
Rothbart- IBQ			x	x	x		
Lab-TAB			x	x	x		
<i>Cognition/language/EF</i>							
BSID			x	x	x		
WPPSI-IV							x
Macarthur Bates					x	x	
NIH Toolbox					x	x	
Executive function					x	x	
<i>Neuroimaging</i>							
MRI		x					x
EEG					x	x	
<i>Sex-typical/dimorphic</i>							
Face preference		x	x				
Toy preference			x	x	x		
Social preference			x	x	x		
Mental rotation			x	x	x	x	
Sex-typical play behavior				x	x		
<b>Lifestyle measures</b>							
Sleep		x	x	x	x	x	x
<i>Diet</i>							
Infant feeding questionnaire		x	x	x			
24-hour dietary recall						x	
Eating behavior						x	

Abbreviations: BSID: Bayley Scales of Infant Development; EEG: Electroencephalogram; EF: Executive function; IBQ: Infant Behavior Questionnaire; MRI: Magnetic Resonance Imaging; NIH: National Institutes of Health; WPPSI: Wechsler Preschool and Primary Scale of Intelligence

**Table 4. Summary of UPSIDE biospecimen collections.**

	Prenatal (trimesters)			Birth	Infant/Child postnatal (months)					
	1	2	3		1	6	12	24	36	48
Blood										
Serum	M	M	M	UC						
Plasma	M	M	M	UC		C	C			C
Whole blood			M							
Red blood cells	M	M	M			C	C			C
Urine	M	M	M		C	C	C			C
Saliva										
Diurnal (5x/day)	M	M	M							
Stress response						C	C	C		C
Oral microbiome	M	M	M			C	C	C		C
Vaginal swab			M							
Placental tissue				P						
Buccal swab			M	C			C	C		
Rectal swab				C	C	C	C	C		
Stool					C	C				
Breast milk					M	M				
Nails							C	C		

M=maternal; C=child; UC=umbilical cord (artery and vein); P=placenta

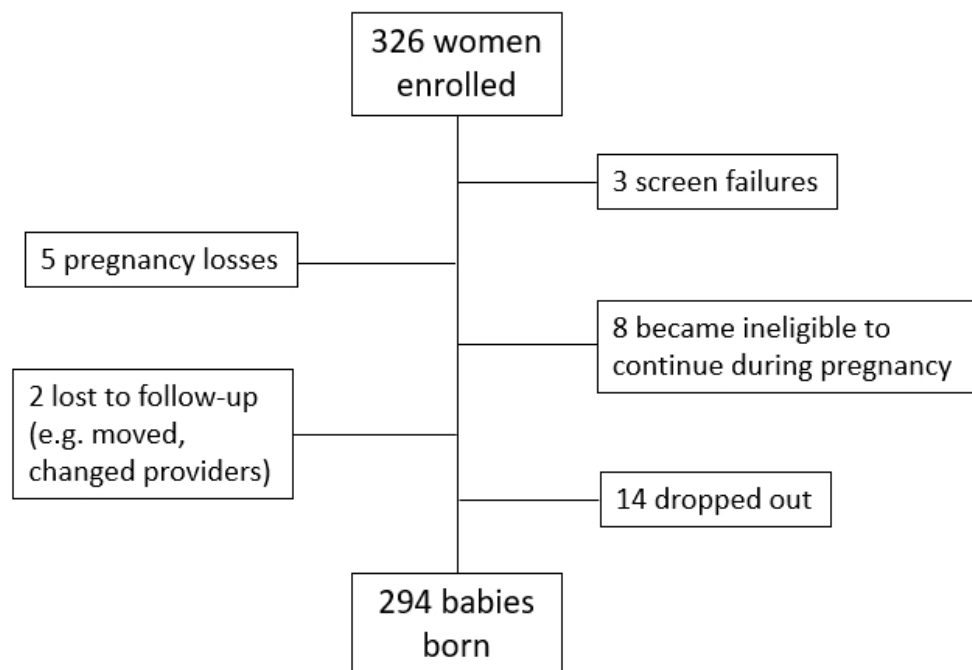
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3 **Figure 1. Consort figure on recruitment and retention in the UPSIDE study (n=326).**  
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**Figure 2. Timing of placental delivery in relation to standard work hours (spontaneous births only).**

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Figure 1. Consort figure on recruitment and retention in the UPSIDE study (n=326).



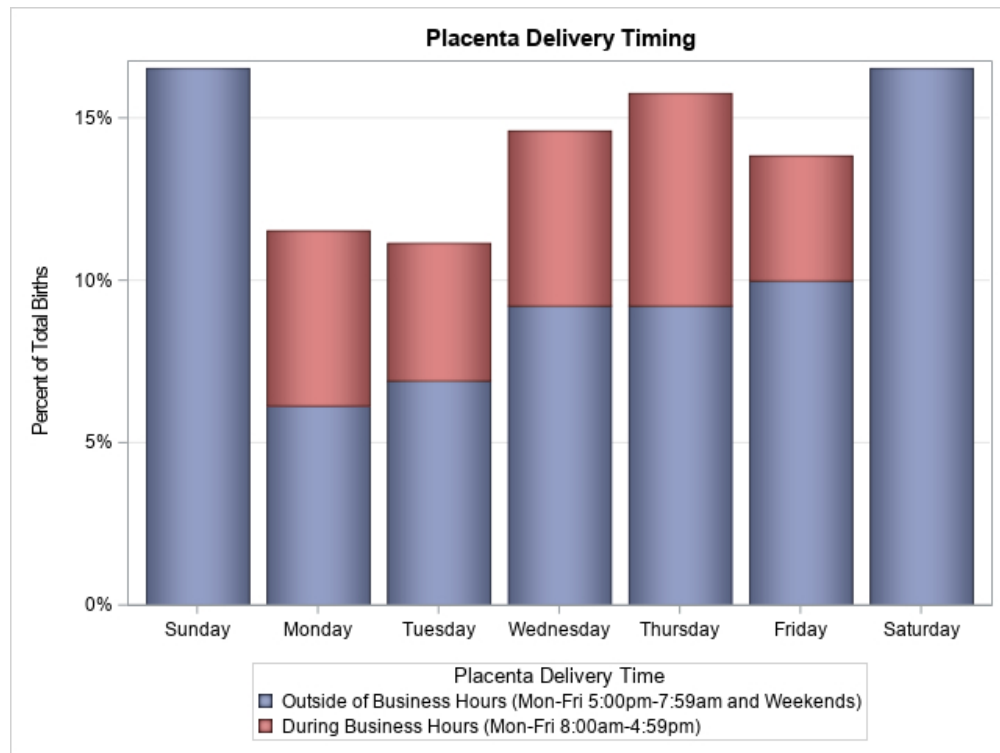


Figure 2. Timing of placental delivery in relation to standard work hours (spontaneous births only).

# BMJ Open

## Cohort profile: Understanding Pregnancy Signals and Infant Development (UPSIDE), a pregnancy cohort study on prenatal exposure mechanisms for child health

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Secondary Subject Heading:	Epidemiology, Paediatrics, Obstetrics and gynaecology
Keywords:	EPIDEMIOLOGY, OBSTETRICS, PAEDIATRICS, PUBLIC HEALTH

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**Cohort profile: Understanding Pregnancy Signals and Infant Development (UPSIDE), a pregnancy cohort study on prenatal exposure mechanisms for child health**

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3 39 **ABSTRACT**

4 40 **Purpose:** Extensive research suggests that maternal prenatal distress is reliably related to  
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6 41 perinatal and child health outcomes – which may persist into adulthood. However, basic  
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8 42 questions remain regarding mechanisms involved. To better understand these mechanisms, we  
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10 43 developed the Understanding Pregnancy Signals and Infant Development (UPSIDE) cohort  
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12 44 study, which has several distinguishing features, including repeated assessments across  
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14 45 trimesters, analysis of multiple biological pathways of interest, and incorporation of placental  
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16 46 structure and function as mediators of child health outcomes.  
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22 48 **Participants: Women with normal risk pregnancies** were recruited at <14 weeks gestation.  
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24 49 Study visits occurred in each trimester and included extensive psychological, socio-  
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26 50 demographic, health behavior, and biospecimen collection. Placenta and cord blood were  
27  
28 51 collected at birth. Child visits (ongoing) occur at birth and 1, 6, 12, 24, 36, and 48 months of age  
29  
30 52 and use standard anthropometric, clinical, behavioral, biological, and neuroimaging methods to  
31  
32 53 assess child physical and neurodevelopment.  
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36 54  
37 55 **Findings to date:** We recruited 326 pregnancies; 294 (90%) were retained through birth.  
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39 56 Success rates for prenatal biospecimen collection were high across all trimesters (96-99% for  
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41 57 blood, 94-97% for urine, 96-99% for saliva, 96% of placentas, 88% for cord blood, and 93% for  
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43 58 buccal swab). Ninety-four percent of eligible babies (n=277) participated in a birth exam;  
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45 59 postnatal visits are ongoing.  
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48 60  
49 61 **Future plans:** The current phase of the study follows children through age 4 to examine child  
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51 62 neurodevelopment and physical development. In addition, the cohort participates in the National  
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53 63 Institutes of Health's Environmental influences on Child Health Outcomes (ECHO) program, a  
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55 64 national study of 50,000 families examining early environmental influences on perinatal  
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3 65 outcomes, neurodevelopment, obesity, and airway disease. Future research will leverage the  
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5 66 rich repository of biological samples and clinical data to expand research on the mechanisms of  
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7 67 child health outcomes in relation to environmental chemical exposures, genetics, and the  
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9 68 microbiome.  
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14 70 **Strengths and Limitations:**

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16 71 • The UPSIDE cohort features intensive, serial biospecimen and questionnaire collection  
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18 72 from the first trimester of pregnancy through age 4 years that will allow us to test  
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20 73 hypotheses regarding the pathways by which maternal distress impacts children's  
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22 74 physical and neurodevelopment during critical and sensitive periods.  
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24 75 • Our comprehensive assessment of the placenta, including both morphometric and  
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26 76 molecular markers, provides novel data on the role that this under-studied organ plays  
27  
28 77 as a mediator of the association between maternal exposures and child outcomes.  
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31 78 • The study is not designed to assess clinical phenotypes in pregnant women (e.g.,  
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33 79 preeclampsia) and children (e.g., autism)..  
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3 81 **INTRODUCTION:**  
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7 83 For several decades, epidemiological studies have provided robust evidence of an association  
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9 84 between maternal prenatal distress and child health outcomes<sup>1-6</sup>. The large and growing  
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11 85 collection of studies that has emerged from both high-income and low/middle-income countries,  
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13 86 suggests that prenatal maternal distress is a plausible illustration of a “developmental  
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15 87 programming effect” on child health outcomes<sup>7-12</sup>. The developmental programming model  
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17 88 proposes that *in utero* exposures instigate an adaptive response in the fetus/child that is carried  
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19 89 forward in development and has persisting effects on behavior and biology<sup>13-18</sup>. Central to this  
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21 90 hypothesis is the concept that early exposures have a privileged – or different – effect on  
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23 91 biological systems than those occurring later in development<sup>19</sup>. In other words, when exposures  
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25 92 occur very early in development, physiology may change (either adaptively or pathologically)  
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27 93 resulting in long-lasting or permanent impacts on health and well-being. A classic example of  
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29 94 this is the “thrifty phenotype” whereby nutrient deprivation during prenatal development may  
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31 95 lead to reduced fetal growth and metabolic changes to conserve energy<sup>20</sup>. In the presence of  
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33 96 subsequent nutrient surplus (characteristic of the modern Western diet), this metabolic  
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35 97 conservation may lead to obesity and metabolic disease<sup>21</sup>. The resulting clinical and public  
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37 98 health implications of this model are substantial because they suggest that the timing of  
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39 99 intervention may be as important as its content. The aim of this paper is to introduce a new  
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43 100 cohort, Understanding Pregnancy Signals and Infant Development (UPSIDE), which includes  
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45 101 several measurement and design advantages to advance our understanding of maternal  
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47 102 prenatal psychosocial distress and child health outcomes.  
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51 104 A key exposure variable in this large collection of studies may be broadly interpreted as prenatal  
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53 105 maternal psychological distress. Assessment of maternal psychological distress can derive from  
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55 106 many sources, including clinical interviews as well as maternal self-report inventories of anxiety,  
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3 107 depressive symptoms, trauma, major life-event stressors, and “pregnancy-specific” worry. The  
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5 108 persistence of reported impacts of maternal psychological distress on child outcomes, despite  
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7 109 variation in exposure measurement, suggests that the association is robust<sup>22-27</sup>. We adopt the  
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9 110 term “distress” when referring in general to this research and identify specific measures in the  
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11 111 research protocol that index this broader construct. The corollary – how maternal experience of  
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13 112 or exposure to distress creates an exposure variable for the fetus – is far less clear and likely  
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15 113 involves multiple, interdependent biological pathways. That is, if distress is indeed a causal  
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17 114 factor, it may operate via neuroendocrine, immune, autonomic, or other physiological  
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19 115 mechanisms<sup>28-31</sup>. Initial research on the mediating mechanisms, based on strong evidence from  
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21 116 experimental animal studies, targeted the hypothalamic-pituitary-adrenal (HPA) axis<sup>32</sup>. The  
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23 117 biological case for its involvement in the stress response (most typically in the form of cortisol,  
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25 118 the downstream product of HPA activation) is certain, its transplacental transfer is well-  
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27 119 established, and the application to placental mechanisms is evident (placental enzyme 11- $\beta$ -  
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29 120 HSD2)<sup>33-34</sup>. Nonetheless, that biological model is too limited. Human studies have not provided  
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31 121 consistent evidence that prenatal maternal distress impacts child development through HPA-  
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33 122 related mechanisms<sup>32-35-39</sup>. Moreover, several lines of research raise alternative mechanisms.  
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35 123 One of the most important of these is maternal inflammation, represented by research on the  
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37 124 Maternal Immune Activation (MIA) model. Research findings show that circulating  
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39 125 proinflammatory markers in pregnancy predict an increased risk of significant  
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41 126 neurodevelopmental problems in the child<sup>40-45</sup>. Other studies indicate that prenatal sex steroids  
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43 127 may also be a plausible predictor of child development<sup>46</sup> and may be confounded with stress  
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45 128 physiology<sup>47-50</sup>. Although many current and past pregnancy cohort studies have examined the  
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47 129 relationship between maternal psychosocial measures and child outcomes, few have gone  
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49 130 beyond the HPA axis to examine additional biological pathways. Accordingly, a first major  
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51 131 methodological and conceptual strength of the UPSIDE study is the assessment of biomarkers  
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3 132 relevant to alternative pathways (e.g. cytokine profiles, steroidogenic activity) across pregnancy  
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5 133 and in multiple biological sample types (e.g. maternal blood, cord blood, placenta).  
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9 135 A second key feature of the UPSIDE study is its focus on and intensive assessment of the  
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11 136 maternal-fetal-placenta unit. Despite the placenta's critical role in transmitting maternal signals  
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13 137 to the developing fetus, direct measurement of the placenta has been notably absent from the  
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15 138 vast majority of studies on prenatal distress and child development<sup>51-52</sup>. There are both practical  
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17 139 and scientific reasons for the limited research that integrates interrogation of the placenta in  
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19 140 studies of prenatal exposures and child outcomes. The practical matter concerns sample  
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21 141 collection and processing, particularly the 24/7 coverage that this requires if spontaneous  
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23 142 deliveries are included. Scientifically, there is variability among studies in which placental  
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25 143 markers are assessed; placenta weight, gene expression, and epigenetics have all received  
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27 144 some attention, almost always in separate reports. In this cohort study, we expand direct,  
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29 145 comprehensive measurement of the placenta to advance the field in several important ways.  
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31 146 First, it is increasingly clear that the placenta contributes to maternal perinatal health<sup>53-55</sup>, with  
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33 147 clear implications for neonatal and, by extension, child health. It is also becoming more widely  
34  
35 148 appreciated that prenatal exposures (including maternal distress but also environmental  
36  
37 149 exposures) may alter placenta structure and function<sup>56-58</sup>. Finally, there is now a growing  
38  
39 150 evidence base linking placenta measures to child outcomes such as obesity and  
40  
41 151 neurodevelopment<sup>59-61</sup>. What has been missing from this field are prospective pregnancy cohort  
42  
43 152 studies that track mother-child dyads from early gestation through early childhood and  
44  
45 153 incorporate placenta mechanisms. To that end, the UPSIDE study includes extensive  
46  
47 154 measurement of placenta structure and function from imaging, histology, and  
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49 155 immunohistochemistry, genetics, and pathology reports.  
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3 157 In addition to these conceptual advances, UPSIDE includes several key design elements that  
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5 158 will inform our study of the multiple physiological pathways by which maternal psychological  
6  
7 159 stress may impact child development. These include: (1) serial maternal questionnaire and  
8  
9 160 biomarker data across all trimesters to examine critical and sensitive windows of gestation; (2)  
10  
11 161 pediatric visits at seven time points from birth to 4 years of age to assess neurodevelopment as  
12  
13 162 well as growth, reproductive development, and HPA axis activity; (3) consideration of potentially  
14  
15 163 important covariates and confounders that are sometimes overlooked in studies of child  
16  
17 164 development (e.g. maternal and child diet, physical activity, sleep).  
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21  
22 166 *Primary aims:* UPSIDE is funded through several major research grants that have informed the  
23  
24 167 design of the study and included activities. The funding stream that started the cohort  
25  
26 168 (R01HD083369) had the over-arching goal of testing the hypothesis that prenatal maternal  
27  
28 169 anxiety programs sex steroid pathways leading to changes in placental structure and function,  
29  
30 170 and ultimately sex differences in physical, neurocognitive, and social behaviors in infancy  
31  
32 171 through 12 months of age. Soon after, additional study activities were funded through the NIH's  
33  
34 172 ECHO program the largest American study of early childhood health and development ever  
35  
36 173 undertaken, with up to 50,000 participating mother-child dyads from cohort studies around the  
37  
38 174 U.S. (UG3/UH3OD023349). The ECHO funding allowed us to expand the contributions of the  
39  
40 175 cohort to consider inflammatory mechanisms, extend child follow-up to age 4, and add a more  
41  
42 176 intensive battery of outcome measures. Additionally, as part of ECHO, data and biospecimens  
43  
44 177 from UPSIDE are harmonized with those of the other participating cohorts in order to address  
45  
46 178 ECHO-wide scientific priorities<sup>62</sup>. With multiple biological pathways of interest now considered in  
47  
48 179 UPSIDE, we are well poised to test competing hypotheses about the biological mechanisms by  
49  
50 180 which maternal distress impacts children's development. The broad Aims that guide research in  
51  
52 181 this cohort are described below.  
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- 182 1. Identify evidence of prenatal maternal distress-related alterations in HPA,  
183 inflammatory, and sex steroid hormone pathways in the placenta and cord blood.
- 184 2. Examine prenatal distress and sex steroid activity (in mother, placenta, and cord  
185 blood) in relation to sex-dependent physical and neurodevelopment.
- 186 3. Examine prenatal distress and inflammatory markers (in mother, placenta, cord  
187 blood, and infant) in relation to measures of neurodevelopment (neurocognitive and  
188 behavioral measurements, brain imaging, and EEG) and child physical development  
189 and adiposity (birth to age 4).

190

## 191 **COHORT DESCRIPTION:**

192

193 **Study setting and recruitment:** From December 2015-April 2019, women were recruited  
194 during their first trimester of pregnancy from outpatient obstetric clinics affiliated with the  
195 University of Rochester. Eligibility criteria included: <14 weeks gestation, age 18 or older,  
196 singleton pregnancy, no known substance abuse problems or a history of psychotic illness, and  
197 ability to communicate in English. Women with major endocrine disorders (such as polycystic  
198 ovary syndrome), high-risk pregnancies, or significant obstetric problems were excluded. Infants  
199 born prior to 37 weeks gestation were not included in postnatal study phases. No screening for  
200 distress was conducted prior to consent; instead we recruited from clinics who serve women at  
201 high psychosocial risk. Women who were recruited and delivered and then had a subsequent  
202 pregnancy during this time period were also invited to participate for the second pregnancy,  
203 towards the goal of examining intra-individual differences in prenatal maternal and placental  
204 biology as a future research direction. The study protocol was approved by the University of  
205 Rochester School of Medicine and Dentistry (URMC) Internal Review Board and Rutgers  
206 University. All participants provided informed consent.

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3 208 **Patient and public involvement:** No patient involvement.  
4

5 209  
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7 210 **Overview of UPSIDE study activities:** Prenatal participation in UPSIDE consisted of face-to-  
8  
9 211 face visits in each trimester, including biospecimen collection and questionnaires. At birth, the  
10  
11 212 placenta and cord blood were collected, and the infant underwent a neonatal physical exam  
12  
13 213 prior to hospital discharge. Additional postnatal visits (ongoing) occur when children are 1, 6, 12,  
14  
15 214 24, 36, and 48 months of age and include biospecimen collection as well as observational and  
16  
17 215 performance-based assessments of the child; parents complete questionnaires on child and  
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19 216 family health and exposures. Child outcome timepoints were chosen based on consideration of  
20  
21 217 several key criteria: 1) developmental milestones and critical windows; 2) coincidence with  
22  
23 218 routine well-child appointments; 3) spacing of visits to allow for repeated measures within  
24  
25 219 domains over time, while minimizing participant burden and loss to follow-up; and 4) constraints  
26  
27 220 of funding timelines. In general, data collection for UPSIDE follows several key principles: (1)  
28  
29 221 repeated measures over time; (2) complementary biospecimen and questionnaire data  
30  
31 222 collection; (3) ability to test multiple/competing hypotheses. Biospecimen collection and study  
32  
33 223 activities are summarized in Tables 1 and 2, respectively, and described in greater detail below.  
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38 225 **Maternal survey measures:** At baseline, participants provided sociodemographic information;  
39  
40 226 time-sensitive data (e.g., employment, marital status) were updated at each study visit.  
41  
42 227 Additional measures relevant to psychological distress, psychosocial risk, and the biological  
43  
44 228 pathways of interest were collected during pregnancy as described below. To assess possible  
45  
46 229 timing effects of exposures as well as changes in maternal distress across pregnancy,  
47  
48 230 assessments were repeated in each trimester and, when applicable, at postnatal visits.  
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52 231 **Key maternal distress measures.** Anxiety, our main measure of maternal distress, was  
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54 232 measured using the Penn State Worry Questionnaire (PSWQ)<sup>63</sup>. This 16-item self-report  
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3 233 instrument targets symptoms of worry (e.g., “my worries overwhelm me”) and has been  
4  
5 234 successfully used with this population in our previous work<sup>64</sup> and others’<sup>65</sup>. We included  
6  
7 235 measures of complementary constructs that capture other aspects of maternal distress that had  
8  
9 236 been used in previous studies. These included scales assessing pregnancy-specific anxiety<sup>3</sup>,  
10  
11 237 depression<sup>66</sup>, domestic abuse and violence<sup>67 68</sup>, global stress<sup>69</sup>, stressful life events<sup>22 70</sup>,  
12  
13 238 adverse childhood experiences (ACEs)<sup>71</sup>, discrimination<sup>72</sup>, aggression<sup>73</sup>, and neighborhood  
14  
15 239 stress<sup>74</sup>. To complement these measures of distress, we additionally assessed social support<sup>75</sup>  
16  
17 240 and relationship satisfaction<sup>76</sup>.

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21 241 **Covariates assessed by maternal surveys.** To complement the extensive maternal distress  
22  
23 242 measures described above, at prenatal visits mothers completed additional measures on related  
24  
25 243 to psychosocial stress and/or child neurodevelopment. These will be used as covariates in  
26  
27 244 models to address primary aims (where applicable) or in secondary data analyses. Specifically,  
28  
29 245 validated measures on physical activity<sup>77</sup>, sleep<sup>78</sup> and diet<sup>79 80 81</sup> were collected at multiple  
30  
31 246 timepoints pre- and postnatally. To assess maternal cognition, an important predictor of child  
32  
33 247 neurodevelopment, participants completed a mental rotation task<sup>82</sup> and an abbreviated Verbal  
34  
35 248 Comprehension subtest of the Wechsler Adult Intelligence Scale IV (WAIS-IV)<sup>83</sup>.

36  
37  
38 249 **Prenatal maternal biospecimen collection and analysis.** UPSIDE collected extensive  
39  
40 250 maternal biospecimens as described below and in Table 1. In addition to the specific analyses  
41  
42 251 described, for all sample types, additional aliquots were banked for future research. **Blood.** In  
43  
44 252 each trimester, a 40mL blood sample was collected and processed to provide aliquots of serum,  
45  
46 253 plasma, cells, and whole blood (3<sup>rd</sup> trimester only) for a variety of analyses. Ongoing analysis of  
47  
48 254 these samples includes (1) sex steroid hormones (estrone, estradiol, estriol, testosterone, and  
49  
50 255 free testosterone) using liquid chromatography with tandem mass spectrometry (LC-MS/MS)<sup>84</sup>;  
51  
52 256 (2) placental corticotropin releasing hormone using radioimmunoassay<sup>85</sup>; (3) immune and  
53  
54 257 related markers [e.g. high sensitivity cytokines, C-reactive protein (CRP), TGF-beta, angiogenic  
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3 258 markers, and Mullerian inhibiting factor (MIF)]. Maternal blood was collected by labor and  
4  
5 259 delivery nursing staff upon admission for delivery for additional assessment of immune markers.  
6  
7 260 Saliva. Participants were trained to collect diurnal saliva for cortisol measurement using the  
8  
9 261 standard passive drool procedures developed by the MacArthur Research Network on  
10  
11 262 Socioeconomic Status and Health<sup>86</sup>. Samples (approximately 1 mL) were collected at home at  
12  
13 263 five pre-determined points across the day (at wake-up, 45 minutes after wake-up, 2.5 hours  
14  
15 264 after wake-up, 8 hours after wake-up, 12 hours after wake-up) on a single day in each trimester  
16  
17 265 (for a total of 5 samples per trimester or 15 samples across the pregnancy). An additional  
18  
19 266 passive drool saliva sample was collected by mothers at face-to-face visits and will be used to  
20  
21 267 assess the oral microbiome. Urine. At each prenatal visit, a urine sample was collected, after  
22  
23 268 which the specific gravity (dilution) and temperature of the sample were measured using a  
24  
25 269 handheld refractometer (National Instrument Company, Inc., USA). Five mL were frozen for  
26  
27 270 future use. Buccal swab. In the third trimester, a buccal cell sample for DNA analysis was  
28  
29 271 collected by swabbing the inside of the participant's cheek (MAWI iSWAB, 250 series) after  
30  
31 272 which samples were stored according to manufacturer guidelines. Vaginal swab. In the third  
32  
33 273 trimester, when a vaginal swab was taken by the provider to test for the presence of  
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35 274 Streptococcus B as part of standard obstetric care, an additional swab was collected for future  
36  
37 275 analysis of the vaginal microbiome.  
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43 277 **Birth biospecimen collection and analysis.** Samples were collected at the time of delivery  
44  
45 278 (usually within 1 hour) and banked at -80°C. Analyses including hormone and immune assays,  
46  
47 279 environmental chemical assessments, and genetics are ongoing.  
48  
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50 280

51 281 Cord bloods. Cord bloods were collected in two venues: (1) mixed cord bloods (up to 25mL)  
52  
53 282 from the delivery room by delivery staff; and (2) fetal arterial and venous bloods (up to 30mL  
54  
55 283 each) drawn from the placental vasculature by trained coordinators immediately following  
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1  
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3 284 delivery. The bloods collected from the cord, umbilical vein, and umbilical artery were placed  
4  
5 285 into additive free tubes, K2-EDTA tubes, and sodium heparin tubes, depending on the volume  
6  
7 286 collected. Peripheral blood mononuclear cells (PBMCs) extracted using Ficoll-Paque and red  
8  
9 287 blood cells (RBCs) reserved from processing cord bloods were stored in liquid nitrogen.  
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11 288  
12  
13 289 **Placental tissue collection.** Fresh core villous tissue was collected by a trained coordinator  
14  
15 290 using a flap technique to leave the maternal decidua surface intact. For RNA analysis, two  
16  
17 291 50mg tissue sections were washed in phosphate buffered saline, placed in cryovials, and flash  
18  
19 292 frozen in liquid nitrogen. About 30g of additional placental tissue was extracted using the same  
20  
21 293 technique and frozen unwashed in liquid nitrogen for other types of analyses.  
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24 294  
25  
26 295 **Placental pathology.** All placentae underwent a detailed pathology examination that included  
27  
28 296 standard gross and histologic protocols as well as novel assessment of placental  
29  
30 297 vascularization patterns on the chorionic plate. Using a standard digital camera with polarizing  
31  
32 298 filters, a trained coordinator took a series of 2D photographs of the fresh tissue prior to core  
33  
34 299 villous specimen sampling. From the 2D photographs, fetal vascular data will be extracted,  
35  
36 300 arterial and venous surface vascular networks mapped, and virtual slices created. Chorionic  
37  
38 301 surface vasculature branching will be further analyzed using computer extraction techniques,  
39  
40 302 yielding continuous measures including number of branch generations, number of branches off  
41  
42 303 base of cord, number of branch points in network, and mean distance from end of artery to end  
43  
44 304 of nearest vein.  
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46 305  
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48 306 Additional photos were taken after removal of the placental specimens to indicate locations of  
49  
50 307 the collections. Weight was collected after removal of the cord and membranes using a  
51  
52 308 standard scale, and cord length was measured (to the nearest 0.1cm) using a tape measure.  
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54 309 Cord twists were counted to measure the twist index. Biopsies of the cord were taken at the  
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3 310 insertion site and 10cm from the insertion site, then placed in cassettes. A section of membrane  
4  
5 311 was cut into a square and rolled, then placed in a cassette. Placentae were reviewed by a  
6  
7 312 pathologist to assess for histology, anomalies, and infections. All tissues were placed into  
8  
9 313 formalin for fixation for at least 72 hours. After fixation, the placentae were sectioned and  
10  
11 314 images were obtained of each section. Biopsies of tissue from four quadrants, plus any  
12  
13 315 additional abnormal tissues that were noted, were collected into cassettes for further analysis.  
14  
15  
16 316 The remaining tissues were retained for future assessments.  
17

18 317  
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20 318 **3D placental imaging.** Three dimensional digital scans of all placentae were collected to  
21  
22 319 assess placental morphology. The custom-built scanner consisted of two webcams mounted on  
23  
24 320 a bar providing binocular view, and a turntable on which the placenta was placed. The scanner  
25  
26 321 took 8 images of the top and bottom of the placenta and the software assembled these resulting  
27  
28 322 16 images into a 3-D shape. Morphometric measures obtained from 3D images include  
29  
30 323 estimated volume, surface area, thickness, shape, and symmetric difference.  
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33 324  
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35 325 **Prenatal and Birth Record Abstraction.** Clinical data was abstracted from the URM C eRecord  
36  
37 326 system. Prenatal record abstraction included medical, surgical, gynecological, and reproductive  
38  
39 327 history, prenatal visit records, ultrasound measurements, and clinical lab values. Delivery chart  
40  
41 328 abstraction included admission date and time, gestational age on admission, labor onset and  
42  
43 329 duration, rupture of membranes, highest intrapartum temperature, group B streptococcus status,  
44  
45 330 maternal white blood cells, delivery date and time, fetal position and mode of delivery,  
46  
47 331 complications, medications, and maternal morbidity. In addition, relevant data from newborn  
48  
49 332 nursery records were abstracted including birth weight and length, head circumference, APGAR  
50  
51 333 scores, admission unit for baby, first recorded temperature, first recorded blood glucose, Kaiser  
52  
53 334 sepsis risk score, cord arterial pH, neonatal resuscitation, and neonatal complications.  
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3 336 **Child study activities (ongoing).** Child visits occur at birth as well as 1, 6, 12, 24, 36, and 48  
4  
5 337 months of age. Consistent with the study emphasis on longitudinal measures over time, many  
6  
7 338 assessments are repeated at multiple time points, as age appropriate. At present, all birth and 1  
8  
9 339 month visits have been completed, whereas 6, 12, 24, and 36 month visits are ongoing, and 48  
10  
11 340 month visits will start in early 2021. Here we provide an overview of the child data collected  
12  
13 341 across all of these timepoints by domain of interest. Activities conducted are displayed by visit  
14  
15 342 timepoint in Table 2. Our primary child outcome measures represent two domains:  
16  
17 343 neurodevelopment and growth. Secondly, we collect data on additional constructs as required  
18  
19 344 for ECHO-wide projects and/or to use as covariates in analyses.  
20  
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22 345

23  
24 346 **Neurodevelopment.** Given the focus on neurodevelopment as a primary outcome, UPSIDE  
25  
26 347 study children participate in an extensive battery of neurodevelopmental assessments that span  
27  
28 348 constructs and method (e.g., cognition and language, temperament and behavior, sex-  
29  
30 349 dependent neurodevelopment, eating behaviors, and neuroimaging) (Table 2). Importantly,  
31  
32 350 although practice effects sometimes occur in older children when measures are closely spaced  
33  
34 351 (i.e. several weeks apart), this is unlikely to occur in children this young with visits spaced many  
35  
36 352 months or years apart.  
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40  
41 354 **Cognition and language.** The Bayley Scales of Infant Development-III (BSID-III), a widely used  
42  
43 355 tool for assessing mental and motor development in infants and toddlers, is administered at 6,  
44  
45 356 12, and 24 months<sup>87</sup>. Of primary interest are the Cognitive scaled-score and the Language  
46  
47 357 scaled-score, including both receptive and expressive communication subtests which assess  
48  
49 358 memory, sensorimotor development, preverbal behaviors and communication and vocabulary  
50  
51 359 development. At 24 months, complementary data on early vocabulary are obtained through the  
52  
53 360 MacArthur-Bates Communicative Development Inventory: Words and Gestures which asks  
54  
55 361 parents to mark on a checklist which phrases, words, and sound effects their child understands,  
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3 362 says, or signs<sup>88</sup>. Executive Function is assessed at 24 and 36 months using age-appropriate  
4  
5 363 standardized tasks that evaluate the child's working memory ("Spin the Pots"), impulse control  
6  
7 364 ("Snack Delay"), and inhibitory control ("Reverse Categorization")<sup>89-91</sup>. At 36 and 48 months we  
8  
9 365 administer additional tasks from the NIH ToolBox Early Childhood Cognition Battery including:  
10  
11 366 Flanker Inhibitory Control and Attention Test (executive function and attention), Dimensional  
12  
13 367 Change Card Sort Test (cognitive flexibility), Picture Sequence Memory (episodic memory), and  
14  
15 368 Picture Vocabulary Test (language development)<sup>92-95</sup>. Finally, at age 4 years, the Wechsler  
16  
17 369 Preschool and Primary Scale of Intelligence (WPPSI-IV) is administered to facilitate calculation  
18  
19  
20 370 of verbal, performance and full scale IQ<sup>96</sup>.

21  
22  
23 371 Temperament and behavior. The Laboratory Temperament Assessment Battery (Lab-TAB) is  
24  
25 372 an observational tool for examining multiple dimensions of infant temperament<sup>97</sup>. We administer  
26  
27 373 Lab-TAB fear tasks involving the presentation of an unpredictable mechanical toy (6, 12, and 24  
28  
29 374 months) and a remote-controlled spider (12 and 24 months). In the Anger/Frustration task (6  
30  
31 375 and 12 months), the infant is allowed to engage with a novel toy and is subsequently interrupted  
32  
33 376 by gentle arm restraint. At 6, 12, and 24 months, mothers complete the Infant Behavior  
34  
35 377 Questionnaire – revised (IBQ-R), a 191-item questionnaire on infant temperament that assesses  
36  
37 378 14 different dimensions (Approach, Vocal Reactivity, High Intensity Pleasure, Smile and  
38  
39 379 Laughter, Activity Level, Perceptual Sensitivity, Sadness, Distress to Limitations, Fear, Falling  
40  
41 380 Reactivity, Low Intensity Pleasure, Cuddliness, Duration of Orienting, Soothability, Social Fear,  
42  
43 381 Attentional Shifting)<sup>98</sup>. At 12 months we also administer the Strange Situation Procedure, widely  
44  
45 382 used to assess parent-infant attachment relationships<sup>99 100</sup>.

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50 383 Sex-dependent neurodevelopment. To address study aims regarding sex steroid pathways and  
51  
52 384 the potentially sex-dependent impacts of maternal distress, we administer a series of  
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54 385 specialized tasks that, in previous research, have demonstrated sex differences even in early  
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3 386 infancy. At 1 and 6 months, infants engage in a task assessing preferences for faces <sup>101</sup>. At 6,  
4  
5 387 12, and 24 months, children complete a series of computer-based tasks assessing preferences  
6  
7 388 for sex-stereotypical toys (e.g. doll versus toy truck) and social stimuli <sup>102 103</sup>. A computerized  
8  
9 389 mental rotation task developed for use in infants is administered at 6, 12, 24, and 36 months<sup>104</sup>.  
10  
11 390 Finally at 12 and 24 months, the child engages in an independent play task designed to assess  
12  
13 391 preferences for stereotypically female, male, and gender neutral toys<sup>105</sup>.  
14  
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16 392  
17  
18 393 Eating behaviors. Of relevance to both neurodevelopment and physical development, eating  
19  
20 394 behaviors are assessed using multiple questionnaires and observational tasks at the child's 36-  
21  
22 395 month visit. The Children's Eating Behavior Questionnaire, Preschool Adapted Liking Survey,  
23  
24 396 and Comprehensive Feeding Practices Questionnaire are completed by the parent to indicate  
25  
26 397 the child's eating behaviors and parental influences, such as food responsiveness, emotional  
27  
28 398 overeating, food preference, food restriction, and family food environment <sup>106-108</sup>. Food  
29  
30 399 reinforcement is an observational task used to examine the reinforcing value of food in children,  
31  
32 400 which indicates motivation to eat <sup>109</sup>. A food ranking task was developed to evaluate the child's  
33  
34 401 ability to indicate his/her food preference by ranking pictures of food items. To complement the  
35  
36 402 eating behavior tasks and provide information on the child's usual dietary intake, at the 36-  
37  
38 403 month visit, the parent completes 24-hour dietary recalls for the child (similar to the one  
39  
40 404 completed by mothers during pregnancy).  
41  
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43 405  
44  
45 406 Neuroimaging. At 1 month of age, magnetic resonance imaging (MRI) was collected on a  
46  
47 407 Siemens Prisma with a 32 channel head coil using a standard protocol that assesses  
48  
49 408 anatomical scans, Diffusion Tensor Imaging, and Resting State Functional Connectivity. MRI is  
50  
51 409 conducted while the infant was in natural sleep. A follow-up MRI will be performed at age 4,  
52  
53 410 which will also include functional assessments. When the child is 24 and 36 months, a  
54  
55 411 electroencephalogram (EEG) assessment is conducted. In the EEG, select stimuli are  
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3 412 presented and the brain's measured response, known as event-related potentials (ERP), is  
4  
5 413 recorded. Our stimulus is an auditory event-related potential, called mismatch negativity (MMN).  
6  
7 414 In the MMN assessment, repetitive sounds are interrupted by an occasional odd sound, that  
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9 415 differs in frequency and duration.  
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11 416  
12  
13 417 **Physical development.** At each visit, child anthropometric measurements are collected by  
14  
15 418 trained research coordinators. In general, measurements are collected in duplicate at each time  
16  
17 419 point, with a third measurement obtained when the first two differ by more than a pre-specified  
18  
19 420 amount (which varied by specific measure and age). Weight, length, and head circumference  
20  
21 421 are measured at every postnatal visit using standard protocols<sup>110</sup>. Weight (to the nearest  
22  
23 422 0.01kg) is measured using a Seca Infant Scale (Model #334). Length (to the nearest 0.1cm) is  
24  
25 423 measured using a Seca Infantometer (Model#416), tape measure, or wall-mounted stadiometer  
26  
27 424 (depending on age). Head circumference is measured by placing a tape measure just above  
28  
29 425 eyebrows and wrapping it around the widest part of the head. Skinfold thicknesses (suprailiac,  
30  
31 426 subscapular, and tricep) are obtained to the nearest 0.1 mm using calibrated Holtain calipers <sup>110</sup>  
32  
33 427 <sup>111</sup>. From birth to 24 months, anogenital distance, a marker of prenatal androgen exposure, is  
34  
35 428 measured to the nearest 0.1 mm using dial Vernier calipers<sup>112</sup>. A second purported measure of  
36  
37 429 prenatal androgen exposure, second to fourth digit ratio (2D:4D) of the child's right hand is  
38  
39 430 measured to the nearest 0.1 mm at 36 and 48 months also using dial Vernier calipers<sup>113</sup>. Finally,  
40  
41 431 at 36 and 48 months, waist circumference is measured to the nearest 0.1 cm by wrapping a  
42  
43 432 tape measure around the body at the level of the umbilicus<sup>110</sup>.  
44  
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46  
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50 434 **Child biospecimen collection.** Postnatal biospecimen collection occurs at each child visit  
51  
52 435 (Table 1). Analysis of immune and HPA axis markers is ongoing, whereas additional analysis of  
53  
54 436 banked biospecimens is pending. **Rectal swab.** At birth as well as 1, 6, 12, and 24 months, a  
55  
56 437 sterile swab applicator is dipped into sterile phosphate buffered saline, inserted into the child's  
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3 438 anal orifice up to the floxed portion of the swab, and stored in a conical tube for future  
4  
5 439 microbiome analyses<sup>114</sup>. Buccal swab. At birth, 12, 24, and 48 months, a buccal specimen is  
6  
7 440 collected for genetic analysis at least 60 minutes after the last recorded feeding. Both cheeks  
8  
9 441 are swabbed using a Mawi iSWAB collection kit (250 series) and the vial is stored according to  
10  
11 442 manufacturer guidelines. Saliva. At 6, 12, 24, and 48 months, saliva is collected to measure  
12  
13 443 cortisol using Salimetrics SalivaBio swabs. To assess cortisol response to a stressor (blood  
14  
15 444 draw at 6 months, strange situation task at 12 months, physical exam at 24 months) a series of  
16  
17 445 swabs are collected: at the start of the visit (6 and 24 months only), pre-stressor, 15 minutes  
18  
19 446 post-stressor, and 30 minutes post-stressor. At 24 and 48 months, an additional saliva swab is  
20  
21 447 collected for assessment of the oral microbiome. Stool. At 1 and 6 months, a stool sample is  
22  
23 448 collected at study visits using standard protocols (or by parents using an at-home collection kit if  
24  
25 449 sample collection at the visit is not possible). Urine. Urine is collected at the 1, 6, and 12 month  
26  
27 450 visits using an Earth's Best chlorine free diaper equipped with either a urine collection bag  
28  
29 451 and/or sterile cotton balls. Blood. At 6, 12, and 48 months, approximately 10mL of blood is  
30  
31 452 collected by a pediatric nurse (4 mL in a K2-EDTA tube and 3 mL in each of 2 sodium heparin  
32  
33 453 tubes at 6 and 12 months, 8mL into a sodium heparin tube, 2mL into a K2-EDTA tube, and 3mL  
34  
35 454 into a tube without additives at 48 months). Nails. At 12, 24, and 36 months, fingernails and  
36  
37 455 toenails are collected at study visits (or by parents using an at-home collection kit). Breastmilk.  
38  
39 456 Mothers who are breastfeeding at 1 and 6 months provide a breastmilk sample (up to 45mL).  
40  
41 457 Mothers collect the sample during the visit (when possible) using a new, sterile Harmony  
42  
43 458 Medela manual breast pump. When that is not possible, mothers bring in a breastmilk sample  
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45 459 that was collected at home.  
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51 461 **Statistical analysis and power calculations.** Complementary analyses are planned to  
52  
53 462 address the aims of the UPSIDE cohort. In general, we will employ longitudinal models to test  
54  
55 463 key hypotheses, examining the mediated routes by which associations may occur. We  
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3 464 anticipate fitting regression models to determine associations between maternal exposures and  
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5 465 child outcomes and will use structural equation modelling to examine potential mediators.  
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7 466 Covariates will differ by the particular analyses of interest and will be included based on a priori  
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9 467 knowledge and/or a LASSO approach depending on the particular analysis.  
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13 469 Power calculations were informed by results from our prior cohort studies and indicated that the  
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15 470 study would be appropriately powered with a sample size of approximately 290 mother-child  
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17 471 dyads. These original power calculations were designed to address hypotheses related to sex  
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19 472 steroid pathways, as those were the first set of funded Aims at the time of cohort establishment.  
20  
21 473 For example, with an anticipated correlation of 0.19, we would have 90% power to detect a  
22  
23 474 significant slope in the regression of maternal anxiety (PSWQ scores) on concentrations of  
24  
25 475 estradiol, an estrogen of primarily placental origin. For our hypothesis on PSWQ scores in relation  
26  
27 476 to anogenital distance (a marker of prenatal sex steroid activity), with an estimated correlation of  
28  
29 477 0.25 between maternal PSWQ scores and girls' AGD, we would have 81% power to detect a  
30  
31 478 slope  $\neq 0$  and 86% power to detect a sex-anxiety interaction (with boys' slope = -0.13). Retention  
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33 479 of 226 children at age 12 months would provide 89% power to detect an association between  
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35 480 maternal PSWQ scores and play behavior in girls, with weaker or no associations expected in  
36  
37 481 boys. These power calculations are provided as illustrative analyses with the recognition that  
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39 482 there will be variation in power based on the particular question under consideration.  
40  
41 483 Additionally, for some highly novel analyses (e.g. maternal serial inflammatory markers in  
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43 484 relation to child MRI data), unfortunately there is a lack of effect size data on which to power the  
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45 485 study.  
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488 **FINDINGS TO DATE:**

489 In total, 312 women were recruited into the study, of whom 14 enrolled for more than one  
490 pregnancy, resulting in a total of 326 study pregnancies (Table 3; Figure 1). Of these, 294  
491 mothers gave birth to an infant in the study. The 32 women who signed informed consent but  
492 did not give birth to a study infant included 3 screen failures, 5 pregnancy losses, 8 who became  
493 ineligible during pregnancy, 2 who were lost to follow up, and 14 who chose to withdraw. Here  
494 we report on the mothers who gave birth to an infant in the study.

495

496 On average, women were  $28.9 \pm 4.7$  years old at recruitment and the majority were White  
497 (61.2%) or Black (25.5%), with 9.9% reporting Hispanic ethnicity. The participants were  
498 socioeconomically diverse with a 34.2% having a high school degree or less, while 25.7% had  
499 obtained a post-college degree. At the time of recruitment 74.9% were employed and 60.3%  
500 were married or living as married. Self-reported alcohol use and smoking during early  
501 pregnancy were both relatively uncommon (3.4% and 7.8%, respectively).

502

503 The cohort was mildly psychosocially risk enriched and overall symptoms were stable across  
504 trimesters with ICC's for the various psychosocial scales ranging from 0.68-0.80 (Table 4). At  
505 baseline, over 50% were characterized as moderate or high anxiety (based on PSWQ scores)  
506 There was a small increase in average EPDS (depression) scores across pregnancy, however  
507 the proportion of women scoring  $\geq 13$  (the most stringent clinical cut-off indicating possible  
508 depression) was stable across pregnancy (9.5-10.6% across trimesters). Women typically  
509 worried less about their fetuses after the first trimester, with slight increases in worry about  
510 delivery over time. In the third trimester, 67.9% of women reported at least one ACE, with 16.7%  
511 reporting four or more. Similarly, the average number of stressful life events during pregnancy  
512 was relatively low ( $2.46 \pm 2.80$ ), however there was great inter-individual variation such that

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3 513 some women reported up to 17 events. By design, this was a low-medical risk cohort at  
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5 514 recruitment therefore relatively few participants had major pregnancy complications including  
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7 515 hypertensive disorders of pregnancy (n=22, 7.5%) and gestational diabetes (n=6; 2.0%). The  
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9 516 average gestational age at birth was 39.5 weeks and only 14 (4.7%) babies were born preterm.  
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11 517  
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13 518 Success rates for biospecimen collection were consistently high across trimesters (blood: 95-  
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15 519 99%; urine 94-97%; saliva 96-100%). In addition, 272 mothers (93%) provided a buccal swab  
16  
17 520 for DNA analysis and 258 mothers (75%) provided a vaginal swab. At birth, the placenta and  
18  
19 521 cord blood were obtained in 96% and 88% of women, respectively. The discrepancy between  
20  
21 522 success in cord blood collection and placenta collection results from the more intensive  
22  
23 523 immediate processing required for the former as even short delays can result in draining or  
24  
25 524 clotting, making collection impossible. Finally, 277 infants (94%) participated in an exam at birth;  
26  
27 525 of these 271 (98%) provided a buccal swab and a rectal swab (for microbial analysis) was  
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29 526 obtained on 261 (94%). One-month visits were recently completed with additional postnatal  
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31 527 visits at 6, 12, 24, 36, and 48 months underway.  
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### 36 529 **STRENGTHS AND LIMITATIONS:**

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39 530 There are several notable strengths of the UPSIDE study. The first is our extensive, rigorous  
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41 531 biospecimen collection starting in the first trimester and continuing throughout pregnancy. This  
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43 532 was made possible by focusing recruitment and prenatal visits primarily at a small set of  
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45 533 obstetric clinics located in close proximity to the labs where processing and storage occurs.  
46  
47 534 Similarly, birth biospecimen collection (placenta and cord blood) is a strength and based on our  
48  
49 535 experience, a large “SWAT” team is needed for around the clock collection and processing of  
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51 536 placentae within 3 hours after birth (ideally within 1 hour). The need for a 24/7 dedicated on call  
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53 537 team is further illustrated by the fact that the majority of study births occurred outside of  
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55 538 business hours (Figure 2). Similarly, we implemented multiple mechanisms to identify when  
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3 539 study participants were admitted for delivery. The successful strategies discussed above  
4  
5 540 allowed UPSIDE to acquire 96% of placentae. Our extensive placental assessments and  
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7 541 biorepository will provide exceptionally rich data on both morphology and molecular biology,  
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9 542 allowing us to address novel questions about the placenta in relation to maternal exposures and  
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11 543 child outcomes.  
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16 545 Another strength of the study is our ongoing intensive longitudinal follow-up of mother-child  
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18 546 dyads with a focus on repeated measures to assess intra-individual changes over time. In the  
19  
20 547 prenatal period, for example, our extensive phenotyping of mothers occurred at three time  
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22 548 points, which will allow us to look at trimester-specific impacts and to potentially differentiate  
23  
24 549 between different domains of psychosocial distress (e.g. anxiety, depression, stressful life  
25  
26 550 events). During the postnatal period, we again adopt this model, with seven visits occurring from  
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28 551 birth to age 4. This intensive visit schedule allows us to assess domains of neurodevelopment  
29  
30 552 (as age-appropriate) over time and facilitates serial collection of relevant biospecimens that may  
31  
32 553 yield mechanistic insights. Our use of standard measures of development and cognition (such  
33  
34 554 as the WPPSI and BSID-III), moreover, is complemented by tools developed to examine more  
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36 555 specific aspects of development that are plausibly linked to our pathways of interest (e.g. social  
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38 556 preferences and sex-typical play behavior in relation to sex steroids).  
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41 557  
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43 558 At the same time, there are several limitations of note. The deliberate recruitment of low-medical  
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45 559 risk pregnancies means that we are underpowered to test hypotheses regarding pregnancy  
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47 560 complications or outcomes. Similarly, our relatively small sample size and overall healthy  
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49 561 population precludes examining pediatric clinical outcomes such as birth defects, autism  
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51 562 spectrum disorders or developmental delays; however, our ability to look at continuous  
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53 563 measures of development will yield insights into neurodevelopmental variation within the typical  
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55 564 spectrum. In addition, given evidence that preterm infants develop along a very different  
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3 565 trajectory than term infants, preterm infants were not included in postnatal follow-up, so we  
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5 566 cannot look at outcomes in this special group. Finally, although the biological and psychosocial  
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7 567 contributions of partners is of great interest and relevance to children's development, our prior  
8  
9 568 work in this population suggested that partner attendance at visits was likely to be low, making  
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11 569 consent and data collection quite difficult. Thus like many pregnancy cohorts, our data on  
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13 570 partners is limited to information provided by the participating women.  
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### 17 18 572 **COLLABORATION:**

19  
20 573 Interested investigators may contact the PIs (EB, TO'C) in writing regarding potential  
21  
22 574 collaborations involving data and/or biospecimens from the UPSIDE study. Potential  
23  
24 575 collaborators will be asked to write a concept proposal for their proposed analysis which will be  
25  
26 576 reviewed by the UPSIDE Executive Committee. After concept proposal approval, collaborators  
27  
28 577 will submit analysis plans and proof of IRB approval to the Executive Committee prior to  
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30 578 receiving data/samples. Requests for collaborations will be considered on an ongoing basis;  
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32 579 however, in general, external collaborations will be started once the primary study aims have  
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34 580 been addressed.  
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### 38 39 582 **FUTURE DIRECTIONS:**

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41 583 As data are cleaned and final outcome data become available, our highest priority is to address  
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43 584 the primary study aims for the multiple projects that support this cohort. Beyond our current  
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45 585 aims, the rich biospecimen collection and intensive phenotyping of the UPSIDE cohort lends  
46  
47 586 itself to a wide range of ancillary studies and future directions. At present, ancillary studies to  
48  
49 587 examine environmental chemicals (synthetic chemicals and metals) and air pollution in relation  
50  
51 588 to placental function, perinatal outcomes, and infant development are in progress. Also  
52  
53 589 underway is a complementary study of participating mothers looking at trajectories of  
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55 590 cardiometabolic health from early pregnancy through three years postpartum. Additional studies  
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3 591 are planned to examine the microbiome (vaginal, oral, gut, and breastmilk) in relation to various  
4  
5 592 endpoints including pediatric oral health and neurodevelopment. As part of the ECHO  
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7 593 consortium, data and biospecimens from our cohort are harmonized with up to 50,000 other  
8  
9 594 participating mother-child dyads from cohort studies around the U.S. with the goal of providing  
10  
11 595 novel insights into factors that shape multiple facets of children's health (including pre-, peri-,  
12  
13 596 and postnatal outcomes, upper and lower airway health, obesity, neurodevelopment, and  
14  
15 597 positive health).  
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20 599 **Author affiliations.** All authors (with the exception of CS) are affiliated with the University of  
21  
22 600 Rochester School of Medicine and Dentistry. The senior author (EB) also holds an appointment  
23  
24 601 at Rutgers School of Public Health. CS's primary affiliation is Placental Analytics.  
25  
26 602

27  
28 603 **Collaborators.** UPSIDE study collaborators include: Jennifer Adibi, Lauren Aleksunes, Mary  
29  
30 604 Caserta, Susan Groth, Philip Katzman, Eva Pressman, Xing Qiu, Zorimar Rivera-Nunez, Kristin  
31  
32 605 Scheible, Ruchit Shah, Lorelei Thornburg, and Sally Thurston.  
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38  
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40  
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42  
43 610 Ntemena Kapula, Meghan Best, Jishyra Serrano, Hannah Murphy, Leena Khoury, Allison  
44  
45 611 Macomber, and Amber Kautz led data acquisition. Emily Barrett and Tom O'Connor led  
46  
47 612 manuscript development with intellectual content for subsections provided by all co-authors. All  
48  
49 613 authors provided final approval for the version to be published and agree to be accountable for  
50  
51 614 all aspects of the work.  
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6  
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9 619 Mitchell, Aurora Newman, Lisa Panisch, Ryan Prawel, Jenelle Putzig, Amanda Rubano, Andrew  
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12  
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16  
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31  
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39 633 **Competing interests.** None declared.  
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43 635 **Patient consent for publication.** Not required.  
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46  
47 637 **Ethics approval.** This study was approved by the University of Rochester School of Medicine  
48  
49 638 and Dentistry Institutional Review Board (#58456) and the Rutgers University Institutional  
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51 639 Review Board (PRO20160001514).  
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56 641 **Provenance and peer review.** Not commissioned; externally peer reviewed.  
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**Data availability statement.** Once complete cohort data has been collected and cleaned, it will be available pending approved concept proposal, analysis plan, and documentation of IRB approval.

For peer review only



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3 647  
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983 **Table 1. Summary of UPSIDE biospecimen collections.**

	Prenatal (trimesters)			Birth	Infant/Child postnatal (months)					
	1	2	3		1	6	12	24	36	48
Blood										
Serum	M	M	M	UC						
Plasma	M	M	M	UC		C	C			C
Whole blood			M							
Red blood cells	M	M	M			C	C			C
Urine	M	M	M		C	C	C			C
Saliva										
Diurnal (5x/day)	M	M	M							
Stress response						C	C	C		C
Oral microbiome	M	M	M			C	C	C		C
Vaginal swab			M							
Placental tissue				P						
Buccal swab			M	C			C	C		
Rectal swab				C	C	C	C	C		
Stool					C	C				
Breast milk					M	M				
Nails							C	C		

984 M=maternal; C=child; UC=umbilical cord (artery and vein); P=placenta

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986 **Table 2. Summary of UPSIDE child assessments.**

	Age at assessment (months) *						
	birth	1	6	12	24	36	48
<b>Anthropometric measures</b>							
Height/weight	x	x	x	x	x	x	x
Head circumference	x	x	x	x	x	x	x
Skinfold thickness	x	x	x	x	x	x	x
Anogenital distance	x	x	x	x	x		
Waist circumference						x	x
Digit ratio						x	x
<b>Neurodevelopmental measures (by domain)</b>							
<i>Temperament</i>							
Rothbart- IBQ			x	x	x		
Lab-TAB			x	x	x		
<i>Cognition/language/EF</i>							
BSID			x	x	x		
WPPSI-IV							x
Macarthur Bates					x	x	
NIH Toolbox					x	x	
Executive function					x	x	
<i>Neuroimaging</i>							
MRI		x					x
EEG					x	x	
<i>Sex-typical/dimorphic</i>							
Face preference		x	x				
Toy preference			x	x	x		
Social preference			x	x	x		
Mental rotation			x	x	x	x	
Sex-typical play behavior				x	x		
<b>Lifestyle measures</b>							
Sleep		x	x	x	x	x	x
<i>Diet</i>							
Infant feeding questionnaire		x	x	x			
24-hour dietary recall						x	
Eating behavior						x	

\* 6, 12, 24, 36 month visits are ongoing and 48 month visits will start in early 2021.

Abbreviations: BSID: Bayley Scales of Infant Development; EEG: Electroencephalogram; EF: Executive function; IBQ: Infant Behavior Questionnaire; MRI: Magnetic Resonance Imaging; NIH: National Institutes of Health; WPPSI: Wechsler Preschool and Primary Scale of Intelligence

992 **Table 3. Baseline characteristics of mother-child dyads participating in UPSIDE**  
 993 **(n=294<sup>1</sup>).**

	Mean (SD)	Min-Max	n (%)
<b>Maternal characteristics<sup>2</sup></b>			
<b>Continuous/ordinal</b>			
Age (years)	28.9 (4.7)	18-41	
Pre-pregnancy BMI (kg/m <sup>2</sup> )	27.2 (6.9)	16.98-49.77	
Poverty to income ratio	3.8 (3.9)	0.04-44.6	
Household size (persons)	3.2 (1.5)	1-11	
Sleep (Pittsburgh Sleep Index)	6.3 (3.5)	0-17	
<b>Categorical</b>			
Race			
White			180 (61.2)
Black			75 (25.5)
Asian			11 (3.7)
Mixed Race			8 (2.7)
Other <sup>3</sup>			20 (6.8)
Hispanic			29 (9.9)
Education			
<High school			8 (2.9)
High school			85 (31.3)
Some college			39 (14.3)
College degree			70 (25.7)
Post-college degree			70 (25.7)
Employed			215 (74.9)
Married/living as married			173 (60.3)
Medicaid status			110 (42.1)
Nulliparous			82 (31.3)
Smoking during pregnancy (any)			23 (7.8)
Alcohol use during pregnancy (any)			10 (3.4)
<b>Paternal characteristics<sup>2</sup></b>			
Age (years)	30.9 (5.6)	18 – 57	
Race			
White			170 (59.9)
Black			79 (27.8)
Asian			5 (1.8)
Other <sup>3</sup>			30 (10.6)
<b>Infant characteristics</b>			
Female			152 (51.7)
Gestational age at birth (weeks)	39.5 (1.6)	27.7-42.7	
Birth weight (g)	3352.8 (495.0)	2195-4654	
Birth length (cm)	51.1 (3.1)	40-60	

994 1 n's for individual variables may differ slightly due to missing data

995 2 At time of enrollment, with the exception of Sleep which was assessed in the second trimester.

996 3 "Other" includes American Indian/Alaska Native and individuals self-reporting as "other".

**Table 4. Psychosocial assessments repeated across pregnancy.**

Scale	Trimester 1	Trimester 2	Trimester 3	ICC
	mean±SD; (range)	mean±SD; (range)	mean±SD; (range)	
Depressive Symptoms (EPDS)	5.56 ± 4.73 (0-21)	5.94 ± 4.93 (0-23)	5.95 ± 5.13 (0-29)	.73
Anxiety Symptoms (PSWQ)	44.41 ± 13.36 (16-77)	44.09 ± 13.72 (17-80)	44.31 ± 13.69 (16-80)	.80
Pregnancy Specific Anxiety				
Worries about the baby	7.97 ± 4.3 (4-20)	7.24 ± 3.77 (4-20)	7.18 ± 3.99 (4-20)	.68
Worries about delivery	6.96 ± 3.64 (3-15)	6.65 ± 3.46 (3-15)	7.19 ± 3.47 (3-15)	.72
Perceived Stress (PSS)	19.9 ± 8.11 (2-42)	20.03 ± 8.1 (0-49)	19.83 ± 8.03 (2-39)	.77

Abbreviations: EPDS: Edinburgh Postnatal Depression Scale; ICC: Interclass correlation coefficient; PSS:

Perceived Stress; PSWQ: Penn State Worry Questionnaire

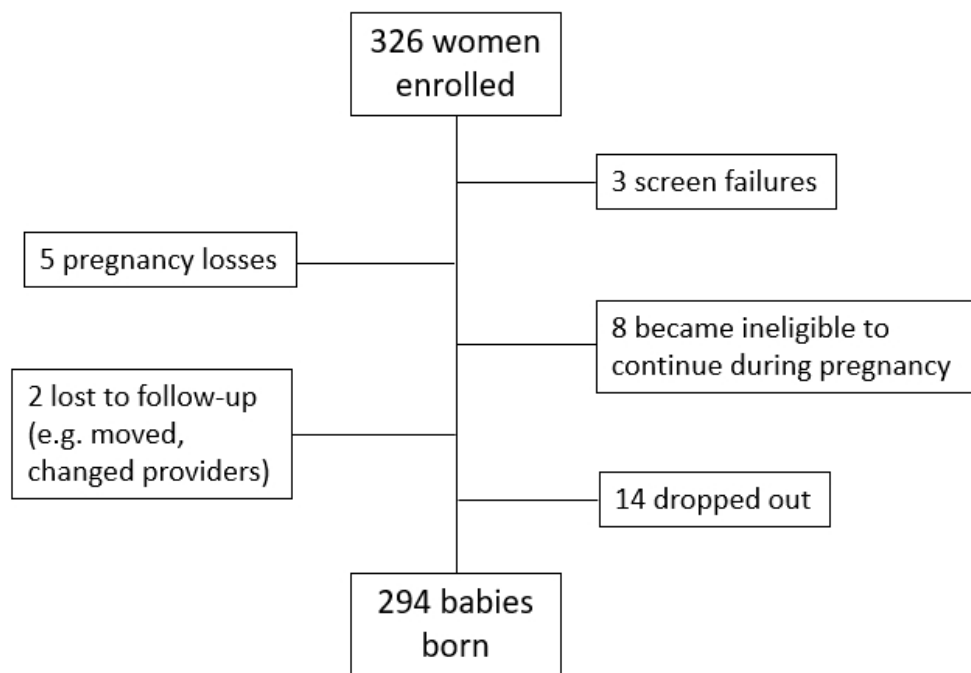
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3 1005 **Figure 1. Consort figure on recruitment and retention in the UPSIDE study (n=326).**  
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**Figure 2. Timing of placental delivery in relation to standard work hours (spontaneous births only).**

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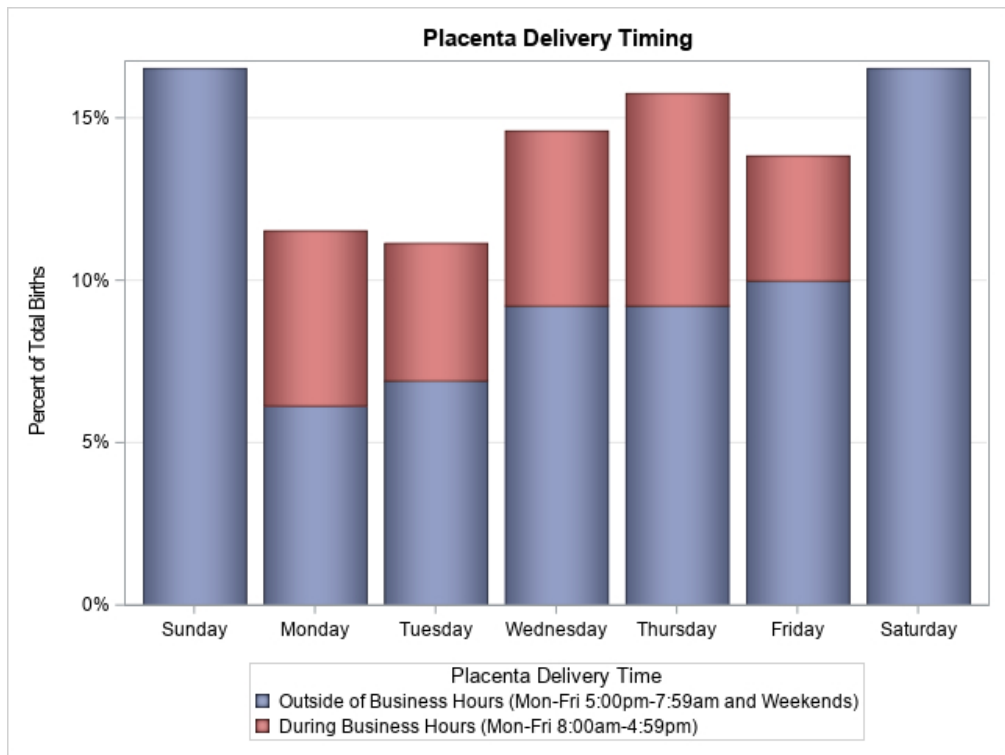


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