Neonatal Intensive Care Unit Network Neurobehavioral Scale Profiles in Full-Term Infants: Associations with Maternal Adversity, Medical Risk, and Neonatal Outcomes

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Objectives To examine healthy, full-term neonatal behavior using the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) in relation to measures of maternal adversity, maternal medical risk, and infant brain volumes.

Study design This was a prospective, longitudinal, observational cohort study of pregnant mothers followed from the first trimester and their healthy, full-term infants. Infants underwent an NNNS assessment and high-quality magnetic resonance imaging 2-5 weeks after birth. A latent profile analysis of NNNS scores categorized infants into neurobehavioral profiles. Univariate and multivariate analyses compared differences in maternal factors (social advantage, psychosocial stress, and medical risk) and neonatal characteristics between profiles.

Results The latent profile analysis of NNNS summary scales of 296 infants generated 3 profiles: regulated (46.6%), hypotonic (16.6%), and fussy (36.8%). Infants with a hypotonic profile were more likely to be male (χ^2 = 8.601; P = .014). Fussy infants had smaller head circumferences (F = 3.871; P = .022) and smaller total brain (F = 3.522; P = .031) and cerebral white matter (F = 3.986; P = .020) volumes compared with infants with a hypotonic profile. There were no differences between profiles in prenatal maternal health, social advantage, or psychosocial stress.

Conclusions Three distinct neurobehavioral profiles were identified in healthy, full-term infants with hypotonic and fussy neurobehavioral features related to neonatal brain volumes and head circumference, but not prenatal exposure to socioeconomic or psychosocial adversity. Follow-up beyond the neonatal period will determine if identified profiles at birth are associated with subsequent clinical or developmental outcomes. *(J Pediatr 2022;246:71-9)*.

The developmental origins of disease theory, which postulates a role for fetal factors on later life outcomes, has increasingly included maternal adversity (characterized by mental health conditions, physical/psychological stress, or economic hardship).[1,2] Recent evidence has linked maternal depression, anxiety, and stress during pregnancy to poor neurobehavior and developmental outcomes, frequently focusing on infants with prematurity or other high-risk factors.[3-9] Limited studies of maternal mental health in full-term infants have often included relatively low exposure to psychosocial adversity (eg, household poverty), generated mixed results, or used socioeconomic status as a covariate rather than an independent contributor to neurobehavior.[5-8,10] Healthy infants with adverse socioeconomic and psychosocial exposures in utero are likely at increased neurodevelopmental risk, yet the impact at birth, particularly on neurobehavior in the neonatal period, has not been thoroughly studied. Standardized neonatal neurobehavioral assessments are frequently used in clinical and research settings to stratify infants who are high risk and optimize early neurodevelopmental interventions.[11,12] Using these tools, atypical neonatal neurologic and neurobehavioral features have been described and linked to adverse cognitive, motor, and psychological outcomes in infancy and childhood.[13-21]

We examined a large cohort of full-term, healthy infants with mothers followed from the first trimester of gestation in a sample enriched for economic disadvantage. We aimed to assess associations between Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNNS) profiles and in

ADI	Area Deprivation Index
eLABE	Early Life Adversity, Biological Embedding, and Risk for Developmental Precursors of Mental Disorders
MMR	Maternal Medical Risk
MRI	Magnetic resonance imaging
NICU	Neonatal intensive care unit
NNNS	NICU Network Neurobehavioral Scale
PMA	Postmenstrual age

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uteroexposure to adversity, measured by maternal socioeconomic advantage and psychosocial stress, maternal medical risk (MMR), and neonatal brain structure.[22-24] We hypothesized that suboptimal infant NNNS scores would be associated with increased prenatal socioeconomic disadvantage and psychosocial stress, medical risk, and abnormal neonatal brain volumes and cortical folding.

Methods

The Early Life Adversity, Biological Embedding, and Risk for Developmental Precursors of Mental Disorders (eLABE) study is an ongoing multiwave, multimethod collaboration following a cohort of pregnant women recruited between 2017 and 2020 for a large-scale March of Dimes study within the Prematurity Research Center at Washington University in St. Louis.[25] Women with a singleton pregnancy, no alcohol or substance use during pregnancy (except for tobacco or marijuana), and no fetal congenital anomalies were enrolled. Women were recruited from 2 obstetric clinics, one primarily serving patients with private health insurance and the other serving patients with public health insurance. After approval by the Washington University Human Research Protection Office, informed consent was obtained from participants, with parental informed consent for infants. Participating women completed assessments during each trimester of pregnancy and at delivery.

There were 399 mother-infant pairs recruited for the eLABE study (**Figure 1**; available at www.jpeds.com), with 60% of the total sample drawn from the clinic serving patients with public health insurance. Mother-infant dyads were excluded if infants did not complete an NNNS assessment between 39 and 45 weeks postmenstrual age (PMA) (n = 26) or met any of the following exclusions: born at less than 37 weeks, birth weight less than 2000 grams, NICU admission for more than 7 days, required respiratory interventions, or had evidence of incidental brain injury on magnetic resonance imaging (MRI) (n = 77).

Measures

The NNNS. The NNNS is a standardized 115-item assessment tool for neonatal neurologic and behavioral examinations.[26] It clusters items into 13 summary scores detailed in **Table I** (available at www.jpeds.com). Two trained, certified, and highly experienced evaluators conducted all NNNS examinations, with 99% (n = 293) conducted by the same evaluator. NNNS examinations were performed in the same quiet, private testing room near the MRI suite.

Maternal Demographics, Socioeconomic Status, and Psychological State. Maternal characteristics, including age, insurance status, highest level of education, and self-reported racial/ethnic background, were obtained through questionnaires during pregnancy. Income-to-needs ratio, total income divided by the federal poverty threshold for household size, was calculated at each trimester. At each trimester, mothers also completed the Perceived Stress Scale and the Edinburgh Postnatal Depression Scale.[27,28] The Area Deprivation Index (ADI) generates a neighborhood disadvantage score from US census poverty, education, housing, and employment indicators and was calculated at birth.[29] Higher ADI values indicate greater disadvantage. The frequency of tobacco and marijuana use was self-reported on questionnaires given to all mothers at each trimester. As part of routine prenatal clinical care, a subset of mothers (n = 124) underwent urine drug screening during pregnancy at one or more time points per hospital guidelines. These clinical drug screens detected metabolites of commonly abused substances, including marijuana, but not tobacco. The Healthy Eating Index, the Stress and Adversity Inventory, and the Everyday Discrimination Scale, were administered once, typically at neonatal assessment.[30-32]

Maternal Social Advantage and Psychosocial Stress. We used 2-factor latent measures of maternal social advantage and psychosocial stress, generated and validated using confirmatory factor analysis and structural equation modeling. The methodology is detailed in **Appendix I** (available at www.jpeds.com). The Maternal Social Advantage score was derived from household income-to-needs ratio, ADI, insurance status, education, and nutrition (Healthy Eating Index), with lower scores indicating greater social disadvantage. The Maternal Psychosocial Stress score was calculated using psychological measures from the Perceived Stress Scale and Edinburgh Postnatal Depression Scale, social experiences of stressful or traumatic life events from the Stress and Adversity Inventory (count/severity), and discrimination from the Everyday Discrimination Scale.

Maternal Medical Comorbidities, Perinatal, and Neonatal Variables. Maternal health was calculated from chart review using a validated Maternal Medical Risk score, which sums weighted comorbidities including advanced age, cardiac disease, and pre-eclampsia.[33] Perinatal variables collected from chart review included the number of prenatal visits, delivery type (vaginal, operative vaginal, or cesarean), and delivery complications. Infant variables from chart review included sex, birth measurements: gestational age, weight, length, occipitofrontal circumference, Apgar scores, diet, and NICU stay length for patients who required a brief (<7 days) NICU admission.

Neonatal Neurological Outcomes. Infant brain MRIs were conducted during natural sleep on the day of NNNS assessment using a Siemens Prisma 3T scanner and a 64-channel Siemens head coil (Siemens). T2-weighted MRI sequence settings were TR = 3200/4500 ms, TE = 563 ms, tissue T2 = 160 ms, voxel size = 0.8 × 0.8 × 0.8 mm^3. Spin echo field maps were acquired with the following settings TR = 8000 ms, TE = 66 ms, voxel size = 2 × 2 × 2 mm^3. Image processing and regional segmentation are described in **Appendix II** (available at www.jpeds.com). Because decreased global brain volumes and cortical folding relate

to socioeconomic disadvantage in this cohort, we measured total brain, cortical and subcortical gray matter, white matter, and cerebellar volumes, and cortical folding (gyrification index).[23]

Statistical Analyses

Analyses used Mplus (v8.5, Muthén & Muthén), SAS (v9.4, SAS Institute), and SPSS (v26, IBM Corp) software. To reduce the dimensionality of 12 NNNS summary scores (excluding habituation), we combined them through latent profile analysis.[13,15,21,34,35] Infants were classified into mutually exclusive phenotypic 'profiles' using probabilistic assignment. One-way ANOVA compared standardized summary score mean differences between profiles. Maternal and neonatal variables were assessed for normality and compared between profiles using ANOVA and Kruskal-Wallis tests (continuous variables) or the χ^2 and Fisher exact tests (categorical variables). ANCOVA assessed differences between profiles and neonatal birth measures (infant sex and gestational age as covariates) and brain volumes (infant sex and PMA at assessment as covariates). Bonferroni adjustment was applied to post hoc pairwise comparisons between profiles.

Results

We included 296 mother-infant pairs with maternal, perinatal, and neonatal characteristics provided in **Table II**. The average maternal age at delivery was 29.0 years. The majority of mothers were Black (61.1%), employed (69.3%), and had a high school degree or higher (87.8%). Approximately one-half of the mothers (49.0%) were uninsured or on government assistance. **Table III** (available at www.jpeds.com) provides descriptive statistics of variables that composed the Maternal Social Advantage and Psychosocial Stress scores. A minority of women endorsed tobacco (38 [12.8%]) or marijuana (37 [12.5%]) use during pregnancy, with 14 (4.7%) who reported the use of both. An additional 39 mothers (13.1%) who did not report marijuana use had urine drug screens positive for tetrahydrocannabinol metabolites during pregnancy, suggesting a level of primary or secondary maternal exposure to a degree that may influence in utero development. Most mothers were healthy, with MMR scores of 0 (43.2%), 1 (31.4%), or 2 (12.2%), whereas fewer mothers had greater measured medical risk, with MMR scores of 3 (7.8%), 4 (4.1%), 5 (1.0%), or 8 (0.3%).

There were 158 male infants (53.4%). The average gestational age and birth weight were 38.9 weeks and 3261 grams, respectively. Most infants (72%) underwent initial assessment (NNNS examination and brain MRI) between 2 and 5 weeks after birth.

NNNS Profiles: Latent Profile Analysis Results

An appropriate sleep state was required to calculate the habituation score, which was only achieved in 4 infants. Therefore, we did not include this score in our analyses. Attention and

Table II. Characteristics of participants: Maternal, perinatal, and neonatal (n = 296)

Maternal demographics	
Age, years, mean ± SD [range]	29.0 ± 5.3 [18.7-41.8]
Race, n (%)	
Black/African American	181 (61.1)
White	104 (35.1)
Other*	11 (3.8)
Employed, n (%)	205 (69.3)
Highest level of education, n (%)	
Less than 12th grade	29 (9.8)
High school degree/GED	72 (24.3)
Some college/vocational school	91 (30.7)
College degree	38 (12.8)
Graduate degree	59 (19.9)
Unknown	7 (2.4)
Insurance status, n (%)	
Medicaid/Medicare/VA/Military	113 (38.2)
Uninsured	32 (10.8)
Individual or group health insurance	151 (51.0)
I/N ratio,† median (Q1-Q3)	1.2 (0.9-3.8)
ADI, median (Q1-Q3)	76.0 (50.0-88.8)
Maternal structural equation model latent constructs, median (Q1-Q3)	
Social advantage score	−0.36 (−0.72 to 0.83)
Psychosocial stress score	−0.22 (−0.79 to 0.44)
Maternal exposures, n (%)	
Any self-reported tobacco use	38 (12.8)
Self-reported use at least once a month	21 (7.1)
Self-reported use at least once a day	17 (5.7)
Any marijuana use/exposure‡	76 (25.7)
Self-reported use at least once a month	15 (5.1)
Self-reported use at least once a day	22 (7.4)
Positive urine drug screen§	61 (20.6)
Depression	47 (15.9)
Anxiety	25 (8.4)
MMR and perinatal characteristics, n (%)	
MMR score	
0	128 (43.2)
1	93 (31.4)
2	36 (12.2)
3	23 (7.8)
≥4¶	16 (5.4)
Prenatal visits during pregnancy	
<10	76 (25.7)
≥10	217 (73.3)
Delivery type	
Vaginal (spontaneous/induced)	208 (70.3)
Operative vaginal (forceps/vacuum)	12 (4.1)
Cesarean	76 (25.7)
Delivery complications**	32 (10.8)
Neonatal characteristics	
Neonatal sex, male, n (%)	158 (53.4)
Gestational age, weeks, mean ± SE [range]	38.9 ± 1.0 [37.0-41.6]
Birthweight, g, mean ± SE [range]	3261.0 ± 486.6 [2200.0-4627.0]
Birth length, cm, mean ± SE [range]	50.3 ± 3.0 [20.5-58.0]
Birth OFC, cm, mean ± SE [range]	34.0 ± 1.6 [29.0-38.5]
1-minute Apgar score, mean ± SE [range]	7.6 ± 1.3 [1.0-9.0]
5-minute Apgar score, mean ± SE [range]	8.8 ± 0.6 [4.0-10.0]
NICU stay, n (%)	16 (5.4)
Length of NICU stay, days, median (Q1-Q3)	2.0 (1.0-4.0)
Diet including breastmilk, n (%)	235 (79.4)
Diet only breastmilk, n (%)	135 (45.6)
Chronological age at assessment,†† days, mean ± SE [range]	22.7 ± 9.0 [3.0-52.0]

(*continued*)

Table II. Continued

PMA at assessment,†† weeks, mean ± SE [range]	42.2 ± 1.3 [39.0-44.9]
Profile assignment	
Hypotonic	49 (16.6)
Fussy	109 (36.8)
Regulated	138 (46.6)

GED, General Educational Development certificate; *I/N*, income-to-needs ratio; *OFC*, occipitofrontal circumference; *VA*, Veterans Affairs.
*Other included Asian (n = 4), Latina (n = 3), unspecified (n = 3), and Asian/White (n = 1).
†I/N ratio is reported as average of I/N from each trimester as there were no differences in log-transformed I/N ratios between the 3 trimesters ($P = .67$).
‡This included mothers with either a urine drug screen positive for marijuana metabolites during pregnancy and/or self-reported use during pregnancy.
§There were 124 mothers (42%) who had urine drug screen data during pregnancy.
¶There were 12 (4.1%) who had a score of 4, 3 (1.0%) had a score of 5, and 1 (0.3%) had a score of 8.
**Delivery complications included shoulder dystocia, maternal fever of >38.0°C, retained placenta, precipitous delivery, arrest of labor, vaginal lacerations, unanticipated surgical extension, and postpartum hemorrhage.
††NNNS assessment and MRIs were conducted on the same day.

handling scores were also state-dependent but were obtained for most infants, with attention scores for 266 infants (90.0%) and handling scores for 284 infants (95.9%). All other scores were obtained in all 296 infants. We fit 2, 3, 4, and 5 profile solutions, with model fit characteristics summarized in **Table IV** (available at www.jpeds.com). Based on the smaller sample size, adjusted Bayesian information criterion, entropy values (as a measure of classification quality), and Vuong-Lo-Mendell-Rubin likelihood ratio test ($P = .0012$), a 3-profile solution provided the best fit. **Figure 2** plots the standardized means of each summary score by profile. Distributions of the NNNS summary scores are plotted in **Figure 3** (available at www.jpeds.com), and the mean values of each NNNS summary score within the 3 identified profiles are provided in **Table V**. There were differences in all summary score means across profiles except for asymmetric reflexes ($F = 2.468$; unadjusted $P = .087$). Based on the pattern of summary scores (described further elsewhere in this article), we labeled these 3 profiles as hypotonic, fussy, and regulated.

A total of 138 infants (46.6%) were characterized by the regulated profile and had the lowest scores (indicating better performance) on handling, arousal, excitability, hypertonicity, and stress/abstinence and the highest scores (also indicating better performance) for attention, self-regulation, and quality of movement. Infants with the fussy profile (n = 109 [36.8%]) had the lowest scores on attention, self-regulation, lethargy, and quality of movement and the highest scores on handling, arousal, excitability, and hypertonicity. Infants with the hypotonic profile (n = 49 [16.6%]) had a nonzero value on the hypotonicity scale and the highest average scores on nonoptimal reflexes and lethargy.

Neonatal Birth Characteristics between Profiles

A comparison of birth characteristics between the profiles is provided in **Table VI**. There was a male predominance in infants with the hypotonic profile. Infants with the fussy profile had significantly smaller birth head circumferences than infants with the other 2 profiles, even when corrected for gestational age and sex. There were no differences in birth weight, birth length, 5-minute Apgar scores, or chronological age at assessment (NNNS and brain MRI)

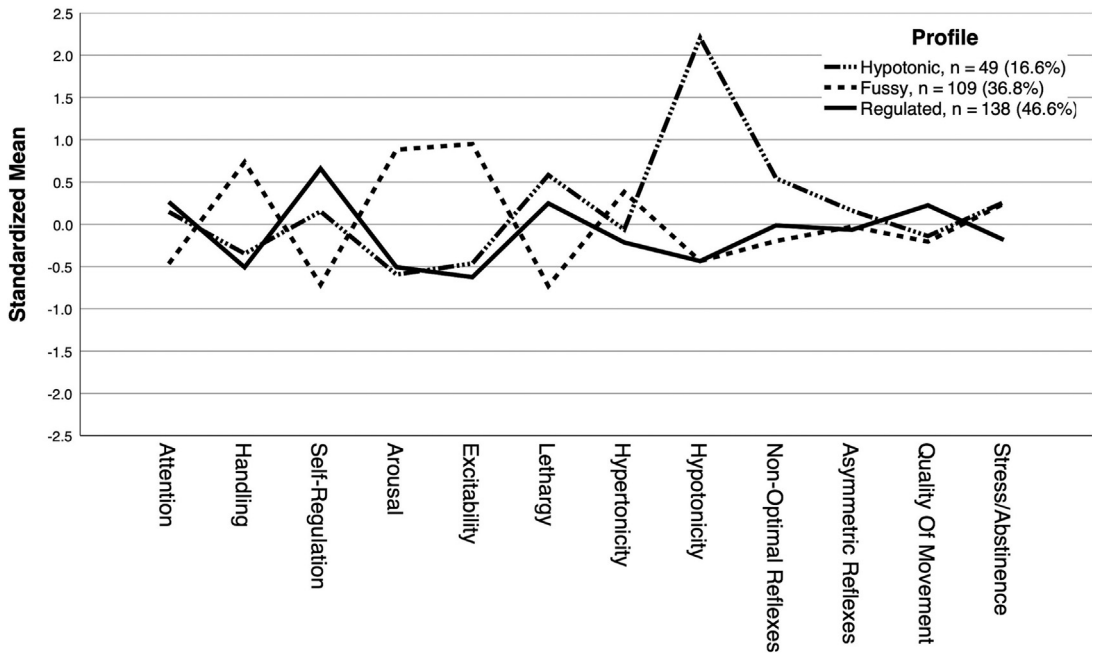

Figure 2. Standardized means of NNNS subscales by profile assignment. The 12 NNNS subscales included in latent profile analysis are labelled along the *x*-axis with the corresponding mean values plotted along the *y*-axis. The hypotonic, fussy, and regulated profiles are denoted by dotted and solid lines.

Table V. Distribution of NNNS summary scores by profile assignment

NNNS summary scores	Profiles			F value	*P* value
	Hypotonic (n = 49 [16.6%])	Fussy (n = 109 [36.8%])	Regulated (n = 138 [46.6%])		
Attention	6.76 ± 1.10*	6.02 ± 1.22*,†	6.88 ± 1.01†	16.90	<.001‡
Handling	0.48 ± 0.24*	0.73 ± 0.17*,†	0.43 ± 0.20†	72.62	<.001‡
Self-regulation	5.86 ± 0.77*,§	5.17 ± 0.59*,†	6.31 ± 0.62§,†	96.28	<.001‡
Arousal	3.68 ± 0.67*	4.74 ± 0.53*,†	3.67 ± 0.47†	139.04	<.001‡
Excitability	3.18 ± 2.09*	6.42 ± 1.70*,†	2.62 ± 1.34†	178.72	<.001‡
Lethargy	3.84 ± 1.69*	1.83 ± 1.32*,†	3.36 ± 1.25†	53.92	<.001‡
Hypertonicity	0.43 ± 0.74	0.73 ± 1.02†	0.30 ± 0.61†	8.78	<.001‡
Hypotonicity	1.04 ± 0.20*,§	0.00 ± 0.00*	0.00 ± 0.00§	3382.64	<.001‡
Nonoptimal reflexes	4.37 ± 1.56*,§	2.86 ± 1.66*	3.29 ± 1.59§	14.81	<.001‡
Asymmetric reflexes	1.82 ± 1.48	1.41 ± 1.26	1.36 ± 1.16	2.47	.087
Quality of movement	4.42 ± 0.59§	4.38 ± 0.61†	4.66 ± 0.67§,†	6.76	.001‡
Stress-abstinence	0.22 ± 0.09§	0.22 ± 0.08†	0.19 ± 0.08§,†	5.41	.005‡

Data reported as mean ± SD.
*Bonferroni-adjusted *P* value < .05 for post hoc pairwise comparisons: between hypotonic and fussy profiles.
†Bonferroni-adjusted *P* value < .05 for post hoc pairwise comparisons: between fussy and regulated profiles.
‡*P* value is statistically significant at threshold of .05 for groupwise comparisons.
§Bonferroni-adjusted *P* value < .05 for post hoc pairwise comparisons: between hypotonic and regulated profiles.

between profile groups. Infants with the hypotonic profile had marginally younger PMAs than infants with the regulated profile.

Maternal Characteristics between Profiles

There were no differences in maternal demographic characteristics between infants with the 3 profiles (**Table VI**). Maternal social advantage and maternal psychosocial stress scores were similar, with no differences in variables that composed the social advantage and psychosocial stress constructs (**Table VII**; available at www.jpeds.com). There were no differences between profiles in multiple maternal variables, including depression, anxiety, or MMR scores. We used post hoc tests to explore prenatal tobacco and marijuana use. Self-reported tobacco use was similar between infant profiles. Out of the 296 mothers in the study who completed questionnaires regarding marijuana use, 124 also underwent urine drug screens. Therefore, we analyzed marijuana use only among the subset of mothers who underwent urine drug screens or self-reported any marijuana use during pregnancy (n = 132). We found no differences between infant profiles in maternal marijuana use.

Neonatal Brain Volumes between Profiles

We analyzed MRI data from 272 infants (91.9%); 24 infants did not have high-quality T2-weighted images. Compared with infants with the hypotonic profile, infants with the fussy profile had smaller total absolute brain and white matter volumes, even after controlling for sex and PMA at assessment. There were no differences in cortical gray matter, subcortical gray matter, cerebellar volumes, or cortical folding between profiles (**Table VI**). Regarding NNNS scores and profile assignment, we note that there were no significant changes in any of these results when including only infants (n = 293) scored by the primary NNNS evaluator (**Appendix III**; available at www.jpeds.com).

Discussion

We investigated the independent contributions of maternal socioeconomic disadvantage and psychological stress during pregnancy on neurobehavioral profiles in healthy, full-term infants and examined the relationship between those profiles and neonatal brain volume measures. We found 3 distinct phenotypes through latent profile analysis: regulated, hypotonic, and fussy. Using these neurobehavioral profiles, we found differences in infant characteristics, including sex, head circumference, and total brain and white matter volumes. Contrary to our hypotheses, differences across profiles between measures of maternal psychosocial stress or socioeconomic disadvantage were not found.

Compared with prior investigations that used the latent profile analysis technique, the distribution of individual summary scores in our profiles is similar.[13,15,21,34,35] Previous studies of healthy full-term infants identified at least one poor neurobehavioral profile with parallel findings to our fussy profile.[21,35] These profiles are characterized by the highest NNNS summary measures of handling, arousal, and excitability and the lowest measures of attention, self-regulation, and lethargy. Sucharew et al also fit a 3-profile solution to a sample of 355 healthy infants—hypotonic (24%), social/easy-going (44%), and high-arousal/difficult (32%), closely paralleling our hypotonic (16.6%), regulated (46.6%), and fussy (37%) profiles.[21] Our study also assessed head circumference and brain volumes. Comparatively, mothers in our study also had more socioeconomic risk factors with lower household incomes and educational achievement, which have implications for the overall generalizability of these studies. Last, we used a multifaceted model to capture socioeconomic disadvantage and psychosocial stress, which may offer a more comprehensive picture of the mother's status compared with the use of individual differences in select characteristics.

Table VI. Comparison of neonatal and maternal characteristics between profiles

Characteristics	Hypotonic (n = 49 [16.6%])	Fussy (n = 109 [36.8%])	Regulated (n = 138 [46.6%])	Test statistic	*P* value
Neonatal characteristics				F-value	
Neonatal sex, n (%)					
Male	35 (71.4)*	58 (53.2)	65 (47.1)*	8.60†	**.014**‡
Female	14 (28.6)	51 (46.8)	73 (52.9)		
EGA at birth, weeks, mean ± SD	38.9 ± 0.9	38.7 ± 1.0	39.0 ± 1.0	2.61	.076
Birthweight,§ g, mean ± SE¶	3258.8 ± 62.3	3207.1 ± 41.7	3303.9 ± 36.9	1.50	.225
Birthweight, g	3291.8 ± 473.5	3171.4 ± 466.95	3320.1 ± 499.2	2.99	.052
Birth length,§ cm, mean ± SE¶	50.6 ± 0.4	50.3 ± 0.3	50.3 ± 0.2	.19	.830
Birth length, cm	50.8 ± 2.6	50.1 ± 2.3	50.3 ± 3.5	.83	.436
Birth OFC,§ cm, mean ± SE¶	34.4 ± 0.2**	33.7 ± 0.1**	34.1 ± 0.1	3.87	**.022**‡
Birth OFC, cm	34.4 ± 1.5**	33.6 ± 1.6**,††	34.2 ± 1.6††	5.72	**.004**‡
5-minute Apgar, mean ± SE	8.6 ± 0.9	8.8 ± 0.5	8.8 ± 0.6	1.35	.262
Chronological age at assessment, days, mean ± SD	20.7 ± 7.3	23.0 ± 8.8	23.3 ± 9.6	1.47	.232
PMA at assessment, weeks, mean ± SD	41.9 ± 1.2	42.0 ± 1.3	42.4 ± 1.3	3.36	**.036**‡
Neonatal brain volumes,‡‡ mean ± SE¶				F-value	
Total brain volume, mm^3	369 196 ± 4469**	355 289 ± 2921**	357 196 ± 4469	3.52	**.031**‡
Cortical gray matter, mm^3	124 111 ± 1648	119 937 ± 1077	120 405 ± 981	2.41	.092
Subcortical gray matter, mm^3	27 877 ± 302	27 083 ± 197	27 204 ± 180	2.53	.081
Cerebral white matter, mm^3	189 901 ± 2540**	181 374 ± 1660**	183 414 ± 1513	3.99	**.020**‡
Cerebellum, mm^3	29 022 ± 409	28 548 ± 268	28 213 ± 244	1.47	.233
Gyrification index (ratio)	1.99 ± .01	1.97 ± .01	1.97 ± .01	1.17	.313
Maternal structural equation model latent constructs, median (Q1-Q3)				H-value§§	
Social advantage score	−0.53 (−0.74 to 0.81)	−0.30 (−0.81 to 0.59)	−0.31 (−0.68 to 1.0)	2.32	.314
Psychosocial stress score	−0.05 (−0.75 to 0.53)	−0.32 (−0.79 to 0.62)	−0.36 (−0.82 to 0.32)	2.46	.292
Prenatal exposures and maternal characteristics, n (%)				χ^2 value	
Any tobacco use (self-reported)	6 (12.2)	12 (11.0)	20 (14.5)	3.39	.494
Any marijuana use/exposure¶¶	19 (70.4)	25 (53.2)	32 (55.2)	2.32	.314
Depression	8 (16.3)	19 (17.4)	20 (14.5)	.40	.818
Anxiety	6 (12.2)	7 (6.4)	12 (8.7)	1.50	.472
Maternal age, years, mean ± SD	28.6 ± 4.9	29.1 ± 5.4	29.2 ± 5.2	.26***	.770
MMR score					
0	25 (51.0)	42 (38.5)	61 (44.2)	6.14	.632
1	16 (32.7)	37 (33.9)	40 (29.0)		
2	5 (10.2)	12 (11.0)	19 (13.8)		
3	1 (2.0)	12 (11.0)	10 (7.2)		
≥4	2 (4.1)	6 (5.5)	8 (5.8)		

EGA, estimated gestational age.
*Bonferroni adjusted *P* value < .05 for post hoc pairwise comparisons: between hypotonic and regulated profiles.
†All test statistics as reported with exceptions as marked: χ^2.
‡*P* value is statistically significant at threshold of .05 for groupwise comparisons.
§Covariates include sex and estimated gestational age (weeks) at delivery.
¶Estimated marginal means adjusted for covariates reported.
**Bonferroni adjusted *P* value < .05 for post hoc pairwise comparisons: between hypotonic and fussy profiles.
††Bonferroni adjusted *P* value < .05 for post hoc pairwise comparisons: between fussy and regulated profiles.
‡‡Covariates include sex and PMA (weeks) at assessment.
§§H-value denotes Kruskal-Wallis test, used for data with a non-normal distribution.
¶¶Analyzed only for 132 mothers with urine drug screens performed (n = 124) or without urine drug screen data who self-reported any marijuana use during pregnancy (n = 8).
***All test statistics as reported with exceptions as marked: F-value for one-way ANOVA.

Our analyses using constructs of maternal psychosocial stress and socioeconomic adversity during pregnancy add to a small body of literature with variable findings.[7,10,13,34] In infants born at full term and preterm, exposure to prenatal or early postnatal maternal depression has been related to increased stress, arousal, excitability, and poor quality of movement on early neurobehavioral assessments.[3-5] Poor neurobehavioral outcomes among full-term infants have also been linked to in utero psychosocial stress.[7,8] Notably, these populations had varied socioeconomic and medical risks. Exposure to maternal psychosocial stress had a stronger neurobehavioral effect on full-term infants with lower levels of maternal economic hardship.[7] Among limited studies analyzing maternal socioeconomic status, high-risk infants born to mothers with greater prenatal socioeconomic risk or on a governmental welfare program also had the poorest neurobehavioral performance.[13,34] However, in a study of healthy full-term infants, maternal insurance status was used as a proxy for socioeconomic status and was not related to any neurobehavioral subscale.[10] In our healthy cohort, we have demonstrated stronger negative impacts of prenatal socioeconomic disadvantage rather than prenatal psychological stress on measures of birth weight and global neonatal brain measures.[22,23] In contrast, neurobehavioral effects are likely influenced by overlapping prenatal factors with different directionality and are modified by exposures such as environmental pollutants, maternal cortisol, or inflammation and resulting epigenetic programming, which were not measured in the current analysis.[5,8,36-38]

We were interested in prenatal exposure to marijuana due to the ability of the active ingredient, delta-9-tetrahydrocannabinol, to readily cross the placenta, the

presence of cannabinoid receptors in the fetal brain, and studies demonstrating early neurobehavioral abnormalities among infants with in utero marijuana exposure.[39-43] We did not find significant differences between infants born to mothers with marijuana use or exposure during pregnancy (26% of the sample had some exposure). This finding may be related to data collection. Although all mothers were asked about self-reported use, only a subset underwent urine drug screens at variable points during pregnancy.

Regarding infant characteristics, we found a male predominance in the hypotonic profile. In studies of full-term infants, hypotonia on the NNNS may denote neurodevelopmental immaturity and has been linked to impaired psychomotor development in the first years of life.[16,21,44] Our results were consistent with studies demonstrating higher scores of hypotonia among males and more males with the most abnormal NNNS scores.[13,16] Compared with infants in our hypotonic profile, infants characterized by our fussy profile had smaller head circumferences, suggesting a parallel with studies that have shown high excitability and related neurobehavioral features among infants with the smallest birth measures (including weight, length, and head circumference).[13,15,34] We extended these findings by examining neonatal brain volumes, demonstrating that infants in the fussy profile also had smaller total brain and cerebral white matter volume when compared with infants in the hypotonic profile. We investigated the relationship between neonatal NNNS scores and brain volumes, focusing on healthy, full-term infants. This contrasts with prior investigations of high-risk populations that linked poor neurobehavioral performance to neonatal brain abnormalities on MRI.[45-50] For example, among infants born preterm, patterns of abnormal neurobehavior have been related to reduced maturation of key white matter tracts, delayed gyral maturation, and severity of white matter, cortical gray matter, and subcortical gray matter injury.[45-48] Similarly, MRI findings of severe brain injury (including subcortical regions) were related to atypical motor scores in full-term infants with neonatal encephalopathy requiring therapeutic hypothermia.[51] Among full-term infants with congenital heart disease, reduced volume of the subcortical gray matter was associated with abnormal neurobehavior.[52] Volumetric measures of fetal brain regions of small for gestational age infants at term have also been related to atypical neonatal behavior.[49,50]

We found differences in total brain and white matter volumes between infants with our fussy and hypotonic neurobehavioral profiles, which align with the known vulnerability of cells in the developing white matter (oligodendrocytes and precursors) to a range of environmental and physiologic exposures.[53,54] We did not find differences in cortical or subcortical gray matter volumes or gyral maturation, in contrast with several studies of infants at high medical risk.[45-52] Those cohorts often included infants with evidence of brain injury on MRI, a specific criterion for exclusion from our analysis. A similar investigation of the brain volumes and neurobehavior of infants born preterm at term-equivalent age demonstrated changes in white matter microstructure related to neurobehavioral scores but no differences in brain volumetric measures.[45] Compared with our cohort, this sample was at substantially higher medical risk with the potential for a variety of postnatal influences from premature birth to term-equivalent evaluation. We interpret our findings conservatively, because we only identified differences in brain volume measures between the fussy and hypotonic profiles. Although these differences may indicate differences in neurodevelopmental trajectories, brain volume measures in both profiles were similar to the regulated profile. Furthermore, the raw differences between the groups were small and may not be clinically meaningful. It is not yet clear if the differences in brain volumes are biologically significant. These relationships may be better elucidated using metrics of brain network functional and structural connectivity, which are being actively investigated, as well as potential associations to longitudinal outcomes.

A strength of this study is that we used 2 latent constructs to characterize maternal social advantage and maternal psychosocial stress that allowed for a multidimensional evaluation of many inter-related variables, a complex issue in research on adversity. This method is a novel approach among studies of neonatal neurobehavior. Second, the NNNS assessments were conducted by 2 trained, certified, and highly experienced evaluators, with 99% performed by the same rater, decreasing the risk of bias in scores. Third, neurobehavioral assessments and neonatal brain MRI scans were standardly conducted during the same visit, allowing for an accurate snapshot of the infant's neurodevelopment. We also acknowledge limitations of our study. First, maternal marijuana and tobacco use during pregnancy were measured by self-reported questionnaires and urine drug screen data in a subset of the sample. Second, because neurobehavioral assessments were primarily conducted between 2 and 5 weeks after birth, there may be confounding unmeasured postnatal influences of profile assignment, such as parental behaviors. Last, although the generation of NNNS profiles categorizes infants into distinct groups based on their score distribution, this technique may blunt individual summary score differences between infants.

Our results underscore the need for further research regarding the in utero influence of psychosocial stress and socioeconomic disadvantage on neonatal neurobehavioral outcomes. Longer-term follow-up may determine if identified neonatal neurobehavioral profiles are related to developmental, cognitive, behavioral, or clinical outcomes in childhood. Further identification of infants at high social risk may, in turn, support targeted early interventions to enhance neurodevelopmental outcomes. ■

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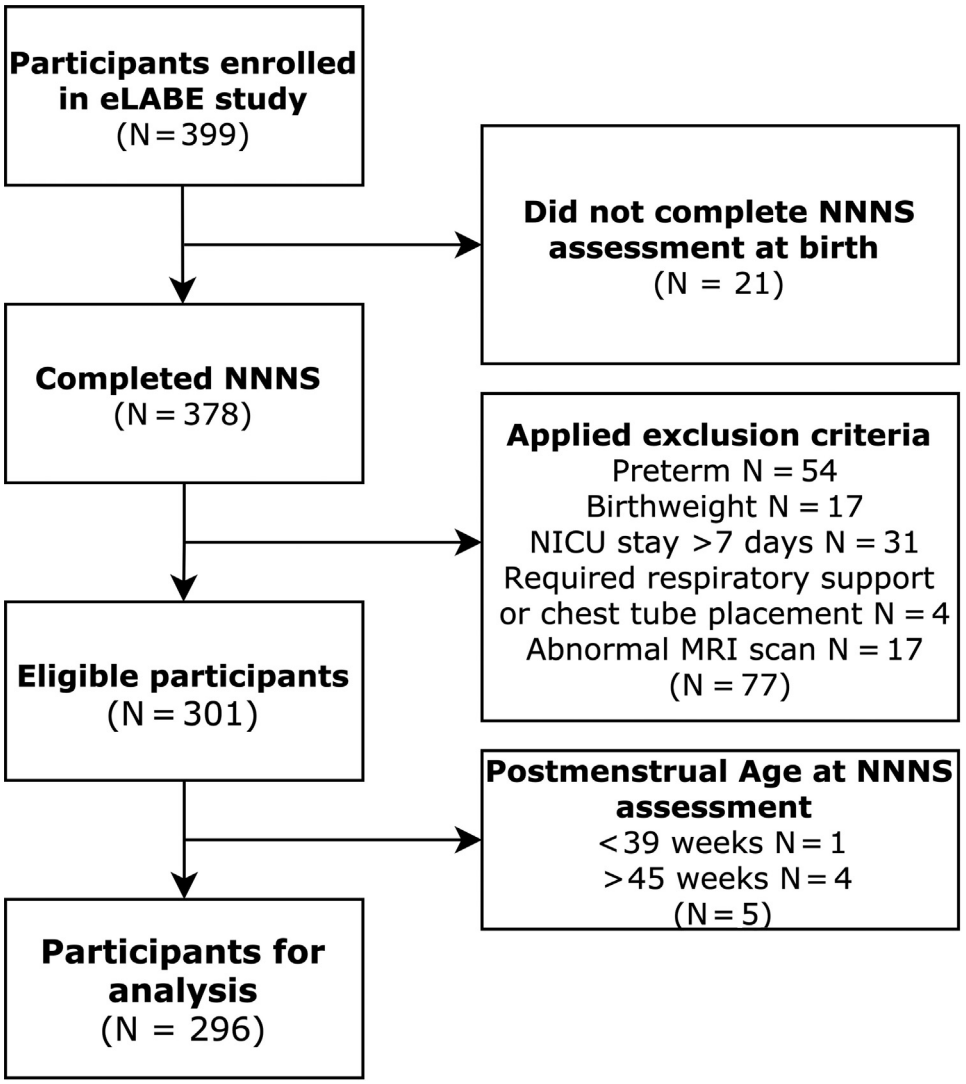

Figure 1. Participant flow from enrollment to inclusion.

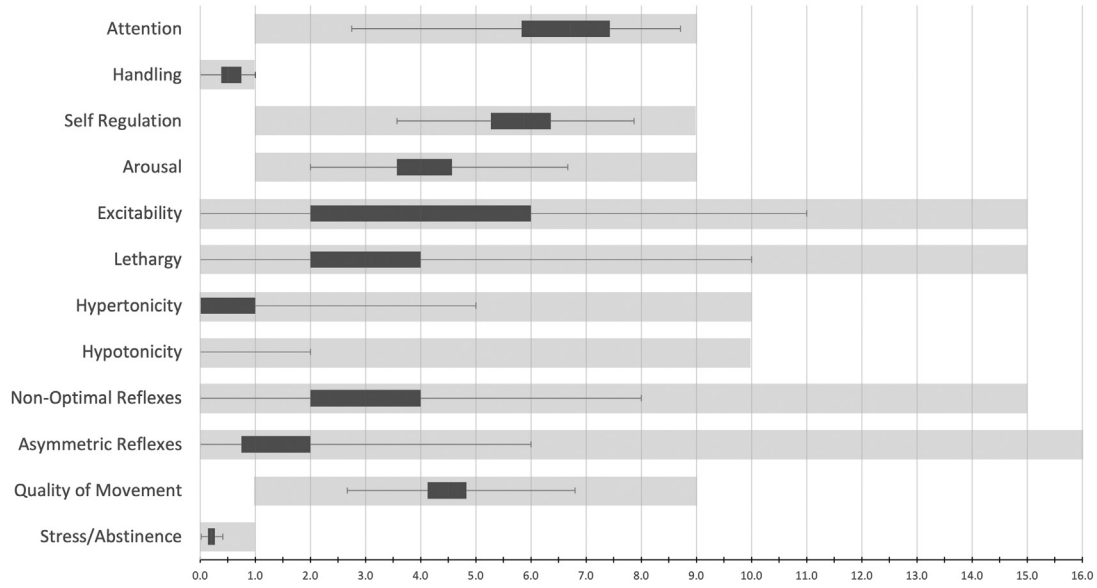

Figure 3. Graphical representation of NNNS summary scores. Light gray indicates the range of possible values for each summary score. The box plot is centered around the sample's median score. The dark gray area defines the 25th and 75th percentile and the whiskers extend to the minimum and maximum scores from the study cohort.

Table I. Descriptions of NNNS summary scores

Summary scores [range of possible values]	This score measures the infant's…	High scores indicate that the infant…
Attention [1-9]	response to animate and inanimate auditory and visual stimuli	has increased alertness and ability to track and maintain response to stimuli
Handling [0-1]	response to various orientation handling strategies to maintain alert and calm state	requires considerable handling and consoling by examiner to maintain an alert and calm state
Self-Regulation [1-9]	ability to regulate physiological state, tone, and activity and respond to cuddling and consoling	is able to self-regulate (ie, relax during cuddling, will successfully quiet self, has smooth motor movements)
Arousal [1-9]	level of arousal, fussiness, and activity during examination	is often crying, fussy, and continuously moving despite attempts at consoling
Excitability [0-15]	physiological state, tone, activity, excitement, and irritability	has increased excitability (ie, jerky movement, irritable fussing) despite attempts at soothing
Lethargy [0-15]	response to stimuli, alertness, physiological state, tone, activity, excitement, and irritability	is underaroused (ie, low reactivity to stimuli, low level of alertness)
Hypertonicity [0-10]	hypertonic response in arms, legs, or trunk or overall tone	is more hypertonic
Hypotonicity [0-10]	hypotonic response in arms, legs, or trunk or overall tone	is more hypotonic
Nonoptimal reflexes [0-15]	any nonoptimal response to reflex elicitation	has more nonoptimal reflexes
Asymmetric reflexes [0-16]	any asymmetric response to reflex elicitation	has more asymmetric reflexes
Habituation [1-9]	ability to maintain sleep state when faced with disturbing stimuli	has better (more rapid) ability to maintain sleep state after encounters with disturbing stimuli
Quality of movement [1-9]	motor control and smoothness of movements, activity level, tremulousness and startles	has more mature movements, with fewer tremors and startles
Stress-abstinence [0-1]	signs of stress or abstinence during examination across seven categories (physiologic, autonomic, CNS, skin, visual, gastrointestinal, state)	has more signs of stress abstinences

A higher score indicates a poorer performance in the handling, arousal, excitability, lethargy, hypertonicity, hypotonicity, nonoptimal reflexes, asymmetric reflexes, and stress-abstinence scales. Adapted from Lester BM, Tronick EZ, Brazelton TB. The Neonatal Intensive Care Unit Network Neurobehavioral Scale Procedures. Pediatrics 2004; 113:679-89.

Table III. Maternal characteristics of variables in SEM

SEM, maternal characteristics (n = 296)	
Maternal social advantage score, median (Q1-Q3)	−0.36 (−0.72 to 0.83)
First trimester I/N, n = 288, median (Q1-Q3) [range]	1.3 (0.9 to 3.8) [0.3 to 12.2]
Second trimester I/N, n = 230	1.5 (0.9 to 4.0) [0.4 to 12.2]
Third trimester I/N, n = 250	1.5 (0.9 to 3.8) [0.4 to 11.8]
ADI	76.0 (50.0 to 88.8) [1.0 to 100.0]
Private insurance, n (%)	151 (51.0)
Healthy Eating Index, mean (SD) [range]	58.5 (10.0) [33.0 to 80.7]
Maternal psychosocial stress score, median (Q1-Q3)	−0.22 (−0.79 to 0.44)
First trimester EPDS, n = 294, median (Q1-Q3) [range], % depressed*	4.0 (1.0 to 7.0) [0 to 25], 18.4
Second trimester EPDS, n = 249	3.0 (1.0 to 7.0) [0 to 26], 20.5
Third trimester EPDS, n = 251	3.0 (1.0 to 6.0) [0 to 25], 15.6
First trimester PSS, n = 292	12.0 (8.0 to 18.0) [0 to 35]
Second trimester PSS, n = 229	13.0 (7.0 to 19.0) [0 to 36]
Third trimester PSS, n = 245	12.0 (7.0 to 17.0) [0 to 36]
Stress and Adversity Inventory (STRAIN), n = 281	
Total count of chronic difficulties (STRAIN-CT)	5.0 (2.0 to 9.0) [0 to 25]
Total severity of chronic difficulties (STRAIN-WT SEV)	15.0 (7.0 to 29.0) [0 to 99]
Everyday Discrimination Scale, n = 271	1.9 (1.3 to 2.6) [1.0 to 5.7]

EPDS, Edinburgh Postnatal Depression Scale; *I/N*, income-to-needs ratio; *PSS*, Perceived Stress Scale; *SEM*, structural equation model; *STRAIN*, Stress and Adversity Inventory.
*Percent depressed are the mothers with an EPDS of ≥10 (clinical cutoff for high risk of depression).

Table IV. Model fit statistics of latent profile analysis

Profiles	Sample-size adjusted Bayesian information criterion	Entropy	Vuong-Lo-Mendell-Rubin likelihood ratio test, *P* value	Bootstrapped parametric likelihood ratio test, *P* value
2	7075.648	1.000	0.7093	<.0001
3	6609.306	0.921	0.0012	<.0001
4	6973.222	0.856	0.5506	<.0001
5	4501.198	0.891	0.2335	<.0001

Table VII. Comparison of maternal variables in SEM model between profiles

	Hypotonic (n = 49 [16.6%])	Fussy (n = 109 [36.8%])	Regulated (n = 138 [46.6%])	H-value*	*P* value
Maternal characteristics (n = 296)					
Social advantage score	−0.53 (−0.74 to 0.81)	−0.30 (−0.81 to 0.59)	−0.31 (−0.68 to 1.0)	2.32	.314
First trimester I/N	1.2 (0.9 to 3.5)	1.2 (0.7 to 3.4)	1.3 (0.9 to 4.2)	2.24	.326
Second trimester I/N	1.7 (0.9 to 3.8)	1.2 (0.9 to 3.9)	1.9 (0.9 to 5.2)	2.41	.300
Third trimester I/N	1.2 (0.8 to 4.5)	1.2 (0.7 to 3.7)	1.7 (0.9 to 5.2)	3.79	.150
ADI	82.0 (61.5 to 90.5)	76.0 (53.5 to 88.0)	70.5 (43.0 to 89.0)	3.45	.178
Private insurance	21 (42.9)	57 (52.3)	73 (52.9)	1.57†	.456
Healthy Eating Index	55.6 ± 9.7	58.5 ± 10.1	59.6 ± 9.9	2.27‡	.105
Psychosocial stress score	−0.05 (−0.75 to 0.53)	−0.33 (−0.79 to 0.62)	−0.35 (−0.82 to 0.32)	2.46	.292
First trimester EPDS	4.0 (1.5 to 8.0)	4.0 (1.3 to 7.0)	4.0 (1.0 to 7.0)	.45	.797
Second trimester EPDS	4.0 (1.0 to 6.0)	4.0 (1.0 to 8.0)	3.0 (1.0 to 6.0)	1.80	.407
Third trimester EPDS	4.0 (0.0 to 6.0)	3.0 (1.0 to 6.0)	2.0 (1.0 to 6.0)	.98	.614
EPDS, average of 3 trimesters	4.0 (1.6 to 7.0)	3.3 (1.8 to 7.2)	3.6 (1.6 to 6.0)	.90	.637
First trimester PSS	15.0 (10.0 to 19.0)	12.0 (7.0 to 19.0)	12.0 (7.3 to 17.0)	1.43	.489
Second trimester PSS	13.0 (8.0 to 20.8)	14.0 (7.3 to 19.8)	11.0 (6.0 to 17.0)	4.73	.094
Third trimester PSS	12.0 (9.0 to 17.8)	12.0 (7.0 to 18.0)	12.0 (7.0 to 16.0)	1.20	.548
PSS, average of 3 trimesters	14.0 (8.8 to 18.7)	13.0 (8.0 to 18.3)	11.7 (7.3 to 16.5)	3.44	.179
STRAIN					
STRAIN-CT	5.5 (2.3 to 10.0)	5.0 (2.0 to 9.0)	5.0 (2.0 to 9.0)	.49	.783
STRAIN-WT SEV	18.5 (9.3 to 31.0)	15.0 (5.3 to 29.8)	15.0 (7.0 to 28.0)	1.71	.425
Everyday Discrimination Scale	2.1 (1.4 to 2.6)	1.9 (1.3 to 2.5)	1.9 (1.2 to 2.6)	.36	.836

STRAIN-CT, Total Count of Chronic Difficulties; *STRAIN-WT SEV*, Total Severity of Chronic Difficulties.
Data are reported as median (Q1-Q3), number (%) or mean ± SD.
*H-value denotes Kruskal-Wallis test, used for data with a non-normal distribution.
†All test statistics as reported with exceptions as marked: χ^2 test.
‡All test statistics as reported with exceptions as marked: F-value for one-way ANOVA.