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# Early Childhood Neurodevelopmental Outcomes in Children with Prenatal Zika Virus Exposure: A Cohort Study in Puerto Rico

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## Abstract

**Objective.—**To describe anthropometric, sensory, and neurodevelopmental outcomes of children who were Zika virus–exposed from birth to 36 months.

**Study design.—**The study cohort included 114 children born to mothers with confirmed and probable Zika virus pregnancy infection in 2016–2017. Children attending study visits from May 2017 through February 2020 underwent physical/neurologic, sensory examinations, and neurodevelopmental assessments with the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) and Ages and Stages Questionnaires, Third Edition (ASQ-3).

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**Results.—**Three of the 114 children (2.6%) had microcephaly (z-score for head circumference ≤ −2) at birth, 19 of 35 (54.3%) had posterior eye abnormalities in retinal images, and 11 of 109 (10.1%) had nonspecific findings on brain ultrasound. Three of 107 children (2.8%) failed hearing screening at birth. Of those children with follow-up data, 17 of 97 (17.5%) failed age-appropriate vision screening. The BSID-III identified developmental delay in at least 1 domain in at least one-third of children, with higher prevalence in the language domain. ASQ-3 screen positive delay peaked at around 24 or 36 months, with some domains showing a decrease at older ages. Correlations among BSID-III and ASQ-3 scores were observed, representing professional and parental perspectives at 24 and 36 months ($r$ = 0.32-0.78; $P$ < .05).

**Conclusion.—**The presence of neurodevelopmental sequelae in early childhood suggests that identification of long-term impairment remains critical to attaining optimal child development. Long-term follow-up highlights vulnerability in the language domain, which likely could be influenced by early intervention, promoting cognitive development and school readiness in exposed children.

### Keywords

congenital Zika virus exposure; prenatal Zika virus exposure; children without microcephaly/ asymptomatic at birth; neurodevelopmental outcomes; developmental delay

## Introduction

Zika virus, a member of the Flaviviridae family, became manifest as a pathogen during an epidemic of febrile rash illness in northeastern Brazil, followed by an increase in infants with microcephaly born to mothers who lived in or visited in the same region during pregnancy.[1–4] Although 4–7% of Zika virus infections during pregnancy result in fetal loss, a critical outcome of Zika virus infection during pregnancy, characterized as congenital Zika syndrome,[5] is identified in 5%–14% of neonates, including 4%–6% with microcephaly.[6] Therefore, 79%–91% are asymptomatic infected neonates without clinically evident birth defects, who can be at risk for medium and long-term sequelae.[6, 7] An array of adverse outcomes have been recognized in asymptomatic infected children over time, including postnatal microcephaly,[7–10] developmental delay,[7, 11–15] autism spectrum disorder,[8] and poor visual function,[16, 17] supporting expert recommendations for follow-up of all exposed children with developmental monitoring and screening.[7, 18–20,21] For pediatric providers in at-risk regions of the world, full knowledge of the spectrum of disease and a high index of suspicion are essential, because most maternal infections are asymptomatic and Zika virus transmission can occur without identifiable outbreaks.[6]

Puerto Rico, a US territory with an estimated population of 3.2 million and a primarily Hispanic ethnic composition (98.7%),[22] experienced the Zika epidemic from January 2016 through December 2017, with a total of 40,630 confirmed cases, including 4,134 pregnant women.[23] The Pediatric Outcomes of Prenatal Zika Exposure cohort study, active since May 2017, aims to describe the spectrum of disease in children born to mothers with confirmed and probable Zika virus infection during pregnancy. Here we report the longitudinal prevalence of anthropometric, sensory and neurodevelopmental outcomes from

birth to 36 months in this group of Hispanic children from Puerto Rico, most of whom were asymptomatic at birth.

## Methods

This prospective cohort includes 114 children of mothers meeting the enrollment criteria born at 2 academic hospital sites in the city of Ponce that serve an estimated population of 477,603 living in the southern region of Puerto Rico.[24] The Centers for Disease Control and Prevention (CDC) reviewed and the Ponce Medical School Foundation Institutional Review Board approved this study. Parental informed consent was obtained in writing. During the Zika epidemic, the Puerto Rico Department of Health Zika Active Pregnancy Surveillance System, in collaboration with the CDC, identified and actively monitored pregnant women for infection during each trimester, as well as their exposed children. Mothers with confirmed and probable Zika virus infection during pregnancy were included after confirmation of their infection status in the birth record and the Zika Active Pregnancy Surveillance System Registry. Confirmed infection was defined as presence of Zika virus RNA in maternal serum on the CDC's Trioplex real-time reverse transcriptase-polymerase chain reaction (PCR) assay. Probable cases were defined by maternal serum positive or equivocal Zika virus immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (Zika virus IgM antibody capture ELISA [MAC-ELISA]) or maternal recent flavivirus infection as evidenced by positive anti-Zika virus IgM MAC-ELISA and positive anti-dengue virus IgM ELISA, based on epidemiologic considerations.[25] For symptomatic pregnant women, the timing of infection was determined from the week of gestation[26] or the trimester coincident with symptom onset as documented in the birth record. Maternal education attainment and other sociodemographic characteristics were obtained through direct interview by trained study personnel.

Enrollment of children in the dynamic cohort occurred at birth or at any age-specific study visit every 6 months. Sixty-two children were enrolled in the neonatal period and 52 were enrolled at a study visit between May 1, 2017, and December 2019 (Figure 1. Assessments were conducted until February 28, 2020. Newborn diagnostic procedures at both study sites followed Puerto Rico Department of Health mandates and CDC guidelines.[18, 19] Trained study personnel performed record extraction of sociodemographic information, medical, obstetric, and delivery history; and childbirth outcomes, including results of automated auditory brainstem response testing, brain ultrasonography, and retinal images with the RetCam3 visualization system (Clarity Medical Systems, Inc), mandated on all exposed newborns. Hospital radiology technicians performed brain ultrasonography in 109 of 114 (95.6%), and a nurse trained in RetCam3 system obtained retinal images in 35 of these114 (30.7%). Pediatric subspecialty experts in radiology and ophthalmology provided the readings to 92 of 109 ultrasound images (84.4%) and 32 of 35 retinal images (91.4%), respectively. Their findings were documented in standardized study forms that specified Zika virus-associated birth defects and allowed for relevant observations that could contribute to the spectrum of disease. Along with microcephaly, these clinically evident retinal imaging and brain ultrasound findings constitute the case definition for symptomatic children at birth.

Study pediatricians performed comprehensive physical and neurologic examinations following structured formats, and trained study personnel obtained anthropometric measures and instrument-based vision screening following American Academy of Pediatrics-recommended procedures.[27] Repeat automated auditory brainstem response testing was performes in 27 children during the first year of life. Analysis of birth head circumference (HC), weight, and length by gestational age was based on INTERGROWTH-21$^{st}$ standards for term infants' and pre-term infants' postnatal growth until 42 weeks.[28] After birth, growth analysis was based on World Health Organization standards for children, correcting for <40 weeks of gestational age, until 24 months.[29] Microcephaly at birth was defined as an HC z-score ≤ −2.00 for sex and gestational age based on INTERGROWTH-21$^{st}$ standards. Zika virus-associated birth defects and neurodevelopmental abnormalities were based on CDC surveillance classification for children (birth to 2 years) born to mothers with any evidence of infection.[7]

Experienced, licensed clinical psychologists administered the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)[30] in Spanish, the primary language of the families, to assess children's neurodevelopment at 24 months and 36 months. Scores <85 (at least 1 SD below the average) on cognitive, language, or motor composite scores indicate neurodevelopmental delay.

Parents completed the Ages and Stages Questionnaire, Third Edition (ASQ-3)[31] to screen for developmental delays in communication, gross motor skills, fine motor skill, problem solving, and personal-social skills at each study visit from age 6 months. The ASQ-3 provides valuable parental perspectives to help identify developmental risks conducive to early intervention. Scores at or below the age-based cutoff score suggest that a child is at risk for developmental delay. Scores in the monitoring zone suggest that the child can benefit from follow-up developmental assessment.

## Statistical Analysis

Descriptive statistics were calculated for sociodemographic and clinical characteristics of the study population. Mean (SD) and percentage were calculated for continuous and categorical variables, respectively. The prevalence of developmental risk or delay as assessed by the BSID-III and ASQ-3, respectively, was calculated at each assessment age. The chi-square test was used to assess whether the prevalence of developmental delay differed significantly within a domain. Summary variables indicating the presence of developmental problems at any time between 6 and 36 months were derived for each domain. Poisson regression with robust error variance was used to test the adjusted associations between maternal age, education, and child sex with developmental problems. Maternal age was dichotomized at the mean, and education was dichotomized as high school or less vs more than high school as the highest level of education attained. Consistency between the parent-reported ASQ-3 and directly assessed BSID-III measures were examined via Pearson correlations. A P-value < .05 denoted statistical significance. Analyses were performed with SAS version 9.4 (SAS Institute). Sample sizes for descriptive analyses reflect the number of people who completed each assessment, ranging from 48 to 114 (Figure 1). Correlations and regression analyses

were separate complete case analyses according to age group and developmental domain examined.

## Results

Mother and child clinical and sociodemographic characteristics are presented in Table 1. The cohort comprised 114 children born of 112 mothers with a mean age of 26 years (Figure 1). Forty-two pregnant women 42 (37.5%) had a laboratory-confirmed infection by PCR and 70 (62.5%) had probable infection with Zika virus IgM-positive results; 21 (18.8%) were positive for both. Fifty-two mothers (46.4%) had symptoms, including 28 infected during the first trimester. Most mothers had public health insurance (n = 90, 80.4%) and an annual household income <$15,000 (n = 80, 71.4%). Forty-eight mothers (42.9%) had an educational level of high school or less. Children were born at a mean gestational age of 38.1 weeks (range, 31–40 weeks); 13 (11.4%) were premature births (31-36 weeks of gestational age). Sixty children (52.6%) were female. Neonatal assessment revealed 3 of 114 children (2.6%) with microcephaly at birth (HC z-score ≤ −2), 3 of 107 (2.8%) with abnormal hearing screening, 19 of 35 (54.3%) with retinal imaging abnormalities, 11 of 109 (10.1%) with nonspecific brain ultrasound findings, and 7 of 114 (6.1%) with neuromotor (movement or tone) abnormalities. Three children with microcephaly had normal brain ultrasound images at birth, 1 child with congenital Zika syndrome was assessed at 2 years by magnetic resonance imaging, which showed a brachycephalic skull configuration with a hypoplastic inferior cerebellar vermix, enlarged ventricular occipital horns, and abnormal (diffusely heterogeneous) white matter. At 30 months, this child had severe microcephaly (HC z-score, −4.57) and serious neurodevelopmental, sensory, and musculoskeletal impairments. A second child had normal HC and development at 24 months, and the third child was lost to follow-up soon after enrollment. Among those without microcephaly who had retinal imaging and brain ultrasound, 34 (97.1%) had no abnormality and thus were considered asymptomatic.

Of the 97 children with follow-up after birth, 3 (3.1%) developed a seizure disorder, and 23 (23.7%) had neuromotor (movement or tone) abnormalities that persisted in 1 or more follow-up visits (Table 2). Seventeen of 97 children (17.5%) who underwent clinical evaluation or instrument-based vision screening had possible visual impairment, including 14 with abnormal retinal images at birth. Twelve of these children were referred for subspecialty confirmation, and 4 were evaluated. All 4 had a confirmatory diagnosis, including hyperopia in 3 and astigmatism in 1. Over time, only 2 of 97 (2.1%) had failure to thrive at any visit. At the end of the study period, 47 participants were considered lost to follow-up (ie, absent on 2 consecutive visits and/or unable to be contacted). Twelve (25.5%) were lost to emigration after the Hurricane María island-wide natural disaster of September 2017 or the 6.4 magnitude earthquake of January 2020 in southern Puerto Rico. Inactive participants were more likely to have private health insurance and mothers with probable (ie, Zika virus IgM) infection (Table 3).

### Neurodevelopmental Outcomes at 24 and 36 Months

Children's performance on the BSID-III at 24 months reflected average mean composite scores on the motor (93.4; SD, 13.7) and cognitive (90.6; SD, 12.7) domains, and below average score on language domain (83.3; SD, 15.0). Mean scores at 36 months were average on all domains, with a slight increase in the language domain scores (86.7; SD, 19.5), although on the lower borderline of the average range (Table 4). Parental reports on the ASQ-3 (n=53) revealed mean scores at 24 months in the cautionary "monitoring" zone for communication, gross motor, fine motor, and personal-social domains (Table 4), indicating that the child likely could benefit from follow up actions. Similarly, mean scores at 36 months (n=47) in problem solving and personal-social domains were in the "monitoring" zone.

### Prevalence of Neurodevelopmental Problems

Figure 2 displays the prevalence of developmental delay and risk according to BSID-III and ASQ-3 domains, respectively, at age 6-36 months. The BSID-III assessment showed high levels of developmental delay, with some change over time. For example, cognitive delay was present in 24.5% at 24 months and in 10.6% at 36 months ($P < .001$); language delay was present in 50.9% at 24 months and in 31.9% at 36 months ($P < .001$); and motor delay was present in 21.2% at 24 months and in 27.7% at 36 months ($P < .001$). Among the domains, a moderate-severe (−2 SD to −3 SD) delay in language at 24 months (17.0%) and 36 months (14.9%) was more prevalent (Figure 3). A finding of interest is developmental delay in at least one domain identified by the BSID-III at 24 or 36 months in 10 of 19 children (52.6%) with abnormal retinal images and in 5 of 11 (45.5%) with nonspecific brain ultrasound findings at birth (data not shown). For the ASQ-3, the prevalence of developmental risk was low in infancy and increased with age, peaking at around 24 or 36 months, with some domains showing lower prevalence of risk at older ages. Specifically, the prevalence of communication delay risk was low at 6 months (1.6%) and increased to 45.3% by 24 months ($P = .21$); by 36 months, the prevalence was 36.9% ($P < .001$). Similarly, the prevalence of gross motor delay risk increased from 6.6% at 6 months to 43.4% at 24 months ($P = .08$) and was greatly reduced by 36 months (8.7%; $P = .85$). A similar pattern was evident for fine motor delay risk: 3.3% at 6 months, 50.9% at 24 months ($P = .36$), and 29.8% at 36 months ($P = .01$). For the problem solving and personal-social domains, developmental risk generally increased with age. For problem solving, 67.4% exhibited developmental risk at 36 months. For personal-social, 56.6% were at risk at 24 months, and 45.7% were at risk by 36 months ($P = .59$).

Overall, BSID-III assessments identified developmental delays in any domain in 33 of 53 children (62.3%) during the second year and 17 of 47 (36.2%) during the third year (Table 2). Results from ASQ-3 identified developmental risk in any domain in 16 of 74 children (21.6%) in the first year, in 29 of 60 (48.3%) in the second year, and in 34 of 61 (55.7%) during the third year.

### Neurodevelopmental Problems According to Sociodemographic Characteristics

Some patterns in developmental delay were noted according to maternal age, education, and child sex as assessed in multivariate Poisson models (Table 5). Children of mothers aged

13–25 years had a significantly lower risk of developmental delay in ASQ-3 communication (relative risk [RR], 0.56; 95% CI, 0.3–0.96) and problem solving (RR, 0.65; 95% CI, 0.46–0.92) compared to children of mothers aged >25 years. Children of mothers with a high-school education or less tended to have a greater risk of BSID-III assessed gross motor delay compared with children of mothers with more education (RR, 2.35; 95% CI, 0.96–5.73), although the difference was not statistically significant. Compared with females, males had significantly higher BSID-III language delay (RR, 2.00; 95% CI, 1.18–3.40) and risk of ASQ-3 fine motor delay (RR, 1.63; 95% CI, 1.11–2.37). Nonsignificant associations also suggested that males may have had higher BSID-III cognitive delay (RR, 2.30; 95% CI, 0.87–6.10), and risk of ASQ-3 communication delay (RR, 1.56; 95% CI, 0.95–2.55).

### Correlations Between ASQ-3 and BSID-III Domains

There was significant agreement between clinically assessed BSID-III and parent-reported ASQ-3 development scores (Table 6). For example, strong and highly significant correlations were observed among BSID-III and ASQ-3 scores when both were assessed at 24 and 36 months ($r$ = 0.32-0.78 $P$< .05).

## Discussion

The study describes pediatric outcomes of prenatal Zika virus exposure in a cohort of 114 Hispanic children born in Ponce, Puerto Rico during and after the Zika epidemic of 2016–2017. Understanding of the neurodevelopmental outcomes associated with prenatal Zika virus exposure is facilitated by a well-characterized study sample and the implementation of specialized longitudinal assessments identifying long-term age-specific impairment, an enhancement to cross-sectional approaches, or when assessments were implemented within a wide age range.[8, 11–15, 32] The high consistency between the 2 assessment tools supports the validity of neurodevelopmental problems in 1 or more domains in at least one-third of children at 36 months (36.2% in the BSID-III, 55.7% in the ASQ-3), compared with a US prevalence for emotional, developmental, and behavioral problems of 10.8% among children aged 0–5 years.[33] In our study the largest proportion of exposed children (97.1%) did not have clinically evident birth defects, that is, no microcephaly or abnormalities in retinal images or brain ultrasound. This finding was similar to the experience in surveillance and prospective cohort studies that enrolled mothers with confirmed or probable Zika virus infection.[7, 8] Consistent with our findings, most studies with follow-up in children without microcephaly after 12 months have reported physical, sensory, or neurodevelopmental abnormalities that become clinically evident as the children grow, leading to recommendations for long-term detailed follow-up.[8, 11, 13–15] Some studies of children without microcephaly at birth failed to show developmental defects on follow-up; nevertheless, the authors suggested the possible need for larger and longer-term studies.[34, 35]

Birth assessments identified 3 children (2.6%) with microcephaly, and among those tested, 19 (54%) had Zika-associated abnormalities in retinal image and 11 (10%) had non-specific brain ultrasound findings that were associated with developmental risk in a study by Mulkey et al.[36] The proportion of children born prematurely and with low birth weight is consistent with the 2016 Puerto Rico rates of 11.4% and 9.7%, respectively.[37]

In mothers with symptomatic Zika, one-half of the infections occurred during the first trimester, when the risk for microcephaly is greatest; nevertheless, the rate of microcephaly in our cohort was lower than the 6%–11% reported in the US Zika Pregnancy Register landmark study of birth defects.[38] Different rates of microcephaly have been reported in surveillance and observational studies from different countries and sites, suggesting that multifactorial etiologies could be involved, including genetic influences and environmental factors not fully defined.[39] Nevertheless, retinal and optic nerve abnormalities were identified in approximately one-half of those tested, and eye abnormalities in the absence of microcephaly and other brain abnormalities have been described as Zika-associated birth defects.[40,7]

Two studies with laboratory-confirmed infection in mothers[8] and neonates[13] that assessed children without microcephaly between 7 and 32 months and between 14.4 and 22 months reported a BSID-III prevalence of developmental delay in 1 or more domains similar to our study, at 34.0% and 38.4%, respectively.[8,13] These results represent children with mild delay within an age range, limiting age-specific comparisons. Hearing screening identified abnormalities in only 2.8% of our tested newborns, a lower rate than reported in other cohorts of Zika-exposed children (4.6%–12%),[10,15,11,8] except for 1 (0%),[13] likely reflecting differences in testing and study populations. Vision screening identified 17.5% of children with possible vision impairment. Vision abnormalities that were identified on follow-up also have been documented in the medical literature.[16,17] Postnatal neuromotor abnormalities were rare, and their significance requires the consideration of other neurologic examination findings.

ASQ-3 results present a tendency of increased prevalence of developmental risk by age, consistent with the paradigm in which the full extent of consequences of early brain insult might not be evident until years have passed and in response to increasing developmental demands,[41] underscoring the effectiveness of developmental screening using appropriate instruments.[42] Of interest are the BSID-III adverse findings by domain and age that peaked at 24 months with some improvement at 36 months, most notably in the communication domain. Our study and others[8, 13] have identified language and communication problems as a common sequela of intrauterine Zika virus exposure, a finding that merits in-depth research to elucidate underlying pathophysiologic mechanisms and their clinical correlates. Language delay can improve with early intervention, home literacy, and a nurturing environment with long-lasting effects on academic, social, and emotional development and well-being. Early intervention (mandated for all exposed children in Puerto Rico) and family responses to study findings during a sensitive period of child development could explain the improvement noted in the older age group, a reassuring prospect that depends on the timeliness and effectiveness of the interventions.

The prevalence of developmental delay in any domain decreased from approximately two-thirds at 24 months to approximately one-third at 36 months. Interpretation of these findings is compounded by several factors that influence outcomes of early brain insult, including brain plasticity compensatory mechanisms, presence or absence of health, good nutrition, security and safety, a nurturing home environment, and timely implementation or not of early education, therapeutic, and rehabilitative interventions.[43,44,45] Nurturing care in

particular has been found to influence child development and could attenuate the effects of adversity.[44]

Strengths of this study include standardized assessments on a reasonably sized population resulting in developmental outcomes at 2 time points for the BSID-III and 6 time points for the ASQ-3 that represent longitudinal and systematic follow-up of complex developmental processes. The validity and comparability of developmental tools representing expert professional and parental perspectives add value to these findings and highlight the importance of parents' observations. In addition, the study design allowed for identification of patterns in developmental delay according to maternal age and education and child sex in the multivariate model that contribute to the published evidence.[11–15] These patterns warrant further exploration and the inclusion of a comparison group to better understand the risk factors involved.

Our study has limitations in maternal diagnosis not confirmed by virus detection but rather by IgM serology in the majority of study participants, and one-half of the mothers had a symptomatic infection. The absence of a control group limited the assessment of developmental risks and precluded measuring the influence of other health and environmental mediators of child development. Although experienced clinical professionals implemented structured assessments, they could not be blinded to the exposure and information bias was possible. Another limitation is that the BSID-III and ASQ-3 testing lack normative data for Puerto Rican children. In addition, some families of children with abnormal retinal images and/or vision screening could not access the subspecialist to confirm the diagnosis for the purposes of this study. The high attrition rate was caused in part by 2 major disasters that forced family mobility and emigration. Among the reasons for emigrating, families reported economic distress and the need for specialized pediatric services. These factors could have affected the ability to enroll and retain children with more severe outcomes.

The recognition of language and communication developmental risks among children without microcephaly alerts pediatric healthcare professionals and families and can contribute to early identification and timely intervention. Furthermore, our study and others suggest that identifying long-term impairment related to early brain injury from Zika virus remains critical and will require follow-up to inform support needs and explore the long-term consequences of prenatal exposure. Future research must elucidate the risks to school readiness and identify effective preventive measures that can improve the health and well-being of children and families.

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## Data sharing statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Abbreviations:

| | |
|---|---|
| **ASQ-3** | Ages and Stages Questionnaire, Third Edition |
| **CDC** | Centers for Disease Control and Prevention |
| **BSID-III** | Bayley Scales of Infant and Toddler Development, Third Edition |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **HC** | Head circumference |
| **IgM** | Immunoglobulin M |
| **PCR** | Polymerase chain reaction |
| **RR** | Relative risk |

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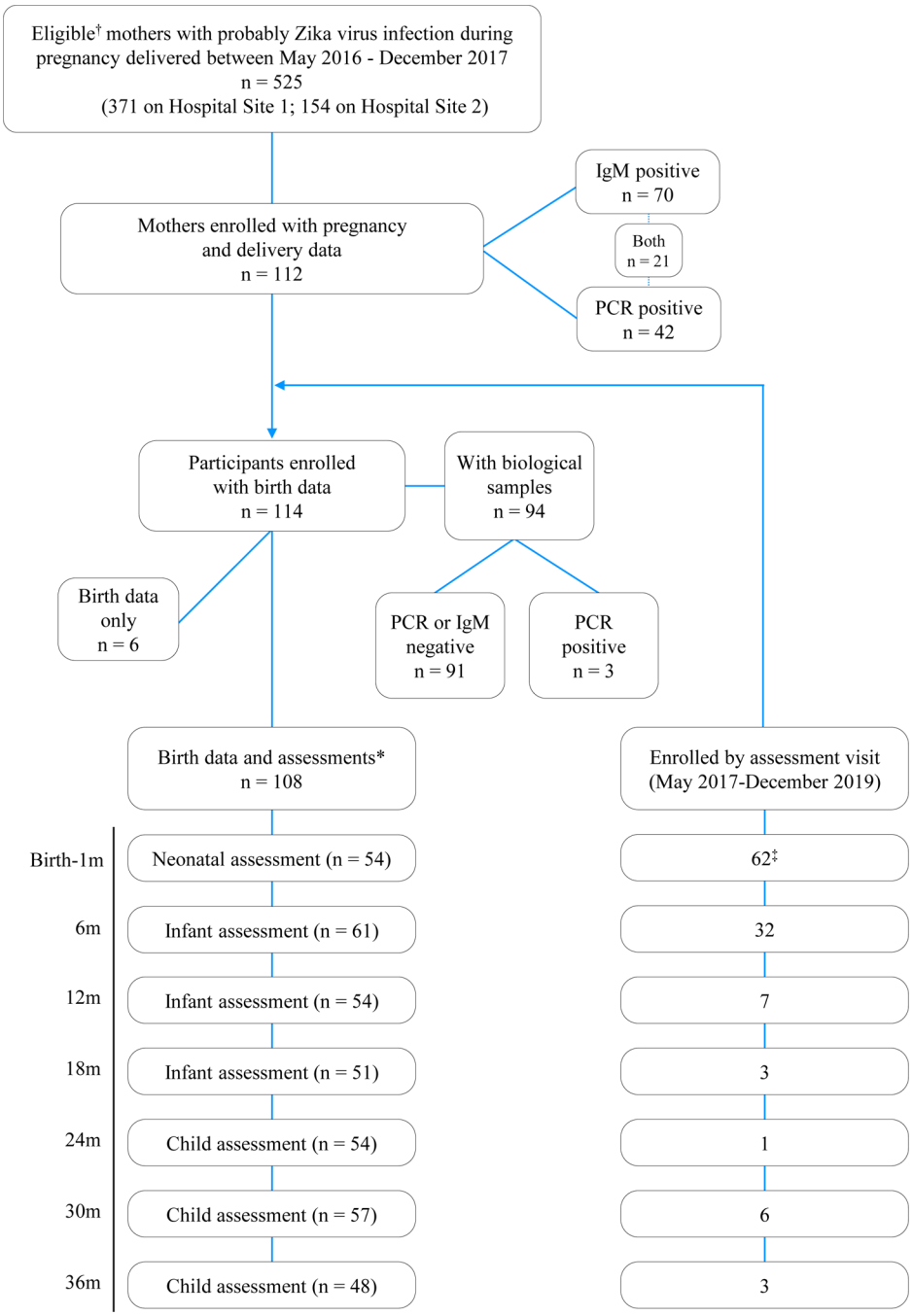


**Figure 1. Dynamic cohort enrollment and compliance with study procedures and assessments, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico 2017–2020.**

†Eligibility: Mothers with positive Zika virus PCR or immunoglobulin M (IgM) antibody at any trimester during pregnancy delivered at 1 of 2 hospital study sites during the Zika epidemic.

*Participants with birth data extracted from neonatal record and assessments performed in the neonatal period and at any of the follow-up-age-specific study visits.

‡Two participants with birth data completed assessments at the 6-month visit.

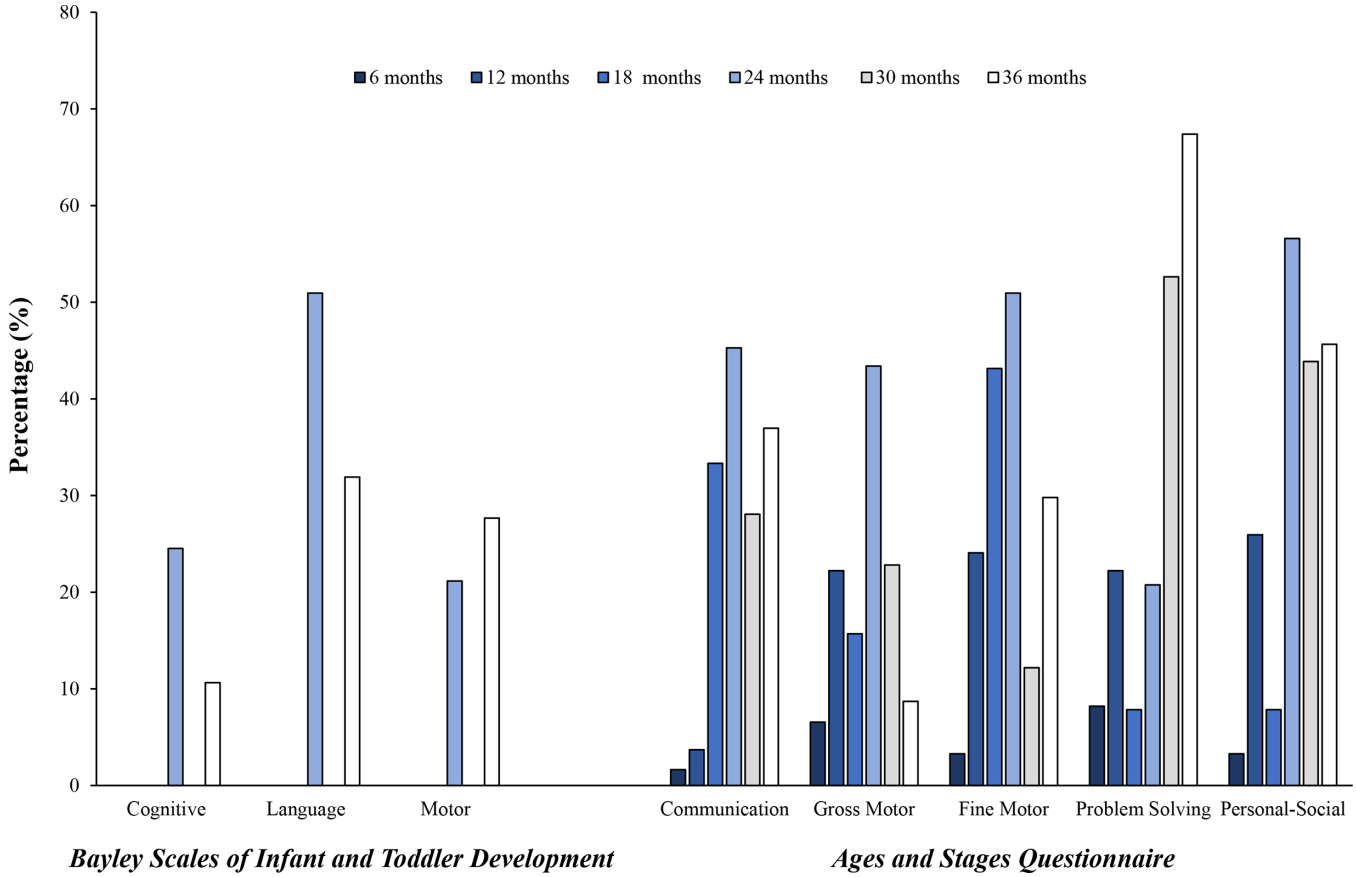


**Figure 2.**

Prevalence of developmental delay or risk by BSID-III and ASQ-3 domains, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020.

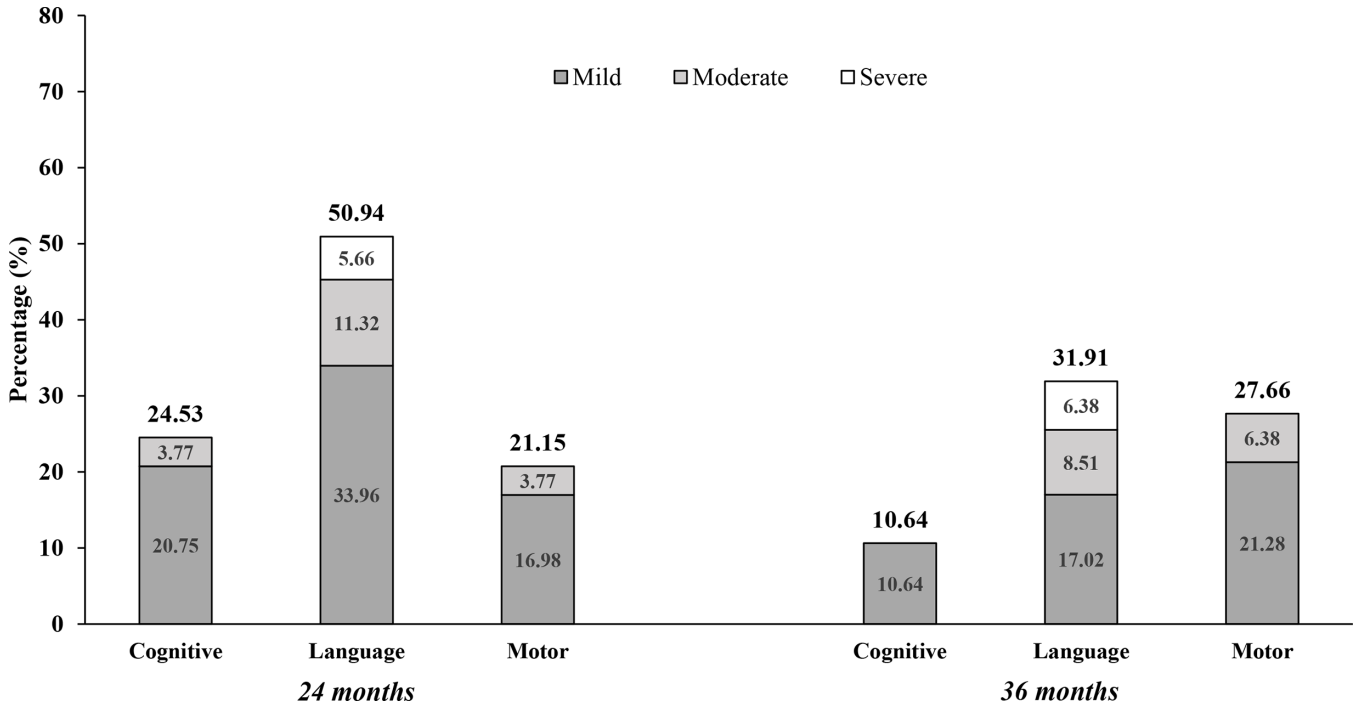


**Figure 3.**

Prevalence of severity of developmental delay on BSID-III domains, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020.

**Table 1.**

Sociodemographic and clinical characteristics at birth of study population, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020.

| Characteristics | Values |
|---|---|
| Maternal | |
| Age, y, mean (SD) | 26.11 (5.39) |
| Age 13–25 y, n/N (%) | 58/112 (51.79) |
| Age 26–40 y, n/N (%) | 54/112 (48.21) |
| Married/living with partner, n/N (%) | 92/112 (82.14) |
| Health insurance, public, n/N (%) | 90/112 (80.36) |
| Educational attainment, n/N (%) | |
| LTHS or HS | 48/112 (42.86) |
| More than HS | 64/112 (57.14) |
| Unemployed, n/N (%) | 69/112 (61.61) |
| Household income < $15,000, n/N (%) | 80/112 (71.43) |
| Prenatal diagnosis, n/N (%) | |
| PCR positive | 42/112 (37.50) |
| IgM positive | 70/112 (62.50) |
| Both | 21/112 (18.75) |
| Symptomatic infection, n/N (%) | 52/112 (46.43) |
| Trimester of symptomatic infection, n/N (%) * | |
| First | 28/52 (53.85) |
| Second | 15/52 (28.85) |
| Third | 8/52 (15.38) |
| Not identified | 1/52 (1.92) |
| Prenatal drug/alcohol/tobacco use, n/N (%) | 1/112 (0.89) |
| Infant | |
| Female sex, n/N (%) | 60/114 (52.63) |
| Gestational age, wk, mean (SD) | 38.06 (1.58) |
| Premature birth (<37 wk), n/N (%) | 13/114 (11.40) |
| Birth weight, kg, mean (SD) | 3.09 (0.51) |
| Birth length, cm, mean (SD) | 49.13 (3.20) |
| Weight for gestational age classification, n/N (%) | |
| PreTSGA | 2/114 (1.75) |
| TSGA | 9/114 (7.89) |
| HC, cm, mean (SD) | 33.50 (1.56) |
| HC *z*-score −1 to −2, n/N (%) | 12/114 (10.53) |
| HC *z*-score −2 to −3, n/N (%) | 3/114 (2.63) |
| ABR, n/N (%) | 3/107 (2.80) |
| Ophthalmologic abnormalities (RetCam) † images | 19/35 (54.29) |
| Nonspecific brain ultrasound findings ‡ | 11/109 (10.09) |

| Characteristics | Values |
|---|---|
| Germinolytic or subependymal cyst | 7/109 (6.42) |
| Choroid plexus cyst | 4/109 (3.67) |
| Lenticulostriate vasculopathy | 1/109 (0.92) |
| Abnormal neuromotor[§] | 7/114 (6.14) |
| Asymptomatic[¶] | 34/35 (97.1) |

*ABR*, abnormal; *HS*, high school; *LTHS*, less than high school; *PreTSGA*, preterm small for gestational age; *TSGA*, term small for gestational age.

[*] One case missing trimester of symptoms.

[†] Optic nerve dysplasia, optic nerve pallor, optic disc hypoplasia, optic disc cupping, and macular pigment mottling.

[‡] Associated to developmental risk.

[§] Tremors, hypotonia, and hypertonia.

[¶] HC *z*-score > −2 and no abnormalities in retinal image and/or no brain ultrasound findings among those with complete testing (n = 35).

**Table 2.**

Structural and functional abnormalities at any time after birth, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020.

| Abnormalities | n/N (%) |
|---|---|
| Microcephaly | 1/97 (1.03) |
| Auditory brainstem response | 0/31 (0) |
| Failure to thrive * | 2/97 (2.06) |
| Possible visual impairment | 17/97 (17.53) |
| Pediatric assessment | 3/97 (3.09) |
| Instrument-based screening | 14/69 (20.29) |
| Contractures | 1/97 (1.03) |
| Seizures | 3/97 (3.09) |
| Motor tone & function | |
| Hypotonia | 32/97 (32.99) |
| Hypertonia | 8/97 (8.25) |
| Both hypotonia/hypertonia | 2/97 (2.06) |
| Abnormal movements † | 7/97 (7.22) |
| Tremors † | 14/97 (14.43) |
| Abnormal posturing † | 7/97 (7.22) |
| Swallowing difficulties † | 8/97 (8.25) |
| Developmental risk or delay | |
| Parental report (ASQ-3), any domain in the first year | 16/74 (21.62) |
| Parental report (ASQ-3), any domain in the second year | 29/60 (48.33) |
| Parental report (ASQ-3), any domain in the third year | 34/61 (55.74) |
| Professional assessment (BSID-III), any domain in the second year | 33/53 (62.26) |
| Professional assessment (BSID-III), any domain in the third year | 17/47 (36.17) |

* Height *z*-scores < −2 and weight or weight/height *z*-scores < −2.

† Parental report.

**Table 3.**

Sociodemographic characteristics of active and inactive study participants by 2020, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020

| Characteristics | N | Active (*n* = 65) n | % | Inactive (*n* = 47) n | % | *P* value* |
|---|---|---|---|---|---|---|
| Maternal characteristics | | | | | | |
| Zika virus diagnosis | | | | | | |
| Immunoglobulin M positive | 70 | 35 | 53.8 | 35 | 74.5 | **.026** |
| PCR pos | 42 | 30 | 46.2 | 12 | 25.5 | |
| Municipality | | | | | | |
| Ponce | 51 | 33 | 50.8 | 18 | 38.3 | .191 |
| Other | 61 | 32 | 49.2 | 29 | 61.7 | |
| Area of residence | | | | | | |
| Rural | 47 | 29 | 44.6 | 18 | 38.3 | .504 |
| Urban | 65 | 36 | 55.4 | 29 | 61.7 | |
| Educational attainment | | | | | | |
| LTHS or HS | 48 | 26 | 40.0 | 22 | 46.8 | .472 |
| More than HS | 64 | 39 | 60.0 | 25 | 53.2 | |
| Household income | | | | | | |
| < $15,000 | 83 | 49 | 75.8 | 34 | 72.3 | .717 |
| ≥$15,000 | 29 | 16 | 24.6 | 13 | 27.7 | |
| Health insurance | | | | | | |
| Private | 22 | 7 | 10.8 | 15 | 31.9 | **.005** |
| Public | 90 | 58 | 89.2 | 32 | 68.1 | |
| Child characteristics | | | | | | |
| Sex | | | | | | |
| Female | 60 | 39 | 58.2 | 21 | 44.7 | .154 |
| Male | 54 | 28 | 41.8 | 26 | 55.3 | |

Significant *P* values are in bold type.

*Pearson's chi-square test

**Table 4.**

Child neurodevelopmental assessment scores according to BSID-III and ASQ-3 domains, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020

| **Tests by age-specific visit** | **Domains, mean (SD)** | | | | |
|---|---|---|---|---|---|
| BSID-III * | Language | | Motor | Cognitive | |
| 24 mo (n = 53) | 83.28 (15.02) † | | 93.40 (13.69) | 90.58 (12.67) | |
| 36 mo (n = 47) | 86.70 (19.46) | | 90.26 (13.20) | 93.19 (8.49) | |
| ASQ-3 | Communication | Gross Motor | Fine Motor | Problem Solving | Personal-Social |
| 6 mo (n = 61) | 54.10 (6.86) | 46.64 (10.48) | 52.62 (7.89) | 52.87 (9.38) | 53.93 (6.96) |
| 12 mo (n = 54) | 49.54 (11.34) | 46.20 (18.50) | 48.15 (10.92) | 45.46 (13.88) | 41.11 (13.86) |
| 18 mo (n = 51) | 31.57 (15.95) | 50.10 (14.23) | 42.75 (12.66) ‡ | 49.22 (11.85) | 47.65 (11.15) |
| 24 mo (n = 53) | 34.34 (20.69) ‡ | 44.15 (14.96) ‡ | 43.87 (14.30) ‡ | 44.19 (15.38) | 40.94 (12.60) ‡ |
| 30 mo (n = 57) | 45.79 (19.15) | 50.79 (11.98) | 49.47 (13.68) | 35.44 (11.74) ‡ | 42.28 (15.50) |
| 36 mo (n = 47) | 45.00 (18.83) | 56.30 (6.87) | 40.00 (18.77) | 36.63 (15.39) ‡ | 43.70 (15.07) ‡ |

* Composite scores.

† Scores in the developmental delay range (−1SD).

‡ Scores within the monitoring zone.

**Table 5.**

Prevalence of any developmental risk or delay from birth to 36 months according to ASQ-3 and BSID-III assessments and multivariate associations with maternal age, education, and child sex, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020

| Domains | Delay | No delay | Risk ratio (95% CI) |
|---|---|---|---|
| ASQ domains * | | | |
| Communication | 40.66 (37) | 59.34 (54) | |
| Maternal age, 13–25 y | 35.14 (13) | 57.41 (31) | 0.56 (0.33 to 0.96) |
| Maternal age, 26–40 y | 64.86 (24) | 42.59 (23) | Reference |
| Education, high school or less | 43.24 (16) | 44.44 (24) | 1.09 (0.67 to 1.79) |
| Education, more than high school | 56.76 (21) | 55.56 (30) | Reference |
| Child sex, male | 54.05 (20) | 35.19 (19) | 1.56 (0.95 to 2.55) |
| Child sex, female | 45.95 (17) | 64.81 (35) | Reference |
| Gross Motor | 40.66 (37) | 59.34 (54) | |
| Maternal age, 13–25 y | 43.24 (16) | 51.85 (28) | 0.77 (0.46 to 1.30) |
| Maternal age, 26–40 y | 56.76 (21) | 48.15 (26) | Reference |
| Education, high school or less | 45.95 (17) | 42.59 (23) | 1.20 (0.71 to 2.00) |
| Education, more than high school | 54.05 (20) | 57.41 (31) | Reference |
| Child sex, male | 37.84 (14) | 46.30 (25) | 0.80 (0.48 to 1.33) |
| Child sex, female | 62.16 (23) | 53.70 (29) | Reference |
| Fine Motor | 53.85 (49) | 46.15 (42) | |
| Maternal age, 13–25 y | 40.82 (20) | 57.14 (24) | 0.71 (0.49 to 1.06) |
| Maternal age, 26–40 y | 59.18 (29) | 42.86 (18) | Reference |
| Education, high school or less | 44.90 (22) | 42.86 (18) | 1.09 (0.75 to 1.59) |
| Education, more than high school | 55.10 (27) | 57.14 (24) | Reference |
| Child sex, male | 55.10 (27) | 28.57 (12) | 1.63 (1.11 to 2.37) |
| Child sex, female | 44.90 (22) | 71.43 (30) | Reference |
| Problem Solving | 61.54 (56) | 38.46 (35) | |
| Maternal age, 13–25 y | 39.29 (22) | 62.86 (22) | 0.65 (0.46 to 0.92) |
| Maternal age, 26–40 y | 62.86 (34) | 37.14 (13) | Reference |
| Education, high school or less | 46.43 (26) | 40.00 (14) | 1.24 (0.90 to 1.70) |
| Education, more than high school | 53.57 (30) | 60.00 (21) | Reference |
| Child sex, male | 46.43 (26) | 37.14 (13) | 1.13 (0.83 to 1.55) |
| Child sex, female | 53.57 (30) | 62.86 (22) | Reference |
| Personal Social | 59.34 (54) | 40.66 (37) | |
| Maternal age, 13–25 y | 40.74 (22) | 59.46 (22) | 1.25 (0.87 to 1.78) |
| Maternal age, 26–40 y | 59.26 (32) | 40.54 (15) | Reference |
| Education, high school or less | 35.19 (19) | 56.76 (21) | 1.38 (0.94 to 2.02) |
| Education, more than high school | 64.81 (35) | 43.24 (16) | Reference |
| Child sex, male | 46.30 (25) | 37.84 (14) | 0.84 (0.61 to 1.17) |
| Child sex, female | 53.70 (29) | 62.16 (23) | Reference |

| Domains | Delay | No delay | Risk ratio (95% CI) |
|---|---|---|---|
| BSID-III domains[†] | | | |
| Cognitive | 21.88 (14) | 78.13 (50) | |
| Maternal age, 13–25 y | 28.57 (4) | 46.00 (23) | 0.54 (0.19 to 1.52) |
| Maternal age, 26–40 y | 71.43 (10) | 54.00 (27) | Reference |
| Education, high school or less | 42.86 (6) | 40.00 (20) | 1.09 (0.43 to 2.80) |
| Education, more than high school | 57.14 (8) | 60.00 (30) | Reference |
| Child sex, male | 64.29 (9) | 38.00 (19) | 2.30 (0.87 to 6.10) |
| Child sex, female | 35.71 (5) | 62.00 (31) | Reference |
| Language | 48.44 (31) | 51.56 (33) | |
| Maternal age, 13–25 y | 35.48 (11) | 48.48 (16) | 0.73 (0.44 to 1.21) |
| Maternal age, 26–40 y | 64.52 (20) | 51.52 (17) | Reference |
| Education, high school or less | 45.16 (14) | 36.36 (12) | 1.17 (0.73 to 1.89) |
| Education, more than high school | 54.84 (17) | 63.64 (21) | Reference |
| Child sex, male | 61.29 (19) | 27.27 (9) | 2.00 (1.18 to 3.40) |
| Child sex, female | 38.71 (12) | 72.73 (24) | Reference |
| Motor | 25.40 (16) | 74.60 (47) | |
| Maternal age, 13–25 y | 37.50 (6) | 44.68 (21) | 0.70 (0.31 to 1.62) |
| Maternal age, 26–40 y | 62.50 (10) | 55.32 (26) | Reference |
| Education, high school or less | 62.50 (10) | 34.04 (16) | 2.35 (0.96 to 5.73) |
| Education, more than high school | 37.50 (6) | 65.96 (31) | Reference |
| Child sex, male | 62.50 (10) | 38.30 (18) | 1.89 (0.78 to 4.59) |
| Child sex, female | 37.50 (6) | 61.70 (29) | Reference |

[*] ASQ scores at or above age-based cutoff scores indicate possible developmental delay.

[†] BSID-III scores meeting thresholds for mild, moderate, or severe delay.

**Table 6.**

Correlations among the ASQ-3 and BSID-III in cognitive, language, and motor domains, Pediatric Outcomes of Prenatal Zika Exposure study, Puerto Rico, 2017–2020

| | BSID-III domains, 24 months | | | BSID-III domains, 36 months | | |
|---|---|---|---|---|---|---|
| **ASQ-3 domains** | **Cognitive** | **Language** | **Motor** | **Cognitive** | **Language** | **Motor** |
| Communication | | | | | | |
| 6 mo, *r* | 0.19 | 0.15 | 0.25 | 0.39 | 0.23 | 0.27 |
| *P* value | .25 | .35 | .13 | .03 | .21 | .14 |
| 12 mo, *r* | 0.15 | 0.13 | 0.09 | 0.19 | 0.10 | 0.17 |
| *P* value | .39 | .46 | .59 | .33 | .61 | .41 |
| 18 mo, *r* | 0.53 | 0.69 | 0.49 | 0.64 | 0.71 | 0.67 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| 24 mo, *r* | 0.47 | 0.71 | 0.48 | 0.68 | 0.75 | 0.73 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| 30 mo, *r* | 0.60 | 0.57 | 0.56 | 0.61 | 0.64 | 0.68 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| 36 mo, *r* | 0.59 | 0.70 | 0.65 | 0.64 | 0.76 | 0.74 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Gross Motor | | | | | | |
| 6 mo, *r* | 0.23 | 0.17 | 0.17 | 0.32 | 0.34 | 0.28 |
| *P* value | .15 | .28 | .30 | .08 | .07 | .14 |
| 12 mo, *r* | 0.43 | 0.37 | 0.48 | 0.44 | 0.35 | 0.41 |
| *P* value | .01 | .03 | .004 | .02 | .07 | .03 |
| 18 mo, *r* | 0.22 | 0.08 | 0.41 | 0.40 | 0.48 | 0.50 |
| *P* value | .15 | .60 | .006 | .03 | .006 | .004 |
| 24 mo, *r* | 0.34 | 0.37 | 0.52 | 0.46 | 0.40 | 0.54 |
| *P* value | .01 | .008 | <.001 | .005 | .01 | <.001 |
| 30 mo, *r* | 0.44 | 0.50 | 0.63 | 0.55 | 0.55 | 0.60 |
| *P* value | .003 | .0005 | <.001 | <.001 | <.001 | <.001 |
| 36 mo, *r* | 0.35 | 0.36 | 0.48 | 0.32 | 0.35 | 0.51 |
| *P* value | .04 | .03 | .004 | .03 | .02 | <.001 |

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| | BSID-III domains, 24 months | | | BSID-III domains, 36 months | | |
|---|---|---|---|---|---|---|
| **ASQ-3 domains** | **Cognitive** | **Language** | **Motor** | **Cognitive** | **Language** | **Motor** |
| Fine Motor | | | | | | |
| 6 mo, *r* | 0.10 | 0.15 | 0.12 | 0.20 | −0.03 | 0.03 |
| *P* value | .53 | .36 | .45 | .29 | .86 | .86 |
| 12 mo, *r* | −0.05 | 0.15 | 0.19 | −0.18 | −0.12 | 0.01 |
| *P* value | .78 | .40 | .27 | .37 | .55 | .94 |
| 18 mo, *r* | 0.29 | 0.42 | 0.33 | 0.45 | 0.39 | 0.50 |
| *P* value | .05 | .004 | .03 | .01 | .03 | .004 |
| 24 mo, *r* | 0.42 | 0.36 | 0.63 | 0.44 | 0.55 | 0.62 |
| *P* value | .002 | .008 | <.001 | .008 | <.001 | <.001 |
| 30 mo, *r* | 0.48 | 0.42 | 0.57 | 0.59 | 0.57 | 0.71 |
| *P* value | <.001 | .004 | <.001 | <.001 | <.001 | <.001 |
| 36 mo, *r* | 0.54 | 0.56 | 0.66 | 0.56 | 0.65 | 0.75 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Problem Solving | | | | | | |
| 6 mo, *r* | 0.20 | 0.10 | 0.10 | 0.44 | 0.26 | 0.20 |
| *P* value | .23 | .53 | .54 | .01 | .17 | .28 |
| 12 mo, *r* | 0.32 | 0.32 | 0.29 | 0.01 | 0.27 | 0.29 |
| *P* value | .07 | .06 | .10 | .96 | .17 | .14 |
| 18 mo, *r* | 0.40 | 0.24 | 0.50 | 0.44 | 0.46 | 0.50 |
| *P* value | .007 | .12 | <.001 | .01 | .008 | .004 |
| 24 mo, *r* | 0.56 | 0.50 | 0.78 | 0.70 | 0.70 | 0.75 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| 30 mo, *r* | 0.60 | 0.65 | 0.53 | 0.49 | 0.63 | 0.55 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| 36 mo, *r* | 0.66 | 0.64 | 0.70 | 0.64 | 0.73 | 0.70 |
| *P* value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |

Sample size for correlations between assessments range from n = 27 to n = 52. Only *P* values < .05 are presented.