Neurodevelopment in normocephalic children with and without prenatal Zika virus exposure

Karen Blackmon ,[1,2] Roberta Evans,[2] Michelle Fernandes,[3,4] Barbara Landon,[2] Trevor Noel,[2] Calum Macpherson,[2] Nikita Cudjoe,[2] Kemi S Burgen,[2] Bianca Punch,[2] Amy Krystosik,[5] Elysse N Grossi-Soyster,[5] Angelle Desiree LaBeaud,[5] Randall Waechter[2,6]

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[1]Department of Psychiatry and Psychology, Mayo Clinic, Jacksonville, Florida, USA
[2]Windward Islands Research and Education Foundation, St. Georges, Grenada
[3]Department of Paediatrics, University of Southampton, Southampton Children's Hospital, Southhampton, UK
[4]Nuffield Department of Women's Productive Health, John Radcliffe Hospital, University of Oxford, Oxford, UK
[5]Department of Pediatrics, Infectious Disease Division, Stanford University School of Medicine, Stanford, California, USA
[6]Department of Physiology, Neuroscience, and Behavioral Sciences, St George's University School of Medicine, St. George's, Grenada

Correspondence to
Dr Karen Blackmon, Psychiatry and Psychology, Mayo Clinic, Jacksonville, Florida, USA; blackmon.karen@mayo.edu

KB and RE are joint first authors.

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ABSTRACT

Objective Zika virus (ZIKV) targets neural stem cells in the developing brain. However, the majority of ZIKV-exposed children are born without apparent neurological manifestations. It remains unclear if these children were protected from ZIKV neurotropism or if they harbour subtle pathology that is disruptive to brain development. We assess this by comparing neurodevelopmental outcomes in normocephalic ZIKV-exposed children relative to a parallel control group of unexposed controls.

Design Cohort study.

Setting Public health centres in Grenada, West Indies.

Patients 384 mother–child pairs were enrolled during a period of active ZIKV transmission (April 2016–March 2017) and prospectively followed up to 30 months. Child exposure status was based on laboratory assessment of prenatal and postnatal maternal serum.

Main outcome measures The INTERGROWTH-21st Neurodevelopment Assessment (INTER-NDA) package and Cardiff Vision Tests, administered and scored by research staff masked to child's exposure status.

Results A total of 131 normocephalic ZIKV exposed (n=68) and unexposed (n=63) children were assessed between 22 and 30 months of age. Approximately half of these children completed vision testing. There were no group differences in sociodemographics. Deficits in visual acuity (31%) and contrast sensitivity (23%) were apparent in the ZIKV-exposed infants in the absence of cognitive, motor, language or behavioural delays.

Conclusions Overall neurodevelopment is likely to be unaffected in ZIKV-exposed children with normal head circumference at birth and normal head growth in the first 2 years of life. However, the visual system may be selectively vulnerable, which indicates the need for vision testing by 3 years of age.

What is already known?

- Zika virus (ZIKV) infection during pregnancy confers an increased risk of microcephaly in the exposed neonate, ranging from 1% to 13%.
- The majority of children with prenatal ZIKV exposure are born without any apparent neurological manifestations, but their risk for postnatal developmental delays is unclear.

What this study adds?

- Normocephalic ZIKV-exposed children do not show global developmental delays at 2 years of age but do show elevated risk for vision deficits.

INTRODUCTION

Prenatal exposure to Zika virus (ZIKV) is associated with elevated risk for brain malformations such as microcephaly, which can predispose children to developmental delays.[1] It remains unclear whether normocephalic ZIKV-exposed children are protected from ZIKV neurotropism during gestation or whether they harbour subtle pathology that might disrupt neurodevelopment. Neuroimaging of ZIKV-exposed children reveals a spectrum of brain abnormalities that vary from severe microcephaly to more subtle and focal malformations.[1 2] In many resource-limited regions where neuroimaging is not available or is cost prohibitive, neurodevelopmental assessment may be the only means available for probing neurotropism in normocephalic ZIKV-exposed children.

Prior case series of normocephalic ZIKV-exposed children suggest an elevated rate of developmental delays on standardised neurodevelopmental assessments[1 3–6]; however, the absence of a parallel local control group of ZIKV-unexposed children is a limiting factor.[7] When study populations culturally differ from normative reference populations, reduced scores can result from test-specific factors (eg, item unfamiliarity) and/or sociodemographic factors (eg, poverty, food insecurity and low parental education). Variance in test performance across cultures calls for increased caution when interpreting standardised scores.[8] The absence of local normative reference groups in many ZIKV-endemic regions makes it important to compare exposed and unexposed groups before drawing conclusions about long-term risk associated with exposure. Such an approach was used in one prior study that found no differences in developmental delay rates between ZIKV exposed and unexposed normocephalic children.[9]

In the current study, we prospectively tracked neurodevelopment in a large cohort of ZIKV exposed and unexposed children who were born during a period of active ZIKV transmission in Grenada, Carriacou and Petite Martinique.[10] We conducted the INTERGROWTH-21st Neurodevelopment Assessment (INTER-NDA),[11] Cardiff

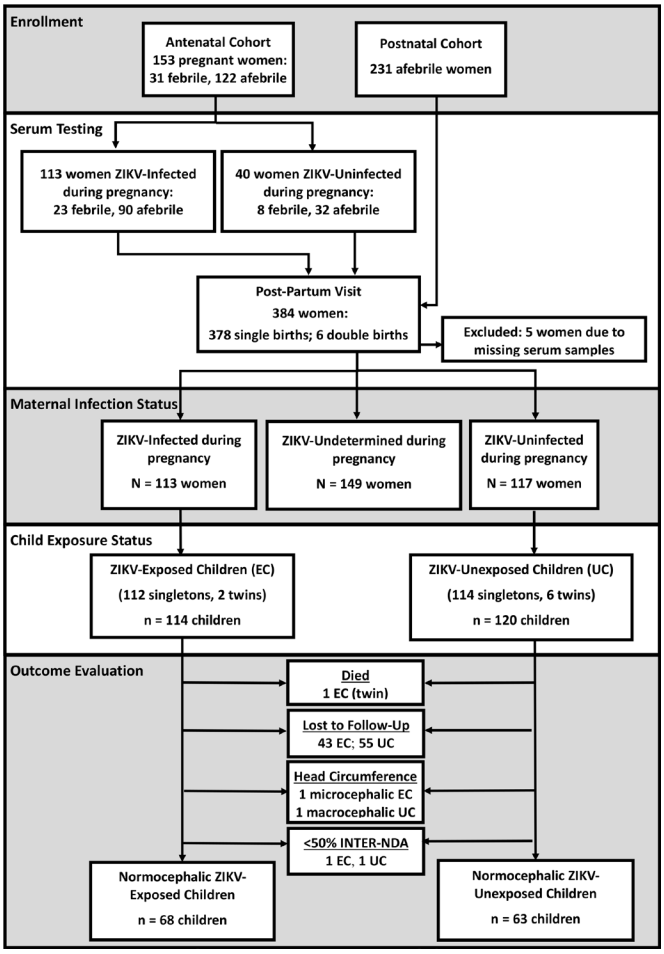

Figure 1 Flow diagram of maternal enrolment, maternal serum testing, maternal Zika virus infection classification, child exposure status and child outcome evaluation. Figure represents original work created by authors.

Visual Acuity Test (CAT)[12] and Cardiff Contrast Test (CCT)[13] when the children were between 22 and 30 months of age to determine whether there were cognitive, motor, language, behaviour and/or vision deficits associated with ZIKV exposure, after accounting for confounding variables such as neonatal complications, household income, parental education and food security.

METHODS

Participants

Mother–child pairs were prospectively enrolled between April 2016 and March 2017. A total of 384 women consented to participate (see figure 1).

Antenatal cohort

A total of 153 pregnant women were enrolled during pregnancy. Maternal serum was collected at a single time point during the prenatal period as well as at a follow-up time point during the postnatal period (0–12 months postpartum).

Postnatal cohort

A total of 231 women were recruited during the postnatal period. Maternal serum was collected at a single time point during the postnatal period (0–12 months postpartum).

Laboratory testing

Maternal serum samples were initially assessed for flavivirus exposure with indirect IgG capture ELISA using pooled dengue virus (DENV) antigen from all four DENV serotypes.[14] Maternal serum samples were further assessed using a multiplexed assay on a nanostructured plasmonic gold (pGOLD) platform (Nirmidas Biotech, Palo Alto, California, USA) for the detection of IgG against ZIKV and DENV antigens.[15] The pGOLD IgG immunoassay was used to cross-validate ELISA results and distinguish ZIKV from DENV exposure, as it has demonstrated sensitivity and specificity to ZIKV greater than 90% and 98%, respectively, in the convalescent phase.[15]

Head circumference classification

Children's occipitofrontal head circumference was measured at birth and at a follow-up visit between 22 and 30 months of age to ensure that none of the children developed late onset microcephaly.[16] Measurements were made by three independent raters and the mean of these measurements was used. Normocephalic was defined as occipitofrontal head circumference between the 3rd and 97th percentile for sex and age in accordance with WHO child growth standards.[17 18]

Sociodemographic measures

We administered a structured interview and questionnaire set to primary caregivers to determine whether ZIKV exposed and unexposed children were similar in terms of household demographics, such as maternal age at delivery, maternal education, household income, maternal social support (Social Support Questionnaire),[19] food security (Food Security Questionnaire),[20] and household stress (Chaos, Hubbub, and Order scale).[21] We assessed child anthropometrics to determine whether the two groups were comparable in physical growth at the time of outcome assessment.[22]

Neurodevelopmental assessment

The INTER-NDA was conducted between the ages of 22 and 30 months. The INTER-NDA is a 37-item measure that combines caregiver report with objective assessment of developmental skill acquisition.[11 23 24] It has been validated as a measure of neurodevelopment in 22–30 month old children,[24] with minimal variance across cultures.[25] Normative standards were created from over 1200 healthy children from Brazil, India, Italy, Kenya and the UK.[11] The INTER-NDA was administered by trained technicians who were masked to the child's serological status and fluent in the local culture and dialect. Cognition, motor and language items are scored on a four-point scale with higher scores indicating more optimal performance. Behavioural items are rated on a three-point scale with higher scores indicating more positive or negative behaviours. Mean index scores were calculated for each child and converted to standardised scores (range=0–100, mean=50). The threshold for normalcy was defined as ≥10th percentile, which translated to a standardised score ≥38.5 for cognition, ≥25.7 for fine motor, ≥51.7 for gross motor, ≥17.8 for language, ≥51.4 for positive behaviour and ≤50.0 for negative behaviour, in accordance with the INTER-NDA standard protocol.[11]

Visual acuity

The CAT[12] was used to assess visual acuity, which is the ability to detect fine visual detail. The CAT involves preferential looking towards two-dimensional pictures that progressively 'vanish' by becoming smaller in outline. Picture cards were presented

to children at a distance of 50 cm. Resulting 'Logarithm of the Minimum Angle of Resolution (LogMAR)' scores were used for analyses. LogMAR scores range from 0.1 to 1.0, with lower scores indicating better visual acuity.[26] The CAT has been validated for use in toddlers.[12] Both vision tests were performed binocularly.

Visual contrast sensitivity

The CCT[13] was used to assess contrast sensitivity, which is the ability to visually detect large but faint objects. Administration is similar to the CAT with vanishing optotypes that progressively decrease in light/dark contrast. Normative estimates for toddlers range from 33.33 to 100, with higher scores indicating better contrast sensitivity.[13]

Inclusion criteria

Children were included in the normocephalic ZIKV-exposed group if: (1) they were born to mothers classified as 'ZIKV-Infected' during pregnancy based on positive prenatal laboratory results for ZIKV, with avidity testing showing infection in the past 6 months[15]; (2) they were normocephalic on birth and postnatal measurements; and (3) they completed at least 50% of the items on the INTER-NDA.

Children were included in the normocephalic unexposed group if: (1) they were born to mothers who were classified as 'ZIKV-Uninfected' during pregnancy, based on prenatal and postnatal negative laboratory results for ZIKV; (2) they were normocephalic on birth and postnatal measurements; and (3) they completed at least 50% of the items on the INTER-NDA.

Statistical analysis

Sociodemographics, birth complications and anthropometrics were compared across the two groups using the χ^2 test for categorical variables and t-tests for continuous variables. Analysis of covariance was used to compare the main outcome measures across groups, with age entered as a covariate. Stratified risk categories were compared across groups using the Fisher's exact test. SPSS V.23 was used for statistical analyses. All hypothesis testing was two sided with a significance threshold of $p<0.05$.

Standard protocol approval, registrations and patient consent

Informed consent was obtained from all mothers who participated in this study. There was no financial compensation.

RESULTS

After application of inclusion criteria, a total of 68 exposed and 63 unexposed children were included in outcome analyses (online supplementary appendix 1; figure 1). There were no differences in sociodemographics between mothers who consented for outcome assessment and those who were lost to follow-up (see table 1).

There were no findings of craniofacial disproportions, arthrogryposis or motor abnormalities in the two groups at birth. This suggests that, in addition to normal head circumference, our sample of ZIKV-exposed infants had no apparent neurological manifestations at birth. The two groups did not differ in the rate of premature births or neonatal complications such as jaundice, eye infections, respiratory infections or need for respiratory assistance (see table 2).

Sociodemographic and anthropometric measures

Comparison of sociodemographic features between the two groups showed no differences in maternal age at delivery, income, maternal education, food security, social support and household stress (see table 2). There were also no group differences in sex distribution, age, head circumference, weight or height (at time of outcome assessment), indicating a similar rate of physical development (see table 2).

Table 1 Sociodemographic characteristics of normocephalic ZIKV exposed and unexposed children who were lost to follow-up

	Children lost to follow-up (n=98) (%)	Children followed (n=131) (%)	χ^2	P value
Household income (monthly Eastern Caribbean dollars)			2.18	0.70
Under 1000	9 (9)	9 (7)		
1001–2000	16 (16)	19 (15)		
2001–3000	19 (19)	25 (19)		
Over 3000	9 (9)	20 (15)		
Unknown/refused	45 (46)	58 (44)		
Maternal education			2.60	0.63
Primary	10 (10)	20 (15)		
Secondary	53 (55)	72 (55)		
College degree	8 (8)	9 (7)		
Graduate degree	4 (4)	8 (6)		
Unknown/refused	23 (23)	22 (17)		

ZIKV, Zika virus.

Neurodevelopmental outcomes

There were no group differences in standardised INTER-NDA scores across cognition, motor, language and behavioural domains (see table 3) or in the number of children classified as being at elevated risk for developmental delays in these domains based on standardised cut-off scores (see table 4).

Visual acuity and contrast sensitivity

A total to 65 children completed visual acuity testing (29 exposed, 36 unexposed) and 54 children completed contrast sensitivity testing (26 exposed, 28 unexposed). Visual acuity and contrast sensitivity were reduced in ZIKV-exposed children relative to unexposed children (see table 3). When compared with the CAT normative standards, 9/29 (31%) ZIKV exposed and 4/36 (11%) unexposed children were below age expectations in visual acuity (see table 4), with more pronounced deficits (logMAR score >0.5) in 3/29 (10%) exposed and 0/36 (0%) unexposed. When compared with CCT normative standards, 6/26 (23%) exposed and 2/28 (7%) unexposed children were below age expectations.

DISCUSSION

In this study, we found no evidence of delays in cognition, language functions, motor skills, or behaviour in normocephalic ZIKV-exposed children at 22–30 months of age, relative to a group of sociodemographically matched unexposed children. However, ZIKV-exposed children did show more deficits in visual acuity and contrast sensitivity, which is consistent with the rate of vision abnormalities found in other cohorts of infants with prenatal ZIKV exposure but without any other central nervous system findings.[27] This suggests that vision may be particularly vulnerable to prenatal ZIKV exposure, even in the absence of other neurodevelopmental delays.

Developmental delays in normocephalic ZIKV-exposed children have been previously reported[1 3–6] but without comparison with local unexposed controls. Use of non-local reference groups can lead to overestimated risk of developmental delays when children are sociodemographically and culturally different

Table 2 Demographic characteristics of the cohort

	ZIKV-exposed children (n=68), n (%)	ZIKV-unexposed children* (n=63), n (%)	χ^2	P value
Child's sex			0.021	0.88
Males	38 (56)	36 (57)		
Females	30 (44)	27 (43)		
Household income (monthly Eastern Caribbean dollars)			1.87	0.76
Under 1000	5 (7)	4 (7)		
1001–2000	9 (13)	10 (17)		
2001–3000	11 (16)	14 (23)		
Over 3000	10 (15)	9 (15)		
Unknown/refused	33 (49)	23 (38)		
Maternal education			2.09	0.72
Primary	11 (16)	8 (13)		
Secondary	35 (52)	35 (58)		
College degree	5 (7)	4 (7)		
Graduate degree	6 (9)	2 (3)		
Unknown/refused	11 (16)	11 (19)		
Food security			2.79	0.42
Food secure	27 (40)	25 (41)		
Food insecure (moderate)	18 (26)	21 (35)		
Food insecure (severe)	8 (12)	7 (12)		
Unknown/refused	15 (22)	7 (12)		
Prematurity			4.05	0.13
Delivery at >37 weeks gestation	53 (78)	51 (85)		
Delivery at ≤37 weeks gestation	3 (4)	5 (8)		
Unknown/refused	12 (18)	4 (7)		
Neonatal complications			0.89	0.64
No complication	48 (71)	45 (75)		
Neonatal complications	17 (25)	14 (23)		
Unknown/refused	3 (4)	1 (2)		
	Mean (SD)	**Mean (SD)**	**t-Value**	**P value**
Gestational age at delivery (in weeks)	39.39 (1.67)	38.80 (2.60)	–1.43	0.16
Mother's age at delivery (in years)	29.15 (5.85)	28.51 (7.13)	–0.55	0.58
Child's age at outcome (in months)	24.65 (1.30)	24.61 (0.92)	–0.18	0.86
Child's head circumference (z-score)†	0.56 (0.89)	0.53 (0.95)	–0.19	0.85
Child's weight (z-score)†	0.48 (1.06)	0.37 (1.19)	–0.54	0.59
Child's height (z-score)†	0.14 (1.05)	–0.08 (0.98)	–1.15	0.25
Household CHAOS Scale	26.79 (7.72)	27.14 (7.72)	–0.21	0.83
Household Social Support Questionnaire	38.08 (10.10)	35.23 (9.42)	–1.39	0.17

*Household variables will sum to n=60, rather than n=63, due to three twin births in unexposed group.
†Z-scores were calculated using WHO Child Growth Standards.
CHAOS, chaos, order, and hubbub; ZIKV, Zika virus.

from those represented by the normative sample.[8] We overcame these limitations by comparison of exposed and unexposed groups that had similar rates of prematurity, neonatal complications, low household income, low parental education and food insecurity. This approach revealed that ZIKV-exposed children perform on par with unexposed children across measures of cognitive, motor, language and behavioural development at 2 years of age.

Nevertheless, there was evidence for deficits in visual acuity and contrast sensitivity. This is consistent with animal studies and human case series that demonstrate a particular vulnerability in the developing brain's visual system to ZIKV infection. In mice, ZIKV targets neural progenitor cells and glial cells in a pattern that suggests local astrocytic release of ZIKV progeny.[28 29] ZIKV appears to have an affinity for the retinal ganglion cells, optic nerve and lateral geniculate nucleus in mice.[28] Ocular abnormalities, such as focal pigmentary changes and chorioretinal atrophy and scarring, are common in children with congenital Zika syndrome and microcephaly[30–32] and are also found in normocephalic ZIKV-exposed children.[27 33] This indicates that eye abnormalities can result from intrauterine ZIKV infection, even without other obvious neurological signs.

Visual acuity deficits are apparent in approximately 90% of infants with congenital Zika syndrome and microcephaly.[34] We show that approximately 31% of normocephalic ZIKV-exposed children have deficits in visual acuity at 2 years of age. It is unclear whether this may be due to retinal anomalies or delays in the maturation of cortical visual functions. The visual system undergoes rapid cortical development in the first 2 years of life,[35] with binocular visual acuity normally reaching adult levels after 5 years of age.[36] Additional follow-up testing with funduscopy is needed to confirm whether early ocular abnormalities may be the primary driver of later visual acuity and contrast sensitivity deficits in our cohort.

Table 3 INTERGROWTH 21st Neurodevelopmental Assessment (INTER-NDA) and Cardiff Vision Test scores

	ZIKV exposed (n=68)	ZIKV unexposed (n=63)		
INTER-NDA domains	Standardised domain score mean (SD; 95% CI)	Standardised domain score mean (SD; 95% CI)	*F**	P value
Cognition	63.71 (13.22; 60.57 to 66.86)	67.17 (12.92; 63.91 to 70.44)	2.280	0.134
Fine motor	91.99 (11.16; 89.25 to 94.95)	93.83 (11.72; 90.97 to 96.68)	0.833	0.363
Gross motor	92.97 (10.67; 90.31 to 95.63)	92.77 (11.49; 90.01 to 95.54)	0.011	0.918
Language	66.20 (19.45; 61.43 to 70.95)	66.10 (20.38; 61.17 to 71.05)	0.001	0.981
Positive behaviour	75.26 (22.13; 70.42 to 79.77)	78.61 (19.57; 73.45 to 83.18)	0.830	0.364
Negative behaviour	26.59 (26.89; 19.15 to 34.04)	18.42 (23.40; 10.74 to 26.46)	2.900	0.091
Cardiff Vision Tests	**ZIKV exposed (n=29)**	**Unexposed (n=38)**		
Visual acuity (logMAR)	0.36 (0.14; 0.31 to 0.41)	0.26 (0.14; 0.21 to 0.30)	9.77	0.003
	ZIKV exposed (n=26)	**ZIKV unexposed (n=30)**		
Contrast sensitivity	42.31 (18.24; 35.17 to 49.74)	50.89 (17.32; 46.70 to 60.26)	4.94	0.03

*With age entered as a covariate.
logMAR, logarithm of the minimum angle of resolution; ZIKV, Zika virus.

In terms of the timing of formal visual testing in ZIKV-exposed children, we recommend assessment prior to school entry; however, assessment of vision in young children can be challenging due to limited attention and cooperation. We were unable to obtain valid vision test results in approximately half of the children who cooperated with other INTER-NDA domains. Assessment around 36 months of age may be the optimal time window to allow for sufficient behavioural maturation to support cooperation with vision test procedures and to identify problems early enough to promote school readiness.

Study limitations include a small sample size, particularly for vision assessments. However, our sample should be considered sociodemographically representative of the larger Grenadian population, given that recruitment took place in public health

Table 4 Developmental delay risk stratification in children with and without prenatal ZIKV exposure

	ZIKV exposed (n=68), n (%)	ZIKV unexposed (n=63), n (%)	χ^2	Fisher's exact p value
Cognition				
Low risk of delay*	64 (94)	61 (97)	0.549	0.682
Med to high risk of delay*	4 (6)	2 (3)		
Fine motor			NA*	NA*
Low risk of delay*	68 (100)	63 (100)		
Med to high risk of delay*	0 (0)	0 (0)		
Gross motor			NA*	NA*
Low risk of delay*	68 (100)	63 (100)		
Med to high risk of delay*	0 (0)	0 (0)		
Language			0.003	1.000
Low risk of delay*	67 (99)	62 (98)		
Med to high risk of delay*	1 (1)	1 (2)		
Positive behaviour			1.621	0.232
Low risk of delay*	55 (81)	56 (89)		
Med to high risk of delay*	13 (19)	7 (11)		
Negative behaviour				
Low risk of delay*	56 (89)	52 (91)	0.182	0.766
Med to high risk of delay*	7 (11)	5 (9)		
	ZIKV exposed (n=29)	**ZIKV unexposed (n=38)**	χ^2	**P value**
Visual acuity (logMAR)			4.424	0.035
Low risk of delay†	20 (69)	34 (89%)		
Med to high risk of delay†	9 (31)	4 (11)		
	ZIKV exposed (n=26)	**ZIKV unexposed (n=30)**		
Contrast sensitivity			3.063	0.080
Low risk of delay†	20 (77)	28 (93)		
Med to high risk of delay†	6 (23)	2 (7)		

NA=not applicable because cell sizes were too small to run proportional analyses.
*Cut-off scores for classification of delay risk (<10th percentile) in cognition, fine motor, gross motor, and language domains were obtained from the INTER-NDA international standardisation sample.[17]
†Cut-off scores for classification of delays in visual acuity and contrast sensitivity were obtained from the Cardiff standardisation samples.[18]
INTER-NDA, INTERGROWTH-21st Neurodevelopment Assessment; logMAR, logarithm of the minimum angle of resolution; ZIKV, Zika virus.

centres and mothers who consented to outcome assessments did not differ sociodemographically from those who were lost to follow-up. Second, the absence of an ophthalmologist on our research team precluded our ability to rule out retinal or optic nerve damage in the children with lower CAT or CCT scores. Given our results, we have now enlisted a paediatric ophthalmologist to perform an examination with funduscopy at follow-up time-points. Third, neuroimaging was not performed; therefore, we could not determine whether ZIKV-associated imaging findings commonly found in normocephalic ZIKV-exposed children were also present in our cohort.[2] Fourth, although it is assumed that the ZIKV strain transmitted by *Aedes aegypti* in Grenada is of the same Asian lineage as that transmitted in Brazil and other South American countries, it is not clear whether virus-specific adaptations may have led to variations in pathogenesis across different regions.[37 38] Fifth, it is difficult to distinguish between mosquito-borne viruses by serology due to cross-reactivity within viral families; however, we used the pGOLD platform, which is specifically designed to minimise cross-reactivity and distinguish between ZIKV and DENV.[15] Finally, by including all pregnant mothers, regardless of symptom status, we were unable to confirm the timing of ZIKV infection. This makes it unclear whether low rates of neurodevelopmental delays may be due to infection occurring later in pregnancy, after a protective zone of mature villous trophoblasts has been established in the uteroplacental circulatory system,[39 40] or other yet to be identified protective factors. However, the presence of visual deficits suggests incomplete protection in at least a subset of the children.

CONCLUSION

Prospective tracking of normocephalic ZIKV-exposed children alongside a parallel group of unexposed children revealed no major delays in cognition, motor skills, language or behaviour at 2 years of age. However, the presence of visual acuity and contrast sensitivity deficits suggests that the visual system may be selectively vulnerable to prenatal ZIKV exposure. Ophthalmological evaluations should be considered essential components of surveillance initiatives in the event of future ZIKV outbreaks, and formal vision testing is recommended in ZIKV-exposed infants around 3 years of age.

Correction notice This article has been updated since it was published online. Author Elysse Grossi-Soyster's surname has been corrected.

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ORCID iD

Karen Blackmon http://orcid.org/0000-0002-2665-7807

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Supplementary Appendix 1

Maternal Laboratory Testing Results

Maternal ELISA and pGOLD results were concordant for flavivirus antigens; with no false negatives or false positives. A total of 113 mothers were classified as "ZIKV-Infected" during pregnancy based on positive laboratory results for anti-ZIKV IgG from serum collected during the prenatal period (prenatal enrollment cohort), with avidity testing confirming infection within the past 6 months [15]. Among the "ZIKV-Infected" women, 90/113 (80%) were asymptomatic; therefore, the trimester of infection was unclear. There were 117 mothers classified as "ZIKV-Uninfected" during pregnancy, based on negative laboratory results for anti-ZIKV IgG from serum that was collected prenatally and postnatally (antenatal cohort) or serum collected during the postnatal period alone (postnatal cohort). There were 149 mothers who were classified as "ZIKV-Undetermined" during pregnancy because they were enrolled during the postnatal period and had positive laboratory results for ZIKV (N=144); or they had negative ZIKV results on prenatal serology but positive ZIKV results on postnatal serology (N=5).

Child Exposure Classification

A total of 132 out of 230 ZIKV-Infected (N=70) and ZIKV-Uninfected (N=62) mothers consented to neurodevelopmental assessments. Two children were excluded from outcome analyses based on abnormal head circumference indicators (microcephaly in 1 ZIKV-exposed child and macrocephaly in 1 unexposed child). A total of 98 children (43 exposed and 55 unexposed) were lost to follow-up and one ZIKV-exposed child (a twin) passed away during the neonatal period. Finally, 1 exposed child and 1 unexposed child were excluded from outcome analyses because they completed less than 50% of the INTER-NDA items. The remaining cohort

included 68 normocephalic ZIV-exposed children and 63 normocephalic unexposed children that met inclusion criteria for our study.

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