



Lee, S. M., Mitchell, R. E., Knight, J. A., Mazzulli, T., Relton, C. L., Khodayari-Moez, E., & Hung, R. J. (Accepted/In press). Early Childhood Cytomegalovirus (CMV) Infection and Children's Neurocognitive Development. *International Journal of Epidemiology*.

Peer reviewed version

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Early Childhood Cytomegalovirus (CMV) Infection and Children's Neurocognitive Development

Journal:	<i>International Journal of Epidemiology</i>
Manuscript ID	IJE-2020-02-0196.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Key Words:	child neurodevelopment, ALSPAC, cytomegalovirus, cognition, perinatal risk factors, birth cohort

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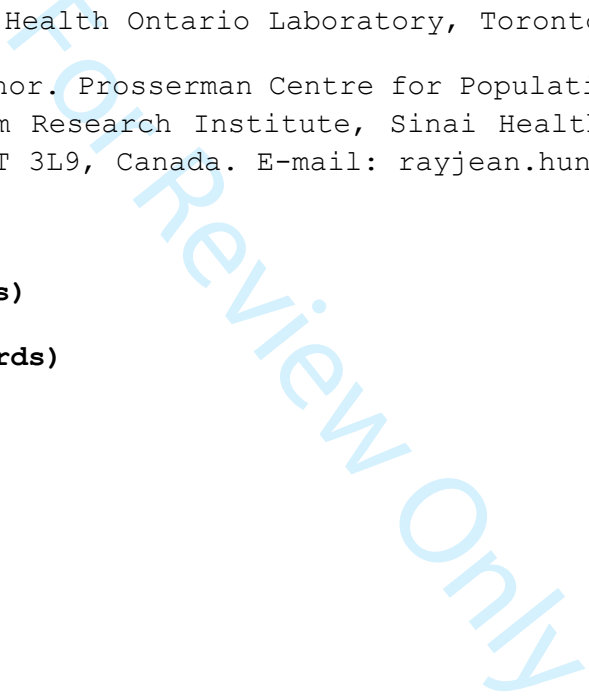
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Abstract (308 words)

Main text (3908 words)



ABSTRACT

BACKGROUND: Despite a clear association seen in congenitally infected children, the effect of postnatal cytomegalovirus (CMV) infection during early childhood on cognitive development has not yet been determined.

METHODS: CMV infection status was obtained based on serological measurements when children were 7 years old. Using population-based longitudinal data, we employed multivariate Poisson regression with a robust variance estimator to characterize the relationship between childhood CMV infection and adverse neurocognitive outcomes in children. Suboptimal neurocognitive outcomes were compared between CMV-positive and CMV-negative children using various cognitive assessments from 8 to 15 years of age. Children were evaluated on the cognitive domains of language, reading, memory, and general intelligence; with a suboptimal score being greater than 2 standard deviations lower than the mean score. Approximate Bayes factor (ABF) analysis was used to determine the level of evidence for the observed associations.

RESULTS: With adjustment for potential confounders, we observed that early childhood CMV infection was associated with suboptimal total intelligence quotient (IQ) at 8 years of age (incident rate ratio (IRR)=2.50, 95% CI 1.35-4.62, ABF=0.08); however, not with suboptimal total IQ at 15 years of age (IRR=0.97, 95% CI 0.43-2.19, ABF=1.68). Suboptimal attentional control at 8 years (IRR=1.74, 95% CI 1.13-2.68, ABF=0.18) and reading comprehension at 9 years (IRR=1.93, 95% CI 1.12-3.33, ABF=0.24) were also associated with CMV infection. Approximate Bayes factor analysis provided strong evidence for the association between CMV infection and total IQ at 8 years and only anecdotal evidence for attentional control at 8 years and reading comprehension at 9 years. All other cognitive measures assessed were not associated with CMV infection.

CONCLUSIONS: In this large-scale prospective cohort, we observed some evidence for adverse neurocognitive effects of postnatal CMV infection on general intelligence during early childhood, however not with lasting effect. If confirmed, these results could support the implementation of preventative measures to combat postnatal CMV infection.

Key words: cytomegalovirus infection, child neurodevelopment, ALSPAC

Key Messages

- Adverse neurological and cognitive effects of congenitally acquired CMV infection have been well established; however the relationship remains unclear for postnatally acquired infection.
- Among a large population-based longitudinal cohort, we observed that early childhood CMV infection is associated with intellectual impairment in later childhood based on total IQ, however not with lasting effect.
- We did not find strong evidence that early childhood CMV infection is associated with the cognitive domains of language, attention, reading, or memory in later childhood.
- If the observed associations are confirmed, it could provide further support for the implementation of preventative measures to combat postnatal CMV infection, particularly within early childhood.

INTRODUCTION

Cytomegalovirus (CMV) is the most common congenital and perinatal infection worldwide (1). It represents a widespread viral infection, but remains largely unrecognised globally (2, 3). CMV may be acquired in utero (congenital CMV infection), during the birth process due to exposure to infected maternal secretions (sometimes referred to as perinatal CMV infection), or after birth (postnatal CMV infection) (4). Postnatally acquired CMV infections, most commonly resulting from transmission through breast milk, but also through blood transfusions or other fluid contact with infected individuals, are significantly more prevalent than congenitally acquired infections (1). However, the majority of what is known about CMV-associated health outcomes is in relation to the congenital form, whose prevalence is approximately 0.6-0.7% in developed countries, and is estimated to be much higher in developing nations (5-9). It is one of the most common causes of congenital sensorineural hearing loss and has been associated with a wide range of adverse neurodevelopmental outcomes such as vision loss,

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2
3 cerebral palsy, microcephaly, as well as motor, behavioural and cognitive
4 deficits (5, 8, 10, 11). It has been estimated that upwards of 60% of
5 the human population is infected with CMV by adulthood, with infection
6 occurring primarily throughout childhood and adolescence (12).
7 Seroepidemiologic studies have shown that CMV antibody prevalence is
8 influenced by age, geography, cultural factors, socioeconomic status,
9 and child-rearing practices (13).
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18 The extent of the health-related consequences associated with postnatally
19 acquired CMV infection is far from clearly understood for the following
20 reasons: (i) the delayed onset of adverse health sequelae makes the
21 establishment of causal associations difficult; (ii) as postnatal
22 infection is largely asymptomatic, it has received little research
23 attention compared to the more severe congenital form; and (iii) while
24 screening tests have recently become available in few countries (e.g.
25 Canada), it is limited to the congenital form of CMV, leaving
26 asymptomatic postnatal infection undetected. In addition, universal
27 newborn CMV screening remains a contentious point and is currently not
28 implemented in most countries, despite community advocacy and the recent
29 consensus report from an international panel of experts (3). As CMV is
30 known to be neurotropic, it is of great importance to comprehensively
31 investigate the effects of postnatally acquired infection.
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48 Previous studies have suggested that CMV infection during early postnatal
49 life could impair complex cognitive skills in later childhood (14, 15).
50 For the present investigation, our primary goal was to comprehensively
51 investigate the association between postnatal CMV infection and a full
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3 spectrum of adverse neurocognitive outcomes, as well as to characterize
4 factors strongly associated with postnatal CMV infection for target
5 surveillance. We hypothesized that children with postnatally acquired
6 CMV infection, when compared to uninfected children, more commonly have
7 impaired neurodevelopment and suboptimal cognitive abilities in later
8 childhood.
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19 **METHODS**

20 **DATA COLLECTION AND STUDY POPULATION**

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25 Population-based data were obtained from the Avon Longitudinal Study of
26 Parents and Children (ALSPAC) cohort (16). ALSPAC is a transgenerational
27 prospective birth cohort study of children born in the former county of
28 Avon in the Bristol area of the UK during 1990-92. It is one of the most
29 detailed studies of its kind in the world, with an immense variety of
30 health information arising from questionnaire and clinical data
31 collection over two decades. It includes genetic, epigenetic, biological,
32 psychological, social and other environmental determinants in relation
33 to a similarly diverse range of health, behavioural and developmental
34 outcomes. The source data and characteristics of the cohort have been
35 described in further detail elsewhere (17, 18). Please note that the
36 study website contains details of all the data that is available through
37 a fully searchable data dictionary and variable search tool (18).
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53 A flow diagram of participant recruitment is presented in Figure 1. Among
54 the 15 589 children enrolled in ALSPAC, 5007 had serological CMV
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3 measurement at 7 years and were included in the study population. We
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5 further excluded children with severe medically-diagnosed neurological
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7 conditions (e.g. epilepsy, cerebral palsy) and those who were not
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9 enrolled in school at either 6 years or 9 years of age, which resulted
10
11 in 4988 children in the analysis. These criteria were considered as they
12
13 may have had a large and undue influence on children's cognitive
14
15 outcomes. The population that had CMV serological data are comparable
16
17 with the overall ALSPAC population in terms of children's gender
18
19 distribution and method of delivery, but different in other aspects
20
21 (Supplementary Table 1), with higher education levels and longer
22
23 breastfeeding duration among other factors.
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26
27 (Figure 1 here)
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29 **EARLY CHILDHOOD CMV INFECTION**

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31
32 The earliest CMV serological data available from ALSPAC with sufficient
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34 sample size for statistical analysis was at 7 years of age. Infection
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36 was measured with three associated variables of CMV IgG antibody titers:
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38 a raw optical density, a ratio-to-standard, and a normalised z-score of
39
40 that ratio-to-standard. Methods used for IgG antibody measurement were
41
42 based on standard enzyme-linked immunosorbent assay (ELISA) techniques
43
44 which were performed previously (19, 20). CMV IgG antibody titers showed
45
46 a bimodal frequency distribution, thus we used finite mixture modelling
47
48 methods that were previously described to determine a cut-off threshold
49
50 value for CMV positivity (21). For purposes of this study, the CMV ratio-
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52 to-standard optical density measurement was used to characterize
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3 infection as it had the most distinct bimodal distribution for threshold
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5 determination.
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8 **NEUROCOGNITIVE OUTCOMES**

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11 Neurocognitive impairment, the primary endpoint we aimed to measure, is
12 clinically defined as a reduction in cognitive functioning in one or more
13 cognitive domains, including memory, attention, learning, language,
14 perception, and social cognition (22). A variety of neurocognitive
15 measures were available from the ALSPAC cohort, and were chosen based on
16 demonstrated ability to characterise different dimensions of cognitive
17 capacity. We chose to evaluate neurodevelopment based on the cognitive
18 domains of intelligence, language, reading, memory, and attention using
19 the Wechsler Intelligence Scale for Children (WISC), the Wechsler
20 Objective Language Dimensions (WOLD), the Neale Analysis of Reading
21 Ability (NARA), the Counting Span Task, and the Test of Everyday
22 Attention for Children (TEA-Ch), respectively. All cognitive assessments
23 were administered by experienced and trained psychologists (16).
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39 The WISC-III was used to characterise children's intellectual ability at
40 8 years as it is the most widely used individual intelligence test
41 worldwide and is well documented in its ability to assess different
42 aspects of intelligence for children between the ages of 6 to 16 (23-
43 25). A subset of the ALSPAC cohort (n=7488) attended the focus clinic at
44 8 years and completed the intelligence assessment using a short form of
45 the WISC-III test. The assessment consisted of subtests designed
46 specifically for measurement of verbal intelligence quotient (IQ),
47 performance IQ, and total IQ, which were calculated from each of the
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3 subtests' total age-scaled scores. An additional intelligence
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5 measurement was taken at 15 years (n=5,509) using the Weschler
6
7 Abbreviated Scale for Intelligence (WASI). This assessment consisted of
8
9 a measurement for vocabulary, matrix reasoning, and total IQ.
10

11
12 The language dimensions of listening comprehension and oral expression
13
14 were measured using WOLD subtests at the focus clinic at 8 years of age
15
16 (n=7488) (26). WOLD consists of individually administered tests designed
17
18 for children aged 6 to 16 years.
19

20
21 TEA-Ch was used to measure different aspects of attention at 8 years and
22
23 11 years, including: selective attention, the ability to divide attention
24
25 between two tasks, and attentional control (27). TEA-Ch has been
26
27 documented as a valuable tool in assessing specific attentional
28
29 operations and is specifically useful in characterising attentional
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31 disorders in children between the ages of 6 and 16 (28). The testing was
32
33 performed at ALSPAC focus clinics at 8 years of age (n=7488) and 11 years
34
35 of age (n=7159).
36

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38 Reading accuracy and comprehension were measured at 9 years using the
39
40 NARA-II, which is a reading assessment based on a series of short
41
42 narratives suitable for children between the ages of 6 and 12 (29).
43
44 Assessment was administered at the ALSPAC focus clinic at 9 years of age
45
46 (n=7725). Words per minute, accuracy and comprehension standardised
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48 scores were calculated. Additionally, a classification of developmental
49
50 dyslexia was provided by ALSPAC using a clinical definition of the
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52 disorder.
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3 Lastly, working memory was tested using the Counting Span Task at 10
4 years of age (n=7563). Working memory was an important cognitive
5 component to assess as it has been implicated as a crucial ability in
6 reading and arithmetic. Working memory tasks require the simultaneous
7 processing and storage of information (30). The Counting Span Task is a
8 widely accepted measure of working memory capacity with documented
9 reliability and validity (31).
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18 For each of the composite scores measuring different aspects of cognitive
19 functioning described above, threshold values were chosen in order to
20 divide subjects into categories of 'suboptimal' and 'normal'
21 neurocognition. A suboptimal threshold value of more than 2 standard
22 deviations (SDs) below the mean score was used for each measurement type
23 (in cases where a high score indicated suboptimal, a threshold value of
24 more than 2 SDs above the median score was used).
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34 Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics
35 and Law Committee and the Local Research Ethics Committees. Consent for
36 biological samples has been collected in accordance with the Human Tissue
37 Act (2004). Informed consent for the use of data collected via
38 questionnaires and clinics was obtained from participants following the
39 recommendations of the ALSPAC Ethics and Law Committee at the time. In
40 addition, this project was approved by the Research Ethics Board at Mount
41 Sinai Hospital.
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51 52 53 54 **CONFOUNDERS** 55 56 57 58 59 60

Potential confounders known to influence cognitive development were selected a priori for inclusion in the regression model, including the child's sex (32), ethnicity (33), gestational age (34), duration of breastfeeding (35), and the quality of home environment (36), as well as the mother's use of potential harmful substances (e.g. tobacco, alcohol, drugs) during pregnancy (37), education levels (as a measure of socioeconomic status) (38), and psychological condition (39). We used an adaptation of the Home Observation Measurement of the Environment (HOME) scoring measure to evaluate aspects of the quantity and quality of social, emotional, and cognitive support made available to the child in their home environment (40). For greater accuracy of this score, we took the average of two measurements which were collected when children were 6 months and 18 months of age. The mother's psychological condition after giving birth was assessed when the child was 8 months old using the Edinburgh Postnatal Depression Scale (EPDS) (41). Lastly, mothers' use of illicit drugs and cigarettes during pregnancy were considered as binary (ever/never), and levels of alcohol use during pregnancy were considered (none/moderate/heavy) by combining data from multiple collection time points and taking the average.

STATISTICAL ANALYSIS

For purposes of target surveillance and prevention, we applied a multivariate Poisson regression with a robust variance estimator to determine factors strongly associated with CMV postnatal infection (measured at 7 years). Exposures investigated included: method of delivery, pregnancy size (i.e., singleton vs twin), infant school

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3 attendance, biological sex, gestational age, birthweight, HOME score,
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5 breastfeeding duration, mother's education, ethnicity, age at delivery,
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7 EPDS, vitamin supplementation during pregnancy, and use of harmful
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9 substances (tobacco, alcohol, drugs) during pregnancy. In order to assess
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11 how factors influence performance on neurocognitive measures
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13 independently of CMV infection and to evaluate the potential for strong
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15 confounding, we also conducted a secondary analysis among CMV-negative
16
17 children where we examined the associations between potential confounders
18
19 and neurocognitive outcomes of interest.
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22 We employed multivariate Poisson regression with a robust variance
23
24 estimator to estimate the incidence rate ratio (IRR), and 95% confidence
25
26 interval (CI) to evaluate the association between CMV seropositivity at
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28 7 years of age and suboptimal cognitive outcomes. The biological sex of
29
30 the child was then assessed as a potential effect modifier by stratified
31
32 analysis.
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35 Imputation was used to limit missingness of the covariates in the
36
37 regression models. When missing, mother's education, breastfeeding
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39 duration and average HOME score were imputed to the median value of each
40
41 variable for those with CMV serological data (i.e., the analysis
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43 subpopulation). Statistical analysis was performed using Stata
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45 statistical and data analysis software version 15.0 (42).
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49 **APPROXIMATE BAYES FACTOR ANALYSIS**

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52 In order to mitigate the potential issue of multiple comparisons, we
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54 estimated the approximate Bayes factor (ABF) based on methods previously
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3 described to assess the noteworthiness of observed associations (43-45).
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5 An ABF can be interpreted as a measure of the strength of evidence in
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7 favour of the alternative hypothesis compared to the null and does not
8
9 depend on the number of comparisons. An ABF of less than 0.1 or greater
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11 than 10 is considered strong evidence for the alternative hypothesis;
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13 0.1-0.33 or 3-10 is moderate evidence; 0.33-1 or 1-3 is anecdotal
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15 evidence; and 1 is no evidence (46).
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21 **RESULTS**

22 ***Study Population and Factors Associated with CMV Infection***

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25 Demographic data on the study population is summarized in Table 1. There
26
27 were 4988 participants that had serological CMV data at 7 years of age
28
29 and met inclusion criteria for the analysis. Overall, 51.8% were boys,
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31 98.3% were from mothers with European ancestry, and mean gestational age
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33 was 39.4 weeks. Of these participants, 864 (17.3%) were classified as
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35 CMV 'positive' (CMV+; infected) and 4124 (82.7%) were classified as CMV
36
37 'negative' (CMV-; uninfected) based on the threshold value computed using
38
39 IgG antibody titers. Children in the CMV+ and CMV- groups were comparable
40
41 on most child and maternal parameters. Table 1 also summarizes the
42
43 mutually adjusted factors associated with CMV infection. As reported in
44
45 previous studies (47), longer breastfeeding duration was shown to be
46
47 associated with CMV infection. Mothers of non-European ancestry were
48
49 found to have a positive association with children's CMV infection when
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51 compared to mothers with European ancestry (IRR=2.34, 95% CI 1.52-3.59).
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3 Furthermore, twin pregnancy was found to have an inverse association with
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5 CMV infection (IRR=0.40, 95% CI 0.17-0.95).

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8 (Table 1 here)
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10 ***Factors Associated with Neurocognitive Outcomes***

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13 Table 2 summarizes the associations between potential confounding factors
14 and the primary outcome of interest, WISC total IQ at 8 years, in the
15 absence of CMV infection. There was strong evidence for associations
16 between HOME score and suboptimal WISC total IQ at 8 years (IRR=0.64,
17 95% CI 0.53-0.77, ABF<0.01). The associations between potential
18 confounders and other cognitive outcomes are summarized in Supplementary
19 Tables 2a-d. In brief, we observed associations between breastfeeding
20 duration (6+ months), alcohol use during pregnancy (>7 units per week)
21 and TEA-Ch attentional control at 8 years (IRR=0.40, 95% CI 0.21-0.75,
22 ABF=0.09; IRR=2.34, 95% CI 1.31-4.18, ABF=0.09, respectively), and
23 child's sex with NARA reading comprehension at 9 years (IRR=0.31, 95% CI
24 0.15-0.63, ABF=0.05).
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39 (Table 2 here)
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42 ***Effects of CMV Infection on Neurocognition***

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45 Table 3 summarizes the results of the association of CMV with children's
46 neurodevelopment. After adjustment for potential confounders, CMV
47 infection was associated with suboptimal WISC total IQ at 8 years
48 (IRR=2.50, 95% CI 1.35-4.62, ABF=0.08) but not with WASI total IQ at 15
49 years (IRR=0.97, 95% CI 0.43-2.19, ABF=1.68). CMV infection was also
50 associated with suboptimal attentional control using the TEA-Ch at 8
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3 years (IRR=1.74, 95% CI=1.13-2.68, ABF=0.18), but not at 11 years
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5 (IRR=1.07, 95% CI 0.65-1.77, ABF=2.33). Based on the NARA at 9 years,
6
7 CMV infection indicated a higher risk for suboptimal reading
8
9 comprehension (IRR=1.93, 95% CI 1.12-3.33, ABF=0.24). All other measures
10
11 of intelligence, attention and reading were not associated with CMV
12
13 infection. Similarly, measures of suboptimal language dimensions and
14
15 working memory were not found to be associated with CMV infection. ABF
16
17 analysis suggested that there was strong evidence for the association
18
19 between CMV infection and lower WISC total IQ at 8 years, but only
20
21 moderate evidence for suboptimal TEA-Ch attentional control at 8 years
22
23 and NARA reading comprehension at 9 years.
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26 (Table 3 here)
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29 We further assessed whether the associations differed by the sex of the
30
31 child. Results of the sex-stratified analysis and we observed no
32
33 differences between boys and girls on any cognitive assessments
34
35 (Supplementary Table 3).
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41 **DISCUSSION**

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44 To our knowledge, this is the largest and longest prospective
45
46 longitudinal analysis investigating neurocognitive outcomes in children
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48 infected postnatally with CMV. Our analysis supported previous
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50 associations reported regarding longer breastfeeding duration and
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52 ethnicity being major influential exposures for postnatal CMV infection
53
54 (48). Our results provide some evidence for adverse neurocognitive
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3 effects of postnatal CMV infection on total IQ during early childhood,
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5 but this effect may be short-lived as subsequent measurement of total IQ
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7 in later adolescence yielded no association. Overall, postnatal CMV
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9 infection did not appear to influence cognitive performance on the
10
11 majority of other measures that were assessed.
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13
14 It is widely recognized that symptomatic congenital CMV infection has
15
16 widespread neurological and developmental consequences (6), however few
17
18 have studied either asymptomatic congenital CMV infection or postnatal
19
20 infection. In particular, previous studies focusing on asymptomatic
21
22 congenital CMV and neurodevelopment have found inconsistent associations
23
24 (49-53), and postnatal CMV infection and neurodevelopment has only been
25
26 studied in higher-risk subpopulations, such as preterm infants to date
27
28 (14, 54-56). Overall, results have been inconclusive and highlight the
29
30 gap in current knowledge related to CMV infection and cognitive
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32 impairment.
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34
35 There are various proposed mechanisms as to how CMV infection may impair
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37 neurological and cognitive abilities. A recent study of postnatal CMV
38
39 infection in preterm infants using sophisticated MRI analyses found that
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41 subtle neural alterations (e.g., regional differences in functional
42
43 activation or disturbances within the neural networks) may explain the
44
45 neurological sequelae in infected individuals (14, 47). It has also been
46
47 hypothesized that the observed association between CMV and intelligence
48
49 could be mediated by leukocytes. Higher CMV IgG titres have been
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51 associated with lower leukocyte telomerase activity and shorter leukocyte
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53 telomere length in healthy adults, while longer leukocyte telomeres were
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3 associated with superior cognitive performance in a large meta-analysis
4 (57-59). Finally, CMV infection has also been shown to modulate
5 inflammatory and anti-inflammatory circulating biomarkers (e.g.,
6 cytokines, receptor antagonists, immune cells, and metabolic markers)
7 and in turn, these neuroimmune factors may increase neurodegeneration
8 and affect cognitive performance (55).
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16 It has been previously concluded that sex modulates neuroimmune factors,
17 cognitive performance, and the relationship between the two domains (50,
18 51, 55). Therefore, we sought to understand how biological sex impacted
19 the associations between CMV infection and neurocognitive measures. The
20 sex-stratified analysis suggested there is no differential effect of
21 postnatal CMV infection on cognition between boys and girls.
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29 It should be noted that cognitive domains are not independent of one
30 another as domains of cognitive functioning are hierarchical in nature,
31 ranging from basic sensory and perceptual processes to higher executive
32 functioning and cognitive control. Therefore, scores on different
33 measures may be inter-related based on the same underlying mechanisms of
34 neurocognitive development. For example, language deficits may be
35 associated with deficits of executive functioning and processing speed
36 (60). Further, interrelationships between reading and intelligence are
37 widely recognized, especially in children (61, 62). For example, we
38 observed correlation between WISC at 8 years of age and NARA reading
39 comprehension at 9 years of age (Pearson correlation= 0.64, $p<0.05$),
40 which is also reflected in their associations with CMV infection. The
41 specific tests of different domains may provide information on each
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3 domain, while the global executive functioning is the general
4 representation that involved each domain (60).
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8 Although we observed an association between CMV infection and
9 neurocognitive impairment using WISC total IQ at 8 years of age, we did
10 not observe the same impairment in WASI total IQ at 15 years of age.
11
12 There are several possible reasons that could explain these differences
13 over time: (i) children scoring sub-optimally at the 8-year measurement
14 were less likely to continue to participate in measurement at the later
15 time point than children who scored within normal range (44% vs. 63%
16 retention, respectively); (ii) cognitive impairment due to CMV infection
17 may not be long-lasting (53, 63). In other words, CMV infection may cause
18 a deficit in cognitive domains during early childhood; however it is
19 possible that infected individuals may compensate cognitively by
20 adolescence; and/or, (iii) CMV seropositivity status at 7 years captures
21 all children who contracted CMV in utero up to age 7. Given the extended
22 lag time needed between CMV infection and adverse neurodevelopment
23 sequelae, the cognitive impairment observed at 8 years is likely due to
24 CMV infection during the toddler time period (critical for brain
25 development), whereas the effect of later CMV infection is likely to be
26 reflected in cognitive measurements at 11 or 15 years old. Therefore,
27 the alternative hypothesis of this observation is that only postnatal
28 infection within infancy or the toddler period, when the brain tissue is
29 under active development, would have an effect on cognitive impairment
30 (as manifested at 8 years), and possibly not CMV infection acquired later
31 on at school age. However, given the timing of the CMV measurement, we
32 are not able to tease out the susceptibility time period in this analysis,
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3 which is a limitation. One related limitation is that we were also not
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5 able to distinguish congenital CMV infection from early childhood CMV
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7 infection. However, congenital CMV infection is rare at merely 0.6-
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9 0.7%, compared to up to 20% prevalence of CMV infection in childhood.
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11 Thus, while we do not expect congenital infection to be driving the
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13 association we observed, we cannot preclude this possibility. It is
14
15 important for our results to be confirmed in a future study where
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17 differentiation between congenital and postnatal CMV is possible. For
18
19 future investigations, it would be best to have multiple earlier
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21 serological measurements of CMV antibodies within the first years of life
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23 in order to identify the timing of infection and the susceptible time
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25 window related to potential adverse sequelae later on.
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29 Another limitation of using data from this specific UK-based cohort was
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31 the ethnic homogeneity of the study population. The total proportion of
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33 mothers with European ancestry recruited eclipsed that of non-Caucasian
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35 races (~97% Caucasian vs ~3% all other ethnicities combined), making a
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37 detailed analysis on ethnic subgroups impossible. Lastly, it is possible
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39 that children's overall health status is related to viral antibody
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41 levels, and it may influence a child's performance on cognitive tests.
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43 We did not have data on the overall health status of children and were
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45 therefore unable to characterise this relationship or account for it in
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47 our modelling. However, it is likely that overall health status would
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49 mediate, rather than confound, the relationship between CMV infection
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51 and suboptimal neurocognitive outcomes. Therefore it is unlikely to
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53 result in a biased association when unaccounted for.
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3 The key strengths of this study are the size of the study population,
4 the length of follow-up and the extent of cognitive assessments that were
5 conducted prospectively. Previous studies examining postnatal CMV
6 infection have primarily focused on much narrower study populations
7 arising from single-centre recruitment. This investigation represents
8 the largest analysis conducted for postnatal CMV infection and children's
9 cognitive outcomes for multiple constructs at multiple time points.
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18 In conclusion, based on a longitudinal study assessing neurocognitive
19 sequelae of children infected postnatally with CMV, we observed a
20 potential increased risk of suboptimal general intelligence associated
21 with infection, although the effect may not be long-lasting. However,
22 the majority of our findings support the null hypothesis that postnatal
23 CMV infection is not associated with adverse neurocognition in later
24 childhood. Nonetheless, our study provides a foundation and motive for
25 further research into the largely understudied relationship between
26 postnatal CMV infection and adverse neurocognitive outcomes, with
27 specific focus on timing of susceptibility. In future investigations,
28 measurements of IgG avidity as well as other antibody types (e.g., IgM)
29 in addition to IgG could help to differentiate active, reactivated, and
30 past CMV infections and shed further insights on CMV sequelae related to
31 the susceptible time window. As CMV is the most wide-ranging and
32 prevalent perinatal infection, and its health consequences are largely
33 unknown, knowledge of its role in neurocognitive development could be
34 beneficial to population health. Knowledge of children's heightened risk
35 for adverse cognitive outcomes associated with postnatal CMV infection
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3 is important when evaluating whether early interventions to mitigate
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5 long-term effects of CMV infection are warranted.
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10 **ACKNOWLEDGEMENTS**

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12
13 We are extremely grateful to all the families who took part in the ALSPAC
14
15 study, the midwives for their help in recruiting them, and the whole
16
17 ALSPAC team, which includes interviewers, computer and laboratory
18
19 technicians, clerical workers, research scientists, volunteers,
20
21 managers, receptionists and nurses.
22
23

24
25 This work was supported by the Lunenfeld-Tanenbaum Research Institute,
26
27 Sinai Health System and the Canada Research Chair to [R.J.H.] from the
28
29 Canadian Institute for Health Research. The UK Medical Research Council
30
31 and Wellcome [Grant ref: 217065/Z/19/Z] and the University of Bristol
32
33 provide core support for ALSPAC. A comprehensive list of grants funding
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35 is available on the ALSPAC website
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39 acknowledgements.pdf) acknowledgements.pdf) .

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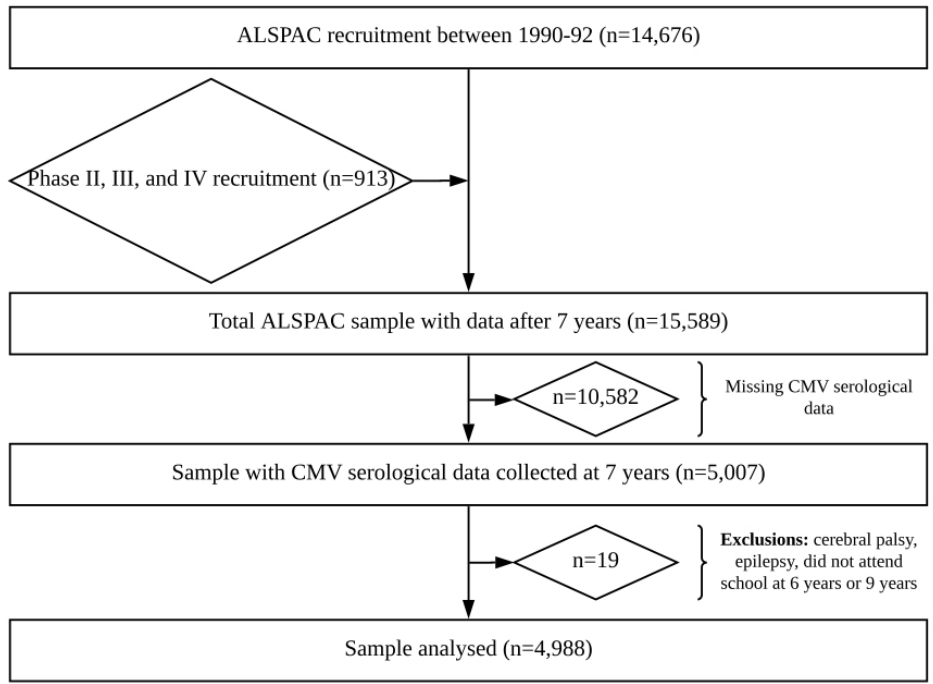


Figure 1. Flow diagram depicting study participant recruitment and exclusions

155x117mm (150 x 150 DPI)

Table 1. Baseline Characteristics of the overall study sample by CMV infection status

	Overall Study Sample (n=4988) ^a		CMV Positive (n=864)		CMV Negative (n=4124)		Incidence-Rate Ratio of CMV infection*		P value
	n	(%)	n	(%)	n	(%)	IRR	(95% CI)	
Child Characteristics									
Sex									
Male	2582	(51.8)	447	(51.9)	2135	(51.8)	1.00	(Reference)	-
Female	2401	(48.2)	415	(48.1)	1986	(48.2)	0.93	(0.78,1.12)	0.44
Gestational age, weeks, mean	39.4	(SD=1.8)	39.4	(SD=1.8)	39.5	(SD=1.8)	0.97	(0.91,1.03)	0.34
Birthweight, grams, mean	3436.1	(SD=545.2)	3429.1	(SD=542.9)	3437.6	(SD=545.8)	1.00	(1.00,1.00)	0.57
Pregnancy size									
Singleton	4866	(97.7)	848	(98.4)	4018	(97.5)	1.00	(Reference)	-
Twin	117	(2.3)	14	(1.6)	103	(2.5)	0.40	(0.17,0.95)	0.04
Breastfeeding duration									
Never	845	(19.7)	87	(11.7)	758	(21.4)	1.00	(Reference)	-
<3 months	934	(21.8)	120	(16.2)	814	(22.9)	1.15	(0.81,1.63)	0.45
3-5 months	777	(18.1)	146	(19.7)	631	(17.8)	1.61	(1.14,2.27)	<0.01
6+ months	1734	(40.4)	388	(52.4)	1346	(37.9)	1.79	(1.30,2.46)	<0.01
Attended infant school/preschool ^b	3851	(85.1)	661	(84.9)	3190	(85.2)	1.04	(0.75,1.45)	0.82
HOME score, mean ^c	9.2	(SD=1.7)	9.4	(SD=1.7)	9.2	(SD=1.6)	1.05	(0.99,1.12)	0.11
Maternal Characteristics									
Maternal age at delivery, years, mean	29.2	(SD=4.6)	29.6	(SD=4.7)	29.1	(SD=4.6)	0.99	(0.97,1.01)	0.28
Method of delivery									
Vaginal, spontaneous	1688	(61.1)	312	(63.3)	1376	(60.7)	1.00	(Reference)	-
Vaginal, assisted	551	(20.0)	96	(19.5)	455	(20.1)	0.94	(0.75,1.18)	0.60
Caesarean	522	(18.9)	85	(17.2)	437	(19.3)	0.85	(0.66,1.10)	0.22
Ethnicity									
European	4433	(98.3)	740	(96.4)	3693	(98.7)	1.00	(Reference)	-
Other ethnic group	75	(1.7)	28	(3.6)	47	(1.3)	2.34	(1.52,3.59)	<0.01
Substance use during pregnancy ^d									
Cigarette use	928	(19.8)	143	(17.9)	785	(20.3)	1.06	(0.83,1.36)	0.64
Alcohol use									
<1 unit per week	2599	(55.6)	426	(53.3)	2173	(56.1)	1.00	(Reference)	-
1-6 units per week	1534	(32.8)	271	(33.9)	1263	(32.6)	1.14	(0.94,1.39)	0.18
≥7 units per week	539	(11.5)	102	(12.8)	437	(11.3)	1.19	(0.91,1.56)	0.21
Illicit drug use	124	(2.7)	31	(3.9)	93	(2.4)	1.41	(0.88,2.25)	0.15
Vitamin supplementation during pregnancy ^e	983	(21.2)	184	(23.2)	799	(20.8)	0.97	(0.78,1.21)	0.80
Mother's Education Level ^f									
None/CSE	577	(12.8)	92	(11.9)	485	(12.9)	1.00	(Reference)	-
Vocational	382	(8.5)	51	(6.6)	331	(8.8)	0.78	(0.49,1.23)	0.28
O-Level	1567	(34.7)	234	(30.2)	1333	(35.6)	0.84	(0.60,1.18)	0.32
A-Level	1224	(27.1)	220	(28.4)	1004	(26.8)	0.93	(0.66,1.31)	0.68
Degree	770	(17.0)	177	(22.9)	593	(15.8)	1.11	(0.76,1.61)	0.59
Mother's EPDS, mean ^g	5.2	(SD=4.5)	5.2	(SD=4.5)	5.2	(SD=4.5)	1.00	(0.98,1.02)	0.66

*All factors were investigated for their association with CMV infection status at 7 years in a single modified Poisson model, therefore mutually adjusting for all other factors under investigation.

^aThe number of observations included in the study sample was dependent on the availability of serological CMV data.

^bInfant school/preschool attendance was assessed as either full-time or part-time attendance when the child was 65 months (5 years, 5 months) of age.

^cHome Observation for Measurement of the Environment (HOME) score was measured when the child was 6 months and 18 months of age, and is a score of 0 to 12 based on the quality of the home environment for child development including cognitive stimulation and emotional support measures, with a higher score indicating greater environment quality.

^dUsers of harmful substances during pregnancy included those who reported any use of cigarettes/tobacco, alcohol, or illicit drugs at any point during pregnancy. Illicit drugs included cannabis, amphetamine, barbiturate, crack, cocaine, heroin, methadone, and ecstasy.

^eMother's vitamin supplementation during pregnancy was assessed at both 18 weeks gestation and 32 weeks gestation.

^fMother's education level at 32 weeks gestation was obtained based on a 5-point UK education scale, including: none/CSE level, vocational level, O-level, A-level, and University Degree level.

^gMother's Edinburgh Post-natal Depression Score was assessed when the child was 8 months of age on a scale of 0 to 29, with a higher score indicating more symptoms of depression.

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Table 2. Associations between confounders and suboptimal total IQ (WISC 8 years) (among CMV-negative children)

Potential Confounders	Incidence-Rate Ratio of suboptimal neurocognitive outcome		P value
	IRR	(95% CI)	
Child Characteristics			
Sex			
Male	1.00	(Reference)	-
Female	0.50	(0.23,1.08)	0.08
Gestational age, weeks, mean	1.10	(0.86,1.41)	0.46
Breastfeeding duration			
Never	1.00	(Reference)	-
<3 months	0.30	(0.08,1.12)	0.07
3-5 months	1.03	(0.41,2.55)	0.96
6+ months	0.53	(0.18,1.56)	0.25
HOME score, mean	0.64	(0.53,0.77)	<0.01
Maternal Characteristics			
Substance use during pregnancy			
Cigarette use	1.44	(0.65,3.16)	0.37
Alcohol use			
<1 unit per week	1.00	(Reference)	-
1-6 units per week	0.45	(0.19,1.10)	0.08
≥7 units per week	0.70	(0.20,2.42)	0.57
Mother's Education Level			
None/CSE	1.00	(Reference)	-
Vocational	1.89	(0.66,5.41)	0.24
O-Level	0.68	(0.24,1.92)	0.47
A-Level	0.30	(0.07,1.39)	0.13
Degree	0.17	(0.02,1.41)	0.10
Mother's EPDS, mean	0.96	(0.89,1.05)	0.38

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Table 3. Associations between CMV infection status and suboptimal neurocognitive outcomes (with adjustment for confounders)

Suboptimal Neurocognitive Outcome*	CMV Positive (infected) (n=864)		CMV Negative (control) (n=4124)		Incidence-Rate Ratio of suboptimal neurocognitive outcome ^a		P value	Approx. Bayes factor
	n/N ^b	(%)	n/N ^b	(%)	IRR	(95% CI)		
Wechsler Intelligence Scale for Children (WISC), 8 years								
Verbal IQ	9/718	(1.3)	54/3436	(1.6)	1.38	(0.70,2.73)	0.36	1.38
Performance IQ	11/717	(1.5)	44/3431	(1.3)	1.77	(0.92,3.39)	0.09	0.65
Total IQ	14/715	(2.0)	41/3423	(1.2)	2.50	(1.35,4.62)	<0.01	0.08
Verbal Comprehension Index	9/713	(1.3)	56/3407	(1.6)	1.10	(0.53,2.29)	0.80	1.76
Perceptual Organization Index	16/682	(2.3)	60/3226	(1.9)	1.66	(0.93,2.98)	0.09	0.69
Freedom from Distractibility Index	9/699	(1.3)	66/3342	(2.0)	0.85	(0.41,1.76)	0.66	1.69
Wechsler Objective Language Dimensions (WOLD), 8 years								
Comprehension Score	15/715	(2.1)	75/3448	(2.2)	0.94	(0.50,1.78)	0.86	1.96
Expression Score	19/710	(2.7)	95/3441	(2.8)	1.04	(0.61,1.76)	0.89	2.30
Expression Score (description, direction, sequencing task)	5/463	(1.1)	26/2293	(1.1)	1.29	(0.49,3.37)	0.61	1.41
Test of Everyday Attention for Children (TEA-Ch), 8 years								
Selective Attention Score	32/694	(4.6)	105/3349	(3.1)	1.43	(0.95,2.15)	0.09	0.78
Dividing Attention Score	18/531	(3.4)	76/2603	(2.9)	1.23	(0.71,2.14)	0.45	1.79
Attentional Control (same world)	27/699	(3.9)	88/3370	(2.6)	1.74	(1.13,2.68)	0.01	0.18
Attentional Control (opposite world)	15/697	(2.2)	48/3368	(1.4)	1.65	(0.90,3.02)	0.11	0.75
Neale Analysis of Reading Ability (NARA), 9 years								
Words per Minute Score	15/653	(2.3)	88/3094	(2.8)	1.05	(0.59,1.87)	0.88	2.13
Accuracy Score	20/654	(3.1)	87/3102	(2.8)	1.28	(0.75,2.16)	0.36	1.64
Comprehension Score	17/654	(2.6)	66/3102	(2.1)	1.93	(1.12,3.33)	0.02	0.24
Developmental dyslexia ^c	22/734	(3.0)	72/3456	(2.1)	1.55	(0.92,2.63)	0.10	0.79
Counting Span Task (Working Memory), 10 years								
Working Memory Global Score ^d	39/677	(5.8)	151/3120	(4.8)	1.35	(0.93,1.97)	0.12	1.04
Working Memory Span Score	8/677	(1.2)	47/3120	(1.5)	0.84	(0.40,1.77)	0.65	1.66
Test of Everyday Attention for Children (TEA-Ch), 11 years								
Selective Attention Score	17/661	(2.6)	92/3156	(2.9)	1.00	(0.56,1.77)	0.99	2.17
Dividing Attention Score	6/650	(0.9)	32/3113	(1.0)	1.18	(0.50,2.77)	0.71	1.56
Attentional Control (same world)	23/646	(3.6)	100/3032	(3.3)	1.07	(0.65,1.77)	0.79	2.33
Attentional Control (opposite world)	26/645	(4.0)	91/3031	(3.0)	1.55	(0.98,2.43)	0.06	0.55
Wechsler Abbreviated Scale of Intelligence (WASI), 15 years								
Vocabulary Score	14/523	(2.7)	63/2438	(2.6)	1.16	(0.60,2.26)	0.66	1.80
Matrix Reasoning Score	19/523	(3.6)	86/2436	(3.5)	1.21	(0.73,2.02)	0.45	1.90
Total IQ	7/523	(1.3)	44/2435	(1.8)	0.97	(0.43,2.19)	0.94	1.68

*Each suboptimal neurocognitive outcome was analysed in a separate multivariate modified Poisson regression model, adjusting for the child's sex, ethnicity, gestational age, duration of breastfeeding, HOME score, mother's education, mother's postnatal psychological condition (using EPDS), and mother's use of tobacco, alcohol, and illicit drugs during pregnancy.

^aIncidence-rate ratio of the suboptimal neurocognitive outcome for those who are CMV positive, compared to those who are CMV negative (i.e., CMV negative is the reference group).

^bProportion of observations with suboptimal neurocognitive outcome, defined by 2 SDs below (or above, if a high score indicates the adverse outcome) the mean score for the entire cohort.

^cThe definition of developmental dyslexia was devised by Julie Williams and Alan Emond and is based on the accuracy component of the NARA II. The equivalent reading age based on the accuracy score is calculated from the NARA II manual and the difference between this and the actual age is calculated. Where the child's reading age is greater than or equal to 30 months (2.5 years) behind the actual age AND the child's WISC Total IQ is greater than or equal to 85 the child is considered developmentally dyslexic.

^dSuboptimal working memory global score was defined by $\leq 5^{\text{th}}$ percentile, as 2 SDs below the mean yielded a threshold that was too stringent (i.e., there were <5 observations in any cell for comparison in the overall sample).

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