Congenital Cytomegalovirus among Children with Cerebral Palsy

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Objectives To determine the proportion of children with cerebral palsy (CP) and cytomegalovirus (CMV) DNA detected retrospectively in their newborn screening cards (NBSC), to compare the proportion of children with CMV DNA in their NBSC across spastic subtypes of CP, and to compare the sex and other characteristics of children with CP and CMV detected on their NSBC with those in whom CMV DNA was not detected.

Study design Retrospective observational study. Data were extracted from patient records on children with CP (birth years 1996-2014) from 2 Australian state CP registers and state-wide paediatric rehabilitation services with consent. NBSCs were retrospectively analyzed for CMV DNA by nested polymerase chain reaction (PCR) using primers against gB. Positive samples were validated using real time PCR for CMV UL83.

Results Of 401 children recruited, 323 (80.5%) had an available NBSC. Of these, 31 (9.6%; 95% CI, 6.8-13.3) tested positive for CMV DNA by nested PCR for CMV gB, of whom 28 (8.7%; 95% CI, 6.1-12.2) also had CMV DNA detected by real-time PCR for CMV UL83. Detection of CMV DNA was significantly associated with epilepsy, but not with clinical or epidemiologic characteristics, including sex and pattern of spasticity.

Conclusions CMV viremia in the newborn period, indicating congenital CMV infection, is highly prevalent among children with CP. Further research is needed to investigate the mechanisms and contribution of congenital CMV to the causal pathways to CP. *(J Pediatr 2017;181:267-71).*

Cytomegalovirus (CMV) is a common herpesvirus that can cross the placenta, infect the fetus and cause damage to the developing brain.[1] Congenital CMV (cCMV) infection has been estimated to occur in approximately 0.7% of newborn infants,[2,3] of whom 10%-15% exhibit signs of infection at birth. These infants are at increased risk of permanent neurodevelopmental disabilities including cerebral palsy (CP). It is estimated that a further 10%-15% of children who are asymptomatic at birth will go on to develop neurologic sequelae beyond the neonatal period, predominantly late-onset hearing loss.[3-5]

CP is the most common physical disabilty of childhood, and has been associated with a number of risk factors, including intrauterine infections such as cCMV.[6] Despite this known association, and estimates of neurologic disability from cCMV, few data describe the prevalence and epidemiology of CP associated with cCMV. Defining the role of cCMV as a risk factor for CP is important because it is the most common intrauterine infection in developed countries, is potentially preventable, and antiviral therapy postnatally can reduce the severity of adverse neurologic outcomes.[7] The recent outbreaks of Zika virus in South America and French Polynesia and their assocation with birth defects including microcephaly further highlight the importance of intrauterine infection in neurodevelopmental disability.[8]

We have reported previously a retrospective population-based study using data from the Australian CP Register (ACPR). We found that 1.5% of children had cCMV (confirmed or probable) reported as an attributable contributing cause of their CP.[9] Here, female children were over-represented, as were younger mothers, and there was a higher prevalence of spastic quadriplegia (73% of children with spastic CP) and severe functional mobility limitations. Similar findings have been reported in small case series in the US and Europe.[10,11] In the absence of a newborn screening program for cCMV in Australia, we hypothesized that our study may have underascertained the proportion of children with CP who had cCMV.

To test our hypothesis, and validate findings from the retrospective population-based study, we aimed to determine the proportion of children with CMV DNA retrospectively detected in their newborn screening card (NBSC) by molecular testing

ACPR	Australian Cerebral Palsy Register
ACT	Australian Capital Territory
cCMV	Congenital cytomegalovirus
CMV	Cytomegalovirus
CP	Cerebral palsy
NBSC	Newborn screening card
NSW	New South Wales
PCR	Polymerase chain reaction

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(polymerase chain reaction [PCR]) among a group of children with CP born in New South Wales (NSW) and the Australian Capital Territory (ACT). Secondary objectives were to use CP register data to compare the proportion of children with CMV DNA on their NBSCs among children with spastic quadriplegia compared with other forms of spastic CP and to compare the sex and clinical profile of children with CP and CMV detected on their NSBC with those in which CMV DNA was not detected.

Methods

Parents or guardians of children (aged less than 18 years) with CP (birth years 1996-2014) from the NSW and ACT CP registers, or the state-wide disability services provider Cerebral Palsy Alliance, or the CP outpatient clinic at the Children's Hospital at Westmead, provided informed consent for NBSC testing for cCMV and extraction of registry data. This study was approved by the NSW Population and Health Services Research Ethics Committee (EC00410), the Cerebral Palsy Alliance Human Research Ethics Committee (EC00402), and by the University of Sydney Human Research Ethics Committee.

Data extracted from the CP Registers included cCMV reported as an attributable cause of CP, sex, gestational age, birthweight, plurality, maternal age at time of birth, parity (live births and stillbirths of 20 weeks or more, excluding comultiples of the case), predominant type of CP at 5 years of age, and functional mobility. Data on symptoms and signs of cCMV in the newborn period were unavailable because they are not part of the CP register dataset. Functional mobility was described using the Gross Motor Function Classification System.[12] Associated impairment data included 5 domains: intellect, (1) no impairment (IQ of less than 70 or so described), (2) mild impairment (IQ 50-69 or so described), and (3) moderate to severe impairment (IQ of less than 35-49 or so described); vision, some impairment, which in this context includes use of glasses; hearing, some impairment, which in this context includes conductive hearing loss; bilateral deafness describes a severe/profound hearing loss for both ears, where conversational speech is inaudible; speech, some impairment describes any expressive speech and language difficulty, and nonverbal is used to describe no or severely limited verbal expressive communication and/or reliance predominantly/exclusively on augmentative and alternative communication strategies and the presence/absence of epilepsy, defined as 2 or more afebrile seizures before 5 years of age; and does not include neonatal seizures. CP register data are obtained from treating specialist clinician records with associated impairments measured by standard methodologies as selected by the treating clinician. Register data are reconfirmed with the treating clinician when the child is 5 years of age. For participating children less than 5 years of age (n = 23/401; 5.8%), data from the initial record of CP registration were used. We deidentified each child's clinical record and NBSC by allocating a unique study number.

NBSC were collected from the NSW Newborn Screening Program at the Children's Hospital at Westmead. The NBSC testing was completed blinded to the child's clinical status. CMV DNA was extracted from 4 punches per NSBC using the QIAamp DNA Micro Kit (Qiagen, Stanford, California)[13] according to the methodology of the National Association of Testing Authorities accredited diagnostic laboratory at Children's Hospital at Westmead, which uses a modification of a published protocol[14] and rigorous attention to prevent contamination, including a minimum of 28 disks punched from a blank filter paper between samples. A 10-μL sample of each extract was subjected to nested CMV DNA PCR testing using 2 primer sets to amplify a segment of the glycoprotein B gene (UL58) with minor modifications to the published protocol and a lower limit of detection was approximately 10 viral copies/mL.[14] CMV-positive DNA and negative controls were included with every reaction, and confirmed the absence of cross contamination. Positive results were validated by a real-time PCR assay for CMV UL83 gene, with human glyceraldehyde-3-phosphate dehydrogenase gene as an internal control as published[15] using a separate punch from the NBSC. The lower limit of detection of this assay was 100 copies/mL, with 1 copy of CMV being roughly equivalent to 0.9 international units.

We compared proportions between groups using the χ^2 or Fisher exact test where appropriate to analyse the crude relationship between characteristics of the children who had positive and negative cCMV test results. We investigated associated impairments between individuals with no impairment and those with the most severe level of impairment only. In a post hoc analysis to measure recruitment bias, we compared the characteristics of participants in this study with those children recorded on population state registers in the ACPR using χ^2 or Fisher extact test where appropriate. $P < .05$ was considered significant. Data were analysed using SPSS Statistics version 23.0.0 (SPSS Inc, Chicago, Illinois).

Results

A total of 401 individuals with CP were recruited, of whom 323 (80.5%) had an available NBSC (**Figure**; available at www.jpeds.com). Of these, 31 (9.6%; 95% CI, 6.8-13.3) tested positive for CMV DNA (cCMV-positive cases) in NBSC by nested PCR for CMV gB, of whom 28 (8.7%; 95% CI, 6.1-12.2) had CMV DNA also detected by real time PCR for CMV UL83.

We next compared the characteristics of participants using extracted registry data, according to their NBSC test result for CMV DNA. There were 140 female children (43.5%) in this study (**Table I**), and a similar proportion of female cases within both the cCMV positive and negative groups (45.2 vs 43.3%; $P = .851$). No differences were found between groups for maternal age ($\bar{x}$ 31 years vs 31 years; $P = .968$) plurality, birthweight, or gestational age (**Table I**).

Even though spasticity was the most common type of CP across both groups, there were no differences in spastic subtypes between the cCMV-positive and cCMV-negative groups (**Table I**). We next compared the functional mobility