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.

Keywords:

disability, registry, newborn screening, neurotropic, virus, development

Abbreviations:

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)

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, 4, 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 (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

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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 co-multiples 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

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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 ($\overline{x}$ 31 years vs 31 years; *P* = .968) plurality, birthweight, or gestational age (Table I).

Table I Characteristics of children with CP the NBSC CMV PCR test result

	Total NBSC tested	**CMV Positive**	**CMV Negative**	***P***
	No. (%)	**No. (%)**	**No. (%)**	
CP cases by CMV test result	323(100)	31(9.6)	292(90.4)	
Sex	n = 322	n = 31	n = 291	.85
Male	182(57)	17(55)	165(57)	
Female	140(43)	14(45)	126(43)	
Gestational age (w)	n = 321	n = 30	n = 291	.75
<37	121(38)	12(40)	109(37)	
≥37	200(62)	18(60)	182(63)	
Mean [SD, range]		36.3(5) 31-41	36.0(5.1) 31-41	
Birthweight (g)	n = 316	n = 30	n = 290	.91
<1500	63(20)	4(13)	61(21)	
1500-2499	54(17)	8(27)	47(16)	
≥2500	199(63)	18(60)	182(63)	
Mean (SD)		2667(973)	2690(1058)	
Range		1693-3639	1631-3747	

Maternal age (y)	n = 278	n = 23	n = 255	.97
≤20	6(2)	1(4)	5(2)	
21-34	199(72)	17(74)	182(71)	
35+		5(22)	68(27)	
Mean (SD), range	73(26)	31.2(4.9), 26-36	31.1(5.1), 26-36	
Parity	n = 254	n = 22	n = 232	.27
0	127(50)	14(64)	113(49)	
≥1	127(50)	8(36)	119(51)	
Functional mobility	n = 290	n = 25	n = 265	.92
GMFCS I-III	218(75)	19(76)	199(75)	
GMFCS IV-V	72(25)	6(24)	66(25)	
Type and topography	n = 279	n = 28	n = 276	.13
Spastic	245[82]	26[92]	223[81]	
Hemiplegia	*114*(*46)*	*14*(*54)*	*100*(*46)*	
Diplegia	*68*(*28)*	*5*(*19)*	*63*(*29)*	
Triplegia	*5*(*2)*	*1*(*4)*	*4*(*2)*	
Quadriplegia	*58*(*24)*	*6*(*23)*	*54*(*23)*	.80
Ataxic	23[8]	0[0.0]	23[8]	
Dyskinetic	17[5]	1[4]	16[6]	
Hypotonic	15[5]	1[4]	14[5]	

Speech	n = 256	n = 23	n = 233	.94
No impairment	95(37)	6(26)	89(38)	
Some impairment	116(45)	14(61)	102(44)	
Nonverbal	45(18)	3(13)	42(18)	
Intellectual impairment	n = 251	n = 22	n = 229	.77
No impairment	151(60)	13(59)	138(60)	
Some impairment	52(21)	6(27)	46(20)	
Moderate/severe impairment	48(19)	3(13)	45(20)	
Hearing impairment	n = 274	n = 24	n = 250	.76
No impairment	238(87)	18(75)	220(88)	
Some impairment	23(8)	3(12)	20(8)	
Bilateral deafness	13(5)	3(12)	10(4)	
Visual impairment	n = 253	n = 23	n = 230	.35
No impairment	161(64)	16(70)	145(63)	
Some impairment	84(33)	7(30)	77(33)	
Functionally blind	8(3)	0(0.0)	8(3)	
Epilepsy	n = 278	n = 25	n = 253	.04**
None	207(75)	15(60)	192(75)	
Resolved	8(2)	0(0)	8(3)	
Epilepsy	63(23)	10(40)	53(21)	

GMFCS, Gross Motor Function Classification System.

() = % of cases excluding unknown/missing data. [%] proportion of CP types excluding unknown/missing.

Italicized data represent subgroups of Spastic CP.

*$P < .05$

Even though spasticity was the most common type of CP across both groups, there were no differences in spastic subytypes between the cCMV-positive and cCMV-negative groups (Table I). We next compared the functional mobility and associated impairments reported for the cCMV-positive and cCMV-negative groups. With the exception of epilepsy, which was over-represented among the cCMV-positive group (40% vs 20.9%; $P < .05$), there were no differences between the 2 groups in relation to any comorbidities examined (Table I). Although proportionally more children in the cCMV positive group had hearing loss and deafness, the difference was not significant.

In a post hoc analysis to investigate possible recruitment bias, we investigated whether differences between the observed assocations (female sex, CP subtype, and comorbidities) with cCMV status in our previous study,[9] and the results from this study could be explained by differences in the study populations. After analyzing key factors (sex, CP type and topography, functional mobility, and associated impairments) we found no significance between these 2 groups (Table II). We also compared the proportion of children with a CP register record of confirmed or probable cCMV with the finding of 1.5% from our previous study of ACPR data.[9] Four children (1.2%) had an existing record of cCMV as an attributable cause of their CP together with reported laboratory evidence to support a diagnosis of probable or confirmed cCMV as previously defined,[9] of whom 2 had CMV DNA detected by retrospective analysis of their NBSC in this study. Last, we reviewed the best available demographic, neonatal, and clinical data (sex, gestational age, birth weight, plurality, parity, functional mobility, and associated impairments) for the Australian Capital Territory and NSW CP Register group as a whole for the same birth years (n = 1881) and the participant group (n = 401). There were no differences observed between the groups.

Table II Characteristics of children with CP**

	Population CP Registers 1993-2003, n (%)	**Children with CP, with NBSC tested for CMV DNA n (%)**	***P***
Registrants	n = 2265	n = 323	
Sex	n = 2265	n = 322	.97
Male	1278(56)	182(56)	
Female	987(44)	140(43)	

CP type and topography	n = 2245	n = 279	.12
Spastic	1913[85]	245[82]	
Hemiplegia	*735*(*38*)	*114*(*46*)	
Diplegia	*689*(*36*)	*68*(*28*)	
Triplegia	*60*(*3*)	*5*(*2*)	
Quadriplegia	*429*(*22*)	*58*(*24*)	.69
Ataxic	145[6]	23[8]	
Dyskinetic	143[6]	17[5]	
Hypotonic	44[2]	15[5]	
Functional mobility	n = 1114[†]†	n = 290	.11
GMFCS I-III	784(70)	218(75)	
GMFCS IV-V	330(30)	72(25)	
Speech	n = 1758[†]†	n = 256	.08
No impairment	701(40)	95(37)	
Some impairment	588(33)	116(45)	
Nonverbal	469(27)	45(18)	
Intellectual impairment	n = 1548[†]†	n = 251	.44
No impairment	960(62)	151(60)	
Some impairment	196(13)	52(21)	
Moderate/severe	392(25)	48(19)	

impairment			
Hearing impairment	n = 1771[†]†	n = 274	.09
No impairment	1559(88.0)	238(87)	
Some impairment	162(9)	23(8)	
Bilateral deafness	50(3)	13(5)	
Visual impairment	n = 1616[†]†	n = 253	.07
No impairment	955(59)	161(64)	
Some impairment	565(35)	84(33)	
Functionally blind	96(6)	8(3)	
Epilepsy	n = 1847[†]†	n = 278	.09
None	1250(68)	207(74)	
Resolved	29(2)	8(2)	
Epilepsy	568(30)	63(23)	

() = % of cases excluding unknown/missing data. [%] proportion of CP types excluding unknown/missing.

Italicized data represent subgroups of Spastic CP.

[*]As reported to the (i) population CP Registers (South Australia, Victoria and Western Australian)[9]compared with the (ii) study participants with CP who had their newborn screening card (NBSC) tested for CMV DNA.

[†]Excludes South ACPR data.

Discussion

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This retrospective study identified that 9.6% (95% CI, 6.8%-13.3%) of children with CP had CMV DNA detected in their NBSC, confirming cCMV infection. This proportion is markedly higher than the proportion of children with CMV detected in the newborn period in the general community (approximately 0.6%).[2, 16] It is also 6 times greater than the proportion of children with CP who have

cCMV reported as an attributable cause to the ACPR (1.5%),[9] and in a recent retrospective study of Caucasian children with CP (1.5%).[13] This finding suggests that children with cCMV as indicated by CMV DNA in blood in the neonatal period (ie, neonatal CMV viremia) occurs frequently among children with CP and suggests that previous reports have underestimated the prevalence of cCMV among this population.

We explored whether other factors, such as recruitment bias, could explain the high prevalence of neonatal CMV viremia in our study population of children with CP. There were no differences between clinical characteristics of the study population and population-based CP registers.[9] Similarly, the proportion of children with confirmed or probable cCMV reported to the register as an attributable cause in this study (1.2%) was comparable with the value reported in our previous study of ACPR data (1.5%).[9] We did not identify cross-contamination of CMV DNA between samples in our testing owing to the rigorous protocols used. However, we cannot exclude the possibility of cross-contamination at the time the NBSC samples were collected and the dried filter cards were stored. However, given that the frequency of cCMV is low in our population (estimated at 0.6%),[2, 16] this occurrence is unlikely. Further, all CMV DNA positive NBSCs were retested using a new card sample and a second testing methodology to further validate these results, with 90% agreement. We defined our cCMV DNA-positive group using the first methodology because this is a highly sensitive, validated diagnostic method. Even if we used the second, less sensitive methodology to define our cCMV population, we will still have identified an unexpectedly high prevalence (8.7%; 95% CI, 6.1-12.2) of cCMV in this group of children with CP.

These findings still may underestimate of the proportion of children with cCMV and CP. We used retrospective NBSC dried blood samples for CMV DNA because it is the only means of testing for cCMV beyond the first 3 weeks of life. However, this method has a low sensitivity for detecting cCMV. It is estimated that only 40% of infants with cCMV have virus in their blood in the early neonatal period when NBSC are collected, compared with the proportion with virus detectable in saliva (>95%) and urine (95%).[17, 18] Although a positive cCMV result from the NBSC can be used to accurately confirm cCMV infection, a negative result does not rule out cCMV, but rather may indicate an absence of CMV viremia or a low viral load at the time of sampling.[18, 19, 20] Notably, of the 4 children in this study whose treating clinicians had previously reported cCMV as an attributable cause of their CP validated by laboratory records, 2 had negative NBSC test results. The likely explanation for this disparity relates to the low sensitivity of retrospective testing for NBSC for cCMV, as explained.

Examination of the registry data in both cCMV-positive and cCMV-negative groups showed that the majority (91.7%) of the cCMV-positive group had spastic CP, similar to our previously published report.[9] However, in contrast with our previous study, children with spastic quadriplegia were not over-represented in the CMV-positive group. Similarly, we found no group differences in relation to maternal characteristics or infant sex. The reported comorbidities across the 2 groups also were similar, with 1 exception; epilepsy was observed in significantly higher proportions among the cCMV-positive group. This finding was not unexpected; epilepsy has been reported frequently as an outcome of cCMV[21, 22, 23] and was over-represented among the cCMV group in previous study.[9] Overall, however, the cCMV-positive group in this study had a much less severe disability profile than the cCMV group reported in our previous paper.[9] This suggests that cCMV is underascertained in the newborn period in the absence of neonatal screening. Infants diagnosed with asymptomatic cCMV infection do not usually develop significant neurologic disabilities, aside from sensorineural hearing loss.[2, 24] In this study, we could not determine if the children were symptomatic for congenital infection in the neonatal period because the CP registers do not include

this specific data field. There were also no available neuroimaging data. Our findings, therefore, suggest that cCMV among infants with mild symptomatic cCMV disease are not always being diagnosed clinically early in life.

Our study has limitations. First, it was not possible to test the NBSCs of all children with CP reported to the 2 state CP Registers, because this requires parental consent. Thus, provision of consent may have introduced a recruitment bias. Further, not all consented participants had an available NBSC. Some cards likely were destroyed after 18 years, as is the legislated standard practice. The impact of including the missing NBSC cases on the clinical profile of the cCMV-positive group is unknown, but we consider it unlikely that their inclusion would have altered our findings greatly because there was not an over-representation of cases of severe CP, or cCMV as an attributable cause, in this group.

This study serves as a timely reminder of the importance of CMV as a common intrauterine viral infection in developed countries and the potential for long-term consequences beyond the newborn period.[8] Additional research is required to better understand the mechanisms involved in cCMV infection as a risk factor for CP and to determine the relative contribution of cCMV on the causal pathways to CP. This information is important to further inform policy and practice for neonatal screening, and the implementation of both primary[4] and secondary prevention[7] strategies. A large prospective study of CMV would overcome many of the limitations of this study and future research should include an examination of the relationship between cCMV and other risk factors among this group of children with cCMV and CP, including but not limited to genetic factors, congenital abnormalities, maternal history, and the presence of neonatal signs of cCMV.

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Appendix

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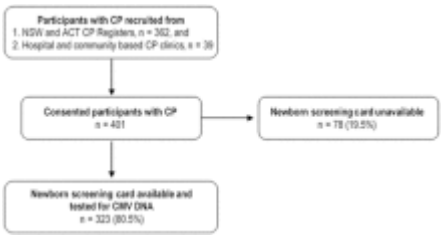

Figure

Participant recruitment and availability of stored newborn screening cards.

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