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## Dried Umbilical Cords in the Retrospective Diagnosis of Congenital Cytomegalovirus Infection as a Cause of Developmental Delays

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**To clarify the impact of congenital cytomegalovirus (CMV) infection on developmental disabilities, 20 children with disabilities of unknown cause were analyzed. Five children were CMV positive and had no clinical manifestations at birth. Intracranial calcification was observed in 4 cases. Thus, congenital CMV infection is a significant cause of developmental disabilities.**

Cytomegalovirus (CMV) is one of the most common causes of intrauterine infection. The prevalence of congenital CMV infection ranges from 0.3% to 2.4% of live births in developed countries. Approximately 10% of infected neonates are symptomatic at birth, with symptoms including petechiae, jaundice, hepatosplenomegaly, and microcephaly. Although most infected neonates are asymptomatic, >10% of these children ultimately develop CMV-linked sensorineural hearing loss (SNHL) and neurologic and behavioral problems, including mental retardation, autism, learning disabilities, cerebral palsy, and epilepsy [1]. Neuroimaging of children with congenital CMV infection has identified a variety of brain abnormalities,

such as intracranial calcification, ventricular enlargement, hydrocephalus, cortical atrophy, and porencephaly [2].

We have taken advantage of the wide availability of dried umbilical cords in Japan for retrospective diagnosis of congenital CMV infection. We found that 15% of cases of severe SNHL could be ascribed to congenital CMV infection and that, in at least one-half of CMV-related cases, SNHL developed after the age of 6 months [3]. The full spectrum of developmental disabilities due to congenital CMV infection has not been fully defined. Thus, our study aimed to clarify the impact of congenital CMV infection on developmental disabilities and to identify clinical features of CMV-associated cases.

We enrolled 20 patients with developmental disabilities of developmental quotient (DQ) score <70, after we excluded patients who had developmental disabilities with known causes, including Down syndrome and other chromosomal abnormalities; genetic defects in metabolism or hormones; difficult delivery, with complications such as fetal distress; and disabilities associated with drug or alcohol use by the mother. On the basis of these criteria, approximately two-thirds of patients were excluded from the study. The Ethical Committee of Asahikawa Medical College and National Institute of Infectious Diseases approved this study, and informed consent was obtained from parents of all enrolled children. Developmental disability was evaluated with use of the Wechsler Intelligence Scale for Children, Third Edition [4], or standard Japanese methods for young children (i.e., the Enjyouji and Kyoto scale [5]). With the Enjyouji method, DQ scales are calculated on the basis of a list of queries in 6 categories (body movements, manipulations with hands, activities of daily living, personal relations, speech skills, and understanding of language) to evaluate whether patients can perform particular skills and physical movements required for ordinary living at the level of their non-developmentally disabled peers. These questionnaires are similar to those in *The Ages and Stages Questionnaires* [6]. Severity of developmental delay was classified into 5 categories: severe, DQ of <35; moderate, DQ of 35–49; mild, DQ of 50–69; borderline, DQ of 70–84; and normal, DQ of ≥85.

Nine of the 20 children had hearing defects identified by the Auditory Brain-stem Response test. At least 8 of the children experienced >1 episode of seizure.

Presence of CMV DNA in dried umbilical cord specimens obtained from the 20 children was assayed by the real-time PCR, as described elsewhere [3]. Five children were CMV positive, and their viral loads are shown in table 1. There was no obvious relationship between the severity of clinical symptoms

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**Table 1. Characteristics of late-onset developmental disability in children with congenital cytomegalovirus (CMV) infection.**

Patient	Sex	Developmental disability		Current age, years	Characteristics at birth		Other findings		CMV diagnosis		CMV genotype				
		Age at onset, months	Severity		Weight, g	HC, cm	Hearing defect	IC	Age at diagnosis, years	CMV load, copies/ $\mu$ g <sup>a</sup>	gB	gN	gO	gH	UL144
1	M	1	Severe	6	2524	31	–	+	1	$4.5 \times 10^3$	1	4c	5	2	A
2	F	5	Moderate	5	2740	32	+	+	1	$1.0 \times 10^3$	1	4b	4	1	C
3	M	10	Mild	4	2720	32	+	+	2	$1.5 \times 10^3$	3	4a	3	1	C
4	F	5	Severe	17	3538	32	+	+	14	$6.5 \times 10^3$	1	1	1a	1	B
5	F	12	Moderate	10	2930	33	–	–	7	$3.2 \times 10^2$	1	2	2b	1	A

**NOTE.** HC, head circumference; IC, intracranial calcification; +, present, –, absent.

<sup>a</sup> CMV genome copy numbers per  $\mu$ g of cellular DNA in dried cord specimens.

and viral load, although the number of cases was insufficient for robust analysis. We also determined the CMV genotypes of the glycoproteins B, N, O, and H and UL144, as described elsewhere [7], to see whether particular CMV genotype(s) were associated with developmental disabilities. All 5 children had different glycoprotein N/glycoprotein O linkage groups [7], and there was no obvious relationship between genotype and clinical outcome (table 1).

All of the 5 children had been delivered after full-term pregnancies, and none had low birth weights. Although the head circumference of patient 1 was 31 cm, which was >1.5 standard deviation less than the mean head circumference of nondevelopmentally disabled peers, the others were in the normal range. Intracranial calcification was detected by computed tomography in 4 of the 5 patients. Porencephaly and intracranial calcification were seen in patient 1. In contrast, patient 2 had less severe developmental disability in the absence of calcification in the brain computed tomography and hearing defect (table 1). The clinical histories of the CMV-positive children are shown below.

**Case reports.** For patient 1, neonatal infection was suspected at birth because of tachypnea and lack of spirit; antibiotics were given for 8 days. The child was discharged from the hospital with no developmental concerns. However, his body weight did not increase well, and spasticity of his extremities gradually emerged 1 month after birth. At 6 years of age, his developmental delay became so severe (mental DQ, 23; motor DQ, 22) that assistance was required for activities of daily living. His hearing ability has been normal, but epileptic seizures have occurred.

For patient 2, no clinical abnormalities, including hearing defects, were observed during the neonatal period. Five months after birth, head control was absent, spasticity of the child's extremities gradually emerged, and a progressive hearing defect developed.

Patient 3 was healthy during the neonatal period. Although head control was seen at 4 months of age, he could not maintain a sitting position at 10 months of age. Hearing loss developed

at around 1 year. The child was hyperactive, probably as a result of unbalanced mental and motor DQs (26 and 81, respectively). He also had epilepsy.

For patient 4, there were no clinical symptoms during the neonatal period. However, head control was absent at 5 months of age, and the child developed mild spasticity of her extremities before the age of 1 year. The first epileptic seizure occurred at 14 months of age. Her mental and motor DQs were 17 and 6, respectively, at 14 years of age. She requires assistance for activities of daily living. She has developed bilateral hearing loss.

For patient 5, there were no clinical abnormalities during the neonatal period. As of 1 year of age, the child could not maintain a sitting position. Her hearing ability has been normal.

**Discussion.** Although this was a small study, the proportion of CMV-associated cases identified in our study was much higher than would have been expected on the basis of the population prevalence of congenital CMV infection. During the neonatal period, none of the 5 children we describe had any clinical manifestations of CMV infection. Their developmental disabilities began to manifest from 1 to 12 months of life, and some of them developed both physical and mental deficits. In addition to the children described here, during the past few years, we have observed 4 children with symptomatic CMV infection who had petechiae, liver dysfunction, intrauterine growth retardation, and other symptoms. Although 3 of these 4 children developed hearing loss and 2 had developmental delays, 1 has been free of sequela for 4 years, indicating that symptomatic infection does not always presage neurological sequela.

Follow-up studies of congenitally infected symptomatic patients by others have demonstrated a high frequency of developmental and/or neurological abnormalities, including microcephaly, psychomotor retardation, seizures, and SNHL [2]. However, developmental delay was documented in <5% of persons with asymptomatic cases [8]. In children with symptomatic congenital CMV infection, microcephaly at birth was the most specific predictor of mental retardation and major motor

disability [9], but the predictor of developmental delay in children with asymptomatic infection remains to be determined.

Analyses of CMV DNA in dried blood spots revealed congenital CMV infections in both of 2 patients who had abnormal white matter lesions and SNHL [10], in 4 of 10 patients with malformations of cortical development [11], and in 1 patient with pachygyria [12]. With use of dried umbilical cord specimens, congenital CMV infection was identified by others in a patient with various central nervous system disorders [13]. To our knowledge, however, our study is the first retrospective study that systematically evaluated the contribution of congenital CMV infection to developmental delay.

Our results suggest that the scope of contribution of neonatal CMV infection to the societal burden of developmental disabilities may have been seriously underappreciated. The Metropolitan Atlanta Developmental Disabilities Surveillance Program found that serious developmental disabilities affect ~2% of school-aged children and are lifelong conditions that incur substantial financial and societal costs. In addition, the National Health Interview Survey in the United States estimated that the prevalence of developmental disability was 0.76%. In Japan, the Governmental Survey in 2006 reported that at least 11,000 patients had severe physical disability due to neurological disorders. Although the exact proportion of developmental disabilities without known etiologies is not officially documented, Yeargin-Allsopp et al. [14] reported that >50% of cases of mental retardation among 10-year-old children had no definite causes, and from our clinical experience, we estimate that ~80% of developmental disabilities, including mild cases, have an unknown etiology. Because congenital CMV infection was detected in 5 of 20 children in our study, the frequency of cases involving developmental disability due to congenital CMV infection could be much greater than has been suspected. A larger study will certainly be required for more accurate ascertainment of the frequency of these events. In conclusion, our study has demonstrated—to our knowledge, for the first time—that asymptomatic congenital CMV infection is a significant cause of developmental disability.

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