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平成19年度「独創性のある生命科学研究」プロジェクト課題 Frequent association of congenital cytomegalovirus infection with developmental disabilities in children who were asymptomatic during neonatal stage (新生児期には無症候であった小児の発育障害にはしばしば先天性サイトメガロウイルス感染症が関与している)

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12) Frequent association of congenital cytomegalovirus infection with developmental disabilities in children who were asymptomatic during neonatal stage

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【Keywords】 congenital CMV infection, developmental disability, hearing loss, intracranial calcification, retrospective diagnosis, dried umbilical cord

[Abstract]

Objective. Cytomegalovirus (CMV) is the most common cause of congenital infection and causes late-onset neurological disorders, such as sensorineural hearing loss. This study aimed to clarify the impact of congenital CMV infection on developmental disabilities and to identify clinical features of CMV-associated cases.

Study design. CMV DNA was assayed by PCR in dried umbilical cord specimens obtained from 20 Japanese children with developmental disabilities of unknown cause. Severity of the disability was scored by Wechsler Intelligence Scale for Children Third Edition (WISC-III) or the standard Japanese methods, and hearing capability was

evaluated by auditory brain-stem response.

Results. Of the 20 cases, 2 severe, 2 moderate, and 1 mild cases were CMV positive. Intracranial calcification and hearing defects were observed in 4 and 3 of the 5 cases, respectively. CMV-associated clinical manifestations were not observed at birth, but their developmental delay became apparent within 1 year after birth.

Conclusion. Congenital CMV infection proved to be associated with a significant proportion of developmental disability cases without other known causes. Many CMV-associated manifestations are late-onset and independent from hearing deficits, thus it is critical to detect congenital infections in the perinatal period.

［Introduction］

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.[1] Approximately 10% of infected neonates are symptomatic at birth, with symptoms including petechiae, jaundice, hepatosplenomegaly, and microcephaly.[2] Although most infected neonates are asymptomatic, more than 10% of these children ultimately develop CMV-linked auditory, neurological and behavioral problems.[3, 4] Neurological problems associated with congenital CMV infection may include mental retardation, autism, learning disabilities, cerebral palsy, and epilepsy.[5, 6] Neuroimaging of children with congenital CMV has identified a variety of brain abnormalities, such as intracranial calcification, ventricular enlargement, hydrocephalus, cortical atrophy, and porencephaly.[7] CMV-associated irreversible damage to the central nervous system and to hearing have also been demonstrated in mouse and guinea pig models.[8, 9]

Retrospective diagnosis for congenital CMV infection depends on the availability of preserved materials that are collected at birth, such as dried blood spots used for genetic screening.[10-12] The amounts of CMV DNA in dried blood spots, especially from asymptomatic cases, are small.[13] We have taken advantage of the wide availability of dried umbilical cords in Japan for retrospective diagnosis of congenital CMV infection.[14] We found that 15% of cases of severe sensorineural hearing loss (SNHL) could be ascribed to congenital CMV infection, and in at least a half of CMV-related cases SNHL developed after the age of 6 months.[15]

The full spectrum of developmental disabilities due to congenital CMV infection has not been fully defined. Thus, this study aimed to clarify the impact of congenital CMV infection on developmental disabilities and to identify clinical features of CMV-associated cases.

［Methods］

Study subjects. Twenty patients with developmental disabilities, developmental quotient (DQ) score; less than 70, were enrolled. Enrollment was based on exclusion of cases of known causes, including Down's syndrome and other chromosomal abnormalities, genetic defects in metabolism or hormones, difficult delivery with complications such as fetal distress, and the mother being under drug or alcohol influence. This study was approved by the Ethical Committee of Asahikawa Medical College and National Institute of Infectious Diseases, and informed consent was obtained from parents of all enrolled cases.

Evaluation of developmental quotient (DQ) and hearing capability. Developmental disability was evaluated by Wechsler Intelligence Scale for Children Third Edition (WISC-III) or standard Japanese methods for young children, i.e. Enjyouji and New-Kyoto-style.[16] In the Enjyouji method, DQ scales are calculated based on a list of queries in 6 categories (body movements, manipulations with hands, activity of daily living, personal relations, speech skills, understandings of language) to evaluate whether patients can perform particular skills and physical movements required for ordinary living at the level of their normal peers.[5] These questionnaires are similar to 'The Ages and Stages Questionnaires' (ASQ). (Paul H Brookes Publishing Co., Baltimore, MD). Severity of developmental delay was classified into five categories: severe DQ<35, moderate DQ 35-49, mild DQ 50- 69, boarder DQ 70-84, and normal DQ>85. Severe cases usually need assistance for daily life, such as taking meals, physical movement, and defecation.

Hearing defects were determined by the Auditory

Brain-stem Response (ABR) test.

Detection of CMV in dried umbilical cords by PCR assay. Approximately 50 μg of total DNA was extracted from 25 mg of dried umbilical cords using QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) according to the vendor's protocol.

Conventional PCR for detection of CMV was done with the following primer set that targets CMV immediate early (IE) gene: 5'-gcTGCGGCATAGAATCAAGGAGCA and 5'- ggTTGGTGGTCTTAGGGAAGGCTGAG (the viral sequence is capitalized). Twenty-five microliter reactions contained 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of dNTPs, 0.4 mM of each primer, 1.25U of Taq polymerase, and 500 ng of extracted DNA. Thermocycling conditions were as follows: 94℃ for 3 min, and 35 cycles of 94℃ for 30 sec, 72℃ for 2 min, and a final extension at 72℃ for 20 min.

The real-time PCR assays for the CMV UL83 gene and for the human albumin gene were performed as described previously.[15] The real-time PCR assays were done blindly at National Institute of Infectious Diseases.

Genotyping. CMV genotyping was done as described[17, 18].

[Results]

Identification of CMV-positive cases. This study included 20 patients with developmental disability who attended to or were referred to the Asahikawa Medical College. Nine of them had hearing defects and at least 8 of them experienced >1 episode of seizure. CMV DNA was detected in the dried umbilical cord specimens obtained from 5 patients both by conventional PCR and real-time PCR (Table 1).

Clinical characteristics of the CMV positive cases. All of the cases were from full-term pregnancies and none had low birth weight. Clinical findings were as follows:

Case 1.[14] Since some clinical manifestations, such as tachypnea and lack of spirit were observed at birth, neonatal infection was suspected and 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 went to so severe (mental DQ 23, motor DQ 22) that assistance was required for daily life. His hearing ability has been normal but epileptic seizure has appeared.

Case 2. No clinical abnormalities, including hearing defects, were observed during the neonatal period. At 5 months after birth, head control was absent, spasticity of her extremities gradually emerged, and a progressive hearing defect developed.

Case 3. The child was normal during neonatal period. Although head control was seen at age of 4 months, he could not maintain a sitting position at 10-months. Hearing loss developed at around 1 year. The child was hyperactive, probably due to unbalanced mental and motor DQs (26 and 81, respectively); he also had epilepsy.

Case 4. There were no clinical symptoms during the neonatal period. However, head control was absent at 5-months, and she developed mild spasticity of her

Table 1. Characteristics of late-onset developmental disability cases with congenital CMV infection.

Case no.	Sex	Developmental disability		Current age	Other findings		CMV diagnosis		CMV genotype				
		onset	severity		hearing defect	intracranial calcification	age	copy no.#	gB	gN	gO	gH	UL144
1	M	1 mo	severe	6 yr	-	+	1 yr	$4.5x10^3$	1	4c	5	2	A
2	F	5 mo	moderate	5 yr	+	+	1 yr	$1.0x10^3$	1	4b	4	1	C
3	M	10 mo	mild	4 yr	+	+	2 yr	$1.5x10^3$	3	4a	3	1	C
4	F	5 mo	severe	17 yr	+	+	14 yr	$6.5x10^3$	1	1	1a	1	B
5	F	12 mo	moderate	10 yr	-	-	7 yr	$3.2x10^2$	1	2	2b	1	A

+:present, -:absent #: CMV genome copy numbers/,,g cellular DNA in dried cord specimens

extremities before the age of 1 year; the first epileptic seizure occurred at 14 months. Her mental and motor DQs were 17 and 6, respectively, at 14 years of age. She necessitates assistance for daily life. She has developed bilateral hearing loss.

Case 5. There were no clinical signs during the neonatal period. As 1 year of age, she could not maintain a sitting position.

Neuroimaging of CMV positive cases. Intracranial calcification was detected by computed tomography (CT) in 4 of the 5 patients. Porencephaly and intracranial calcification were seen in the case 1 (Fig. 1). In contrast, case 2 had less severe developmental disability in the absence of calcification in the brain CT and hearing defect.

Relationship of viral load and genotypes with clinical symptoms. Viral loads in dried umbilical cord specimens are shown in Table 1. There was no obvious relationship between the severity of clinical symptoms and the viral loads, although the number of cases is insufficient enough for robust analysis. To see whether particular CMV genotype(s) might be associated with developmental disabilities, genotypes of the glycoprotein B (gB), gN, gO, gH, and UL144 genes were determined. All five cases have different gN/gO linkage groups[18], and there was no obvious relationship of genotypes with clinical outcome (Table 1).

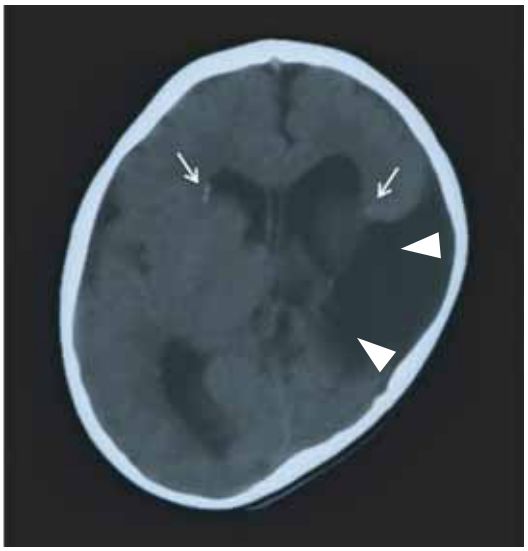

Figure 1. The brain computed tomography of Case 1 showed porencephaly (arrowhead) and intracranial calcification (arrow) at age of 4 months.

[Discussion]

In this study, we identified congenital CMV infection in 5 out of 20 children with developmental disability. Although this was a small study, the proportion of CMV-associated cases was much higher than would have been expected based on the population prevalence of congenital CMV infections. During neonatal period, none of the 5 children had any CMV-associated clinical manifestations. Their developmental disabilities began to manifest from 1 to 12 months of life, and some of them have developed both physical and mental deficits. This progressive development of disability in children who were congenitally infected but asymptomatic as newborns provides further evidence for the need of implementing newborn screening programs for congenital CMV infection.[13] Separately from the cases described above, during the last few years, we have observed 4 children with symptomatic CMV infections who had petechiae, liver dysfunction, intrauterine growth retardation, and other symptoms. Although 3 of them developed hearing loss and 2 had developmental delays, one of them 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 cases demonstrated a high frequency of developmental and/or neurological abnormalities, including microcephaly, psychomotor retardation, seizures, and SNHL.[7, 19, 20] However, developmental delay was documented in less than 5% of asymptomatic cases.[21-27] Viral loads in blood have been reported to be associated with hearing loss, systemic CMV diseases, and long-term outcome,[20, 28] and DNAemia <1000 copies per 10^5 polymorphonuclear leukocytes has a negative predictive value of 95%.[23] Viral DNA loads in umbilical cord specimens of the developmental disability cases described in this study were not apparently higher than for asymptomatic cases we analyzed previously.[13, 15] Previous studies indicated that infant outcome was related neither to trimester of maternal infection nor to duration of urinary excretion.[21, 30] In children with symptomatic congenital CMV infection, microcephaly at birth was the most specific predictor of mental retardation and major motor disability,[29] but the predictor of developmental delay

in asymptomatic cases remains to be determined.

Involvement of congenital CMV infection in children with neurological abnormality has been identified by demonstration of CMV DNA in dried blood spots. For example, such analyses revealed congenital CMV infections in both of two cases with abnormal white matter lesions along with SNHL,[12] 4 of 10 cases with malformations of cortical development,[31] and a case with pachygyria[32]. We demonstrated that dried umbilical cords that are kept as family heirlooms in Japan have advantages for retrospective diagnosis of cases with SNHL.[15] Using this method, congenital CMV infection in a case with various central nervous system disorders was identified by others.[33] To our knowledge, 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 infections 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 approximately 2% of school-age children and are lifelong conditions that incur substantial financial and societal costs[35]. In addition, the National Health Interview Survey in the US estimated that prevalence of developmental disability was 0.76%. [36] In Japan, the Governmental Survey in 2006 described that there were at least 11,000 patients with severe physical disability due to neurological disorders. Although the exact proportion of developmental disability without known etiologies is not officially documented, Yeargin-Allsopp et al. reported that over 50% of mental retardation children among 10-year-old had no definite causes[34], and from our clinical experience the proportion of cases with unknown etiology is estimated around 80% of all with developmental disability including mild cases. Since congenital CMV infection was detected in 5 out of 20 cases in this study, the frequency of cases with developmental disability due to congenital CMV infection could be much greater than previously suspected; a larger study will be certainly required to more accurately ascertain the frequency of these events.

In conclusion, this study demonstrated at first time that asymptomatic congenital CMV infection is a significant cause of developmental disability.

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