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A cohort study of the universal neonatal urine screening for congenital cytomegalovirus infection

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All authors meet the ICMJE authorship criteria.

Running title: Universal neonatal urine screening

Abstract

Objectives

This prospective cohort study aimed to evaluate the efficacy of the universal neonatal urine screening, followed by diagnosis, workup and antiviral therapy for symptomatic congenital cytomegalovirus (CMV) infection to reduce neurological impairments and sequelae.

Methods

Neonates born in three facilities underwent the universal urine screening of PCR analyses for CMV-DNA. Neonates with symptomatic congenital CMV infection (cCMV) received oral valganciclovir (VGCV) of 32 mg/kg/day for six weeks or six months, and were evaluated for neurological outcomes including developmental quotient (DQ) and hearing function at around 18 months of corrected age.

Results

cCMV was diagnosed in 56 (0.48%) of 11,736 neonates, consisting of 23 neonates with symptomatic and 33 with asymptomatic cCMV. The incidence of cCMV in the general perinatal medical center (0.69%) was higher than that in the primary maternity hospital (0.23%, $p<0.01\%$). Twenty of the 23 infants with symptomatic cCMV received VGCV therapy, and 19 underwent neurological assessment. Eight neonates (42%) had severe sequelae of DQ <70, bilateral hearing dysfunction, and/or epilepsy. Four neonates (21%) had mild sequelae of DQ

70-79 or unilateral hearing dysfunction only, and seven (37%) showed normal development without any impairment.

Conclusions

This study on a large scale demonstrated that a series of universal neonatal urine screening, diagnosis, workup, and VGCV therapy for neonates with symptomatic cCMV may decrease neurological impairments, because 58% of the treated infants had normal development or mild sequelae. The universal urine screening likely identifies subclinical symptomatic cCMV. Mothers with fetuses of cCMV seem to be selectively transferred to perinatal medical centers before deliveries.

Keywords: Congenital infection; Cytomegalovirus; Neonate; Pregnancy; Urine screening

Introduction

Cytomegalovirus (CMV) is the most common mother-to-child transmission in humans. The prevalence of congenital CMV infection (cCMV) in neonates is 0.2–2.0% [1], and approximately 20% of neonates with cCMV have symptoms. These clinical manifestations include fetal growth restriction (FGR), low birth weight, and central nervous system and multiple organ involvement with petechiae, hepatomegaly, splenomegaly, jaundice, pneumonia, and encephalitis. Approximately 70–90% of infants with symptomatic cCMV develop neurological sequelae including hearing dysfunction, neuromuscular disorder, psychomotor retardation, ocular abnormality, delayed language development, and intellectual disability [2–4]. In addition, 10–15% of infants with asymptomatic cCMV develop long-term sequelae, such as progressive sensorineural hearing difficulty and mental retardation [3, 5].

Randomized controlled trials demonstrated that intravenous injections of ganciclovir (GCV) 12 mg/kg/day for six weeks for neonates with symptomatic cCMV involving central nervous system abnormalities improved the hearing function in 17% of the infants. The proportion of worsening hearing dysfunction in infants administered GCV therapy (21%) was lower than that in infants with no therapy (68%) [6]. In addition, intravenous injections of GCV 12 mg/kg/day for six weeks for neonates with symptomatic cCMV decreased the psychomotor delay scores of the Denver II test at 6 and 12 months of age as compared with infants with no therapy [7]. A randomized placebo-controlled trial demonstrated that oral valganciclovir

(VGCV) of 32 mg/kg/day for six months, as compared with six weeks, modestly improved the hearing function and the developmental outcome in the longer term [8]. These randomized controlled trials demonstrate that GCV and VGCV therapies for neonates with symptomatic cCMV have the efficacy to improve the neurological outcome of the affected infants.

However, it is unclear whether the universal neonatal urine screening for CMV, followed by a series of procedure that includes, diagnosis of neonates with cCMV, workup for symptoms, and VGCV therapy for neonates with symptomatic cCMV, is effective in improving the neurological outcome of the affected infants. Therefore, a prospective cohort study on a large scale was conducted to evaluate the efficacy of the universal neonatal urine screening, followed by a series of procedure for cCMV.

Methods

Study design and participants

The institutional review board at Kobe University Hospital and the research ethics committee at Hyogo Prefectural Kobe Children's Hospital and Nadeshiko Ladies Hospital approved this multi-center prospective cohort study (reference numbers: 923 and 1214). Written informed consent was obtained from all participants. From November 2009 to March 2018, newborns who were born at a general perinatal medical center, Kobe University Hospital where high-risk pregnancies are managed; a regional perinatal medical center, Hyogo Prefectural Kobe Children's Hospital; and a primary maternity hospital, Nadeshiko Ladies Hospital, in Kobe, Japan, underwent the universal screening of polymerase chain reaction (PCR) tests for CMV-DNA in the urine. Congenital infection was diagnosed with the detection of CMV-DNA in their urine. All newborns who had positive results for CMV-DNA in the urine were referred to Kobe University Hospital, and received a workup for the diagnosis of cCMV and its symptoms. In the primary maternity hospital, women with high-risk pregnancies were referred or transferred to the university hospital and regional perinatal medical centers. The high-risk pregnancies included maternal complications, fetal abnormalities, FGR, hypertensive disorders of pregnancy (HDP), multiple pregnancies, and preterm deliveries before 34 gestational weeks.

Procedures

Urine samples were collected from newborns on filter paper within one week after birth and the presence of CMV-DNA was assessed, as described previously [9]. The urine-filter based assay used in the present study and different in-vitro diagnostic assays by the regulatory authorities yielded identical results [10]. The presence of cCMV was confirmed by positive PCR results using the liquid urine samples [11]. All newborns with cCMV received a workup to identify the symptoms of congenital infection. Ophthalmoscopy, cerebral ultrasound, physical and neurological examinations, head computed tomography, head magnetic resonance imaging, and repeated automated auditory brainstem response tests were performed.

Symptomatic cCMV disease is defined by the following findings: microcephaly (less than the 10^{th} percentile of head circumference at birth); small for gestational age (SGA) (<−2.0 standard deviation score of birthweight and/or birth height); hepatitis (≥100 U/L of serum aspartate aminotransferase); thrombocytopenia (<100,000/μL); abnormalities on brain imaging by ultrasonography, computed tomography, and magnetic resonance imaging (cortical dysplasia, white-matter abnormality, ventricular dilation, and calcification); ocular complications such as chorioretinitis; and abnormal brainstem auditory-evoked potential (BAEP) (no detection of V-wave at 30 dB at ≥37 gestational weeks of corrected age and at 40 dB at 34–36 gestational weeks of corrected age).

The oral VGCV of 32 mg/kg/day was administered for six weeks during hospitalization between November 2009 and June 2015. From July 2015, oral VGCV of 32 mg/kg/day was administered for six months according to a report [8]. The VGCV therapy was discontinued temporarily when the neutrophil count was <500/mm^3 and resumed when it recovered.

The neurodevelopmental outcomes including DQ, hearing dysfunction, blindness, and epilepsy were evaluated at around 18 months of corrected age. No impairment was defined as DQ ≥80 and no hearing dysfunction; Mild sequelae were defined as unilateral hearing dysfunction or DQ of 70–79; and severe sequelae were defined as DQ <70, bilateral hearing dysfunction requiring hearing aids, blindness, or epilepsy requiring anti-epileptic drugs. DQ was assessed using the Kyoto Scale of Psychological Development [12]. DQ was calculated as follows: DQ = (developmental age obtained from the Kyoto Scale of Psychological Development / corrected chronological age) × 100.

Statistical analysis

The chi-squared test was used to compare the frequencies of cCMV in three facilities. Fisher's exact test was used to compare between neonatal symptoms and outcomes. Mann-Whitney U test was used to compare age of neonates. Statistical significance was considered present at a *p*-value of less than 0.05.

Results

A flowchart of the universal neonatal urine screening and antiviral therapy for symptomatic cCMV is shown in Figure 1. During the study period, 11,736 neonates underwent PCR tests for CMV-DNA in the urine. In this cohort study, 56 (0.48%) neonates were diagnosed with cCMV, including 23 neonates with symptomatic cCMV and 33 with asymptomatic cCMV. Twenty-nine of the 33 infants with asymptomatic cCMV underwent neurological assessment at around 18 months of corrected age, excluding two infants with anomalies and two who moved out of Kobe. One infant with asymptomatic cCMV later developed unilateral hearing loss and autism. The early diagnosis of cCMV for this patient led to the early detection of hearing difficulty. This patient used hearing aids during infancy, and began a language therapy early than usual. On the other hand, 20 of 23 neonates with symptomatic cCMV received VGCV therapy, excluding one with early neonatal death, one with an anomaly and one with no parental consent. Nineteen of the 20 infants with symptomatic cCMV underwent neurological assessment at around 18 months of corrected age. One infant with myotonic dystrophy was excluded from this study.

Table 1 shows the incidence of cCMV in three facilities. The incidence of cCMV in the general perinatal medical center, Kobe University Hospital (0.69%), was higher than that in the primary maternity hospital (0.23%, $p<0.01\%$). The frequency of symptomatic cCMV in

Kobe University Hospital (17/32) was higher than that in the primary maternity hospital (1/10, $p<0.05\%$).

Table 2 shows the neurological outcomes in 19 neonates with symptomatic cCMV who received VGCV therapy. The symptoms of cCMV, detected by workups in the 19 neonates, included abnormalities on brain imaging (n=17), abnormal BAEP (n=15), thrombocytopenia (n=8), SGA (n=6), microcephaly (n=5), hepatitis (n=5), ocular complications (n=5). The universal urine screening could detect neonates with subclinical symptomatic cCMV including Case 15, Case 17, Case 18, and Case 19. Neurological assessment demonstrated that 8 of the 19 neonates (42%) had severe sequelae of DQ <70, bilateral hearing dysfunction, and/or epilepsy. Four neonates (21%) had mild sequelae of DQ 70-79 or unilateral hearing dysfunction, and seven neonates (37%) showed normal development without any impairment.

The frequency of neonates who had microcephaly among neonates with severe sequela (5/8) was higher than that among neonates with mild sequela or normal development (0/11, $p<0.01$). The frequency of neonates who had restricted symptoms of ocular complication, abnormality on brain image, or abnormal BAEP among neonates with severe sequela (0/8) was lower than that among neonates with mild sequela or normal development (7/11, $p<0.05$).

The median 9 (range 3-43) day-old at cCMV diagnoses among 8 neonates with severe sequela was not different from median 8 (range 1-34) day-old among 11 neonates with mild sequela or normal development ($p=0.62$). The median 12 (range 5-77) day-old at

commencement of VGCV therapies among 8 neonates with severe sequela was not different from median 11 (range 7-39) day-old among 11 neonates with mild sequela or normal development (p=0.87). There were no differences in frequencies of neonates who started VGCV therapy before 14 day-old between neonates with severe sequela (5/8) and neonates with mild sequela or normal development (7/11, p=1.00). There were no differences in frequencies of neonates who received VGCV therapy for 6 weeks between neonates with severe sequela (5/8) and neonates with mild sequela or normal development (7/11, p=1.00).

Discussion

This cohort study of the universal urine screening for CMV in 11,736 neonates found that the total incidence of cCMV in three facilities was 0.48%, and for the first time demonstrated that the incidence of cCMV in the general perinatal medical center (0.69%) was higher than that in the primary maternity hospital (0.23%). The proportion of symptomatic cCMV among all neonates with cCMV born in the general perinatal medical center (53%) was also higher than that in the primary maternity hospital (10%). The incidence of cCMV (0.51%) and the proportion of symptomatic cCMV (36%) in the regional perinatal medical center were at an intermediate level. These results suggest that mothers with fetuses of cCMV are selectively referred or transferred to the perinatal medical centers before deliveries as high-risk pregnancies, presumably because of fetal abnormality and/or obstetric complications. Recent

prospective cohort studies found that ultrasound fetal abnormality [13], multiple pregnancy, threatened miscarriage, threatened premature labor, and fever/flu-like symptoms during pregnancy [14, 15] were risk factors for cCMV. These were considered clinical findings prenatally predictive of births with cCMV. Pregnant women with these complications in primary maternity hospitals are likely referred or transferred to perinatal medical centers. Therefore, to detect neonates with symptomatic cCMV who need VGCV therapy, the universal neonatal urine screening for CMV in perinatal medical centers seems to be more cost-effective than that in primary maternity hospitals. The early diagnosis of cCMV can lead to the early commencement of antiviral therapies for infants with symptomatic cCMV to reduce neurological impairments and sequelae. This cohort study also found that restricted symptoms of ocular complication, abnormality on brain image, and abnormal BAEP were associated with better outcome, while a symptom of microcephaly was associated with severe sequela of neonates with symptomatic cCMV despite VGCV therapy.

In this study, 23 of the 56 neonates with cCMV (41%) were symptomatic. The proportion of symptomatic cCMV among all the neonates with cCMV seems to be higher than the epidemiological frequency, probably because 7,369 of the 11,736 participating neonates (63%) were born in two perinatal medical centers, and Kobe University Hospital actively receives high-risk pregnancies, including cases with suspected cCMV and fetal abnormality in this region. Another reason may be because workups for symptoms in neonates with cCMV

who were diagnosed based on the universal neonatal urine screening can effectively detect subclinical symptoms of abnormal brain images and ocular complications. Coincidently, only 1 (3.8%) of the 26 infants with asymptomatic cCMV developed neurological impairments of unilateral hearing loss and autism, and it was a very low incidence as compared with the 10 – 15% reported previously [3, 5]. Thus, subclinical cases may have been identified by the universal neonatal urine screening, and the true utility of the universal screening may be to identify subclinical symptomatic cCMV. Indeed, the present study demonstrated 21% (4/19) of neonates with cCMV had subclinical symptoms, and they would be found by the universal urine screening.

It is known that 70–90% of infants with symptomatic cCMV have neurological impairments with severe sequelae [2-4]. Several reports of intravenous GCV and oral VGCV therapies for neonates with cCMV found the effects of these therapies on the reversal of hearing loss or the prevention of its deterioration [6, 8, 16,17]. However, most of the reports concerned the effect on hearing impairment. The present cohort study on a large scale for the first time demonstrated that a series of the universal neonatal urine screening for CMV, diagnosis of neonates with cCMV, workup for symptoms, and early commencement of VGCV therapy for neonates with symptomatic cCMV may decrease neurological impairments of infants with cCMV. This is because 37% of the treated infants showed normal development without any neurological impairment, and 21% had mild sequelae of unilateral hearing dysfunction only or

DQ 70-79. The universal neonatal urine screening for CMV, followed by a series of procedures for cCMV, is likely effective in improving the neurological outcomes of infants with symptomatic cCMV. Recently, a cohort study of CMV screening for pregnant women and their newborns demonstrated that maternal antibody screening using CMV IgG, IgG avidity index, and IgM could identify pregnancies with cCMV due to maternal primary CMV infection accounting for 30% of cases; however, it overlooked those caused by non-primary CMV infection, which accounted for 70% [18].

These results provide useful information for clinical practitioners. However, the present study has some limitations. This is not a randomized placebo-controlled trial, but an observational cohort study. Therefore, the efficacy of VGCV therapies has not been rightly evaluated. The six-week and six-month VGCV therapies were mixed in this study. Since the six-month treatment with VGCV proved more effective than the six-week treatment [8], the treatment method had to be changed halfway in 2015.

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Potential conflicts of interest

All authors report no potential conflicts of interest.

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Figure legend

Figure 1 Flowchart of universal neonatal urine screening and antiviral therapy for symptomatic congenital CMV infection

CMV, cytomegalovirus; VGCV, Valganciclovir

Figure 1 Flowchart of universal neonatal urine screening and antiviral therapy for symptomatic congenital CMV infection

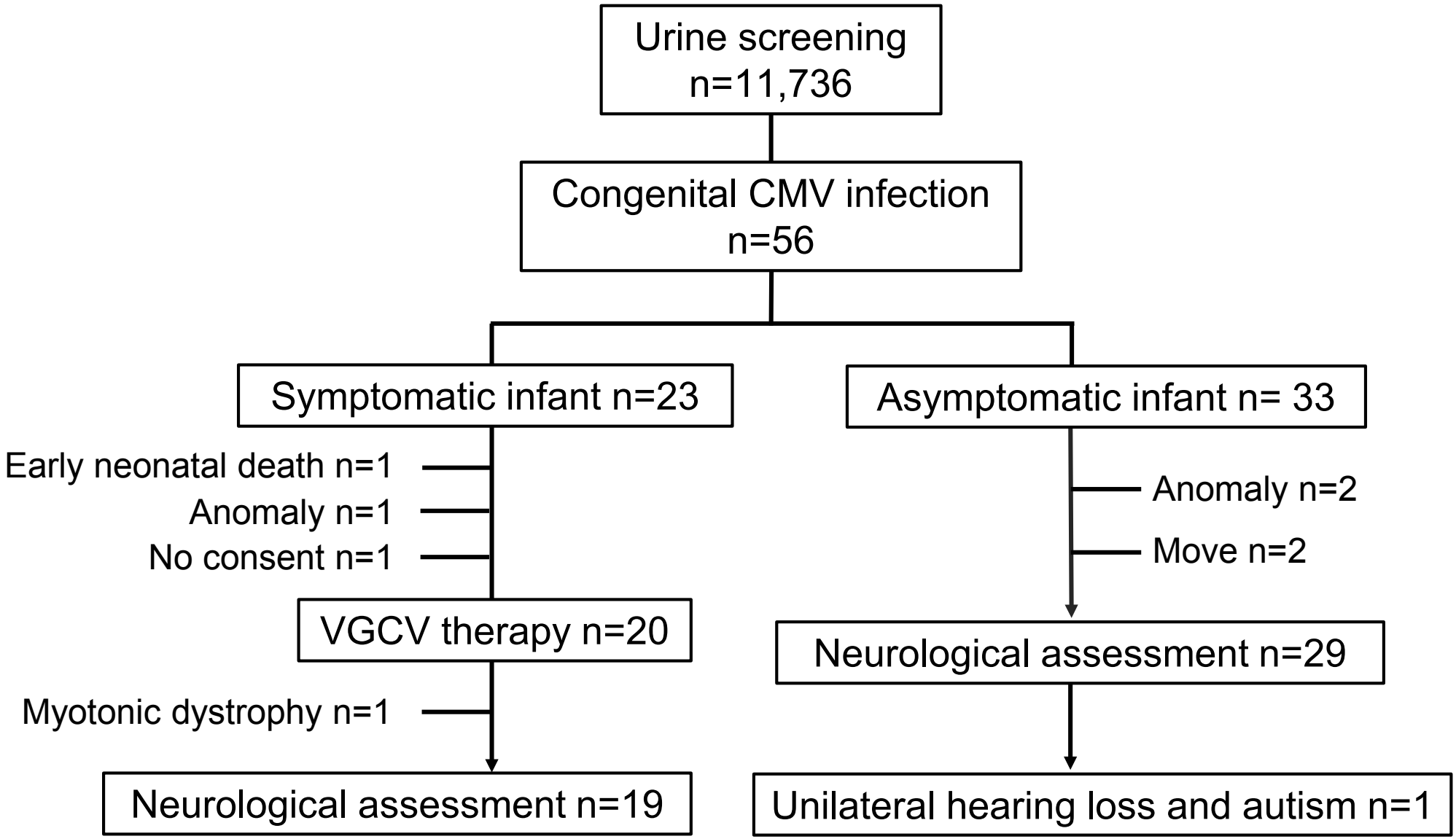

Table 1 Frequencies of congenital cytomegalovirus infection in three facilities

Facility	Total number of neonates	Number of neonates with congenital infection (%)	Number of neonates with asymptomatic infection	Number of neonates with symptomatic infection
University hospital	4,628	32 (0.69) *a	15	17 *b
Perinatal medical center	2,741	14 (0.51)	9	5
Primary maternity hospital	4,367	10 (0.23) *a	9	1 *b
Total	11,736	56 (0.48)	33	23

*a $p<0.01$ and *b $p<0.05$

Table 2. Neurological outcome in 19 neonates with symptomatic congenital cytomegalovirus infection who received VGCV therapy

Case	Neonatal symptoms							Neurological outcome				Outcome
	SGA	Microcephaly	Thrombocytopenia	Hepatitis	Ocular complication	Abnormality on brain image	Abnormal BAEP	DQ <70	DQ 70-79	Hearing dysfunction	Epilepsy	
1		●			●	●	●	●		Bilateral	●	Severe sequela 42%
2	●	●	●	●	●	●	●	●		Bilateral	●	
3	●	●	●			●	●	●				
4	●	●	●	●		●	●	●		Bilateral		
5	●					●	●	●		Bilateral		
6			●			●	●	●		Bilateral		
7	●	●		●		●	●		●	Bilateral		
8			●		●	●	●			Bilateral		
9			●			●	●		●			Mild sequela 21%
10			●	●		●	●			Unilateral		
11						●	●			Unilateral		
12						●	●			Unilateral		
13							●					Normal development 37%
14	●			●		●	●					
15					●							
16						●	●					
17					●	●						
18						●						
19			●			●						

VGCV, Valganciclovir; SGA, small for gestational age; BAEP, brainstem auditory-evoked potential; DQ, developmental quotient