Screening for congenital cytomegalovirus infection using newborn urine samples collected on filter paper: feasibility and outcomes from a multicentre study

Shin Koyano,1 Naoki Inoue,2 Akira Oka,3 Hiroyuki Moriuchi,4 Kimisato Asano,5 Yushi Ito,6 Hideto Yamada,7 Tetsushi Yoshikawa,8 Tatsuo Suzutani,9 for the Japanese Congenital Cytomegalovirus Study Group

ABSTRACT

Background: As congenital cytomegalovirus (CMV) infection causes significant clinical consequences not only at birth but also later as neurological sequelae, it is critical to establish a strategy for screening congenitally infected newborns. Previous studies have identified an insufficient sensitivity in screening methods based on the use of dried blood spots (DBSs).

Objectives: To evaluate the feasibility of the authors' recently developed method for large-scale screening for congenital CMV infection and to identify risk factors for congenital infection.

Methods: More than 21 000 newborns were enrolled at 25 sites in six geographically separate areas of Japan. Urine was collected onto filter cards placed in the diapers, which were then analysed by quantitative PCR using the filter disc directly as a template. Clinical and physical findings of the newborns were extracted from their medical records. CMV strains from the cases and their siblings were genetically compared. Viral loads in urine samples collected on filter paper were evaluated for CMV infection using newborn urine samples collected on filter paper: feasibility and outcomes from a multicentre study.

Results: Congenital CMV infection was identified in 0.31% (95% CI 0.24% to 0.39%) of the newborns, and 30% of the cases (20/66) had typical clinical manifestations and/or showed abnormalities in brain images at birth. Although the positive predictive value of our screening was 94%, the lack of any comparison with a gold standard assay prevented calculation of the negative predictive value. Almost two-thirds of the cases had siblings, suggesting a transmission problem of the same level as Down’s syndrome.

Conclusions: Implementation of an effective large-scale screening programme for congenital CMV infection is feasible. Siblings are the major risk factor for congenital CMV infection, which emphasises the need for education of mothers-to-be as well as vaccine development.

ARTICLE SUMMARY

Article focus

- It is critical to establish a strategy for screening newborns infected congenitally with cytomegalovirus (CMV), as they are at risk for the development of neurological sequelae. However, because of the insufficient sensitivity of newborn CMV screening based on dried blood spots, the feasibility of an alternative approach using urine specimens needs to be evaluated.
- Not only population-based epidemiological studies, but also prospective molecular studies, are necessary to clarify the transmission routes of congenital CMV infection.

Key messages

- Collection of urine specimens onto filter cards placed in the diapers made large-scale screening for congenital CMV infection feasible and effective without compromising detection sensitivity.
- One out of every 300 newborns is congenitally infected with CMV, and 30% of the cases were symptomatic in the society where the CMVseroprevalence is 70%, indicating that the frequency of congenital CMV infections is a medical problem of the same level as Down’s syndrome.
- Our direct molecular evidence indicates that siblings are the major risk for congenital CMV infection.

Strengths and limitations of this study

- This multicentre large-scale screening study demonstrates that our urine-filter-based method is robust and reliable. However, the lack of any comparison with a gold standard assay prevented the determination of the exact frequency of false-negative results.
- Serological studies on mothers need to be combined with newborn screening to confirm whether transmission predominantly occurs via maternal primary infection.
Screening of Congenital CMV Infection

INTRODUCTION

Congenital cytomegalovirus (CMV) infection occurs in 0.2–2% of births in developed countries and causes significant clinical consequences not only at birth but also later as neurological sequelae, including sensorineural hearing loss (SNHL) and developmental delay.1 Our retrospective studies demonstrated that 12–15% of cases with severe SNHL and 25% of cases with developmental delay of unknown cause were associated with fetal congenital CMV infection; half of the sequelae were of late onset.2 3 Early identification of congenital CMV infection may lead to new treatment options with antiviral agents.4 In addition, early intervention in infants with SNHL enables language development to reach a level comparable with that of audiologically normal peers.5 As many cases have late-onset SNHL, the ability of standard newborn hearing screening to detect congenital CMV-associated SNHL is limited.6 Therefore, it is important to establish newborn congenital CMV screening programmes.

Traditionally, diagnosis of congenital CMV infection has been performed by the culture of CMV from urine specimens collected within 3 weeks of birth. As the collection of liquid urine specimens is laborious, recent studies have examined the utility of dried blood spots (DBSs) for congenital CMV diagnosis.7 8 The arguments in support of DBS-based assay rely on the presence of infrastructure for DBS collection and their cost efficiency. However, several studies have found that DBS-based assays are of limited sensitivity.9 10 Virus loads in blood, particularly from asymptomatic cases, are very low,11 12 which necessitates the use of large DBS discs, efficient DNA extraction and sensitive PCR assays.13 14 To overcome the limitations associated with DBS-based assays, we developed a quantitative PCR (qPCR) assay using urine specimens collected on filter discs that can be used directly as a PCR template without additional purification or elution steps.15

In this study, we established a multicentre congenital CMV screening programme and tested specimens from 21 272 newborns to evaluate the feasibility of our assay for a relatively large-scale screening programme, to learn the prevalence of congenital CMV infection and diseases, and to identify risk factors for congenital CMV infection in Japan.

METHODS

Study design

The collection and use of human-subject materials was approved by the Ethical Committee on Human Subjects of each participating institute. We obtained informed consent from the parents of all enrolled newborns. The screening method and study outline are illustrated in figure 1. The National Institute for Infectious Diseases (NIID) in Tokyo distributed filters with ID numbers to all study sites. The ID numbers consisted of five digit numbers, indicating <area><study site within the area><three digits for each newborn ID at the site>. We collected urine on a 2 cm × 5 cm piece of FTA-Elute filter cards (Whatman) inserted into the diaper of each newborn within 4 days of birth and before discharge. After removal from the diaper, the urine filter was dried and mailed to NIID, where it was received within 1–2 days of shipment. The qPCR described in the following section was performed shortly after receipt of the specimens (mean of 1.1 ± 1.2 days). Approximately 70% of distributed filter cards were recovered for screening. The main reasons for the non-return of filters were (1) that they were used to demonstrate urine collection techniques to the nurses and paramedicals; (2) repeated urine collection owing to insufficient urination prior to diaper change; (3) too much stool on the filter; and (4) misplacement of the initial filter. All filters were accounted for as the NIID sent each study site an Excel file containing information regarding filter ID, date of receipt of the specimens, and test results after each qPCR run, and each study site added the results to the newborns’ medical records. There were about 20 occasions on which the study sites emailed or called NIID because of a typographical error in the filter ID in the file.

When the test result was positive, physicians contacted the parents to arrange an immediate clinic visit for the collection of urine and blood specimens from the newborns to confirm congenital CMV infection. Specimens for confirmation were collected at the age of 15.8 ± 3.8 days (range 7–21 days). Virus isolation, virus load measurement and serology were performed using these specimens. In addition to urine specimens, dried umbilical cord specimens were obtained from 23 of the cases for further confirmation.

Figure 1 Screening method and study design. ABR, auditory brainstem responses; CMV, cytomegalovirus; DBS, dried blood spot; GCV, ganciclovir; VGCV, valganciclovir.
We obtained separate informed consent to use DBS specimens from the cases retrospectively for our study, and requested the incorporated foundations handling DBSs for sending the specimens. DBS specimens were retrieved from 12 of 13 cases, 11 of which were asymptomatic, and one had SNHL at birth. Blood specimens were obtained from mothers and urine specimens from siblings.

We established 25 study sites in six geographically separate areas of Japan. The study sites serve rural, rural–urban, and urban/metro societies. More than 99.9% of all babies born at the sites were recruited for enrolment, and 1.3% of their parents refused participation. A few infants severely ill in neonatal intensive care units (NICUs) were excluded owing to difficulties in the collection of urine. Enrolment was carried out from April 2008 to September 2010. The sites included primary obstetric clinics and municipal hospitals (type 1 sites), as well as university-associated and national hospitals (type 2 sites) that care for the general population of pregnant women as well as patients referred from type 1 clinics. Pregnant women are able to select clinics/hospitals according to their preference. Type 2 sites are generally large in scale, have a NICU and employ specialists in most clinical areas. Type 1 sites include Mori Hospital, Tomakomai City Hospital, Sapporo Tokusyukai Hospital, Nihonmatsu Hospital, Yamaguchi Hospital, Toyokawa City Hospital, Wakamiya Hospital, Palmore Hospital, Hanamizuki Ladies Clinic, Fujita Clinic, Fuchi Ladies Clinic, Miura Clinic for Women and Children, Miyamura Hospital and Takara Maternity Clinic. Type 2 sites include National Fukushima Hospital, National Centre for Child Health and Development, Iwaki Kyoritsu Hospital, Hyogo Prefectural Kobe Children’s Hospital and Kariya Toyota General Hospital, and hospitals at Asahikawa Medical University, Fukushima Medical University, Hyogo Prefectural Kobe Children’s Hospital, and Kariya Toyota General Hospital, and hospitals at Asahikawa Medical University, Fukushima Medical University, Hyogo Prefectural Kobe Children’s Hospital, and Kariya Toyota General Hospital.

### qPCR for screening

We punched a 3 mm diameter disc from the urine filters, washed this with 200 μl of water and then transferred it into 50 μl of qPCR reaction mixture in a well of a 96-well plate. Only one filter disc can be submerged into the reaction mixture after brief centrifugation (400 g, 30 s). The qPCR assay for the CMV UL84 fragment and thermal cycling conditions were described previously. Although it is ideal to include an internal control to ensure the absence of PCR inhibition, we did not do so in this study for the following reasons: (1) although we initially spiked irrelevant DNA/primer/probe into the qPCR reaction mixture for screening (n=200 urine-filters) to check the efficiency of qPCR, no cases of inhibition were observed; (2) our previous study demonstrated a close correlation in viral load measurements between the urine-filter-based qPCR assay and the common qPCR assay using DNA samples purified from liquid urine; and (3) to reduce cost as far as possible. To confirm positive results in the screening qPCR, DNA samples were recovered from four 3 mm diameter filter discs by incubation at 95°C for 30 min in 50 μl of water containing 100 ng of carrier DNA, and 5 μl aliquots of the recovered DNA samples were used for qPCR. Simple mathematical consideration of the detection efficiencies (15~20% in qPCR containing urine-filter vs 60% in qPCR using eluted DNA samples) and the number of used filter discs indicates that both qPCR assays detect almost equivalent amounts of CMV DNA. The cost for our test itself, including the filter paper, reagents, disposables and technical labour for the assay, was <750 Yen (~£5.50, €7 or US$9) per study participant.

### DNA preparation from specimens other than urine filters

DNA samples were purified from liquid urine using a Viral RNA kit (Qiagen, Hilden, Germany), and from whole blood using a QIAamp DNA Mini kit (Qiagen) according to the manufacturer’s instructions. DNA was purified from dried umbilical cord and DBS specimens as described previously. The efficiency of DNA recovery from DBS specimens was >90%.

### Clinical and audiological evaluation

Clinical data for newborns, including birth weight, gestational age (GA), clinical manifestations and abnormal laboratory findings, were extracted from their medical records. The mental and physical development of infected infants has been closely monitored at outpatient clinics. Audiological testing was carried out using auditory brainstem responses and/or auditory steady-state responses, and brain imaging was carried out by CT and/or magnetic resonance images. Although interpretation of mild abnormalities in brain images is sometimes difficult, our Study Group contains a specialist with long experience in paediatric neurology and brain imaging. Medical records of mothers were examined for abnormalities during pregnancy. We defined ‘typical clinical manifestations’ as any of microcephaly, chorioretinitis, SNHL or a combination of petechiae, hepatosplenomegaly and jaundice. Intrauterine growth restriction (IUGR) and any single, mild manifestation were discounted as ‘typical clinical manifestations.’ ‘Symptomatic’ cases were defined as those exhibiting any typical clinical manifestations and/or abnormalities in their brain images.

Prior to the initiation of screening, our Study Group prepared a tentative guideline for antiviral treatment based on the same protocol and inclusion/exclusion criteria used in the published clinical trial. Treatment was performed based on the criteria and informed consent.

### Strain analysis and serology

Sequences of the polymorphic regions of the gN, UL144, and UL146 genes of CMV strains were determined as described previously. In contrast to European countries, only 10% of obstetric clinics/hospitals in
Japan conduct routine CMV serology for pregnant women. In this study, two study sites performed a CMV-specific IgG test for all pregnant mothers (n=4877), and some sites tested CMV-IgG of pregnant women with any risk factors (n=524) at 10–20 weeks of pregnancy. Each of those sites used one of the following additional tests; CMV-IgG avidity test for seropositives at 19 weeks of pregnancy, CMV-IgG test for seronegatives at 35–36 weeks of pregnancy, and CMV-IgG and -IgM tests for seronegatives during the middle and late terms of pregnancy. All of their babies except for referrals were enrolled for CMV screening after birth. Serology tests for CMV-specific IgG and IgM were carried out at a commercial laboratory (SRL, Tokyo, Japan) using EIA kits produced at DENKA SEIKEN Co. (Niigata, Japan). The CMV-IgM kit is based on the IgM-captured sandwich method using CMV-specific antibodies for detection. The CMV-IgG avidity test was carried out at Aisenkai Nichinan Hospital as described previously.19

**Statistical methods**

Statistical significance was evaluated using the chi-squared test. The Mann–Whitney U test was used to analyse differences in virus loads between two groups. We used a website (http://www.measuringusability.com/wald.htm) to calculate adjusted Wald 95% CIs.

**RESULTS**

**Prevalence of congenital CMV infection**

Seventy of 21272 newborns were positive in the initial urine-filter screening, and all of these were confirmed positive by the second PCR using DNA samples recovered from the urine filters. The positive results were re-examined with urine specimens collected in liquid form. In two cases, dried umbilical cord specimens were used for the confirmation, as there was a delay in urine-filter collection. Among the 70 cases positive by the second PCR using DNA samples recovered from asymptomatic cases (supplementary figure), Virus loads in whole blood, but not urine, from the symptomatic cases were statistically higher than those from asymptomatic cases (supplementary figure). Virus loads in the urine were 16- to 22 000-fold higher than those in the blood.

| specimens (5.8×10⁴/ml). CMV was successfully cultured from all available urine specimens obtained from the screening-positive cases (n=50). In addition to the urine specimens, dried umbilical cord specimens from the cases (n=23) were used for further confirmation. In all, congenital CMV infection was confirmed in a total of 66 newborns (0.31%; 95% CI 0.24 to 0.39%) (table 1). There were no statistically significant differences in the prevalence of congenital CMV infection among the six geographical areas, although life styles ranged from metropolitan to traditional (data not shown).

Type 2 sites have more patients referred by primary clinics/hospitals (type 1 sites), and some type 2 sites enrolled newborns who required NICU care. Thus, there was a difference between the type 1 and type 2 sites in the proportion of newborns with a birth weight <2500 g (5.1% vs 13.5%) but not in that of newborns small for GA (SGA) (5.1% vs 5.9%). The prevalence of congenital CMV infections differed approximately twofold between the two types of study sites (table 1).

**Characteristics of CMV-infected newborns**

Typical clinical manifestations, defined as any of microcephaly, choriorretinitis, SNHL or a combination of petechiae, hepatosplenomegaly and jaundice, were observed in 22.7% (15/66) of the infected newborns (table 1). SNHL was observed in 53% (8/15) of those cases. Abnormalities in brain images, including intracranial calcifications, ventricular dilation and abnormal lesions, were identified in 10 out of 58 cases. The proportion of cases with typical clinical manifestations and/or with the abnormalities in brain images was 30.3% (20/66). As we did not include cases with a single, mild clinical manifestation and because brain imaging was not available for eight cases, the proportion of so-called ‘symptomatic’ cases was likely to have been underestimated. Cases identified at type 2 sites had more frequent clinical manifestations and abnormalities.

Virus loads in whole blood, but not urine, from the symptomatic cases were statistically higher than those from asymptomatic cases (supplementary figure). Virus loads in the urine were 16- to 22 000-fold higher than those in the blood.

**Table 1** Comparison of prevalence and clinical outcomes between types of study sites

<table>
<thead>
<tr>
<th>All sites (n=25)</th>
<th>Type 1 sites* (n=14)</th>
<th>Type 2 sites† (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of screened newborns</td>
<td>21 272</td>
<td>14 642</td>
</tr>
<tr>
<td>No of congenitally infected cases</td>
<td>66</td>
<td>35</td>
</tr>
<tr>
<td>Prevalence of congenital infection, % (95% CI)</td>
<td>0.31 (0.24 to 0.40)</td>
<td>0.24 (0.16 to 0.32)</td>
</tr>
<tr>
<td>Abnormalities identified within 3 months after birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: typical clinical manifestations at birth</td>
<td>22.7% (n=15)</td>
<td>8.6% (n=3)</td>
</tr>
<tr>
<td>B: abnormalities in brain images (CT/MRI)</td>
<td>15.2% (n=10)</td>
<td>11.4% (n=4)</td>
</tr>
<tr>
<td>A and/or B</td>
<td>30.3% (n=20)</td>
<td>17.1% (n=6)</td>
</tr>
<tr>
<td>Treated with antiviral drugs</td>
<td>9.1% (n=6)</td>
<td>0.0%</td>
</tr>
<tr>
<td>Detectable cytomegalovirus-IgM in blood of the cases</td>
<td>50.8% (30/59)</td>
<td>51.6% (16/31)</td>
</tr>
</tbody>
</table>

*Primary obstetric clinics and municipal hospitals.
†University-associated and governmental hospitals that care for general populations as well as patients referred from type 1 clinics.
Of the 66 confirmed cases, six newborns, who exhibited clinically obvious symptoms, including IUGR, petechiae, hepatosplenomegaly and jaundice, accompanied by the following abnormalities, SNHL (n=4), chorioretinitis (n=1) and abnormal brain images (n=3), were treated with ganciclovir (GCV) and/or valganciclovir (VGGC) immediately after diagnosis. In spite of the intensive treatment, one infant died. In addition, one child with late-onset SNHL was treated with VGGC at 5 months after birth, and an improvement in SNHL was observed.20

Newborns with congenital CMV frequently have siblings

The general characteristics of the newborns enrolled in this study are summarised in table 2. It is likely that the uninfected participants represent our average national population, since the 2009 National Vital Statistics reported by the Ministry of Health, Labour, and Welfare in Japan indicates that the average mothers’ age, the average birth weight and the proportion of newborns with a birth weight of <2500 g are 31.0 years, 3000 g and 7.7%, respectively, similar to the values observed in our study population.

Mothers’ age and GA of the infected newborns were similar to those of the uninfected newborns. However, the average birth weight of the infected newborns was slightly less than that of the uninfected newborns. The differences in the proportion of newborns with a birth weight of <2500 g and that of SGA were statistically significant, with the differences being more significant in symptomatic cases.

One of the most important findings of this study is that infected newborns were more likely to have siblings (63.6% vs 48.8%, p<0.02). Among the 42 cases with siblings, 34 had one sibling, seven had two, and one had three.

Comparison of CMV strains between cases and siblings

We collected urine specimens from the siblings of 34 infected newborns. Twenty-five of the siblings’ specimens contained sufficient CMV DNA for PCR amplification of the polymorphic regions of the gN, UL144 and UL146 genes. Eighty-four per cent (21/25) of the pairs had identical DNA sequences in these genes (table 3). The mother of one of the four newborns for whom the sibling strain was not identical is a nurse working in the department of internal medicine. There was no significant linkage of particular genotype(s) with clinical outcomes. In addition to the 21 pairs, three additional pairs, symptomatic cases identified at hospitals outside the study sites and their siblings, were infected with the same strains (data not shown).

Technical issues

The first issue is the fact that the detection of CMV-specific IgM is insensitive for newborn congenital CMV screening, since CMV-specific IgM was detectable in only half (30/59) of the congenital CMV cases (table 1). The second issue is the choice of specimens for screening. Viral load estimates obtained from urine-filters were in good agreement with those determined from liquid urine specimens (figure 2). Viral load estimates from the DBS specimens from the cases (n=12) also showed fair agreement with those determined using blood specimens. Importantly, the total CMV DNA load in three or four discs of DBS specimens was far less than that in a single urine-filter disc, and three out of 12 DBS specimens were negative in the PCR assay, the sensitivity of which was previously demonstrated to be >90%. Finally, adverse events associated with filter screening were observed in 0.15% of the study population. These were mostly minor skin irritations (ie, redness of skin), but four babies, including two premature newborns with a body weight <1000 g, experienced severe perianal skin erosions. Mistakes such as placing the filter directly onto the skin rather than underneath the top layer of the diaper and leaving the diaper unchanged for an excessive period were the probable cause.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of general characteristics between the uninfected screened newborns and the identified congenital cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected screened newborns</td>
</tr>
<tr>
<td></td>
<td>n=18330*</td>
</tr>
<tr>
<td>Mean±SD of mothers’ age (years old)</td>
<td>31.2±4.8</td>
</tr>
<tr>
<td>Mean±SD of gestation age</td>
<td>39w1.9±1w2.1d</td>
</tr>
<tr>
<td>Mean±SD of birth weight (g)</td>
<td>3002.9±385.2</td>
</tr>
<tr>
<td>Proportion of low birth weight (&lt;2500 g), % (95% CI)</td>
<td>7.3 (6.9 to 7.7)</td>
</tr>
<tr>
<td>Proportion of those small for gestational age‡, % (95% CI)</td>
<td>5.3 (5.0 to 5.6)</td>
</tr>
<tr>
<td>Proportion of the cases with elder sibling(s), % (95% CI)</td>
<td>48.8 (48.1 to 49.5)</td>
</tr>
</tbody>
</table>

*Uninfected newborns whose information was available from 17 study sites in all six areas.
†Abnormalities include typical clinical manifestations and abnormalities found in brain imaging (see the text).
‡<−1.5SD in the distribution in the Japanese standard list of birth weight, which is based on sex, number of pregnancy and gestational age. d, days; w, weeks.
DISCUSSION

There were three major outcomes of our study. First, we demonstrated that the collection of urine specimens in diapers followed by high-throughput qPCR is feasible for large-scale screening for congenital CMV. Second, we provided additional evidence that virus loads in the blood of symptomatic cases are higher than those in cases who are asymptomatic at birth12 21 and that virus loads in the urine are several orders of magnitude higher than those in blood,11 thereby enabling urine-based screening to detect more congenital CMV cases. Finally, we found that (1) two-thirds of congenital CMV cases had siblings, (2) most of the siblings were actively excreting large amounts of CMV in their urine, and (3) most of the sibling pairs were excreting identical strains of CMV. The prevalence of congenital CMV infection, one out of every 300 newborns, and the presence of clinical manifestations in 30% of the infected infants indicate that congenital CMV infection is a serious medical problem at a level similar to that of Down’s syndrome, as demonstrated in other studies.1 22 We found that the urine-filter collection was not a burden, either on the newborns or on clinic/hospital staffs, once they understood the work flow, although it required extra labour in comparison with DBS-based screening. Since most babies in Japan are delivered at clinics/hospitals and stay for 5–6 days, we cannot predict whether our approach is applicable for babies delivered at home. However, in one of our studies, we provided 30 mothers with filters and an illustration showing how to collect urine from their 1–2-year-old children at home, and found that most mothers were able to send the urine filters back without any trouble, suggesting that home collection is possible. The use of an auto-puncher and a bar-code system may increase the assay throughput and reliability, although we did not try this, owing to limited resources.

We should note that one of the limitations of our study is the lack of any comparison with a gold-standard screening method, which made it impossible to identify false-negative cases, although the higher viral loads in urine are expected to increase the sensitivity of our

Table 3 Comparison of cytomegalovirus strains between cases and their siblings

<table>
<thead>
<tr>
<th>No</th>
<th>Genotypes of case</th>
<th>Sequences between case and sibling(s)</th>
<th>Genotypes of sibling(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gN</td>
<td>UL144</td>
<td>UL146</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>B</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>C</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>A</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>B</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>B</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>3a</td>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>3a</td>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>3a</td>
<td>C</td>
<td>12</td>
</tr>
<tr>
<td>11</td>
<td>3b</td>
<td>C</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>4a</td>
<td>A</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>4a</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td>14</td>
<td>4a</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td>15</td>
<td>4a</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>4a</td>
<td>C</td>
<td>12</td>
</tr>
<tr>
<td>17</td>
<td>4b</td>
<td>A</td>
<td>9</td>
</tr>
<tr>
<td>18</td>
<td>4b</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>4b</td>
<td>B</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>4b</td>
<td>C</td>
<td>11</td>
</tr>
<tr>
<td>21</td>
<td>4c</td>
<td>B</td>
<td>9</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>B</td>
<td>12</td>
</tr>
<tr>
<td>23</td>
<td>2</td>
<td>B</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>3a</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>4c</td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>B</td>
<td>9</td>
</tr>
<tr>
<td>27</td>
<td>3a</td>
<td>B</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>3a</td>
<td>C</td>
<td>12</td>
</tr>
<tr>
<td>29</td>
<td>3a</td>
<td>NA</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>3a</td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td>31</td>
<td>3b</td>
<td>B</td>
<td>5</td>
</tr>
<tr>
<td>32</td>
<td>4a</td>
<td>A</td>
<td>8</td>
</tr>
<tr>
<td>33</td>
<td>4c</td>
<td>B</td>
<td>11</td>
</tr>
<tr>
<td>34</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
筛检方法。检出率为94%时，即意味着筛选阳性结果的假阳性率为6%。这些结果强调了使用额外材料的重要性。我们认为，这并不局限于我们的研究。主要的挑战是如何在可能的情况下，使用额外材料来诊断出进一步的感染。我们希望，这个挑战不是唯一的问题。

图2：比较PCR检测在1 mL全血或尿液中的病毒载量（x轴）与基于干血斑（DBS）或尿液滤纸的PCR检测（y轴）的比较。封闭和开放的圆圈分别表示基于DBS检测和基于尿液滤纸检测的全血标本和尿液标本的比较结果。

我们的研究发现，婴儿期的CMV感染病例更多，且更容易感染同一血系的其他儿童。这可能与家庭传播有关。在日语中，CMV初次感染的婴儿与CMV感染的婴儿在家庭中的比例更高。因此，我们推断，CMV初次感染的婴儿更可能将CMV感染传播给其他家庭成员。

一个重要的问题是母系—胎儿传播是否发生在初次感染后，还是在反应性或再感染后与不同血系的病毒有关。最近的一项分析表明，初次感染和复发性感染的平均比例分别为32%和1.4%，分别占总数的71.2%，在不同地理位置之间有较大差异。

另外，不同地区的CMV基因组序列数据受限制。为了验证CMV基因组序列的保守性，我们采用了一种更稳健的方法，通过限制酶片段长度多态性（RE-PCR）分析。结果表明，CMV基因组序列在不同地理位置之间有较大差异。

妨碍我们研究母系—胎儿传播的原因之一是CMV初次感染的婴儿在家庭中可能更易感染同一血系的其他儿童。因此，我们希望通过直接验证，可以了解家庭传播在CMV感染中的作用。考虑到这一点，我们发现27%（29/108）的1岁时有CMV感染者。

在日语中，CMV初次感染的婴儿与CMV感染的婴儿在家庭中的比例更高。因此，我们推断，CMV初次感染的婴儿更可能将CMV感染传播给其他家庭成员。
Thus, we think that at least half of the congenital CMV infections we identified most likely occurred during maternal primary infection.

Our study suggests that young children in a family are the major risk factor for transmission to their pregnant mothers. A significant proportion of pregnant mothers may be constantly exposed to CMV at home since, as described above, at least a quarter of young children excrete 0.5–1 l of urine containing >10,000 copies/ml of CMV daily, often for >2 years. Because of this, development of a vaccine to prevent CMV must become a national and international priority. A recent clinical trial using gB subunit vaccine provided a promising direction.35 In the mean time, serological screening coupled with educational counselling of seronegative mothers-to-be can decrease seroconversion.36,37

Our study clearly demonstrates the feasibility of implementing large-scale and effective screening programmes that will enable strategies for timely clinical intervention and treatment that minimise the damage caused by congenital CMV-associated sequelae.

Author affiliations
1Department of Paediatrics, Ashikawa Medical University, Hokkaido, Japan
2Department of Virology I, National Institute of Infectious Diseases, Tokyo, Japan
3Department of Paediatrics, Kyorin University, Tokyo, Japan
4Department of Paediatrics, Nagasaki University, Nagasaki, Japan
5Department of Obstetrics and Gynecology, Fukushima Medical University, Fukushima, Japan
6Department of Maternal and Perinatal Services, National Centre for Child Health and Development, Tokyo, Japan
7Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Hyogo, Japan
8Department of Pediatrics, Fujita Health University, Aichi, Japan
9Department of Microbiology, Fukushima Medical University, Fukushima, Japan

Acknowledgements
We dedicate this article to K Fujieda, who originally administered the study group before passing away in March 2010. We would like to thank our medical and nursing colleagues as well as the newborns and their parents who agreed to take part in this study. We also thank P Pellett (Wayne State University, Detroit, Michigan) and T Kawana (Teikyo University), for their intellectual input, and M Tsuda and Y Fukui, for their technical assistance with PCR screening and virus isolation. We would like to thank the following members of the Japanese Congenital Cytomegalovirus Study Group members for their contributions: T Nagamori (Ashikawa Medical University), H Asanuma (Tomakomai City Hospital), Y Mori (Mori Hospital), M Oshima (Sapporo Tokushukai Hospital), T Imanura, K Ikuta, A Sato, K Fujimori (Fukushima Medical University), M Owa (Nihonmatsu Hospital), R Suzuki (National Fukushima Hospital), T Honda (Iwaki Kyoritsu Hospital), K Goishi, Y Mizuno (Tokyo University), S Yamaguchi (Yamaguchi Hospital), K Tsukamoto, S Fujihara, H Nakamura, T Kubo, H Taiji, S Harada (National Centre for Child Health and Development), S Yamada (National Institute of Infectious Diseases), T Ohashi (Saitama Children’s Medical Centre), M Morizane, A Sonoyama, I Morioke (Kobe University Graduate School of Medicine), T Funakoshi (Hyogo Prefectural Kobe Children’s Hospital), M Ohashi (Wakamaya Hospital), K Shimogaki (Palmore Hospital), H Nakai, K Tanaka, S Go (Fujita Health University), M Morichu, H Masuzaki (Nagasaki University), T Hamasaki (Hanimizuki Ladies Clinic), A Fujita (Fujita Clinic), T Fuchi (Fuchi Ladies Clinic), K Miura (Miura Clinic for Women and Children), Y Miyamura (Miyamura Hospital), M Morisaki (Takara Maternity Clinic), T Minematsu (Asienkai Nichinan Hospital). All the listed individuals are part of the Study Group and have not received any compensation.

Funding
This work was supported by Grants for the Research on Child Development and Diseases (H20-Kodomo-007; H23-Jisei-1ppan-001) from the Ministry of Health, Labour and Welfare, Japan.

Competing interests
None.

Patient consent
Obtained from the parents.

Ethics approval
Ethics approval was provided by the Ethical Committee on Human Subjects of each participating institute.

Contributors
SK, NI and K Fujieda (deceased) designed the study. SK and K Fujieda (deceased) coordinated operations at the study sites. SK and NI had full access to all of the data in this study and take responsibility for the integrity of the data and accuracy of the data analysis. SK, AO, HM, KA, YI, HY and KN obtained newborns and acquired medical data. NI conducted all initial screening, and TS performed serological tests. NI drafted the manuscripts, and all others provided important intellectual input for revision of the manuscript.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data sharing statement
Technical details of the screening assay and some of the raw dataset for the tables presented in the manuscript are available from the corresponding author at ninoue@nih.go.jp. Consent for data sharing was not obtained from the parent(s), but the presented data are anonymised, and the risk of identification is low.

REFERENCES
Screening for congenital cytomegalovirus infection using newborn urine samples collected on filter paper: feasibility and outcomes from a multicentre study

Shin Koyano, Naoki Inoue, Akira Oka, Hiroyuki Moriuchi, Kimisato Asano, Yushi Ito, Hideto Yamada, Tetsushi Yoshikawa, Tatsuo Suzutani and for the Japanese Congenital Cytomegalovirus Study Group

BMJ Open 2011 1: originally published online July 29, 2011
doi: 10.1136/bmjopen-2011-000118

Updated information and services can be found at:
http://bmjopen.bmj.com/content/1/1/e000118

These include:

Supplementary Material
Supplementary material can be found at:
http://bmjopen.bmj.com/content/suppl/2011/08/12/bmjopen-2011-000118.DC1

References
This article cites 36 articles, 8 of which you can access for free at:
http://bmjopen.bmj.com/content/1/1/e000118#BIBL

Open Access
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. See: http://creativecommons.org/licenses/by-nc/2.0/ and http://creativecommons.org/licenses/by-nc/2.0/legalcode.

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Epidemiology (2045)
- Infectious diseases (549)
- Paediatrics (607)
- Screening (epidemiology) (11)
- Screening (public health) (11)
- Immunology (including allergy) (28)

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/
Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/