Cytomegalovirus Genotype Distribution Among Congenitally and Postnatally Infected Patients: Association of Particular Glycoprotein (g)B and gN Types With Symptomatic Disease

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Background. Human cytomegalovirus is a leading cause of congenital infection, and there are limited data on prognosis markers in disease development. We aimed to study 3 virology targets (glycoprotein [g]B, gN, and UL144) to assess their correlation with congenital infection and various organ system involvement.

Methods. Forty-eight congenital cases and 58 postnatally infected children were included (2003–2014). Genotyping for the 3 targets and distribution among the cohorts were investigated, and the relationship between the gB, gN, and UL144 types with clinical manifestations in congenital infection was also studied.

Results. All of the genotypes were similarly represented among cohorts, and the most prevalent were the UL144B, gB1, and gN1 genotypes. The gB2 genotype was associated with abnormal image findings by ultrasound and/or magnetic resonance in congenital infection (odds ratio [OR], 6.2; 95% confidence interval [CI], 1.1–34.3; P = .036); the gN1 genotype was associated with an elevated risk of developing neurological disorders (OR, 7.0; 95% CI, 1.1–45.9; P = .043). Both gN1 and gB2 were independent factors for symptomatic infection. Statistical analyses showed no association between any UL144 genotype and disease severity.

Conclusions. All of the genotypes can be involved in congenital infection, although the gB2 and gN1 genotypes might be associated with a more serious illness.

Keywords. congenital infection; cytomegalovirus; gB; genotypes; gN; UL144.

Human cytomegalovirus (CMV) is a leading cause of congenital infection worldwide. In industrialized countries, it affects an average 0.6%–0.7% of newborns, and approximately 10%–15% of these present with symptoms at birth [1, 2]. Disease severity in symptomatic children is variable, ranging from mild symptoms to severe sensorineural hearing loss (SNHL) and long-term neurological sequelae [3–5].

Primary infections during pregnancy could lead to intrauterine transmission of CMV, although a transmission in previously seropositive women due to a reactivation or reinfection is the major route [6–8]. It is not typically possible to recognize CMV seroimmune status or to establish the moment of acquisition for documented primary infection because universal screening during pregnancy does not exist in most countries. In addition, there is no clear disease severity correlation between newborns born from women who are primarily or not primarily infected [4, 5].

Some viral factors have been postulated to be CMV pathogenicity markers [9–12]. Envelope glycoproteins are implicated in the recognition and input to the host...
cell, playing a potential role in the virulence of the various clinical isolates. Glycoprotein (g)B and gN are involved in the initial attachment of the virion to the cell surface, and the polymorphisms in these genes (UL55 and UL73, respectively) have been widely studied. Previous reports have associated particular gB or gN genotypes with severe congenital CMV (cCMV) infection, but information is scarce and is contradictory between studies [13–20]. There is also emerging interest in the study of other viral regions involving the host immune response. This interest is applied to UL144, a truncated tumor necrosis factor-α-like receptor gene, which contributes to the ability of CMV to escape immune clearance [9]. The association between the UL144 CMV genotype and the outcome in congenital infection has been investigated, but the heterogeneity between studies and the small number of congenital cases included have had inconsistent results [21–25].

We aimed to study the viral UL55, UL73, and UL144 genes to assess their correlation with cCMV infection and the involvement of various organ systems in CMV disease. The circulating strains of viral CMV in our geographic area were also studied, and a group of children with postnatal primary infection was included for this purpose.

MATERIAL AND METHODS

Study Population and Clinical Isolates

A retrospective study was conducted at Hospital Universitario 12 de Octubre, a tertiary care facility in Madrid, Spain, whose virology laboratory is a reference laboratory for the Southern area of Madrid, and which routinely receives samples from other hospitals (Hospital Universitario Severo Ochoa and Hospital Universitario de Getafe). The subjects involved in the study were identified from the virology laboratory database. We reviewed all of the positive CMV results from urine cultures and positive polymerase chain reaction (PCR) detections (amniotic fluid, fetal blood, and dried blood spots) from 2003 to 2014. Patients were included based upon virological results, detailed clinical records, and a specimen available at the moment of the study (kept frozen at −80°C). Approval from our hospital’s ethics committee was obtained, and informed consent was given by the source hospitals.

A total of 48 congenital cases were included in the study, consisting of 36 newborns and 12 infected fetuses. Demographical and clinical data from all congenitally infected patients were recorded, including sex, gestational age at birth, presence of abnormal image findings by ultrasound (US) and/or magnetic resonance (MR) compatible with cCMV infection, clinical signs, neurologic abnormalities, SNHL, chorioretinitis, and laboratory findings at birth.

Included in the study were maternal age, human immunodeficiency virus (HIV) infection and other immunodeficiencies, treatment with CMV-specific hyperimmune globulin (HIG) during pregnancy, and primary maternal CMV infection, as defined by seroconversion and/or positive immunoglobulin (Ig)M and IgG with low CMV-specific IgG avidity.

Congenital CMV infection was confirmed by virus isolation from the patient’s urine or saliva within the first 3 weeks of life (conventional cell culture and shell-vial culture [Vircell CMV-MAb, Granada, Spain] in MRC-5 cell lines) and/or by real-time PCR detection in amniotic fluid, fetal blood, or dried blood spots [26]. Viral load quantification in the blood and amniotic fluid was performed using the CMV R-gene test (Argene, Verhailes, France).

A group of 58 postnatally infected children from the same period of time was also included in the study. Laboratory and clinical records were reviewed. Cytomegalovirus infection was diagnosed based on consistent clinical symptoms (fever, mononucleoside syndrome) and the presence of specific CMV IgM in the patient’s serum, as well as the isolation of the virus from the patient’s urine. Among the 58 patients, 12 were younger than 4 months of age, and congenital infection was discharged in these cases because a previously negative urine CMV culture was available.

Clinical Definitions

Abnormal image findings in cranial US or cranial MR at birth or during pregnancy were defined as the presence of hydrocephalus, ventriculomegaly, periventricular cysts, white matter abnormalities, cerebral or cerebellar hypoplasia, hippocampal dysplasia, neuronal migration abnormalities, calcifications, ischemic lesions, lissencephaly, and microcephaly. All of the children infected with cCMV underwent a fundoscopy and cranial US at birth. All of the children with clinical symptoms and/or abnormal cranial US underwent cranial MR.

Neurologic abnormalities were defined as the presence of seizures, hypotonia, paresis, or spasticity.

Abnormal physical findings at birth were defined as the presence of microcephaly (head circumference <2 standard deviations below the mean for age and birth weight [World Health Organization]), hepatosplenomegaly, petechiae, and/or purpura.

Hearing function was assessed by the brainstem auditory evoked response (BAER) test, and SNHL was defined as a unilateral or bilateral hearing threshold of >20 decibels in the test.

The abnormal laboratory findings were defined as a hemoglobin value <9.5 g/dL (<8 g/dL in preterm infants), a neutrophil count <1000 cells/mm³, a platelet count <100 000 cells/mm³, alanine aminotransferase levels of >80 UI/L, or direct bilirubin levels of >2 mg/dL.

Symptomatic congenital CMV disease at birth was defined as the presence of at least 1 of the following: (1) abnormal physical exam, (2) abnormal image findings in US or MR, (3) SNHL, (4) neurologic abnormalities, (5) chorioretinitis, or (6) laboratory abnormalities. The presence of isolated intrauterine growth retardation, small for gestational age at birth, preterm delivery, or lenticulostriate vasculopathy in cranial US were not considered to be symptomatic criteria.
Characterization of Glycoprotein B, Glycoprotein N, and UL144 Genomic Variants

Genomic viral DNA was extracted from 200 µL of the specimens, which were stored at −80°C, using the NucliSENS easy-MAG instrument (Biomérieux Diagnostics, Marcy l’Etoile, France). Three nested PCR targeting the UL55 (gB), UL73 (gN), and UL144 genes were performed according to published protocols [27–29]. The amplicons were sequenced by using Big Dye 3.1 sequencing technology (3130 Genetic Analyzer; Applied Biosystems, Austin, TX). Genotypes for each target were determined by comparing the divergence of each amplicon with the reference sequences previously submitted to GenBank via the neighbor-joining tree-building method.

The sequences obtained were deposited at GenBank under the following accession numbers: KR992723-KR992835 for gB sequences; KR992836-KR992947 for gN sequences; and KR992948-KR993060 for gN sequences.

Statistical Analysis

The data were collected and analyzed using SPSS software version 15.0 (SPSS, Chicago, IL). Genotype distribution among congenitally and postnatally infected patients, the relationship between the gB, gN, and UL144 genotype, and the outcome of CMV congenital infections were analyzed using a 2-tailed χ² test or Fisher’s exact test for categorical variables and the Mann–Whitney U test for continuous variables. Logistic regression analysis was used to assess the associated risk between particular genotypes and the variables of the study. An unadjusted and adjusted (for treatment with HIG during pregnancy) odds ratio (OR) and associated 95% confidence interval (CI) were obtained. A P value <.05 was considered statistically significant.

RESULTS

Clinical Data on Congenitally Infected Patients

The clinical records of the 36 congenitally infected newborns are summarized in Table 1. We found a 50% proportion of symptomatic neonates. Sensorineural hearing loss, neuroimaging findings, and neurologic abnormalities were found in 83.3%, 83.3%, and 66.7% of the symptomatic patients, respectively. The most common pathologic image findings found were white matter disease (66.7%), intracranial calcifications (38.9%), periventricular cysts (27.8%), ventricular adhesions (16.7%), and hydrocephalus (16.7%). Of symptomatic newborns in the study, 72.2% were treated with antiviral therapy (ganciclovir or valganciclovir).

Regarding the 12 cases of pregnancies that were terminated, 1 was a spontaneous abortion, and the rest were interrupted on the basis of pathologic ultrasound findings (N = 10) and/or alterations in the cord blood analytical parameters (thrombocytopenia and elevated β₂ microglobulin) (N = 8). The primary image abnormalities found in the fetus were hepatomegaly (66.7%), cardiomegaly (41.7%), ascites (41.7%), splenomegaly (33.3%), hydrops (33.3%), periventricular cysts (33.3%), central nervous system (CNS) parenchymal lesions (16.7%), and cerebral ischemic damage (16.7%).

Pregnancy Information

Primary CMV infection was confirmed in 45.8% of the pregnant women, whereas it was not possible to determine the moment of acquisition in the remaining 54.2% of the cases. The women had a median age of 32.5 years (interquartile range [IQR], 27.0–35.5), 88.9% were born in Spain, and 11.1% were from South America. Four mothers (8.3%) were infected with HIV, but no transmission to the fetus occurred in those cases. Nine (19.6%) mothers with a positive CMV result in amniotic fluid decided to be treated with HIG to prevent the development of symptomatic congenital CMV infection.

Table 1. Clinical and Laboratory Findings at Birth and Pathologic Obstetric Evaluations From Newborns Congenitally Infected With Cytomegalovirus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>18 (50.0)</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>38.0 (IQR, 37.0–41.0)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2755.0 (IQR, 2250.0–3295.0)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48.0 (IQR, 45.0–50.0)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.0 (IQR, 31.75–35.0)</td>
</tr>
<tr>
<td>Neuroimaging findings (%)</td>
<td>15 (41.7)</td>
</tr>
<tr>
<td>Sensorineural hearing loss (%)</td>
<td>15 (41.7)</td>
</tr>
<tr>
<td>Chorioretinitis (%)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Microcephaly (%)</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Small for gestational age (%)</td>
<td>5 (13.9)</td>
</tr>
<tr>
<td>Cardiomegaly (%)</td>
<td>4 (11.1)</td>
</tr>
<tr>
<td>Hyperechogenic bowel lesions (%)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Amniotic fluid levels altered (%)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Petechiae (%)</td>
<td>6 (16.7)</td>
</tr>
<tr>
<td>Hepatomegaly (%)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Splenomegaly (%)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Neurologic abnormalities (%)</td>
<td>12 (33.3)</td>
</tr>
<tr>
<td>Laboratory findings (%)</td>
<td>3 (8.3)</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; MR, magnetic resonance; US, ultrasound.

a Data expressed as medium (IQR).

b Neuroimaging findings in cranial US or cranial MR were defined as the presence of hydrocephalus, ventriculomegaly, periventricular cysts, white matter abnormalities, cerebral or cerebellar hypoplasia, hippocampal dysplasia, neuronal migration abnormalities, calcifications, ischemic lesions, lissencephaly, and microcephaly.

c Neurologic abnormalities were defined as the presence of seizures, hypotonia, paresis, or spasticity.

d Laboratory findings were defined as a hemoglobin value <9.5 g/dL (<8 g/dL in preterm infants), a neutrophil count <1000 cells/mm³, a platelet count <100,000 cells/mm³, alanine aminotransferase levels of >80 UI/L, or direct bilirubin levels of >2 mg/dL.
We did not find any combination of particular gB/gN, gB/UL144, or gN/UL144 genotypes among clinical isolates. We also reported the displacement of circulating viral strains over years. An increasing trend in the number of congenital cases occurred until 2011 (Figure 1).

Genotype Association With Congenital Infection

We aimed to study the involvement of particular CMV genotypes in the development of symptomatic disease at birth, as well as its association with image findings, SNHL, neurological disorders, and analytical parameters. The statistical analysis showed no association between any UL144 genotype and specific clinical manifestations. However, regarding the UL55 gene, the gB2 genotype was associated with the presence of abnormal findings in image studies at birth ($P = .046$). After including in the analysis the fetuses from interrupted pregnancies with CNS abnormalities on fetal US, significant differences remained ($P = .030$). In the logistic regression analysis, the OR (adjusted by HIG treatment during pregnancy) for gB2 and abnormal image findings were 6.2 (95% CI, 1.1–34.3; $P = .036$) (Table 3).

On the other hand, gB4 was linked with a better prognosis, and no children or fetuses with this genotype presented abnormal findings (Table 3). The gN1 genotype was also associated with abnormal findings ($P = .015$). It was not possible to calculate the OR and 95% CI for this association (Table 3). Patients with the gB4 genotype presented higher platelet counts at birth (324 200 cells/L vs 187 600 cells/L; $P = .067$).

The study of the UL73 gene showed an association between the gN1 genotype and neurologic findings at birth ($P = .029$); in the logistic regression analysis, adjusted OR was 7.0 (95% CI, 1.1–45.9; $P = .043$) (Table 3). The gN1 genotype was also associated with a lower platelet count at birth compared with other gN variants (120 400 cells/L vs 238 550 cells/L; $P = .037$). The gN1 genotype was not associated with abnormal image findings ($P = .081$) (Table 3).

Finally, we did not find any association between a particular gB or gN genotype and SNHL.

**DISCUSSION**

Although cCMV infection is a major cause of long-term sequelae in children worldwide, in most countries there is no current strategy for general screening during pregnancy or in newborns. Moreover, most cases are asymptomatic at birth; however, even in these cases, the rate of long-term sequelae is important [30, 31]. In this scenario, it is of paramount importance to establish sensitive and accurate prognostic markers to guide the management of congenitally infected patients.

As with other viral infections, organ system involvement caused by CMV is a balance between the pathogenicity of the strain and the host’s immune ability to neutralize the infection [32]. The viral load in blood or urine has been reported to be a prognostic marker of CMV infection. The levels of viral load in
Figure 1. Distribution of viral glycoprotein (gB and gN) and UL144 genotypes over a 12-year study period (2003–2014). (A) The gB genotype distribution in congenital cases. (B) The gB genotype distribution in postnatal group. (C) The gN genotype distribution in congenital cases. (D) The gN genotype distribution in postnatal group. (E) UL144 genotype distribution in congenital cases. (F) UL144 genotype distribution in postnatal group.

Table 3. Association of Viral Genotypes and Outcome in Congenital Infection

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>P Value</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal image findings&lt;sup&gt;a&lt;/sup&gt;</td>
<td>gB2</td>
<td>6.3 (1.2–33.4)</td>
<td>.030</td>
<td>6.2 (1.1–34.3)</td>
<td>.036</td>
</tr>
<tr>
<td></td>
<td>gB4</td>
<td>.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.015</td>
<td>.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.015</td>
</tr>
<tr>
<td></td>
<td>gN1</td>
<td>4.5 (1.8–24.0)</td>
<td>.081</td>
<td>3.9 (1.7–22.3)</td>
<td>.123</td>
</tr>
<tr>
<td>Neurological disorders&lt;sup&gt;b&lt;/sup&gt;</td>
<td>gB2</td>
<td>5.0 (1.9–26.5)</td>
<td>.059</td>
<td>4.5 (1.8–24.6)</td>
<td>.082</td>
</tr>
<tr>
<td></td>
<td>gB4</td>
<td>0.5 (0.4–4.6)</td>
<td>.504</td>
<td>0.4 (0.4–4.7)</td>
<td>.510</td>
</tr>
<tr>
<td></td>
<td>gN1</td>
<td>7.9 (1.2–49.8)</td>
<td>.029</td>
<td>7.0 (1.1–45.9)</td>
<td>.043</td>
</tr>
<tr>
<td>SNHL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>gB2</td>
<td>3.0 (1.6–15.3)</td>
<td>.186</td>
<td>2.4 (1.4–13.9)</td>
<td>.314</td>
</tr>
<tr>
<td></td>
<td>gB4</td>
<td>.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.062</td>
<td>.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.062</td>
</tr>
<tr>
<td></td>
<td>gN1</td>
<td>4.7 (1.8–29.0)</td>
<td>.092</td>
<td>3.7 (1.5–25.2)</td>
<td>.179</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; MR, magnetic resonance; OR, odds ratio; SNHL, sensorineural hearing loss; US, ultrasound.

<sup>a</sup> Data from 46 patients (36 congenitally infected newborns and 10 fetuses from pregnancies interrupted by pathological image findings).

<sup>b</sup> Data from 36 congenitally infected newborns.

<sup>c</sup> Data adjusted for treatment with CMV-specific hyperimmune globulin during pregnancy.

<sup>d</sup> It was not possible to calculate OR (95% CI) because none of the patients with the gB4 genotype presented abnormal image findings in US/MR.

<sup>e</sup> It was not possible to calculate OR (95% CI) because none of the patients with the gB4 genotype presented SNHL at birth.
urine have been observed to be higher in congenital than in acquired infection [15], and 1 study has recently reported that CMV DNAemia could predict CMV sequelae in asymptomatic congenitally infected newborns [33]. Further studies with larger and homogeneous populations are required to establish a viral load cutoff, because there is evidence that DNAemia and urine excretion could be high and prolonged during CMV infection [34]. In our case, data on the CMV DNA viral load in urine were not available because we used viral cultures and did not quantify samples. For amniotic fluids, fetal blood, and dried blood spots, the viral load values obtained varied among specimens, ranging from 10^3 logarithm values in fetal blood to 10^7 logarithm values in amniotic fluid. The number of determinations was low, and we could not estimate the association with CMV disease.

The linkage between specific envelope glycoproteins and CMV disease has been previously investigated, and data on genotype distribution among congenitally infected patients are variable between studies [13, 15, 17–19]. As we report, the gB1 type has been found to be the most prevalent in congenital infection [14, 16, 17]. We also identified this genotype as the predominant variant among postnatally infected children, which indicates it is the primary genotype circulating in our geographic area. We found that genotype distribution among cohorts was similar, and that all gB genotypes can be involved in congenital infection, as other authors have also reported [13–15, 17]. Genotype 5 is a much less common circulating genotype, and we only determined 1 patient to be postnatally infected by this variant, so we cannot conclude that there is an association of this genotype with congenital infection. Yan et al [16] reported greater frequencies of the gB3 genotype in congenital than in acquired infection, and they found this variant to be associated with SNHL among congenital cases. Gandhoke et al [20] reported that the gB2 genotype was associated with CNS disease and SNHL among CMV-infected babies, although the study design and the criteria to define the study population (congenital and noncongenital cases) were not clearly established, thus the results should be carefully considered.

Our research revealed that the gB2 genotype was associated with abnormal image findings in the fetus and newborns with congenital infection, whereas gB4 might be associated with a lower risk of abnormal image findings. However, the number of infants with the gB4 genotype is small and the study is not sufficiently powered to assess this association. Abnormal cranial image findings in fetuses and newborns with cCMV infection are well known prognostic factors of long-term sequelae, including SNHL [35–37], and, to our knowledge, this is the first report that finds a relationship with a particular gB genotype.

Regarding gN types, we found a similar distribution of genotypes among cohorts, with gN1 being the most prevalent in congenital infection and gN3a the most prevalent in the postnatal group. Other authors found the gN4c or gN3a genotypes the most representative in congenital infection [17, 19]. Although the frequencies are similar to ours, the differences could reflect geographical distribution and the time of the studies. We found all genotypes both in congenital and acquired infection, although genotype 4b showed a trend toward a higher prevalence among noncongenital patients (P = .038), suggesting a reduced ability of this type to be transmitted in utero.

Some authors have documented an association between particular gN genotypes and disease severity in congenital infection. Pignatelli et al [19] found the gN1 and gN3a genotypes more frequently represented in asymptomatic patients with a favorable long-term outcome, and the gN4 genotypes more frequently represented in patients with symptoms at birth and more sequelae. Paradowska et al [18] also reported the detection of the gN2 or the gN4 genotypes in the children who were most seriously affected. Our results do not confirm prior observations, and, in contrast, we found the gN1 genotype more frequently in newborns with neurological disorders and lower platelet counts at birth.

We also investigated the UL144 gene due to its role in the virus’s ability to escape the immune response. Previous studies have reported an association between UL144 genotypes and congenital infection [21, 23, 24], and other authors have reported no linkage between the congenital picture and disease severity [16, 22, 25, 29]. We did not find any association between a particular UL144 genotype and symptomatic disease. The distribution of UL144 genotypes in our population is consistent with previously reported data: the B genotype is the primary genotype represented both in congenital and acquired infection. However, we found higher frequencies of A, C, and A/C genotypes than other studies reported [16, 22, 23].

Genomic variation among CMV strains and intrahost viral diversity have recently been characterized through next-generation sequencing [38, 39], and the extensive variability found could be comparable to many RNA viruses. Mixed populations of the virus have been documented in congenitally infected fetuses and newborns as well as in pregnant women [14, 17, 39–41]. Our study characterizes the primary circulating genotypes in the affected patients, and these genotypes are hypothetically the most important viral populations that could ultimately lead to disease severity.

**CONCLUSIONS**

Although the data presented here are compelling, this study has some limitations. First, this is a retrospective research, and due to the patient’s inclusion criteria and the lack of a current strategy to identify congenital cases, we obtained a high rate of symptomatic infections among live-born infants (50%), which does not represent the prevalence in Madrid (Spain). We have probably detected the most serious infections. Second, the information about the moment of the infection during pregnancy was
Miller WE, Zagorski WA, Brenneman A, et al. US28 is a potent activator of viral targets that we have investigated, gB seems to be the most appropriate because we have found some evidence about certain genotypes and the abnormal image findings present in the infected patients. Further studies are warranted to analyze the evolution of patients in time, both symptomatic and asymptomatic, and the implication of genotypes during the follow up.

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Potential conflicts of interest.

All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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