




# Cytomegalovirus Genotype and Virulence in Infants with Congenital Infection

Hong-bo Hu<sup>1</sup> Jian-gang Wu<sup>2</sup> Jian-jun Sun<sup>2</sup> Qiao-ying Peng<sup>3</sup> Xiao-peng Shang<sup>4</sup> 

<sup>1</sup>Department of Laboratory, Maternal and Child Health Hospital of Hubei Province, People's Republic of China

<sup>2</sup>Department of Laboratory, First People's Hospital of Guangshui, People's Republic of China

<sup>3</sup>Department of Neonatology, Maternal and Child Health Hospital of Hubei Province, People's Republic of China

<sup>4</sup>Department of Infectious Disease, First People's Hospital of Guangshui, People's Republic of China

Address for correspondence Xiao-peng Shang, MD, Department of Infectious Disease, First People's Hospital of Guangshui, Guangshui, Hubei 432700, China (e-mail: xiaopengs0910@163.com).

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## Abstract

**Objective** Cytomegalovirus (CMV) virulence may depend on genetic variability in several regions of the genome. This study aimed to assess specific CMV genotypes' association with the severity of symptomatic congenital CMV disease at birth.

**Methods** CMV glycoprotein B (gB), glycoprotein N (gN), glycoprotein H (gH), and UL144 strains were identified by nested polymerase chain reaction, restriction fragment length polymorphism, and heteroduplex mobility assay single-stranded conformation polymorphism in 50 infants infected congenitally and 25 asymptomatic infants.

**Results** gN1 ( $p=0.010$ ) and UL144-B ( $p=0.034$ ) genotypes were associated, by logistic regression, with reduced risk of developing symptomatic congenital CMV infection. gN1 ( $p=0.020$ ) and gN3 ( $p=0.022$ ) genotypes were associated with reduced risk of severe symptomatic disease. Conversely, gB1 ( $p=0.018$ ) was the most virulent genotype and was associated with severe symptoms.

**Conclusion** An association among gB1, gN1, gN3, and UL144-B genotypes of CMV and severity of congenital CMV disease might exist. gB, gN, and UL144 genotypes could be important virological markers of infant infection.

## Keywords

- ▶ CMV genotypes
- ▶ symptomatic CMV disease
- ▶ virulence
- ▶ congenital infection

## Introduction

Some infants develop symptoms, sometimes severe, from congenital cytomegalovirus (CMV) infection. The host immune system affects the outcome of infection to some extent, but the CMV strain sequence polymorphisms may also shape outcomes and tissue tropism.<sup>1</sup> Several envelope glycoproteins, encoded by *UL55*, *UL73*, and *UL75*, including glycoprotein B (gB), glycoprotein N (gN), and glycoprotein H (gH), respectively, have been evaluated in clinical CMV iso-

lates because of their influence on tissue tropism and virulence.<sup>2–4</sup> The UL144 polymorphisms are concentrated in the 5' end of the gene, especially in cysteine-rich domain 1 (CRD1), that may bind the B- and T-lymphocyte attenuator proteins. This domain's activity may vary among polymorphisms, leading to a range of clinical symptoms and prognoses.<sup>5–7</sup> Genetic polymorphisms of all these genes can be used to classify CMV strains and may affect the infectivity or pathogenicity of CMV.

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Few validated risk factors are available for outcome prediction or response to treatment, and little progress has been made in the treatment of infants with congenital disease. Additional research on congenital CMV infection should be focused on carefully designed randomized trials and multicenter prospective cohort studies. To this end, the gB, gN, gH, and UL144 genotypes of CMV strains isolated from different clinical specimens of infants with symptomatic congenital CMV infection were studied, focusing on viral genes involved in virulence. The incidence of CMV genotypes was determined to serve as a baseline for direct comparison and support epidemiological research.

## Materials and Methods

Infants at two hospitals with positive CMV infection and treated between January 2017 and December 2019 were included in the study. Demographical and clinical data were recorded, including gender, gestational age at birth, clinical signs, presence of abnormal imaging findings by ultrasound (US) or magnetic resonance (MR) compatible with CMV infection, neurologic abnormalities, failed hearing screen or proven sensorineural hearing loss (SNHL), chorioretinitis, and laboratory findings. Patients suffering from Epstein-Barr virus or other viral infections were excluded.

Symptomatic congenital CMV disease at birth is defined as the presence of at least one of the following conditions: (1) abnormal physical examination; (2) abnormal imaging findings by US or MR; (3) neurologic abnormalities; (4) abnormal visual examination; and (5) laboratory abnormalities.<sup>8,9</sup> Asymptomatic congenital CMV infection is defined as no apparent abnormalities to suggest congenital CMV disease and normal hearing. Moderately to severely symptomatic CMV disease is defined as multiple manifestations attributable to CMV infection, including thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth

restriction, hepatitis (raised transaminases or bilirubin), or central nervous system involvement such as microcephaly, radiographic abnormalities consistent with CMV central nervous system disease, abnormal cerebrospinal fluid (CSF) indices for age, chorioretinitis, SNHL, or the detection of CMV DNA in CSF. Mildly symptomatic CMV disease is defined as an occurrence with one or two isolated manifestations of CMV infection that are mild and transient (e.g., mild hepatomegaly or a single measurement of low platelet count or raised levels of alanine aminotransferase).<sup>9</sup>

Patients were detected for CMV infection using serological tests (immunoglobulin M [IgM]), viral culture, and real-time polymerase chain reaction (PCR) for blood, urine, or CSF samples or a combination of these media. CMV IgM was assayed with an enzyme-linked immunosorbent assay kit following the manufacturer's instructions (DiaSorin S.p.A., Italy). Urine samples were collected and cultured using the shell vial culture method (Chemicon, Temecula, California, United States). A quantitative fluorescence CMV-DNA kit was used to assess CMV-DNA following the manufacturer's instructions (Daan Gene Company of Zhongshan University, China). DNA level > 10<sup>3</sup> copies/mL indicated the presence of viral genome and was considered positive. CMV gB, gN, gH, and UL144 genotypic analyses were used by nested PCR, restriction fragment length polymorphism, and heteroduplex mobility assay single-stranded conformation polymorphism, as previously reported<sup>10-13</sup> (► **Table 1**).

Statistical analyses used SPSS version 21.0 software (SPSS, Inc., Chicago, Illinois, United States). Genotype distribution among congenitally infected patients, relationships among the gB, gN, gH, and UL144 genotypes, and severity of CMV disease were analyzed using chi-square test for ratio comparisons. Logistic regression analysis was used to assess risk for symptomatic diseases or severity of symptomatic CMV diseases associated with particular genotypes. Multiple comparisons between the mild and moderate to severe

**Table 1** Methodological approaches to discriminate gB, gN, gH, and UL144 genotypes

Genotype	Method	Primers	Restriction endonucleases
gB	PCR-RFLP	External primers: 5'-GGC ATC AAG CAA AAA TCT-3' Antisense: 5'-CAG TTG ACC GTA CTG CAC-3' Internal primers: 5'-TGG AAC TGG AACGTT TGG C-3' Antisense: 5'-GAA ACG CGC GGC AAT CGG-3'	<i>Hin f I</i> <i>Rsa I</i>
gN	PCR-RFLP	UL73-105725F: (5'-GGC GGT GGT GTG ATG GAG -3') UL73-106122R: (5'-TTC TGG AAG CAG CAA TGT CG -3')	<i>Scal</i> <i>SalI</i> <i>SacI</i>
gH	PCR-RFLP	External primers: 5'-AGG TAT TGA CAG ATC AAT GG-3' Antisense: 5'-CTC CTT CTC TCG GGT GTA AC-3' Internal primers: 5'-TGG TGT TTT CAC GCA GGA A-3' Antisense: 5'-CCA CCT GGA TCA CGC CGC TG-3'	<i>HhaI</i>
UL144	HMA-SSCP	External primers: 5'-TCG TAT TAC AAA CCG CGG AGA GGA T-3' Antisense: 5'-ACT CAG ACA CGG TTC CGT AA-3' Internal primers: 5'-TCG TAT TAC AAA CCG CGG AGA GGA T-3' Antisense: 5'-TAC GGT GTT ATT AGT GGA AGT G-3'	\

Abbreviations: gB, glycoprotein B; gN, glycoprotein N; gH, glycoprotein H; HMA-SSCP, heteroduplex mobility assay single-stranded conformation polymorphism; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

**Table 2** Distribution of CMV genotypes and clinical features in infants with symptomatic congenital CMV infection

Symptomatic congenital CMV infection (moderately to severely symptomatic CMV disease)								
No	Gender	Clinical manifestation	Diagnosis	Specimen type	Genotype			
					gB	gN	gH	UL144
1	F	Intrauterine growth restriction, respiratory distress, raised levels of conjugated hyperbilirubinemia	PCR	Urine	gB1	gN1	gH1	UL144-A
2	F	Pneumonia, thrombocytopenia, anemia, hepatosplenomegaly	PCR	Urine	gB1	gN2	gH1	UL144-A
3	M	Sepsis-like, intrauterine growth restriction, hepatitis	PCR	Urine	gB1	gN2	gH2	UL144-B
4	F	Respiratory distress, anemia, hepatosplenomegaly	PCR	Urine, blood	gB2	gN4	gH1	UL144-A
5	M	Respiratory distress, raised levels of conjugated hyperbilirubinemia, thrombocytopenia	PCR, culture	Urine	gB2	gN3	gH2	UL144-A
6	M	Sepsis-like, respiratory distress, anoxic brain damage, raised levels of conjugated hyperbilirubinemia	PCR, culture	Urine	gB3	gN4	gH1	UL144-A
7	F	Sepsis-like, respiratory distress, pneumonia, anemia	PCR	Urine	gB3	gN4	gH1	UL144-A
8	M	Respiratory distress, anemia, hepatosplenomegaly	PCR	Urine	gB1	gN3	gH2	UL144-C
9	F	Respiratory distress, raised levels of conjugated hyperbilirubinemia, intrauterine growth restriction	PCR	Urine	gB1	gN4	gH1	UL144-A
10	F	Sepsis-like, respiratory distress, raised levels of conjugated hyperbilirubinemia	PCR	Urine	gB1	gN1	gH1	UL144-A
11	M	Neutropenia, thrombocytopenia, anemia, hepatosplenomegaly	PCR	Urine	gB2	gN4	gH1	UL144-B
12	M	Neurologic abnormalities	Serodiagnosis, PCR	Blood, CSF	gB1	gN2	gH1	UL144-C
13	F	Pneumonia, hepatosplenomegaly, raised levels of aminotransferase	PCR	Urine	gB1	gN4	gH1	UL144-B
14	M	Hepatitis, anemia, hepatosplenomegaly	PCR	Urine	gB2	gN4	gH1	UL144-B
15	M	Respiratory distress, thrombocytopenia, anemia, hepatosplenomegaly	PCR	Urine	gB1	gN4	gH2	UL144-A
16	M	Respiratory distress, intrauterine growth restriction, hepatitis	PCR	Urine	gB1	gN4	gH1	UL144-A
17	M	Neurologic abnormalities	PCR	CSF, urine	gB2	gN3	gH1	UL144-C
18	F	Sepsis-like, respiratory distress, anemia, hepatosplenomegaly	PCR, culture	Urine	gB1	gN1	gH1	UL144-A
19	F	Intracranial hemorrhage, pneumonia, anemia, raised levels of aminotransferase	PCR	Urine	gB1	gN4	gH1	UL144-B
20	M	Sepsis-like, respiratory distress, hemangioma	PCR	Urine	gB2	gN4	gH1	UL144-A
21	F	Respiratory distress, anoxic brain damage, pneumonia, anemia	PCR	Urine	gB1	gN4	gH1	UL144-A
22	M	Respiratory distress, raised levels of conjugated hyperbilirubinemia, chorioretinitis	PCR	Urine	gB3	gN4	gH1	UL144-A

(Continued)

**Table 2** (Continued)

Symptomatic congenital CMV infection (moderately to severely symptomatic CMV disease)								
No	Gender	Clinical manifestation	Diagnosis	Specimen type	Genotype			
					gB	gN	gH	UL144
23	M	Sepsis-like, respiratory distress, cholestasis, cardiomegaly, anemia	PCR, culture	Urine	gB3	gN4	gH2	UL144-B
24	F	Raised levels of conjugated hyperbilirubinemia, raised levels of alanine aminotransferase	PCR	Urine, blood	gB1	gN3	gH1	UL144-A
25	F	Sepsis-like, thrombocytopenia, anemia, hepatosplenomegaly	PCR	Urine, blood	gB1	gN4	gH2	UL144-B
26	F	Respiratory distress, raised levels of conjugated hyperbilirubinemia, thrombocytopenia	PCR	Urine	gB1	gN2	gH1	UL144-A
27	M	Sepsis-like, respiratory distress, raised levels of conjugated hyperbilirubinemia	PCR	Urine	gB1	gN1	gH1	UL144-B

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; gB, glycoprotein B; gN, glycoprotein N; gH, glycoprotein H; PCR, polymerase chain reaction.

symptoms and asymptomatic infants were not included in the analysis. A *p*-value of less than 0.05 was considered statistically significant.

## Results

Fifty immunocompetent infants with symptomatic congenital CMV infection were analyzed. Twenty-seven were diagnosed with moderate to severe symptomatic CMV disease, and the remainder were diagnosed with mild symptomatic CMV disease. Further, 25 infants with asymptomatic CMV infection were served as a baseline for direct comparison. Baseline characteristics, laboratory data, and genotypes of CMV are summarized in **Tables 2 and 3**.

The incidence of gB genotypes in symptomatic neonates was gB1, 46.0%, 23/50; gB2, 30.0%, 15/50; and gB3, 24.0%, 12/50. Notably, a significantly higher incidence of gB1 (63.0%, 17/27) was observed in moderate to severe CMV infections than in mild infections (chi-square = 6.918, *p* = 0.031) (**Table 4**).

The overall incidence of individual genotypes in the study cohort was gN1, 22.0%, 11/50; gN2, 8.0%, 4/50; gN3, 26.0%, 13/50; and gN4, 44.0%, 22/50. gN1 (14.8%, 4/27) and gN3 (14.8%, 4/27) were less prevalent in moderate to severe CMV disease (chi-square = 14.278, *p* = 0.003) (**Table 4**).

gH1 and gH2 genotypes were found in 70.0% (35/50) and 30.0% (15/50) of infants, respectively. The prevalence of gH genotypes between symptomatic and asymptomatic infants was not statistically significant, and these genotypes were correlated with disease severity (**Table 4**).

The incidence of UL144 genotypes in patients with symptomatic infection was UL144-A, 52.0%, 26/50; UL144-B, 40.0%, 20/50; and UL144-C, 8.0%, 4/50. Significantly greater prevalence (68.0%, 17/25) of UL144-B was observed in asymptomatic infants (chi-square = 6.119, *p* = 0.047) (**Table 4**).

The gN1 (*p* = 0.010) and UL144-B (*p* = 0.034) genotypes were associated with a reduced risk of developing symptomatic congenital CMV infection. Similarly, the gN1 (*p* = 0.020) and gN3 (*p* = 0.022) genotypes were associated with a reduced risk of severe symptomatic disease. Conversely, gB1 (*p* = 0.018) was the most virulent genotype, associated with severe manifestations in congenital CMV infection (**Table 5**).

## Discussion

gB is a polyprotein cleaved at position 460 within a cleavage zone (CLZ). Its variability is produced by variation and possible recombination at the CLZ and N-terminal region.<sup>14</sup> The four gB genotypes currently identified reflect alterations in the CLZ region.<sup>14</sup> Our study shows that the prevalence of gB genotypes in symptomatic and asymptomatic infants is not different. In contrast, a relationship exists between gB genotypes and outcome; gB1 variants are associated with more severe infection. Critical gB-specific nonneutralizing antibodies exhibit genotype-specific gB recognition on the cell surface. This group of nonneutralizing gB-specific monoclonal antibodies typically bind preferentially to gB2 and gB4.<sup>15</sup> We speculate that gB1-producing CMV strains may elicit a severe immunopathological response that leads to tissue damage and disease manifestations.

Previous studies demonstrated that CMV gN1 and gN3 variants are less virulent genotypes than gN2 and gN4 and are associated with a reduced risk of some symptoms.<sup>16-18</sup> Our results confirmed that the gN1 and gN3 variants are less virulent and demonstrate that both genotypes might display a decreased risk of severe symptomatic CMV disease. The gN1 genotype is a typical AD169-like glycoprotein that is immunologically separate from other CMV clinical genotypes.<sup>16,17,19</sup> The obtained results suggest that the gN1

**Table 3** Distribution of CMV genotypes in infants with symptomatic and asymptomatic congenital CMV infections

Group settings												
Symptomatic congenital CMV infection (mildly symptomatic CMV disease)						Asymptomatic congenital CMV infection						
No	Gender	Clinical manifestation	Genotype				No	Gender	Genotype			
			gB	gN	gH	UL144			gB	gN	gH	UL144
1	M	Raised levels of conjugated hyperbilirubinemia	gB2	gN4	gH1	UL144-A	1	F	gB1	gN1	gH2	UL144-B
2	M	Raised levels of conjugated hyperbilirubinemia	gB2	gN3	gH1	UL144-B	2	M	gB3	gN1	gH1	UL144-A
3	M	Hepatomegaly	gB1	gN3	gH2	UL144-B	3	M	gB3	gN4	gH1	UL144-B
4	M	Raised levels of alanine aminotransferase	gB3	gN1	gH2	UL144-A	4	M	gB1	gN4	gH1	UL144-A
5	F	Low platelet count	gB1	gN4	gH2	UL144-C	5	F	gB1	gN3	gH2	UL144-B
6	M	Neutropenia	gB1	gN4	gH1	UL144-B	6	M	gB1	gN1	gH2	UL144-B
7	F	Hepatomegaly	gB2	gN3	gH1	UL144-B	7	M	gB2	gN3	gH2	UL144-A
8	F	Raised levels of alanine aminotransferase	gB3	gN4	gH1	UL144-A	8	F	gB2	gN3	gH1	UL144-A
9	F	Hepatosplenomegaly	gB3	gN3	gH2	UL144-A	9	F	gB3	gN1	gH1	UL144-B
10	M	Low platelet count	gB2	gN1	gH1	UL144-B	10	F	gB2	gN1	gH1	UL144-B
11	F	Low platelet count	gB3	gN3	gH2	UL144-A	11	M	gB3	gN3	gH1	UL144-B
12	M	Raised levels of alanine aminotransferase	gB1	gN1	gH1	UL144-B	12	F	gB1	gN1	gH1	UL144-B
13	M	Raised levels of conjugated hyperbilirubinemia	gB2	gN1	gH1	UL144-A	13	M	gB3	gN4	gH1	UL144-A
14	M	Raised levels of alanine aminotransferase	gB3	gN1	gH2	UL144-B	14	M	gB1	gN1	gH2	UL144-B
15	F	Raised levels of alanine aminotransferase	gB3	gN1	gH2	UL144-A	15	F	gB1	gN3	gH1	UL144-B
16	F	Neutropenia	gB2	gN4	gH1	UL144-B	16	F	gB1	gN3	gH1	UL144-B
17	F	Low platelet count	gB2	gN1	gH2	UL144-A	17	F	gB2	gN1	gH1	UL144-B
18	M	Raised levels of conjugated hyperbilirubinemia	gB2	gN4	gH1	UL144-B	18	F	gB1	gN1	gH2	UL144-A
19	F	Anemia	gB3	gN3	gH2	UL144-B	19	F	gB1	gN4	gH2	UL144-B
20	F	Raised levels of alanine aminotransferase	gB1	gN3	gH1	UL144-A	20	M	gB3	gN1	gH1	UL144-B
21	M	Raised levels of alanine aminotransferase	gB3	gN3	gH1	UL144-B	21	M	gB1	gN3	gH2	UL144-A
22	M	Hepatosplenomegaly	gB2	gN4	gH1	UL144-B	22	F	gB2	gN3	gH1	UL144-B
23	M	Hepatomegaly	gB1	gN1	gH1	UL144-A	23	F	gB1	gN4	gH2	UL144-B
							24	M	gB3	gN1	gH1	UL144-A
							25	M	gB1	gN1	gH1	UL144-B

Abbreviations: CMV, cytomegalovirus; gB, glycoprotein B; gN, glycoprotein N; gH, glycoprotein H.

genotype of CMV might be an important virological marker of asymptomatic infection in infants.

Nahar et al<sup>3</sup> described that the gH2 variant of CMV was significantly more prevalent than the gH1 variant in ulcerative colitis active patients with severe and moderate symptoms, which possibly indicates more virulence. Another study has demonstrated that gH1 represents the more virulent genotype, compared with gH2, and is

associated with SNHL. However, a virulence difference between these genotypes is not observed for other clinical symptoms.<sup>20</sup> No genotypes of gH in the present study are confirmed to be associated with virulence. At least two explanations are possible: (1) a specific CMV genotype may show strong virulence in some CMV-related diseases, while in other CMV-related diseases or asymptomatic infants, it may not show corresponding virulence

**Table 4** Distribution of CMV genotypes in moderately to severely infection and mildly infection among infants infected congenitally

Group	gB1	gB2	gB3	Total	Chi-square	p-Value	Chi-square	p-Value	
Asymptomatic, n (%)	13 (52.0)	5 (20.0)	7 (28.0)	25					
Symptomatic, n (%)	23 (46.0)	15 (30.0)	12 (24.0)	50	0.855	0.652			
Moderate to severe CMV infection, n (%)	17 (63.0)	6 (22.2)	4 (14.8)	27	1.368	0.505	6.918	0.031	
Mild CMV infection, n (%)	6 (26.1)	9 (39.1)	8 (34.8)	23	3.712	0.156			
Group	gN1	gN2	gN3	gN4	Total	Chi-square	p-Value	Chi-square	p-Value
Asymptomatic, n (%)	12 (48.0)	0 (0)	8 (32.0)	5 (20.0)	25				
Symptomatic, n (%)	11 (22.0)	4 (8.0)	13 (26.0)	22 (44.0)	50	8.555	0.036		
Moderate to severe CMV infection, n (%)	4 (14.8)	4 (14.8)	4 (14.8)	15 (55.6)	27	14.278	0.003	9.390	0.025
Mild CMV infection, n (%)	7 (30.4)	0 (0)	9 (39.1)	7 (30.4)	23	1.627	0.443		
Group	gH1	gH2	Total	Chi-square	p-Value	Chi-square	p-Value		
Asymptomatic, n (%)	16 (64.0)	9 (36.0)	25						
Symptomatic, n (%)	35 (70.0)	15 (30.0)	50	0.276	0.600				
Moderate to severe CMV infection, n (%)	21 (77.8)	6 (22.2)	27	1.201	0.273	1.691	0.193		
Mild CMV infection, n (%)	14 (60.9)	9 (39.1)	23	0.050	0.823				
Group	UL144-A	UL144-B	UL144-C	Total	Chi-square	p-Value	Chi-square	p-Value	
Asymptomatic, n (%)	8 (32.0)	17 (68.0)	0 (0)	25					
Symptomatic, n (%)	26 (52.0)	20 (40.0)	4 (8.0)	50	6.119	0.047			
Moderate to severe CMV infection, n (%)	16 (59.3)	8 (29.6)	3 (11.3)	27	8.843	0.012	2.883	0.237	
Mild CMV infection, n (%)	10 (43.5)	12 (52.2)	1 (4.3)	23	2.004	0.367			

Abbreviations: CMV, cytomegalovirus; gB, glycoprotein B; gN, glycoprotein N; gH, glycoprotein H.

characteristics and (2) study populations may differ. The study population in the earlier reports was from patients with active ulcerative colitis and SNHL. This population differs substantially from the population in the present study.

We also investigated the *UL144* gene because the protein has a role in allowing CMV to escape the immune response. Waters et al indicated that detection of UL144-A and UL144-C was associated with severe congenital CMV infection and was more likely to lead to long-term CMV-associated clinical

**Table 5** Factors associated with outcome of CMV infection and severity of CMV disease

Factors	Group settings	Genotype	B	p-Value	OR (95% CI)
Outcome of CMV infection <sup>a</sup>	Symptomatic congenital CMV infection	gN1	-1.720	0.010	0.179 (0.049-0.657)
	Asymptomatic congenital CMV infection	UL144-B	-1.156	0.034	0.315 (0.108-0.917)
Severity of CMV disease <sup>a</sup>	Mildly symptomatic CMV disease Moderately to severely symptomatic CMV disease	gB1	1.740	0.018	5.695 (1.349-24.048)
		gN1	-2.144	0.020	0.117 (0.019-0.711)
		gN3	-1.985	0.022	0.137 (0.025-0.752)

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; gB, glycoprotein B; gN, glycoprotein N; OR, odds ratio.

<sup>a</sup>By backward LR binary logistic regression.

manifestations.<sup>21</sup> Similarly, Arav-Boger et al showed that UL144-A and UL144-C are related to congenital CMV symptoms, and UL144-C was identified only in symptomatic patients.<sup>22</sup> Our findings are consistent and further demonstrate that the UL144-B genotype may be associated with asymptomatic congenital infection. CRD1 shows greater variation in UL144-A and UL144-C but is better conserved in UL144-B than the Towne strain.<sup>6</sup> This finding could be a reason for the enhanced virulence of UL144-A and UL144-C.

Our study has some limitations. Some uncertainty exists in the grouping of disease severity in children. Nine of 27 patients exhibited sepsis-like syndrome. However, other pathogens can cause sepsis-like symptoms, including certain viruses, bacteria, and fungi. Such pathogens were not tested or detected. The existence of coinfection might aggravate clinical symptoms, thus causing errors in grouping and statistical deviation.

## Conclusion

In summary, our study demonstrated a relationship between the gN1 and UL144-B genotypes and asymptomatic congenital CMV infection after birth. Further, detection of the gB1, gN1, or gN3 genotypes may help define this disease's manifestations in children. This study suggests that the gB, gN, and UL144 genotypes may be valuable virological markers of congenital CMV disease. Our data will help predict the severity of congenital CMV infection and help formulate preventive measures.

### Conflict of Interest

None declared.

## References

- Mei C, Yang W, Wei X, Wu K, Huang D. The unique microbiome and innate immunity during pregnancy. *Front Immunol* 2019; 10:2886
- Hu H, Peng W, Peng Q, Cheng Y. Cytomegalovirus genotype distribution among congenital and perinatal infected patients with CMV-associated thrombocytopenia. *Fetal Pediatr Pathol* 2020:1–10
- Nahar S, Hokama A, Iraha A, et al. Distribution of cytomegalovirus genotypes among ulcerative colitis patients in Okinawa, Japan. *Intest Res* 2018;16(01):90–98
- Hu H, Cheng Y, Peng Q, Chen K. Clinical features, treatment courses, and distribution of cytomegalovirus genotypes among thrombocytopenia patients aged younger than 12 months. *Am J Perinatol* 2020:10
- Bitra A, Nemčovičová I, Picarda G, et al. Structure of human cytomegalovirus UL144, an HVEM orthologue, bound to the B and T cell lymphocyte attenuator. *J Biol Chem* 2019;294(27): 10519–10529
- Guo G, Zhang L, Ye S, et al. Polymorphisms and features of cytomegalovirus UL144 and UL146 in congenitally infected neonates with hepatic involvement. *PLoS One* 2017;12(02):e0171959
- Chen HP, Jiang JK, Chan CH, et al. Genetic polymorphisms of the human cytomegalovirus *UL144* gene in colorectal cancer and its association with clinical outcome. *J Gen Virol* 2015;96(12):3613–3623
- Luck SE, Wieringa JW, Blázquez-Gamero D, et al; ESPID Congenital CMV Group Meeting, Leipzig 2015. Congenital cytomegalovirus: a European expert consensus statement on diagnosis and management. *Pediatr Infect Dis J* 2017;36(12):1205–1213
- Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect Dis* 2017;17(06):e177–e188
- Yu ZS, Zou CC, Zheng JY, Zhao ZY. Cytomegalovirus gB genotype and clinical features in Chinese infants with congenital infections. *Intervirology* 2006;49(05):281–285
- Guo S, Yu MM, Li G, Zhou H, Fang F, Shu SN. Studies on genotype of human cytomegalovirus glycoprotein H from infantile clinical isolates. *Zhonghua Er Ke Za Zhi* 2013;51(04):260–264
- Chen HP, Lin JC, Yang SP, et al. The type-2 variant of human cytomegalovirus glycoprotein N (gN-2) is not the rarest in the Chinese population of Taiwan: influence of primer design. *J Virol Methods* 2008;151(01):161–164
- He R, Ruan Q, Xia C, et al. Sequence variability of human cytomegalovirus UL144 open reading frame in low-passage clinical isolates. *Chin Med Sci J* 2004;19(04):293–297
- Grosjean J, Hantz S, Cotin S, et al. Direct genotyping of cytomegalovirus envelope glycoproteins from toddler's saliva samples. *J Clin Virol* 2009;46(Suppl 4):S43–S48
- Goodwin ML, Webster HS, Wang HY, et al. Specificity and effector functions of non-neutralizing gB-specific monoclonal antibodies isolated from healthy individuals with human cytomegalovirus infection. *Virology* 2020;548:182–191
- Paradowska E, Jabłońska A, Studzińska M, et al. Distribution of cytomegalovirus gN variants and associated clinical sequelae in infants. *J Clin Virol* 2013;58(01):271–275
- Pignatelli S, Lazzarotto T, Gatto MR, et al. Cytomegalovirus gN genotypes distribution among congenitally infected newborns and their relationship with symptoms at birth and sequelae. *Clin Infect Dis* 2010;51(01):33–41
- Mujtaba G, Khurshid A, Sharif S, et al. Distribution of cytomegalovirus among neonates born to infected mothers in Islamabad, Pakistan. *PLoS One* 2016;11(07):e0156049
- Pignatelli S, Rossini G, Dal Monte P, Gatto MR, Landini MP. Human cytomegalovirus glycoprotein N genotypes in AIDS patients. *AIDS* 2003;17(05):761–763
- Paradowska E, Jabłońska A, Studzińska M, et al. Cytomegalovirus glycoprotein H genotype distribution and the relationship with hearing loss in children. *J Med Virol* 2014;86(08):1421–1427
- Waters A, Hassan J, De Gascun C, et al. Human cytomegalovirus UL144 is associated with viremia and infant development sequelae in congenital infection. *J Clin Microbiol* 2010;48(11):3956–3962
- Arav-Boger R, Battaglia CA, Lazzarotto T, et al. Cytomegalovirus (CMV)-encoded UL144 (truncated tumor necrosis factor receptor) and outcome of congenital CMV infection. *J Infect Dis* 2006; 194(04):464–473