

# HUMAN CYTOMEGALOVIRUS gB1 GENOTYPES AMONG CHILDREN WHO LIVE AT THE PHAYATHAI BABIES' HOME IN NONTHABURI, THAILAND

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**Abstract.** We conducted a survey of human cytomegalovirus (HCMV) genotypes among 176 children aged 1 month to 5 years living at Phayathai Babies' Home in Nonthaburi Province, Thailand to determine the prevalence of HCMV glycoprotein B (gB) genotype. The study was conducted on urine samples using nested polymerase chain reaction and restriction fragment length polymorphism; the HCMV gB1 genotype was found in 89% of subjects, much higher than previous reports. Our results show a high proportion of HCMV gB1 infected children in this population.

**Keywords:** HCMV, children, genotype, Thailand

## INTRODUCTION

Human cytomegalovirus (HCMV) is a  $\beta$ -herpesvirus (human herpesvirus 5) and a major cause of morbidity and mortality in immunocompromised patients, recipients of solid-organ transplants, AIDS patients, and cancer patients (Polprasert *et al*, 2011; Watcharananan *et al*, 2012). HCMV infects humans world-wide with a prevalence rate of 50-90% depending on the socioeconomic status and personal hygiene of the individual (Scholz *et al*, 2003; Pass, 2005). HCMV is present in the body fluid of infected persons, including blood, saliva, urine, cervical secretions, semen and

breast milk (Cannon *et al*, 2011). HCMV may infect infants when passing through the birth canal or upon consuming breast milk from an infected mother (Kurath *et al*, 2010). Newborns often have no symptoms but are at high risk for developing vision, hearing, and neurological problems later in life (Buonsenso *et al*, 2012).

The HCMV envelope glycoprotein B (gB) (UL55) plays a role in viral entry, cell to cell spread and is a major target for neutralizing antibodies (Vanarsdall, *et al*, 2008). HCMV can be classified using nested polymerase chain reaction and restriction fragment length polymorphism (nested PCR-RFLP) based on the highly variable region on protease cleavage sites of the gB gene which exhibits four patterns; gB1, gB2, gB3 and gB4 (Chou and Dennison, 1991).

In Thailand, the seroprevalences of HCMV range from 70.7-100% among

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pregnant women and adult blood donors (Likitnukul *et al*, 2003). HCMV gB genotypes were determined by Bhattarakosol and Chantaraarphonkun (2007) from HCMV-DNA obtained during 2000 - 2004; they found mixed gB genotype in 35%, gB1 in 33%, gB3 in 15% and gB2 in 11%. However, the distribution of HCMV gB genotypes among Thai children has not been reported. This information is important for understanding the epidemiology of HCMV infection in Thailand. In this study, we determined the prevalence of HCMV and gB genotypes among Thai children at Phayathai Babies' Home in Nonthaburi Province, Thailand. We also conducted phylogenetic analysis of the HCMV US28 gene to study genetic diversity (Gong and Padhi, 2011).

#### MATERIALS AND METHODS

We obtained 176 urine samples from Thai children residents of Phayathai Babies' Home, Nonthaburi, Thailand from January 2007 to March 2008. Phayathai Babies' Home is a home for abandoned, orphaned and homeless children. The samples were obtained from children aged 1 month to 5 years, comprised of 69 females and 107 males. This study was approved by the Mahidol University International Review Board (MU-IRB2010/137.1705).

The urine samples were processed with the Axygen<sup>®</sup> AxyPrep body fluid viral DNA/RNA miniprep kit (Axygen<sup>®</sup> Biosciences, SanDiego, CA). The gB genotype was determined using nested PCR-RFLP analysis as described by Chou and Denison (1991). The PCR products were digested with restriction enzymes *Hinf*I and *Rsa*I (New England Biolabs, Hertfordshire, UK) and analyzed with 12% polyacrylamide gel electrophoresis.

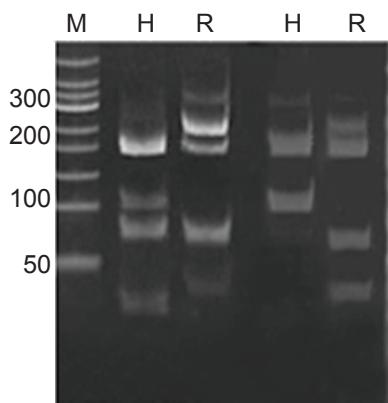
Four gB genotypes were distinguished by different patterns of fragment lengths. A mixed gB genotype was also identified by the fragment length patterns. The PCR products were subjected to nucleotide sequencing analysis (Macrogen, Seoul, Korea). The genotypes of the HCMV were confirmed by homology to published gB genotype sequences from the GeneBank: gB1 (accession numbers: M60927 and M60929); gB2 (accession numbers: M60931 and M60932); gB3 (accession numbers: M60933 and M60934) and gB4 (accession numbers: M60924 and M60926) using the Clustal W program.

The amplified gB products of known gB genotypes were inserted into the pGEM<sup>®</sup>T Easy cloning vector. The plasmids containing a fragment of gB gene were screened by blue-white colony selection and restriction digestion. The selected plasmids were named pgB1, pgB2, pgB3 and pgB4. Plasmid concentration was quantified by spectrophotometer. Different concentrations of plasmids were used to performed PCR amplification. The products were analyzed with 1.7% agarose gel electrophoresis.

The final nucleotide tree was rendered with FigTree V1.3.1 and Molecular Evolutionary Genetics Analysis (MEGA) 4.1.

#### RESULTS

Of the 176 samples examined, 103 (58.5%) were positive for HCMV; 55.1% (38/69) in females and 60.7% (65/107) in males. There were no significant differences by gender. All 4 gB genotypes were found in this population. The gB1 genotype was the most prominent genotype (92/103, 89%), followed by gB2 (3/103, 3%), gB4 (3/103, 3%) and gB3 (2/103, 2%). A mixed gB genotype was seen in



	gB1	gB2	gB3	gB4
<i>HinfI</i>	202,67, 36	202,100	202,97	202,67, 36
<i>RsaI</i>	239,66	239,63	195,63, 41	195,66, 44

Fig 1—Restriction digestion pattern of the mixed gB genotypes. M, DNA molecular weight marker (500 bp marker); H, *HinfI* and R, *RsaI* digestion.

3% (3/103). The mixed patterns included gB2+gB3 and a combination of more than 2 gB genotypes (Fig 1). To study the variations in these samples, phylogenetic analysis was performed on the US28 gene, which is a chemokine-like receptor of the subfamily of G protein-coupled receptors. The phylogenetic analysis classified this population into 4 groups (Fig 2).

To determine the sensitivity of distinct gB amplification, varying amounts of the plasmids containing fragments of the gB gene were used as a template for PCR. The plasmid concentration ranges tested were  $10^5$  to  $10^8$  copies of the gB gene. The gB1, gB3 and gB4 genotypes were detected at a concentration of  $10^6$  copies of the gB gene per microliter (Table 1) while the gB2 genotype was detected at a concentration of  $10^5$  copies per microliter. Thus, the test was more sensitive at detecting the gB2 genotype than the other genotypes.

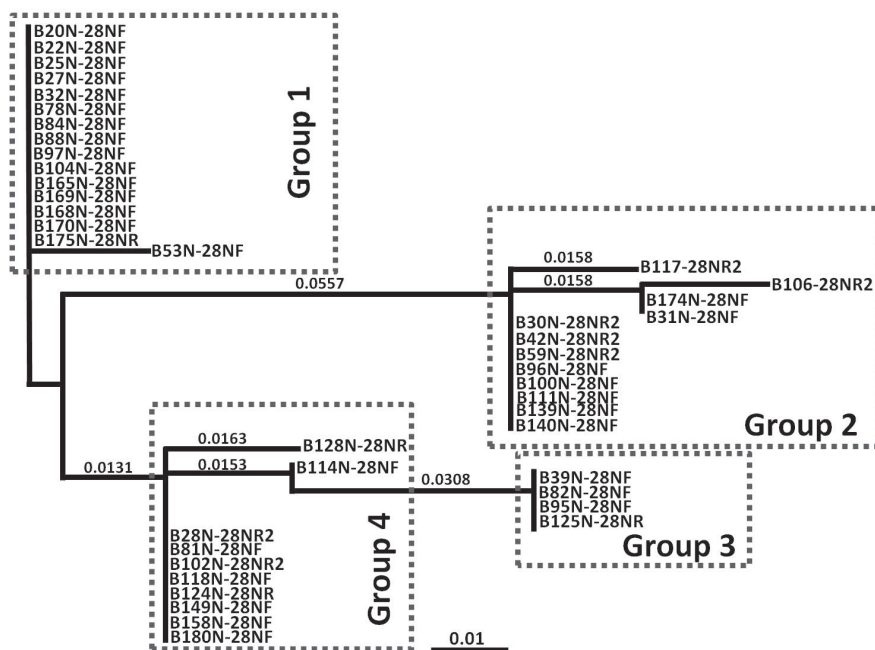


Fig 2—Phylogenetic analysis of the HCMV US28 gene.

Table 1  
Comparison of PCR sensitivity of different gB genes.

DNA	copies of gB gene			
	10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>
gB1	pos	pos	pos	neg
gB2	pos	pos	pos	pos
gB3	pos	pos	pos	neg
gB4	pos	pos	pos	neg

pos, positive; neg, negative.

## DISCUSSION

Seropositivity with HCMV is high among Thai children (33.3%) (Tantivanich *et al*, 1999). Children at day care centers are at high risk of contracting HCMV infection (Joseph *et al*, 2006). The HCMV gB1 genotype was the most common type found in this study, similar to previous studies among children (Barbi *et al*, 2001; Arista *et al*, 2003). The prevalence of the gB1 genotype in our study was higher than previous studies (30-63.5%) (Zawilińska *et al*, 2011; González-Ramírez *et al*, 2012). gB genotypes are classified by nested PCR-RFLP, which is based on the sensitivity of the PCR, which in turn depends on DNA quality, the PCR conditions and specific binding primers. The PCR technique may preferentially amplify a particular genotype over others although the same reagents and conditions were used to conduct the test. This test was the most sensitive in detecting the gB2 genotype (10-fold greater sensitive) which could be the result of more efficient primer binding. Mixed gB genotypes were also observed in our study, similar to previous studies among infants (Renzette *et al*, 2011; Ross *et al*, 2011).

It is unclear whether different HCMV genotypes are associated with different

clinical outcomes. Manuel *et al* (2009) studied 239 patients with HCMV and reported they found no associations between specific genotypes and symptoms of infection. However, Pignatelli *et al* (2011) found genetic variability in HCMV may be involved in pathogenicity and the prognosis among newborns congenitally infected with HCMV. A case report of a mixed infection with gB2/gB3 infection treated with gancyclovir showed dynamic patterns of infection (González-Ramírez *et al*, 2012).

The predominant genotype identified among the children in our study who live at the Phayathai Babies' Home, Nonthaburi, Thailand was gB1. Our study also found mixed infections. Phylogenetic analysis of the US28 gene showed 4 groups.

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