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Characterization of human cytomegalovirus *UL145* and *UL136* genes in low-passage clinical isolates from infected Chinese infants

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

Human cytomegalovirus (HCMV) is a leading cause of morbidity and mortality in immunocompromised individuals. The unique long b' (ULB') region of HCMV contains at least 19 open reading frames (ORFs); however, little is known about the function of *UL145* and *UL136*. We characterized *UL145* and *UL136* in low-passage clinical isolates from Chinese infants.

Material/Methods:

The clinical strains of HCMV were recovered from the urine from HCMV-infected infants. Human embryonic lung fibroblasts (HELFs) were infected with clinical isolates of HCMV, and the viral DNA and mRNA for *UL145* and *UL136* were analyzed by polymerase chain reaction (PCR) and sequencing techniques. We also predicted the structure and function of *UL145* and *UL136* proteins.

Results:

Sixty-two Chinese infants infected with HCMV were recruited into this study and the clinical isolates were recovered from the urine. Two strains among the low-passage isolates, D2 and D3, were obtained. The *UL145* and *UL136* sequences were deposited with GenBank under accession numbers of DQ180367, DQ180381, DQ180377, and DQ180389. The mRNA expression of both *UL145* and *UL136* was confirmed by reverse transcription (RT-PCR) assays. *UL145* was predicted to contain 1 protein kinase C phosphorylation site, 2 casein kinase II phosphorylation sites and a zinc finger structure. *UL136* was predicted to contain a protein kinase C phosphorylation site, *N*-myristoylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site and tyrosine kinase II phosphorylation site. Both *UL145* and *UL136* are highly conserved.

Conclusions:

UL145 may act as an intranuclear regulating factor by direct binding to DNA, while *UL136* may be a membrane receptor involving signal transduction.

Key words:

human cytomegalovirus (HCMV) • *UL145* • *UL136* • clinic isolate • Chinese infant • sequence

Abbreviations:

HCMV - human cytomegalovirus; ORF - open reading frame; PCR - polymerase chain reaction; UL - unique long

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BACKGROUND

Human cytomegalovirus (HCMV), a prototype member of the β -herpesvirus family, is a double-stranded DNA virus, with a genome of ≈ 235 kb encoding approximately 165 functional genes [1–3]. The linear viral DNA core of HCMV located in an icosahedral nucleocapsid is enveloped by a proteinaceous matrix ('tegument'), which is enclosed in a lipid bilayer envelope that contains a number of functionally important glycoproteins such as unique long 83 (UL83), UL82, UL32 and UL99 [4]. HCMV is readily transmitted by direct contact via exposure to body fluids such as saliva, urine, feces, semen, tears and breast milk. The seroprevalence of HCMV infection in human populations ranges from 30% to 90% in developed countries and more than 90% in developing countries [5,6]. In general, infection with HCMV remains benign with no clinical manifestations, or is associated with a self-limited mononucleosis-like syndrome only. While in immunocompromised individuals, such as people with HIV infection or advanced cancer, organ transplant, or bone marrow stem cell transplant, HCMV can be reactivated from latency and is a leading cause of morbidity and mortality. Additionally, congenital HCMV infections are a leading cause of birth defects and infections in children, occurring in 1% to 2% of all live births [6]. Currently available drugs for the treatment of HCMV infection in the immunocompromised host are mainly targeted at the viral DNA polymerase, including ganciclovir, its oral prodrug valganciclovir, cidofovir, and foscavir [7].

The HCMV genome is one of the largest and most complex genomes among the human herpesviruses [3]. A substantial portion of the HCMV genome encodes proteins with the potential to determine virulence through cell tropism, immune evasion, molecular mimicry, or interference with host chemokines. The ULb' region of HCMV genome was found in Toledo and several other low-passage clinical isolates, but was not found in laboratory AD169 strain (X17403) and Towne [8]. The fact that AD169 has attenuated virulence and different tropism for endothelial cells than low-passage clinical isolates suggest that the products of ULb' genes determine the clinical outcomes of HCMV infection.

A small number of HCMV genes have been analyzed in clinical isolates from Chinese neonates, such as *UL128* [9], *UL130* [9], *UL138* [10–12], *UL139* [13], *UL143* [14], *UL145* [15], and *UL148* [16]. However, little is known about the function of both *UL145* and *UL136* genes located in the ULb' region. In this study we investigated the sequence variations and expression of *UL145* and *UL136* in clinical isolates from HCMV-infected Chinese newborns. We also predicted the structure and function of *UL145* and *UL136* proteins using bioinformatics approaches.

MATERIAL AND METHODS

Reagents and instruments

Human embryonic lung fibroblasts (HELFs) were obtained from American Type Culture Collection (ATCC, Manassas, Virginia). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco, and Nonidet P40 from Promega. rTaq, Ex Taq, Moloney murine leukemia virus (M-MLV) buffer, LA Taq, gelatum reclaiming kit, plasmid extraction

kit, and pMD18-T vectors were all purchased from Takara Co. (Tokyo, Japan). The engineered bacterial strain JM109 was from Gene Co., the electrophoresis apparatus from Amersham Pharmacia Biotech (Buckinghamshire, UK), and gelatum imaging system from Multi Genius Co. (Syngene, Cambridge, UK).

Subject selection

Sixty-two infants infected with HCMV were recruited into this study from the Women and Children's Hospitals in Guangzhou from October 2001 to March 2002. Ethics permits were obtained from corresponding institutional human ethics committees. All clinical strains were found to contain detectable HCMV DNA as confirmed by a polymerase chain reaction (PCR) method. Clinical isolates were passaged less than 10 times through HELF cells and stored until use at -70°C for less than 1 week. Urine samples were collected from the infants and 500,000 U/L penicillin 2 ml and 100,000 U/L mycostatin 2 ml were added.

Viral inoculation of HCMV strains

All clinical strains were recovered from urine samples of infants infected with HCMV. Fresh urine (10 ml) from each of the newborns was centrifuged at 1100 g for 15 min. HELFs provided by ATCC were grown in DMEM medium (Gibco) supplemented with 10% fetal calf serum, and used at passage 10 to 20 for HCMV inoculation. An aliquot of urinary supernatant (100 μl) was inoculated into confluent HELF cells (5×10^6) cultured in DMEM medium with 2% fetal calf serum, and adsorbed at 37°C for 30 min. Inoculated fluid was then removed and cells were cultured at 37°C for 24 hr, and 48 hr after the cytopathic effects extended to $>80\%$ of the cells, cells were collected after treatment by 2.5% pepsin and centrifugation at 1000 g for 5 min. Typical cytopathic effects included increased cell size, rounding of infected fibroblasts, and the appearance of granular or dense intracytoplasmic and intranuclear inclusion bodies. The cell pellet was resuspended in culture medium with 10% fetal calf serum, and 10% dimethyl sulfoxide was added, mixed by gentle shaking and stored at -70°C until analysis within the next 10 days.

Viral DNA extraction

To extract viral DNA from infected HELF cells, 20% Nonidet P40 was added to 1 ml virus-infected cell suspension at a 1% final concentration, and vortexed for 30 sec. Protease K (200 $\mu\text{g/ml}$) and sodium dodecyl sulfate (SDS, 0.5%) were added to the mixture and kept at 37°C for 5 hr. The mixture was first purified by phenol and then by chloroform/isoamyl alcohol (24:1, v/v) before precipitation by sodium acetate and absolute alcohol. The resultant pellet was washed with 70% alcohol, centrifuged and dried, and then dissolved in TE buffer [10 mM Tris/HCl pH 8.0 and 1 mM ethylenediaminetetraacetic acid (EDTA)].

Viral DNA amplification by PCR and sequencing of the *UL145* and *UL136* genes

To amplify the *UL145* gene, the PCR reaction mixtures contained 1 μl Ex Taq, 5 μl 10 \times PCR buffer, 6 μl deoxy-nucleotide triphosphate (dNTP) mixture and 2.5 μM for each

Table 1. Paired primer sequences of multiplex PCR for assays of HCMV genes.

Gene	Nucleotide sequence (5'→3')	Primer length (bp)	GC content	Annealing temperature (T _m , °C)	Product length (bp)
<i>LA (US14)</i>					
<i>LA-F</i>	CCTGTCACCCGCTGCTATATTGC	23	52.2%	63.9	209
<i>LA-R</i>	CCACGCAGCGGCCCTTGATGTTT	22	50.0%	60.2	209
<i>IEA</i>					
<i>IEA-F</i>	ATTGTACTCTGAGGATAAGCGGG	23	60.9%	73.7	401
<i>IEA-R</i>	CATTGGTGGTCTTAGGGAAGGC	22	54.5%	62.6	401
<i>UL145</i>					
<i>UL145-F</i>	CTGGCTCATTACTACTCTTCAT	23	43.5%	55.3	401
<i>UL145-R</i>	TCTGTGCAGGTTCTCATCATCA	22	40.9%	55.2	401
<i>UL136</i>					
<i>UL136-F</i>	GAGGAACTCTTGGAAACAGGC	20	55.0%	56.1	1019
<i>UL136-R</i>	TCAGCACGAGCATCACGC	18	61.1%	59.1	1019

IE – immediate early; IEA – immediate early antigen; F – forward; LA – late antigen; R – reverse; UL – unique long; US – unique short.

one, 5 µl template, 1 µl (10 pmol/µl) for *UL145-F*/*UL145-R* primers designed by Primer Premier 5.0 (Table 1), and 31 µl sterile deionized water. The mixture was incubated at 94°C for 4 min followed by 30 cycles of incubation at 94°C for 1 min, 53°C for 30 sec, and 72°C for 30 sec. Thereafter, the mixture was incubated for at 72°C 10 min. The PCR products were separated in 1.5% agarose gels.

The PCR incubation for *UL136* contained 1 µl LA Taq, 5 µl 10×PCR Buffer, 6 µl dNTP mixture and 2.5 µM for each one, 5 µl template, 1 µl (10 pmol/µl) for *UL136-F*/*UL136-R* primers, and 31 µl sterile deionized water. The PCR conditions were 94°C 4 min, followed by 30 cycles of 94°C for 1 min, 53°C for 30 sec, and 72°C for 30 sec. The 30 cycles were followed by 72°C for 10 min. The PCR production was loaded in 1.2% agarose gels. After electrophoresis, the 2 gene PCR products were purified using a gel reclaiming kit (Takara Co., Tokyo, Japan), then the fragments were cloned into the vector pMD18-T (Takara Co., Tokyo, Japan). The recombinants were transformed into engineering bacteria *E. coli*, JM109. The solid LB agar plate treated with ampicillin was used to screen the recombinants.

DNA sequencing

The plasmids and PCR products were subject to gel electrophoresis. Bacterial culture fluid containing positive recombinants was sequenced using an ABI 3730 automated sequencer (Applied Biosystem, Carlsbad, CA). The *UL145* and *UL136* genes were sequenced bilaterally to ensure accurate alignments of nucleotide position. Each sequence was verified in a separate amplification with negative control (water). The sequences were submitted to the GenBank sequence database provided by the National Center for Biotechnology Information (NCBI).

Analysis of nucleotide and amino acid sequences of *UL145* and *UL136*

Using BioEdit 7.0.1 (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>), Clustalx V2.0 (<http://www.clustal.org/>) and MEGA (Molecular Evolutionary Genetics Analysis) 4 (<http://www.megasoftware.net/>), we conducted alignment of nucleotide and amino acid sequence, construction of cladogram and analysis of physical and chemical property of proteins, respectively. We applied nucleotide-nucleotide BLAST (blastn) and protein-protein BLAST (blastp) to analyze nucleic acid sequence and amino acid sequence homology, respectively. ProtParam (<http://au.expasy.org/prosite/>) was used to predict the biological function of *UL145* and *UL136* proteins.

Viral RNA extraction and determination of *UL145* and *UL136* mRNA by reverse transcription polymerase chain reaction (RT-PCR)

The HCMV-infected cells were centrifuged at 3000 *g* for 25 min. The supernatant was treated with Trizol (1 ml per 1×10⁷ cells) for RNA extraction. Progressing formaldehyde denatured agarose electrophoresis confirmed RNA integrity and lack of DNA contamination. The 50-µl RNase-free RNA purification system contained 20 µl total viral RNA, 10 µl 10×DNase I buffer, 5 µl DNase I (RNase-free), 1 µl RNase and 14 µl diethyl pyrocarbonate (DEPC)-treated water.

Paired RT-PCR primers were designed for *UL145* and *UL136* (Table 2). The incubation was performed in a waterbath at 37°C for 30 min, and then mixed with 50 µl DEPC-treated water. It was purified with phenol/chloroform (1/1, v/v) and then with chloroform/isoamyl alcohol (24:1, v/v). The pellet was dissolved with 20 µl DEPC-treated water, followed by formaldehyde-denatured agarose electrophoresis. The

Table 2. Paired primers of RT-PCR for HCMV *UL145* and *UL136* genes.

Gene	Sequence (5'→3')	Length	GC content	Annealing temperature (T _m value, °C)
<i>UL145</i>				
<i>UL145-F</i>	ATGTACGGCGTCTGGCTCATT	22 bp	54%	56
<i>UL145-R</i>	TCAATCTTCACTTCCACCCATCG	23 bp	47%	54
<i>UL136</i>				
<i>UL136-F</i>	GAATGTCGGCTACGGGTGT	19 bp	57.9%	57.3
<i>UL136-R</i>	TGTCTCGCAACTGTCTCTG	19 bp	57.9%	57.3

F – forward; R – reverse; UL – unique long.

Table 3. Disease distribution of the 62 HCMV-infected infants.

Disease	Number of cases
Neonatal jaundice	23 (37.1%)
Infant hepatitis syndrome	11 (17.7%)
Microcephaly	6 (9.7%)
Cerebral dysgenesis	5 (8.1%)
Cerebral palsy	5 (8.1%)
Mental retardation	2 (3.2%)
Epilepsy	2 (3.2%)
Congenital deafness	2 (3.2%)
Hearing impairment	2 (3.2%)
Hydrocephalus	2 (3.2%)
Hemolytic anemia	1 (1.6%)
Premature and low birthweight	1 (1.6%)
Total	62 (100.0%)

RNase-free PCR canoula 10 µl purified RNA of HCMV and 2 µl IEC-F/IEC-R (Table 1). After incubation at 65°C for 15 min, the mixture was placed on ice for 10 min. The viral RNA was further incubated with 5 µl dNTP, 5 µl 5× M-MLV buffer, 1 µl M-MLV, 0.5 µl RNasin (a ribonuclease inhibitor purified from human placenta) and 1.5 µl DEPC-treated H₂O at 42°C for 1 hr followed by incubation at 95°C for 10 min, and then was stored at –20°C.

Statistical analysis

Data of proportions were analyzed using the χ^2 test and a *P* value of <0.05 was considered significant.

RESULTS

Clinical characteristics of the study population

Out of 62 cases, 33 were male and 29 female. The average age of infected infants was 46 days, ranging from 3 to 148

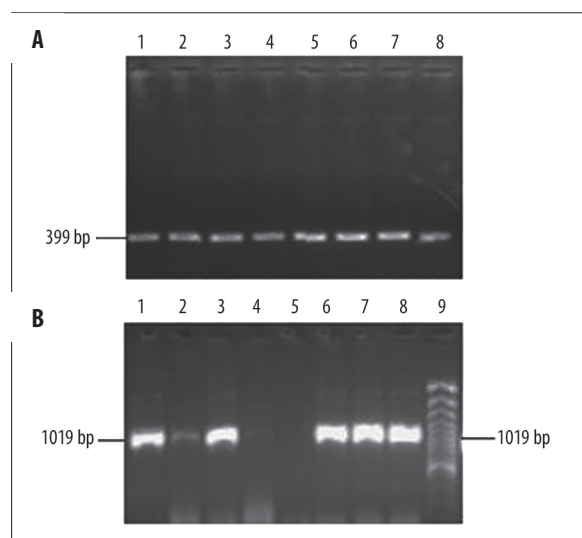


Figure 1. PCR products for the recombinant HCMV plasmids cloned from low-passage D2 and D3 isolates for *UL145* (A) and *UL136* (B). A band of 399 bp was detected in Plot A and a band of 1019 bp was found in Plot B. Lanes 1–4: DNA products of recombinant plasmids from D2 isolates; Lanes 5–8: DNA products of recombinant plasmids from D3 isolates. Lane 9 is a DNA marker.

days. There were 27 cases aged from 3 to 14 days, 12 cases from 14 to 28 days, and 23 cases from 28 to 148 days. The 62 HCMV clinical strains were from 23 infants with jaundice, 11 with hepatitis syndrome, 6 with microcephaly, 5 with cerebral dysgenesis, and 5 with cerebral palsy (Table 3).

HCMV low-passage clinical strains and analysis of the *UL145* and *UL136* genes

Among the 62 low-passage clinical isolates of HCMV, specific DNA bands were detected by PCR assays, while no viral DNA bands were found in the negative control. Two HCMV-specific conservative genes, late antigen (*LA*) and immediate early antigen (*IEA*), were detected in all HCMV-infected isolates. Two specific bands 209 and 401 bp long were detected in our PCR assays (data not shown). These 2 bands were not found in negative controls. Of 62 low-passage clinical strains, 2 (D2 and D3) were further analyzed. Their sequences have

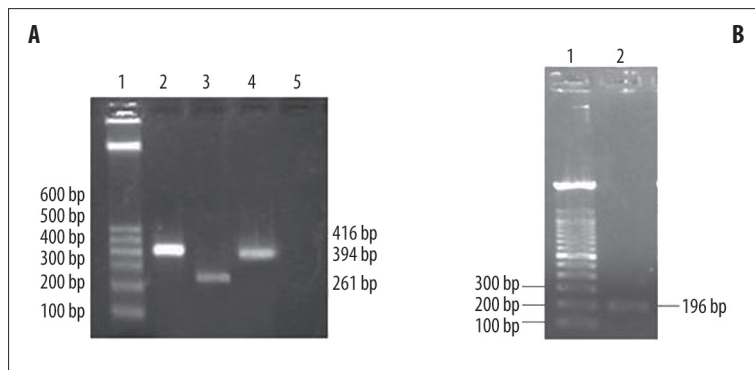


Figure 2. The mRNA expression of the *UL145* (A) and *UL136* (B) genes in D2/D3 isolates determined by RT-PCR. Plot A: Lane 1: DL100 DNA marker; Lane 2: PCR product of IE gene; Lane 3: RT-PCR product of IE gene; Lane 4: PCR product of the *UL145* gene; and Lane 5: negative control. Plot B: Lane 1: DL100 DNA marker; Lane 2: PCR product of the *UL136* gene.

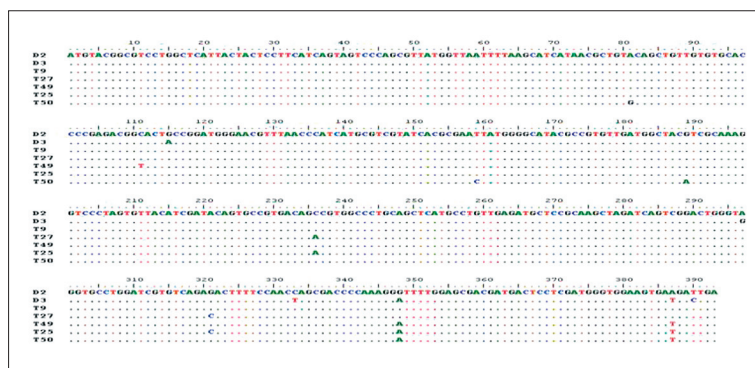


Figure 3. Comparison of the open reading frame (ORF) of the *UL145* gene among various strains.

been deposited into GenBank under accession numbers of DQ180367, DQ180381, DQ180377, and DQ180389.

The *UL145* and *UL136* genes were successfully amplified by PCR methods and sequenced from the D2 and D3 strains (Figure 1). The *UL145* PCR product was 399 bp long, and that for *UL136* was 1019 bp in length.

The *UL145* and *UL136* genes were highly conserved in all clinical strains. The nucleotide and amino acid identity was >96.3%. The clinical strains from infants with jaundice and anomalies in the central nervous system were distributed randomly in the 2 genes. There was no significant relationship between the clinical signs and symptoms and the *UL145* and *UL136* variations.

mRNA expression, homology analysis and functional prediction of HCMV *UL145* and *UL136* genes

The mRNA of the *UL145* and *UL136* genes in D2 and/or D3 isolates was determined using an RT-PCR technique (Figure 2). A specific band of 196 bp for *UL136* mRNA and 394 bp for *UL145* mRNA was detected in our RT-PCR assays.

The *UL145* open reading frame (ORF) sequence of D2 and D3 strains had high homology with 10 strains from GenBank, including T9, T8, 9J, 20M, T27, T49, U253, 8J, T25 and T50 (Figure 3). The *UL136* ORF sequence of D2 and D3 showed high homology with 11 strains (4J, 51C, 39J, 33J, 63J, 22M, 10J, 32C, 29C, 27C, and Toledo) (Figure 4).

The *UL145* gene cloned from the D2 and D3 isolates was 393-bp long (GenBank accession No.: DQ180367). The sequence of *UL145* cloned in this study showed high conservation,

and the aberration rate was 0.7–1.5% compared to other reference strains ($n=10$). Its predicted amino acid sequence also showed high conservation, and the aberration rate was 0.7–2.2% compared to the reference strains ($n=11$).

The *UL136* gene cloned from the D2 isolates was 723-bp long (GenBank accession No.: DQ180377). *UL136* was deduced to encode a protein of 240 amino acids. Compared to other reference strains, the sequence of *UL136* was highly conserved, with aberration rates of 1.8–2.9% (Figure 5). A total of 30 out of 1019 nucleotides in *UL136* from the D2 isolates showed variations. In terms of amino acid sequence, the protein encoded by the *UL136* gene from the D2 isolates had 14 variations and the mutation rate was 1.6–3.7% compared to other strains.

We studied the genetic evolution and sequence variations of *UL136* when the *UL136* gene of Toledo was employed as the root using the MEGA 4 program (<http://www.megasoftware.net/>). A phylogenetic tree was constructed using the nucleotide sequence of the *UL136* gene from the D2 isolates and 11 of those previously published in GenBank. The cladogram is shown in Figure 6. Three groups (clades) were found from the tree, but HCMV strains did not cluster preferentially based on the measure of divergence. A further analysis of the phylogenetic tree of *UL145* and *UL136* did not show any preferential clustering of clinical isolates (data not shown). The assessment of the genetic distance in HCMV strains in different groups of patients revealed an even distribution of viral sequences.

The deduced amino acid numbers were 131 for *UL145* and 241 for *UL136*. On-line secondary structure anticipation and predicted isoelectric point (IP) of proteins encoded by the

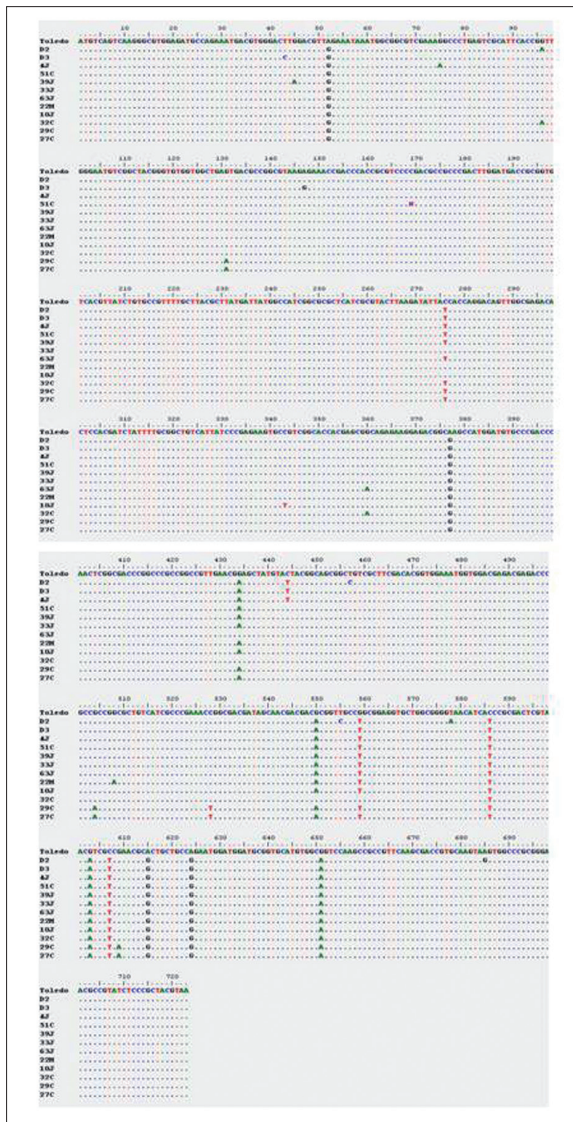


Figure 4. Comparison of the open reading frame (ORF) of the UL136 gene among various strains.

UL145 gene from various strains is shown in Table 4 and Table 5, respectively. In the predicted secondary structure of UL145, 53 amino acids participated in α -helix formation, 23 residues formed extended strands, and 55 residues generated random coils. UL145 was predicted to contain 1 protein kinase phosphorylation site located at residues 64–66 and 2 casein kinase II phosphorylation sites located at residues 119–122 and 128–131. It might also contain a zinc finger at positions of 37 to 88.

An on-line anticipation of the post-translationally modified sites of the UL136 protein showed that it might contain a protein kinase C phosphorylation site, an *N*-myristoylation site, a cAMP- and cGMP-dependent protein kinase phosphorylation site and a tyrosine kinase II phosphorylation site. Compared to UL136 in Toledo strain, the UL136 protein in our D2 strain was predicted to contain an additional protein kinase C phosphorylation site located at residues 151–153, a tyrosine kinase phosphorylation site located at residues 141–149, and an amidation site located at residues

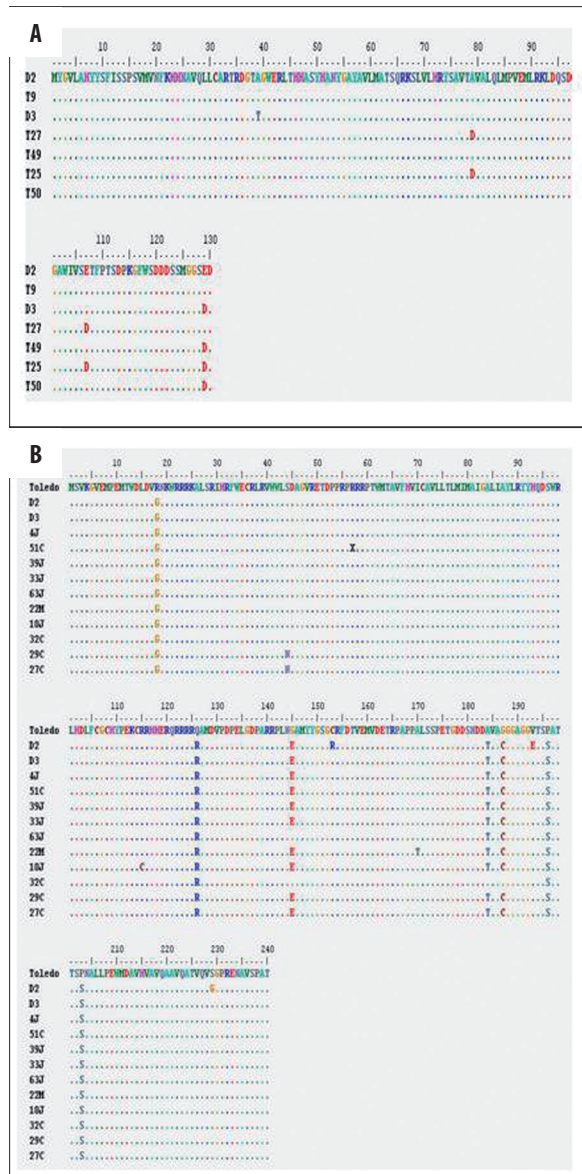


Figure 5. Comparison of amino acid sequences encoded by HCMV UL145 (A) and UL136 (B) genes. The deduced amino acid number was 131 and 241 for UL145 and UL136, respectively.

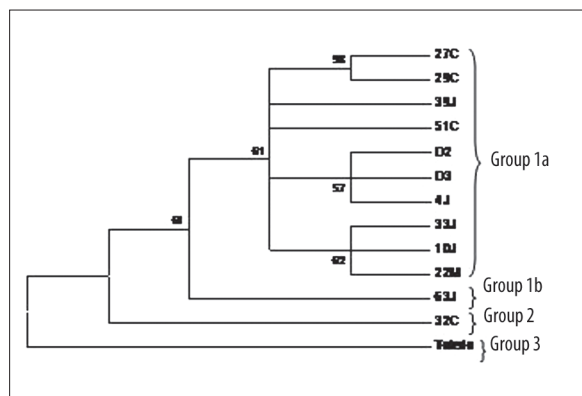


Figure 6. The cladogram of UL136 sequences from various HCMV strains.

Table 4. Predicted amino acid number of secondary structures in HCMV UL145 protein.

Isolate	D2	D3	T9	T27	T49	T25	T50
α -Helix	53	52	53	44	53	44	53
Extended strand	23	23	23	26	23	26	23
Random coil	54	55	54	60	54	60	54

Table 5. Predicted isoelectric point (IP) values of HCMV UL145 protein.

Isolate	D2	D3	T9	T27	T49	T25	T50
IP	6.64	6.64	6.64	6.35	6.64	6.35	6.64

Table 6. Predicted amino acid number of secondary structures in HCMV UL136 protein.

Strain	4J	51C	39J	33J	63J	22M	10J	32C	29C	27C	D2	D3	Toledo
α -Helix	106	106	106	106	99	106	103	99	106	106	107	106	99
Extended strand	18	18	18	18	19	18	18	18	18	18	17	18	18
Random coil	116	116	116	116	122	116	119	123	116	116	116	116	123

Table 7. Predicted isoelectric point (IP) values of HCMV UL136 protein.

Strain	4J	51C	39J	33J	63J	22M	10J	32C	29C	27C	D2	D3	Toledo
IP	8.20	7.66	8.20	8.20	8.52	8.20	7.63	8.58	8.20	8.20	8.26	8.20	8.85

151–154. The predicted secondary structure of UL136 from D2 isolates contained α -helices formed from 107 amino acids, β -folding formed from 17 residues and random coils generated from 116 residues, which were different among various strains. The predicted IP values and amino acid number of secondary structures of the UL136 protein in various strains are shown in Table 6 and Table 7, respectively.

DISCUSSION

HCMV infection can cause an array of damaging clinical effects in the fetus, neonate and immunocompromised patients [2]. When occurring during pregnancy, HCMV can lead to neonate intrauterine infection, and 10% of infected neonates show symptomatic diseases and congenital birth defects such as fetal anomaly, deafness, mental retardation, microcephaly and hydrocephalus [17]. Congenital HCMV infection causes severe morbidity and mortality in newborns and is the major infectious cause of deafness and developmental anomalies in the central nervous system of children [17]. In this study, we have isolated 62 low-passagen strains from HCMV-infected Chinese infants. Among these HCMV-infected Chinese infants, 37.1% (23/62) of them developed jaundice and 30.6% (18/62) of them had developmental defects in the central nervous system.

Currently, the mechanism for the reactivation and pathogenesis of HCMV is not fully understood; however, it has been suggested that the host's cellular immune responses may be associated with the genes related to the viral ability of avoiding organism immune aggression, virulence, and viral tissue and cell tropism [2]. The ULb' region, the unique structure of HCMV low-passagen clinic isolates, is thought to determine the pathogenicity of HCMV. Several genes in this region have been found to encode products that can determine the interactions of HCMV-host and the clinical outcomes. Among 19 putative ORFs within this region, some functional genes have already been identified: *UL146*, encoding a viral α -chemokine (CXC-1) that induces calcium mobilization, chemotaxis, and degranulation of neutrophils [18]; *UL144*, encoding a putative tumor necrosis factor (TNF) receptor [19]; *UL141*, encoding a modulator of the natural killer (NK) cell-activating ligand CD155 [20]; and *UL142*, encoding an MHC class I-like molecule that inhibits NK cellular lysis [21]. The *UL131A-128* locus encodes products determining endothelial, epithelial, and dendritic cell tropism [22–24]. In addition, putative chemokine motifs have been shown in *UL130* and *UL128* [23,25,26]. The ability to infect endothelial cells and leukocytes is a nonessential virus-encoded function and is characteristic of clinical HCMV isolates, but it is not found in reference laboratory strains such as AD169, Towne, and Davis [27].

In AD169 and Towne, the loss of endothelial cell tropism and leukocyte transmissibility was associated with mutation of the *UL131A*, *UL130*, and *UL128* genes [23]; extensive fibroblast propagation of an endothelial cell tropic clinical isolate (VR6110) resulted in a tropism-deficient variant, probably due to the deletion of *UL132-130* within the ULb' region [28]. *UL128*, *UL130*, and *UL131* may participate in the entry of HCMV into epithelial and endothelial cells, since their mutations abolished the ability of epithelial and endothelial cell tropism [23,24]. It appears that *UL128*, *UL130*, and *UL131* must all bind simultaneously onto gH/gL, 2 viral glycoproteins, to form functional complexes that can mediate entry into epithelial and endothelial cells [29]; however, data are limited about the function of *UL145* and *UL136*.

This study demonstrated that all the strains contained *UL145* and *UL136* as determined by PCR and sequencing analysis. Both *UL145* and *UL136* were shown to be highly conserved. Compared with other genes such as *UL144* and *UL146* that are lesser conserved, *UL145* and *UL136* may be essential for HCMV replication and spread in various organs and for various damaging clinical effects of HCMV infection. Further studies are needed to explore the role of both *UL145* and *UL136* in the pathogenesis of HCMV infection.

We predicted the properties of the proteins encoded by *UL145* and *UL136* using a bioinformatics approach. The theoretical IP of *UL145* was about 6.4, indicating that *UL145* was an acidic protein; whereas that of *UL136* was about 8.2, indicating that *UL136* was an alkaline protein. The result also showed that amino acid numbers and IP values varied among different strains. The polymorphism of *UL145* and *UL136* would affect the physico-chemical properties of their coded proteins.

A prediction of the post-translational modification site of *UL145* suggested a lack of terminal signal peptide and membrane-spanning domain. Thus, *UL145* may not be a secretory protein. Similar to *UL141*, *UL142* and *UL147*, *UL145* appears to contain PKC and casein kinase II phosphorylation sites. The amino acid sequence, ranging from 37 to 88, may contain a zinc finger structure. The *UL145* protein may interact with nucleic acid by direct binding.

In this study, *UL136* was predicted to contain a protein kinase C phosphorylation site, *N*-myristoylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site and tyrosine kinase II phosphorylation site. These kinds of sites are related to membrane receptor-mediated signal transduction. Thus, the *UL136* protein may participate in signaling pathways.

Compared to known sequences of *UL136* from other strains in GenBank, *UL136* is highly conserved. Compared to the Toledo strain, there were 18 different nucleotides in *UL136* from the D2 isolates, which were dispersed within the coding regions located at positions 52, 96, 276, 377, 434, 444, 457, 550, 555, 559, 578, 586, 603, 607, 615, 624, 651, and 685. This would result in 6 substitutions in the amino acid residues in *UL136*, namely 126Q→R, 145G→E, 184A→T, 193V→E, 196P→S and 229S→G. Changes of amino acid residues at these positions are not supposed to significantly affect the function of *UL136*.

CONCLUSIONS

In conclusion, both *UL145* and *UL136* are highly conserved and may be functionally important for the proliferation and spread of HCMV. *UL145* may act as an intranuclear regulating factor by direct binding to DNA, while *UL136* may serve as a membrane receptor involved in signal transduction. Further investigation is warranted to determine their role in clinical pathogenesis of HCMV infection and their therapeutic potential by interfering with the action of *UL145* and *UL136*.

REFERENCES:

1. Davison AJ, Dolan A, Akter P et al: The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J Gen Virol*, 2003; 84: 17–28
2. Crough T, Khanna R: Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev*, 2009; 22: 76–98, Table of Contents
3. Dolan A, Cunningham C, Hector RD et al: Genetic content of wild-type human cytomegalovirus. *J Gen Virol*, 2004; 85: 1301–12
4. Chen DH, Jiang H, Lee M et al: Three-dimensional visualization of tegument/capsid interactions in the intact human cytomegalovirus. *Virology*, 1999; 260: 10–16
5. Cannon MJ, Schmid DS, Hyde TB: Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol*, 2010; 20: 202–13
6. Syggelou A, Iacovidou N, Kloudas S et al: Congenital cytomegalovirus infection. *Ann N Y Acad Sci*, 2010; 1205: 144–47
7. Andrei G, De Clercq E, Snoeck R: Drug targets in cytomegalovirus infection. *Infect Disord Drug Targets*, 2009; 9: 201–22
8. Cha TA, Tom E, Kemble GW et al: Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *J Virol*, 1996; 70: 78–83
9. Sun ZR, Ji YH, Ruan Q et al: Structure characterization of human cytomegalovirus *UL131A*, *UL130* and *UL128* genes in clinical strains in China. *Genet Mol Res*, 2009; 8: 1191–201
10. Grainger L, Cicchini L, Rak M et al: Stress-inducible alternative translation initiation of human cytomegalovirus latency protein pUL138. *J Virol*, 2010; 84: 9472–86
11. Petrucelli A, Rak M, Grainger L, Goodrum F: Characterization of a novel Golgi apparatus-localized latency determinant encoded by human cytomegalovirus. *J Virol*, 2009; 83: 5615–29
12. Tey SK, Goodrum F, Khanna R: CD8+ T-cell recognition of human cytomegalovirus latency-associated determinant pUL138. *J Gen Virol*, 2010; 91: 2040–48
13. Qi Y, Mao ZQ, Ruan Q et al: Human cytomegalovirus (HCMV) *UL139* open reading frame: Sequence variants are clustered into three major genotypes. *J Med Virol*, 2006; 78: 517–22
14. Wang B, Li YQ, Su HH et al: Human cytomegalovirus *UL143* gene polymorphisms in low-passage clinical isolates in Guangzhou. *J Trop Med*, 2008; 8: 327–31
15. Hu JJ, Wang B, Su HH et al: Polymorphism analysis of HCMV *UL145* gene of the clinical isolates from Guangzhou congenitally infected infants. *Maternal & Child Health Care of China*, 2010; 25: 2862–65
16. Wang B, Li YQ, Hu JJ et al: Identification and expression of human cytomegalovirus *UL148* mRNA of low-passage clinical isolates in Guangzhou. *Med J China*, 2008; 88: 1693–96
17. Ross SA, Boppa SB: Congenital cytomegalovirus infection: outcome and diagnosis. *Semin Pediatr Infect Dis*, 2005; 16: 44–49
18. Penfold ME, Dairaghi DJ, Duke GM et al: Cytomegalovirus encodes a potent α -chemokine. *Proc Natl Acad Sci USA*, 1999; 96: 9839–44
19. Benedict CA, Butrovich KD, Lurain NS et al: Cutting edge: a novel viral TNF receptor superfamily member in virulent strains of human cytomegalovirus. *J Immunol*, 1999; 162: 6967–70
20. Tomasec P, Wang EC, Davison AJ et al: Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus *UL141*. *Nat Immunol*, 2005; 6: 181–88
21. Wills MR, Ashiru O, Reeves MB et al: Human cytomegalovirus encodes an MHC class I-like molecule (*UL142*) that functions to inhibit NK cell lysis. *J Immunol*, 2005; 175: 7457–65

22. Gerna G, Percivalle E, Lilleri D et al: Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131-128 genes and mediates efficient viral antigen presentation to CD8+ T cells. *J Gen Virol*, 2005; 86: 275–84
23. Hahn G, Revello MG, Patrone M et al: Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. *J Virol*, 2004; 78: 10023–33
24. Wang D, Shenk T: Human cytomegalovirus virion protein complex required for epithelial and endothelial cell tropism. *Proc Natl Acad Sci USA*, 2005; 102: 18153–58
25. Akter P, Cunningham C, McSharry BP et al: Two novel spliced genes in human cytomegalovirus. *J Gen Virol*, 2003; 84: 1117–22
26. Novotny J, Rigoutsos I, Coleman D, Shenk T: In silico structural and functional analysis of the human cytomegalovirus (HHV5) genome. *J Mol Biol*, 2001; 310: 1151–66
27. Gerna G, Percivalle E, Baldanti F, Revello MG: Lack of transmission to polymorphonuclear leukocytes and human umbilical vein endothelial cells as a marker of attenuation of human cytomegalovirus. *J Med Virol*, 2002; 66: 335–39
28. Grazia Revello M, Baldanti F, Percivalle E et al: *In vitro* selection of human cytomegalovirus variants unable to transfer virus and virus products from infected cells to polymorphonuclear leukocytes and to grow in endothelial cells. *J Gen Virol*, 2001; 82: 1429–38
29. Ryckman BJ, Rainish BL, Chase MC et al: Characterization of the human cytomegalovirus gH/gL/UL128-131 complex that mediates entry into epithelial and endothelial cells. *J Virol*, 2008; 82: 60–70