Genetic variability of HCMV strains isolated from Polish pregnant women, their fetuses and newborns

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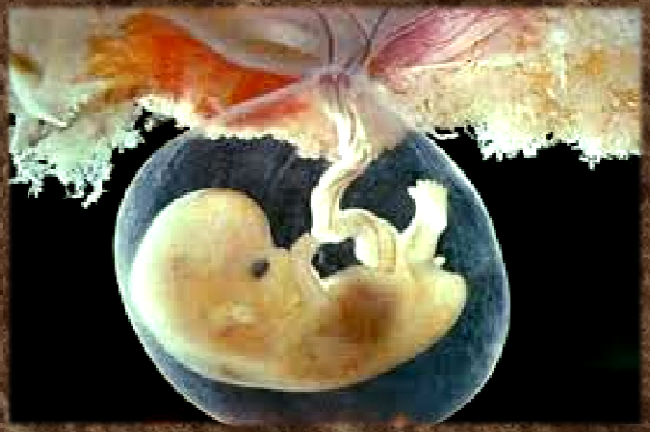
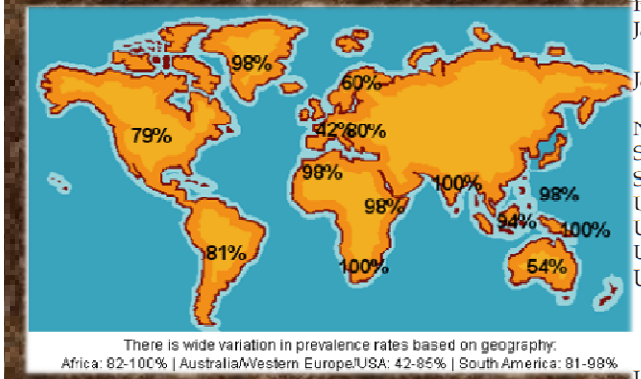


Seroprevalence of HCMV infections

Prevalence from 40% to 100% varying between continents and countries

The most common etiologic agent of intrauterine infections in fetuses (0.2% to 2.2% of all live births) and a major cause of infection induced hearing loss and mental retardation



There is wide variation in prevalence rates based on geography:
Africa: 82-100% | Australia/Western Europe/USA: 42-85% | South America: 81-98%

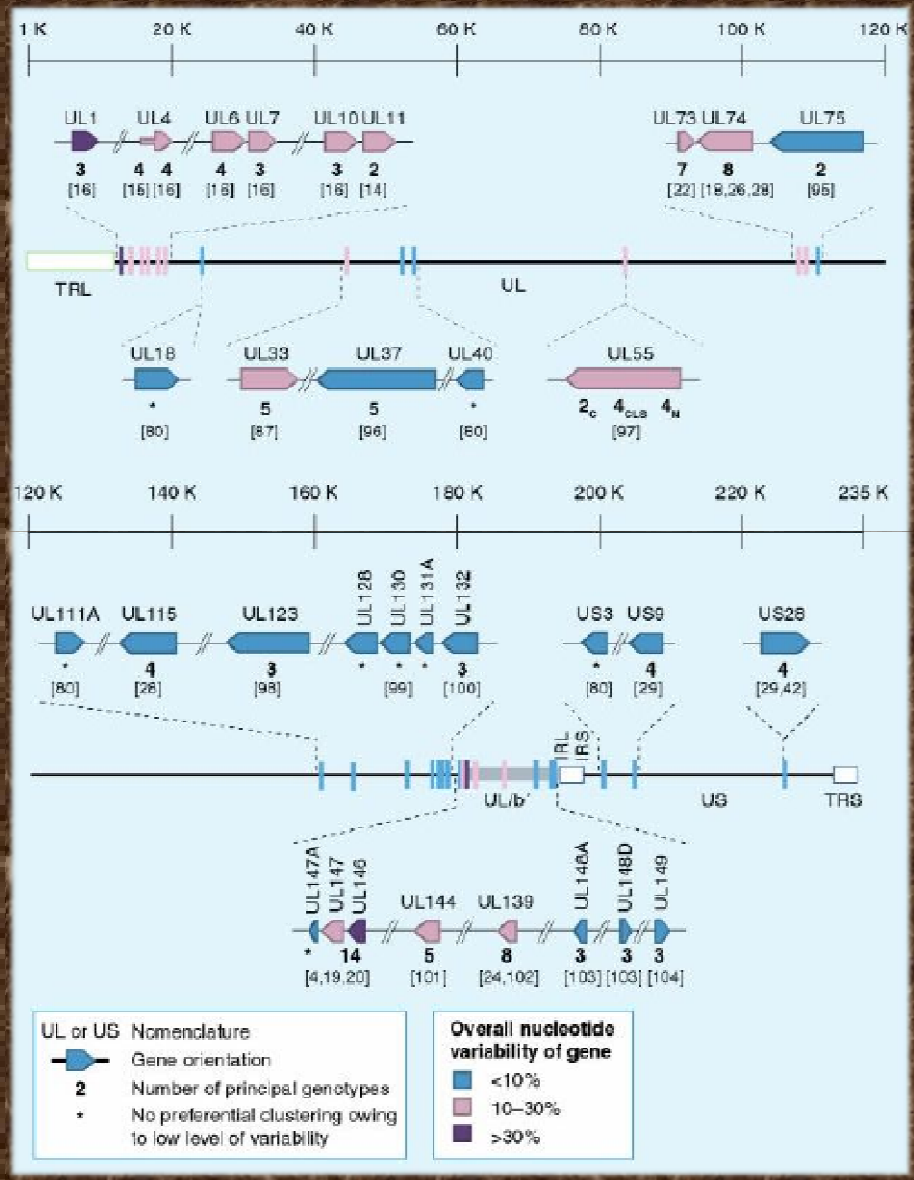
http://www.abbottdiagnostics.com.au/Your_Health/Infectious_Diseases/CMv/

Country	Study years	Population/sampling method	Risk factors for seroconversion	CMV seroprevalence in sampled population	Number of seroconverters	Number of seronegatives followed	Annual seroconversion rate (estimated mean follow-up)[a]
Belgium [39]	1988–1990	Pregnant women	None reported	51%	20	861	4.0% (30 weeks)
Belgium [40]	1996–2003	Pregnant women	None reported	57%	44	3098	2.5% (30 weeks)
Finland [41]	2000	Pregnant women	None reported	56%	1	244	0.7% (30 weeks)
France [42]	1992–1999	Pregnant women	Hospital of birth	45%	152	10780	2.4% (30 weeks)
France [43]	1995–1996	Pregnant women	None reported	44%	5	2320	1.1% (10 weeks)
Italy [44]	1992–1999	Pregnant women	None reported	71%	7	299	4.1% (30 weeks)
Japan [18]	Pre-1983	Pregnant women	<1 Previous pregnancy	94%	10	77	22.5% (30 weeks)
Japan [17]	1989–1997	Pregnant women	CMV-seropositive husband	84%	8	122	14.8% (23 weeks)
Norway [45]	1973–1974	Pregnant women	None reported	40%	15	1204	2.2% (30 weeks)
Scotland [46]	1975–1978	Pregnant women	None reported	54%	13	1841	1.2% (30 weeks)
Sweden [47]	1977–1979	Pregnant women	None reported	72%	14	1175	2.1% (30 weeks)
UK [48]	Pre-1973	Pregnant women	Asian ethnicity	67%	11	270	7.1% (30 weeks)
UK [49]	1975–1979	Pregnant women	None reported	57%	14	1608	1.5% (30 weeks)
UK [50]	1975–1982	Pregnant women	None reported	58%	32	3716	1.8%
UK [51]	1999	Pregnant women. Aged <30 or have a pre-school aged child	None reported	58%	1	152	1.1% (30 weeks)
US-Alabama [52]	1978–1984	Pregnant women	Middle/upper income (higher number of previous pregnancies)	54%	77	4692	2.5%
			Low income	77%	19	507	6.8%
US-Alabama [53]	Pre-1983	Pregnant women	Urban middle/upper income	60%	29	2056	1.2%
			Rural	70%	4	245	1.6%
			Urban lower income	85%	4	328	2.2%

Hyde TB et al. (2010) *Rev Med. Virol.* 20: 311-326

Genetic variability of HCMV

Approximately 20 HCMV ORFs encoding viral envelope glycoproteins B (gB, *UL55*), H (gH, *UL75*) and N (gN, *UL73*), and chemokines and chemokine receptors, as well as TNF-α receptor (pUL144, *UL144*)



Puchhammer-Stöckl E and Görzer I. (2011) *Future Virol.* 6(2): 259-271

HCMV glycoprotein B (gB)

The major HCMV envelope protein, important for viral replication *in vivo* and *in vitro* as well as host cell entry, cell-to-cell virus transmission, and fusion of infected cells

The site at position 460 cleaved by cellular endoprotease

The region between 448 and 481 codons including the area of the highest genetic variability of *UL55*

ORF	Gene product	Function	Essential for replication *in vitro*	Structural	Immune/response		Genotypes	Overall variability (%)[a]	Main polymorphic regions
					Neutralising antibodies	Cell mediated			
UL55	gB (gC-I)	Attachment, fusion, virus spread	Yes	Yes	+	+	gB-1, gB-2, gB-3, gB-4 (rare gB-5, gB-6, gB-7)	9.5	aa27-68; aa181-195; aa311-322; aa387-399; aa448-481

Pignatelli S et al. (2004) *Rev Med Virol.* 14(6):383-410

HCMV *UL55* genotypes in congenital cytomegaly

The gB1 genotype of HCMV most commonly observed in congenital cytomegaly worldwide

Eur J Clin Microbiol Infect Dis (2012) 31:1335–1345
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ARTICLE

Distribution of UL144, US28 and UL55 genotypes in Polish newborns with congenital cytomegalovirus infections

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Abstract Human cytomegalovirus (HCMV) is the most common congenital infection. HCMV strains display genetic variability in different regions. Distribution of HCMV genotypes in the population of congenitally infected newborns from Central Poland and viral load in newborns' blood is described and discussed. HCMV determined. Most of the newborns had identical virus genotype, gB2 (96%), UL144 B1 (88%) and US28 A2 (84%). These genotypes were detected in all newborns with asymptomatic congenital infection. The occurrence of UL144 B1 or US28 A2 genotypes in the babies examined was significant in comparison to other genotypes (p=0.0002 and

PRZEGL EPIDEMIOL 2011; 65: 409 - 413 **Problemy zakażeń**

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RÓŻNORODNOŚĆ GENOTYPOWA UL55 SZCZEPÓW WIRUSA CYTOMEGALII IZOLOWANYCH OD NOWORODKÓW I NIEMOWLĄT HOSPITALIZOWANYCH W POLSCE POŁUDNIOWEJ

UL55 GENOTYPE DIVERSITY OF CYTOMEGALOVIRUS STRAINS ISOLATED FROM NEWBORNS AND INFANTS HOSPITALIZED IN SOUTHERN POLAND

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STRESZCZENIE

W badaniach nad zakażeniami cytomegalowirusem (HCMV) coraz częściej zwraca się uwagę na różnice w patogenności, tropizmie i wirulencji tego wirusa w zależności od jego genotypu.

Celem pracy była ocena występowania poszczególnych genotypów gB kodowanych w regionie UL55 genomu HCMV, w populacji noworodków i niemowląt z Polski południowej.

ABSTRACT

Studies on cytomegalovirus (HCMV) infections more often draw attention to the differences in tropism, pathogenicity and virulence of the virus depending on its genotype.

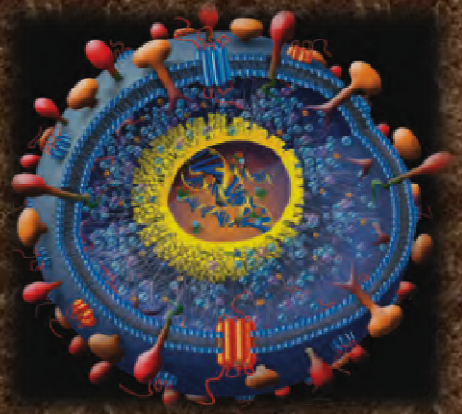
The aim of this study was to assess the individual gB genotypes which are encoded in UL55 region of HCMV genome in a population of newborns and infants from Southern Poland.

Single gB1 genotype of HCMV identified in congenital infections from Southern Hungary

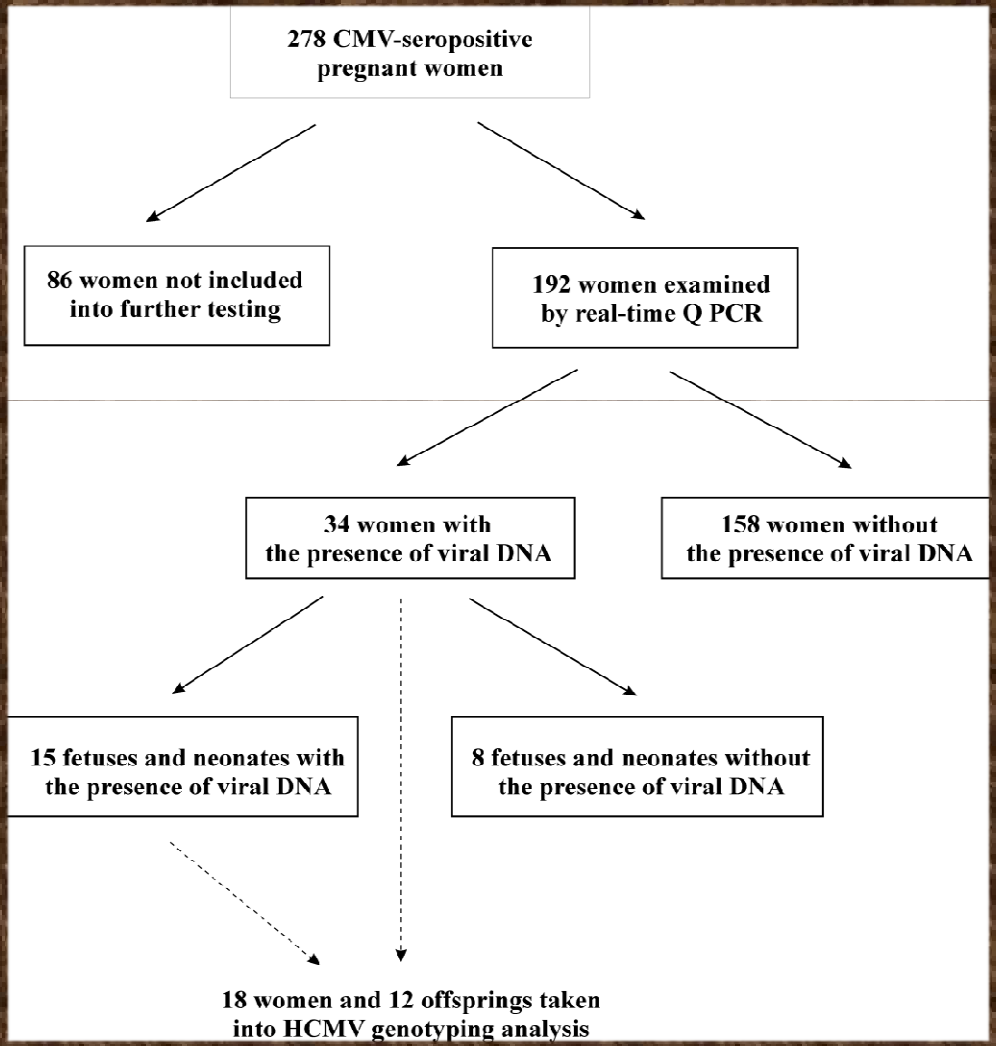
Co-infections with various HCMV gB strains observed in infants from the U.S. and China

Aims of study:

- Determination of HCMV *UL55* genotypes in pregnant Polish women, their fetuses and newborns
- Estimation of the impact of viral genotypes on both the transplacental transmission of the virus and disease outcome in the offsprings



Materials and Methods: Collection of clinical specimens



Patients' classification for molecular testing

Serological screening:

Anti-CMV IgG and IgM tests (Diasorin/Biomedica, Italy) based on Chemiluminescence Immunoassay (CLIA) between 2009 and 2011 years

Tests based on an Enzyme-Linked Fluorescence Assay (ELFA) between 2012 and 2013

Determination of IgG avidity

Clinical symptoms observed in pregnant women and their fetuses:

Flu-like symptoms in mothers

Ultrasound markers in fetuses:

ventriculomegaly, hydrocephaly and fetal hydrops as well as intrauterine growth restriction, ascites, pericardial effusion, cardiomegaly and the presence of hyperechogenic foci in different organs like the fetal brain, liver and pancreas

Preparation of DNA to further molecular studies:

Nucleic acid extraction with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and storage at -20°C

HCMV DNA detection and quantification

Real-time Q PCR assay for *UL55* gene fragment of length 150 bps with forward and reverse primers and TaqMan probe of the following sequences: 5'-GAGGACAACGAAATCCTGTTGGGCA-3', 5'-TCGACGGTGGAGATACTGCTGAGG-3', and 5'-6-FAM-CAATCATGCGTTTGAAGAGGTAGTCCA-TAMRA-3'

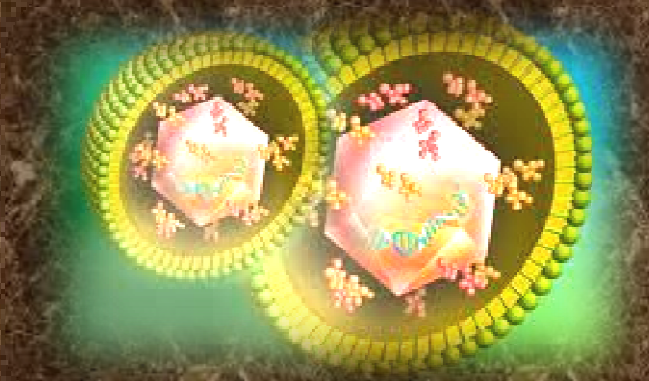
Absolute quantification analysis using standard curves for serial 10-fold HCMV DNA dilutions from 10^5 to 1 viral copy

Genotyping of HCMV strains

Real-time PCR assay to amplify DNA fragments of lengths between 72 and 79 bp, dependent on HCMV UL55 genotype

Single reactions performed in two separate tubes for gB1 and gB3 as well as gB2 and gB4 genotypes

Serial dilutions of HCMV reference strains Towne (gB1; ATCC: VR-977) and AD-169 (gB2; ATCC: VR-538) used in calibration curves



Results: HCMV load and *UL55* genotype in pregnant women

Patient No.	Clinical specimen	HCMV load(*)	*UL55* genotype
1.	PBMC	1.23×10^2	gB3
2.	plasma	1.31×10^3	gB2
	PBMC	51	
3.	PBMC	46	gB2
4.	urine	1.5×10^3	gB2
5.	plasma	5.59×10^2	gB1-gB2
6.	PBMC	5.82×10^2	gB1-gB2
	urine	8.17×10^3	
7.	whole blood	1.08×10^3	gB1
	urine	4.28×10^2	gB1-gB2
8.	plasma	1.81×10^2	gB2
	urine	1.32×10^3	
	plasma[3]	1.98×10^2	
	whole blood[3]	1.66×10^2	gB2-gB3
	urine[3]	7.16×10^3	
9.	plasma	1.36×10^2	
	whole blood	9.67×10^2	gB2
	urine	8.74×10^2	
10.	whole blood	4.88×10^2	gB2
	urine	1.14×10^3	
11.	whole blood	6.90×10^2	gB2
	urine	2.35×10^2	
12.	whole blood	2.78×10^2	gB2
	urine	2.41×10^4	
13.	whole blood	1.14×10^3	gB2
	urine	8.05×10^3	
14.	PBMC	28	
	whole blood	1.28×10^2	gB2
	urine	1.49×10^2	
15.	whole blood	1.30×10^2	gB2
	urine	4.10×10^2	
16.	whole blood	1.59×10^2	gB2
	urine	2.48×10^2	
17.	urine	1.51×10^2	gB4
18.	urine	5.35×10^2	gB4

(*) - HCMV load per 1 mL of study fluid (blood, plasma, urine) or 5×10^5 cells (PBMC)

HCMV load, *UL55* genotype and cytomegaly outcome in fetuses and newborns

Patient No.	Clinical specimen	HCMV load(*)	*UL55* genotype	Outcome
1.**	amniotic fluid cells	4.32×10^6	gB2	Symptomatic
	plasma	6.15×10^3		
	urine	9.33×10^5	gB1-gB2	
2.**	amniotic fluid cells	8.2×10^4/1 x 10^5 cells	gB2-gB3	Symptomatic (death)
	ascitic fluid	1.54×10^2		
	brain	52.6***	gB3	
	kidney	42.4***		
3.	amniotic fluid cells	1.86×10^5/1.4×10^5 cells	gB1-gB2	Asymptomatic
4.	whole blood	4.49×10^2	gB1-gB2	Asymptomatic
	plasma	2.11×10^3		
5.	whole blood	6.61×10^3	gB1-gB2	Asymptomatic
6.	whole blood	1.55×10^2	gB1-gB2	Asymptomatic
7.	amniotic fluid cells	2.98×10^4/5×10^4 cells	gB2	Symptomatic (death)
	ascitic fluid	6.66×10^3		
	brain	1.40×10^3***		
	kidney	3.31×10^2***		
	liver	3.18×10^3***		
8.	amniotic fluid and whole blood of child	1.34×10^3	gB2	Symptomatic
9.	amniotic fluid	2.19×10^2	gB2	Symptomatic
10.	amniotic fluid	6.36×10^2	gB2	Asymptomatic
11.	amniotic fluid	1.47×10^3	gB2	Asymptomatic
12.	brain	7.82×10^3***	gB1	Symptomatic (death)
	kidney	4.11×10^3***		
	liver	7.01×10^3***		

(*) - HCMV load per 1 mL of study fluid;

** - HCMV loads identified in fetal and neonatal body fluids that were included in our previous study [4]

*** - HCMV load per one cut section of paraffin-embedded tissue

Maternal *vs.* fetal and neonatal infections with HCMV

Study group	Total No. tested*	HCMV *UL55* genotype (No. tested (%)**)							
		Single				All single infections	Multiple		All multiple infections
		gB1	gB2	gB3	gB4		gB1-gB2	gB2-gB3	
Pregnant women	18	0 (0)	11 (61.1)	1 (5.55)	2 (11.1)	14 (77.8)	3 (16.7)	1 (5.55)	4 (22.2)
Fetuses	9	1 (11.1)	6 (66.7)	0 (0)	0 (0)	7 (77.8)	1 (11.1)	1 (11.1)	2 (22.2)
Newborns	5	0 (0)	1 (20)	0 (0)	0 (0)	1 (20)	4 (80)	0 (0)	4 (80)
Congenital infections	12	1 (8.3)	5 (41.7)	0 (0)	0 (0)	6 (50)	5 (41.7)	1 (8.3)	6 (50)
Symptomatic fetuses and newborns	6	1 (16.66)	3 (50)	0 (0)	0 (0)	4 (66.7)	1 (16.66)	1 (16.66)	2 (33.3)
Asymptomatic fetuses and newborns	6	0 (0)	2 (33.3)	0 (0)	0 (0)	2 (33.3)	4 (66.7)	0 (0)	4 (66.7)

* No. tested – number of tested patients; ** % - part of all the patients included in each study group.

Maternal *vs.* fetal and neonatal infections with HCMV

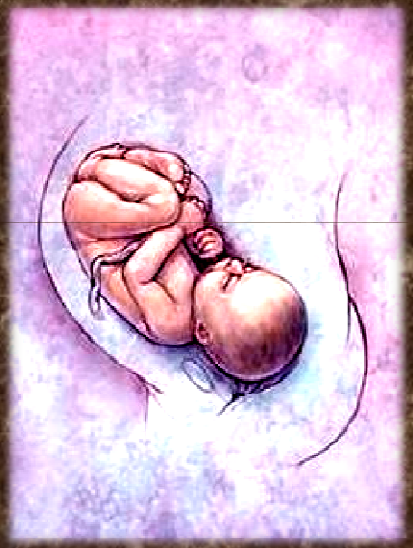
Significant correlation between genotypes of HCMV identified in the pregnant women and their congenitally infected offsprings

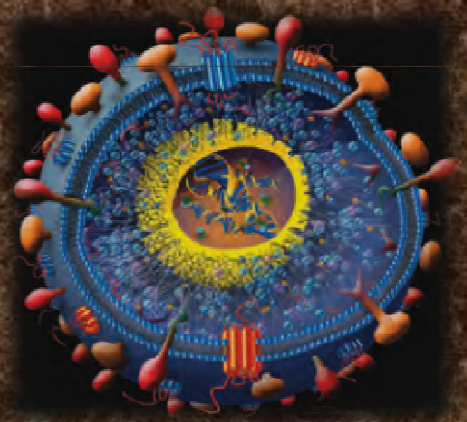
Maternal genotype	Fetal and neonatal genotype		Total
	gB2	gB1-gB2	
gB2	4	0	4
gB1-gB2	0	4	4
gB2-gB3	1	0	1
Total	5	4	9

$\chi^2 = 9$; $P \leq 0.050$

Conclusions

- Infections with single and multiple HCMV strains occur in pregnant women, as well as in their fetuses and neonates, both with the asymptomatic and symptomatic infections.
- HCMV infections, identified in mothers, seem to be associated with the viral genotypes in their children.





Thank You for Your attention!