

Congenital CMV infection and Hearing Loss

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Disclosures

- Received one time consulting fee as a member of the GSK CMV Vaccine Advisory Board
- Do not intend to discuss unlabeled or commercial products other than issues related to treatment of congenital CMV infection

Outline

- Historical perspective
- Background
- CMV-related hearing loss
 - Natural history of CMV-related hearing loss
 - Risk factors for CMV-associated SNHL
 - Symptomatic congenital CMV infection
 - Maternal Immunity
 - Pathogenesis of CMV-related SNHL
- Interim results from the CHIMES Study

Outline

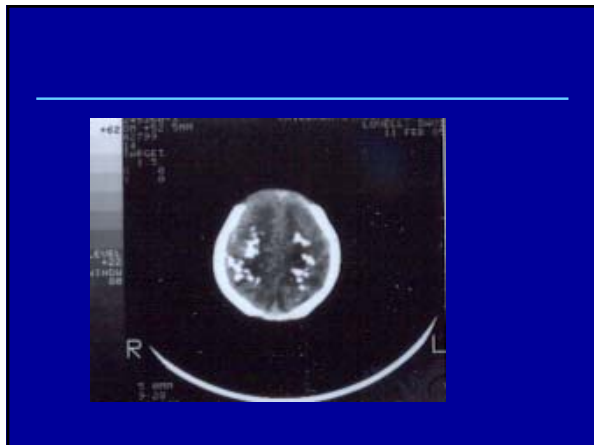
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Historical Perspective

- 1904 Ribbert described protozoan like cells in organs of an infant with presumed cong. Syphilis
- 1921 Goodpasture and Talbot hypothesized that the swollen cells (cytomegalia) were host cells injured by a virus
- 1926 The term *salivary gland virus* was used to describe infectious agents present in the salivary glands of guinea pigs
- 1952 *Cytomegalic Inclusion Disease* (CID) as a clinical entity was described

Historical Perspective

- 1954 Smith successfully propagated the mouse SGV
- 1955 Isolation and propagation of a new virus by three independent groups; Smith, Rowe, and Weller
- 1956 Weller first isolated the virus from an infant with CID
- 1960 The name "*Cytomegalovirus*" was proposed by Weller
- 1962 First published report of virologic and clinical findings in CID by Weller



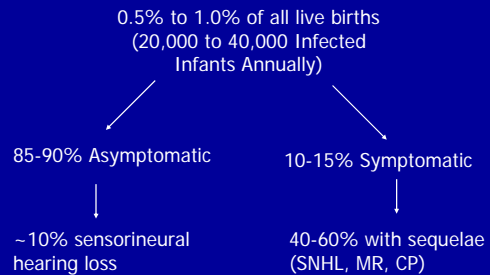
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- ### CMV related hearing loss
- 1964 Medearis first described deafness in children with CID
 - 1969 McCracken, et al. 4/13 surviving infants (31%) had hearing loss; all diagnosed after the first year; 1 at 40 months of age
 - 1973 Initial reports of asymptomatic or subclinical congenital CMV infection
 - 1974 Reynolds et al., Dahle et al. 4/16 children (25%) with subclinical CMV infection had definite bilateral or unilateral SNHL

CMV related hearing loss

- Hanshaw, et al. 1976
5/40 (13%) children with clinically silent or asymptomatic CMV infections had bilateral severe HL
- Stagno, et al. 1977
7/51 infants (14%) with asymptomatic congenital CMV infection had hearing loss; progressive loss indicated in 1 infant (between 21 to 37 months)
- Dahle, et al. 1979
Progressive HL observed in 4/12 (33%) of children with congenital CMV infection

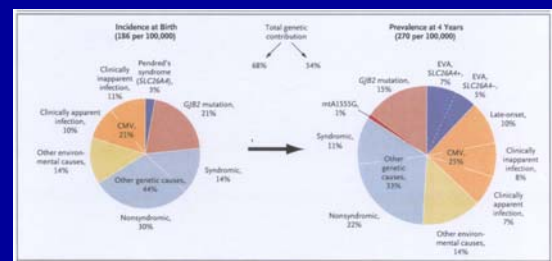
Congenital CMV infection



CMV-related hearing loss - Summary

- 22 – 65% Symptomatic children will have hearing loss
- 6-23% Asymptomatic children will have hearing loss
- SNHL following congenital CMV infection may be present at birth or delayed onset
- Variable degree of hearing loss - unilateral high frequency loss to profound bilateral loss
- Frequent progression (≥ 10 dB deterioration) and fluctuation of hearing loss

Estimates of Causes of Deafness at Birth and Four Years in the United States



Morton and Nance. Newborn Hearing Screening – A Silent Revolution. *New Engl J Med* 354: 2151-64, 2006

CMV related Hearing loss

In a cohort of ~12,000 children in Sweden who were screened for congenital CMV infection and monitored for hearing outcome:

10/12,000 (0.08%) had bilateral, profound HL

4 with HL due to congenital CMV infection

4 with HL due to genetic causes

2 with HL of unknown etiology

Harris et al. *Ear Hear*, 1984

UAB Longitudinal Study of CMV Related Hearing Loss

	Asymptomatic	Symptomatic
Total number of children	651	209
Number with hearing loss	48 (7.4%)	85 (41%)
Characteristics of Loss		
Unilateral	25 (52%)	28 (33%)
Bilateral	23 (48%)	57 (67%)
High frequency loss only (4000 – 8000 Hz)	18 (38%)	11 (13%)

Dahle et al. *J Am Acad Audiol*. 11: 283-290, 2000

UAB longitudinal study of CMV related hearing loss

Finding	Asymptomatic (N=651)	Symptomatic (N=209)
Delayed onset loss	18 (38%)	23 (27%)
Median age of delayed onset (range)	44 Mo (24-182)	33 Mo (6-197)
Progressive loss	26 (54%)	46 (54%)
Median age of progression (range)	51 Mo (3-186)	26 Mo (2-209)

Dahle et al. *J Am Acad Audiol.* 11: 283-290, 2000

UAB Longitudinal study of CMV related SNHL Degree of loss

Degree of Loss	Asymptomatic	Symptomatic
Mild (21 – 45 dB HL)	17%	12%
Moderate (46 – 70 dB HL)	15%	13%
Severe (71 – 90 dB HL)	17%	31%
Profound (>90 dB HL)	51%	44%

Dahle et al. *J Am Acad Audiol.* 11: 283-290, 2000

Cumulative incidence of SNHL according to age

Age	Asymptomatic	Symptomatic
Birth – one month	25.5%	43.5%
Three months	31.4%	55.3%
Six months	43.1%	67.1%
Two years	47.1%	82.4%
Three years	58.8%	88.2%
Four years	72.5%	89.4%
Six years	86.6%	95.3%
Seven – 15 years	100%	100%

Dahle et al. *J Am Acad Audiol.* 11: 283-290, 2000

What proportion of children with CMV related hearing loss will be detected on newborn hearing screen?

Risk criteria based neonatal auditory screening was not successful in identifying HL due to congenital CMV infection

Only 17.6% of children with SNHL due to congenital CMV infection were identified by risk criteria based neonatal auditory screening at UAB between 1985-1998

Hicks, et al., 1993
Fowler, unpublished data

Estimated number of children with CMV-related hearing loss in the U.S.

32,000 (0.8%) infants are born each year in the US with congenital CMV infection

3.9% will have HL at birth

Assume universal hearing screening

1,248 children with congenital CMV infection & HL will be identified before hospital discharge

0.31 per 1000 children

1,408 children with congenital CMV infection born each year will develop hearing loss later

0.35 per 1000 children

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Risk factors for CMV related SNHL

- Symptoms at birth
 - Disseminated infection at birth
 - Neuroimaging abnormalities
 - IUGR
- Virus burden
- Maternal immunity

Clinical impact of congenital CMV infection according to condition at birth

Sequela	Incidence of sequelae	
	Symptomatic (10-15%)	Aysmptomatic (85-90%)
Death	10%	0
Hearing loss	40-60%	7-15%
Mental retardation	45%	2-10%
Cerebral palsy	35%	<1%
Chorioretinitis	15%	1-2%

Risk Factors for HL in Symptomatic Infants

	Adjusted OR (95% CI)
Private Health Insurance	2.8 (1.4-6.2)
IUGR	2.2 (1.1-4.9)
Petechiae	2.8 (1.2-6.0)

Adjusted for: race, insurance status, IUGR, petechiae, HSM, jaundice, microcephaly, seizures, thrombocytopenia & referral status

Rivera, et al., *Pediatrics*, 2002

Maternal immunity

- Provides significant protection against intrauterine transmission
 - Transmission rate drops from 20-60% in primary infection to 1-2% in non-primary infection
- Lower incidence of SNHL and other sequelae following non-primary infection (Fowler, 1992)
- Protects from transfusion acquired CMV disease in premature infants
- Current vaccine strategies are focused on preventing primary maternal infection

Maternal immunity only provides partial protection

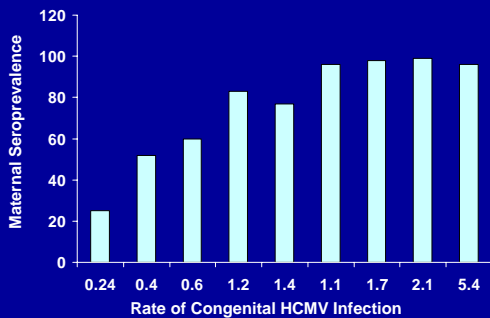
- Congenital CMV infection in consecutive births to same mother
- Congenital infection rates are directly proportional to seroprevalence rates
- More frequent in offspring of low-income, young women

Prevalence of Congenital CMV Infection

Location	No. studied	% Congenital CMV infection	Seroprevalence rates
Manchester, England	6,051	0.24	25
Aarhus-viborg, Denmark	3,060	0.4	52
Hamilton, Canada	15,212	0.42	44
Birmingham (upper SES)	8,545	0.6	54
Birmingham (Low SES)	2,579	1.4	77
Houston (upper SES)	461	0.6	50
Houston, TX (Low SES)	493	1.2	83
London, England	720	0.69	58
Abidjan, Ivory Coast	2,032	1.38	100
Santiago, Chile	118	1.7	98
Ribeirão Preto, Brazil	8,047	1.1	96
Ballabgarh, India	423	2.1	99
Sukuta, The Gambia	741	5.4	96

Modified from Stagno S, et al. *Clin Obstet Gynecol*. 1982;25:563-576.

Rate of Congenital Infection Increases With Maternal Seroprevalence



Symptomatic infection following non-primary maternal CMV infection

- Thought to occur exclusively following primary maternal infection
- Case report:
 - CC was born in 1993 with microcephaly, jaundice, hepatosplenomegaly and intracranial calcifications
 - His mother had CMV IgG antibodies at the time of delivering her 1st child in 1990

Symptomatic infection following non-primary maternal infection

- A study of 246 congenitally infected children between 1991 and 1997:
 - Of the 47 symptomatic infants, 8 were born to immune mothers and 8 were born following primary maternal CMV infection
- These results suggested:
 - Symptomatic infection follows non-primary maternal infection more often than has been thought
 - Newborn disease is not milder
- Is the outcome in these children different?

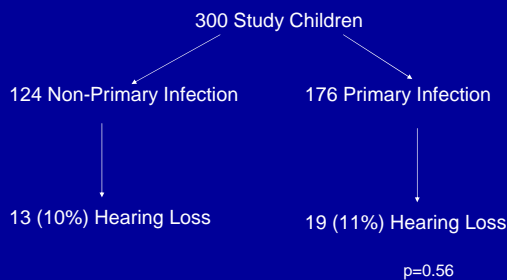
Boppana et al., *Pediatrics* 1999

Hearing loss following non-primary maternal infection

- 300 children with congenital CMV were identified between 1980 and 2000
- Maternal infection classified by serology
- Audiologic evaluations done at 3-8 wks, 6, 12, 18, 24, 30 mo and annually

Ross et al. *J Pediatr*, 2006

Type of maternal infection and hearing loss



Ross et al. *J Pediatr*, 2006

Type of maternal infection and hearing loss

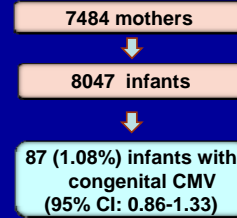
Hearing loss characteristic	Non-Primary (N=13)	Primary (N=19)	P value
Bilateral	23%	42%	0.36
Delayed	31%	53%	0.29
Progressive	15%	63%	0.01
Hi-Frequency	38%	19%	0.42
Fluctuating	31%	43%	0.72
Severe/Profound	23%	63%	0.04
Age of Hearing Loss Diagnosis (mo)			
Mean (±SD)	39 (±53)	13 (±21)	0.16
Median (range)	7 (0-182)	5 (0-76)	0.16

Ross et al. *J Pediatr*, 2006

Conclusions

- Rate of hearing loss similar in children born following primary and non-primary maternal infection
- Hearing loss is less severe and less likely to progress in the non-primary group
- Maternal immunity seems to provide some but not complete protection against damaging fetal infection

Birth Prevalence of Congenital CMV Infection in Ribeirão Preto, Brazil



Demographic Characteristics

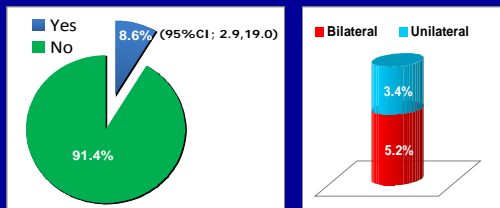
Finding	CMV-uninfected (n=7960)	CMV-infected (n=87)	P
Mean maternal age (yrs)	25.6 ± 6.6	23.9 ± 7.1	0.023
Male gender (%)	4072 (51.1)	54 (62.1)	0.12
Twin infants (%)	370 (4.6)	10 (11.5)	0.007
Mean birth weight	2993 ± 709	2671 ± 724	<0.001
Gestational age (wks)	37.9 ± 2.7	37.7 ± 2.7	0.42
Gestational age <37 wks (%)	1442 (18.1)	23 (26.4)	0.13
SGA (%)	852 (10.7)	21 (24.1)	<0.001

Newborn Findings

Finding	Positive/total examined (%)
Jaundice (direct bilirubin >2 mg/dL)	4/87 (4.6)
Petechiae	4/87 (4.6)
Hepatosplenomegaly	4/87 (4.6)
Purpura	2/87 (2.3)
Microcephaly	0/85 (0.0)
Seizures	1/87 (1.1)
Cranial CT scan abnormalities	3/79 (3.8)
Thrombocytopenia (<100,000/mm ³)	4/41 (10.0)
Elevated ALT or AST (>130 IU/L)	4/20 (20.0)
At least one clinical abnormality	7/87 (8.1)

Sensorineural Hearing Loss in 58 Infants

- Tested at a median age of 21 mo (3-63 mo)

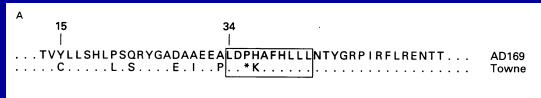


Mussi-Pinhata MM, et al. Clin Infect Dis. 2009;49:522-528

Sources of non-primary maternal infection

- Reactivation of persistent/latent infection?
- Reinfection by new strain of virus?
 - Weller recognized antigenic diversity of CMVs
 - Children in daycare
 - STD clinic attendees
 - Immunocompromised hosts
 - Transplant recipients and HIV infected patients
 - Congenital Infection
 - Arav-Boger et al. JID, 2002; 186: 1057
 - Congenital infection rates vary with SES

Sequence Variation in AP86 gH Epitope Allows Detection of Strain Specific Antibody Responses



Congenital Infection following non-primary maternal infection is associated with reinfection

TABLE 3. COMPARISON OF STRAIN-SPECIFIC ANTIBODY RESPONSES AGAINST GLYCOPROTEIN H IN SERIAL SERUM SAMPLES FROM MOTHERS WITH PRECONCEPTIONAL IMMUNITY AGAINST CMV, ACCORDING TO WHETHER THEIR INFANTS HAD CONGENITAL CMV INFECTION.

ACQUISITION OF NEW ANTIBODY SPECIFICITIES BETWEEN PREGNANCIES	MOTHERS OF INFECTED INFANTS (N=16)	MOTHERS OF UNINFECTED INFANTS (N=30)
	no. (%)	
Yes	10 (62)	4 (13)*
No	6 (38)	26 (87)

*P<0.001 for the comparison with the mothers of infected infants.

Boopana et al. N Engl J Med 344: 1366, 2001

Maternal reinfection and congenital CMV infection - Conclusions

- Reinfection may be associated with intrauterine transmission and severe fetal infection
- Reinfections may occur more often in populations with increased CMV exposure
- Strain-dependent immunity may have a protective role

Unresolved issues

- The exact frequency of reinfection in healthy people is not known
- Significance of reinfection
- Other factors associated with intrauterine transmission in women with preexisting immunity
- The role of strain-specific immunity

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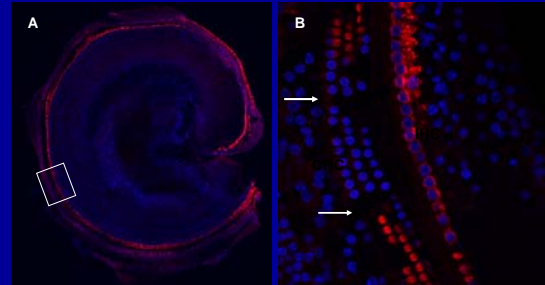
CMV related hearing loss Pathology and pathogenesis

- Limited human temporal bone studies
 - Virus replication or the presence of viral antigens in structures of inner ear
 - CMV can readily infect epithelium of inner ear
 - Lack of significant inflammatory infiltrates
 - Delayed host immune response?
 - Delayed infection of inner ear?

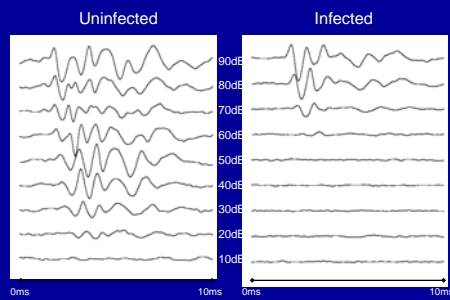
Pathogenesis of hearing loss

- Animal studies (guinea pig model)
 - Disseminated fetal infection
 - CMV antigens in epithelium and neural cells of inner ear
 - ~28% of infected animals with abnormal auditory function
 - Intact immune function is required for developing hearing loss

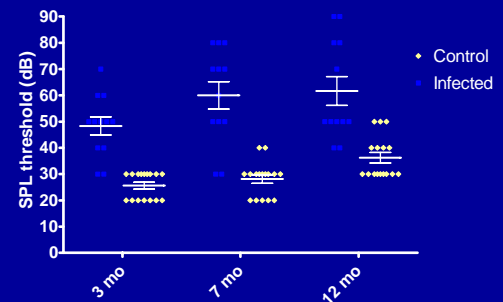
Neonatal MCMV Infection Model Cochlear Whole Mount



Representative ABR tracings from 3 month old MCMV infected and age-matched uninfected control animals



Hearing loss in mice infected neonatally with MCMV



Neonatal MCMV Infection Model Summary

- BALB/c mice, infected peripherally with MCMV at birth, develop systemic infection followed by dissemination to the CNS
- Infected animals demonstrate significant, but variable hearing loss (half of ears have an abnormal ABR at 3 months)
- Hearing loss appears to be progressive

Pathogenesis of CMV related hearing loss

- Both human and animal studies support a model that includes virus infection and host inflammatory responses leading to both acute (viral mediated) and chronic (virus and host derived) damage
- Other:
 - Host factors
 - Genetic susceptibility
 - Stage of differentiation
 - Virologic determinants
 - Tissue tropism
 - Virulence factors
 - Genetic polymorphisms

Uncertainties and gaps in knowledge

- Exact contribution of CMV in newborn and early childhood hearing loss
 - Few population based studies and lack of studies in different population groups
- Better methods to screen newborns
- Predictors of hearing loss in asymptomatic infection
- Understanding of the pathogenesis
 - Host factors: Immune response, genetic susceptibility
 - Virologic determinants: Tropism, polymorphisms
- Better predictors of outcome

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CHIMES Study



Overall Objectives

- Define the long-term audiologic outcome in children with congenital CMV infection
- Determine the clinical validity and utility of CMV screening:
 - in the detection of hearing impairment in the newborn
 - In the prediction of hearing impairment with onset during infancy or in the early years of life

NIDCD Multicenter CHIMES Study

Study Population:

Between March 2007 and March 2009, 43,557 infants born at seven medical centers in different geographic regions of the United States were enrolled in the NIDCD CHIMES study.

Study hospitals included:

- University Hospital, Birmingham, AL
- Mississippi Medical Center, Jackson, MS
- Saint Peter's University Hospital, New Brunswick, NJ
- Carolinas Medical Center, Charlotte, NC
- Good Samaritan Hospital, Cincinnati, OH
- Magee Women's Hospital, Pittsburgh, PA
- Parkland Hospital, Dallas, TX.

The study population reflects the underlying newborn population of the respective medical centers.

Overall prevalence of congenital CMV infection

0.51% (95% CI, 0.44 – 0.58)

NIDCD Multicenter CHIMES Study

Congenital CMV Infection Prevalence

	% (95% CI)
UAB, Birmingham, AL	0.9% (0.7 – 1.2%)
Med Center, Jackson, MS	1.1% (0.8 – 1.5%)
Saint Peters, New Brunswick, NJ	0.2% (0.1 – 0.4%)
Carolinas, Charlotte, NC	0.6% (0.4 – 0.8%)
Good Samaritan, Cincinnati, OH	0.5% (0.3 – 0.7%)
Magee, Pittsburgh, PA	0.4% (0.2 – 0.5%)
Parkland, Dallas, TX	0.3% (0.2 – 0.5%)

Congenital CMV Infection Prevalence

	% (95% CI)
Multiracial	0.9% (0.4 – 1.8%)
Asian	0.08% (0.01 – 0.3%)
Black	1.1% (0.9 – 1.3%)
Hispanic	0.3% (0.2 – 0.4%)
White, Non Hispanic	0.3% (0.2 – 0.4%)

Comparison of Saliva and DBS Results

DBS PCR	Saliva DEAFF		Total
	P	N	
P	55	10	65
N	59	20322	20381
Total	114	20332	20446

Total positive: 124 (saliva, DBS or both)

DBS PCR Results

- Sensitivity: 48.3% (38.8–57.8)
- Specificity: 99.9% (99.9–100)
- PPV: 84.6% (73.4–92.4)

Comparison of DEAFF and PCR Results

Saliva PCR	Saliva DEAFF		Total
	P	N	
P	85	8	93
N	0	17644	16644
Total	85	17652	17737

Total positive: 93 (DEAFF, PCR, or both)

Saliva PCR in Newborn CMV Screening

- Sensitivity: 100% (95.8 - 100)
- Specificity: 99.9% (99.8 - 99.9)
- PPV: 91.4% (83.8 - 96.2)
- NPV: 99.6% (99.5 - 99.7)

CHIMES study – Interim findings

- Race, ethnicity and maternal age contribute to differences in the prevalence of congenital CMV infection in the United States.
- Black infants & multiracial infants have an increased risk of congenital CMV infection.
- Hispanic infants have a lower rate of congenital CMV infection than white non Hispanic infants.
- Offspring of young women < 20 years have the highest prevalence of congenital CMV infection.
- DBS PCR has unacceptably low sensitivity
- Saliva PCR has excellent sensitivity and specificity

The CHIMES Study Investigators & Personnel

Sponsor: NIDCD



University of Alabama at Birmingham Suresh Boppana Karen Fowler William Britt Mirjam Kempf David Kimberlin Faye MacCollister Shannon Ross Masako Shimamura Nitin Arora Amrita Bey Belinda Blackstone Valisa Brown Alice Brumbach Nazma Chowdhury Steven Fabres-Cordero Monique Jackson Noelle Le Lievre Emily Mixon Zdenek Novak Misty Purser Julie Woodruff	University of Mississippi Medical Center April Palmer Kathy Irving Della Owens Suzanne Roark Mindy Ware St Peters University Hospital Robert Tolan Kristina Feja Maria Class Marci Schwab Carolinas Medical Center Amina Ahmed Eddie Cox Julie Courtney Nubia Flores Molly Ricart Lisa Schneider Jennifer West	Cincinnati Children's Medical Center David Bernstein Dan Choo Kurt Schibler Kate Catalanotto Linda Jamison Patty Kern Maureen Sullivan-Mahoney Stacie Wethington Pittsburgh Children's Hospital Marian Michaels Diane Sabo Jena Colaberdino Noreen Jeffrey Anne Maracek Gretchen E. Probst Cheryl Rosenberg	University of Texas Southwestern Medical Center Pablo Sanchez Gregory L. Jackson Asuncion Mejias Peter S. Roland Oscar Rosado Angela G. Shoup Elizabeth K. Stehel Cathy Boatman Jessica Esquivel Kathy Katz-Gaynor April Lehr Kristine E. Owen David Sosa Jessica Santoyo Lizette Torres Fiker Zeray
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- ### Recap
- Congenital CMV infection is a leading cause of hearing loss in children in both developed and developing world
 - CMV-related hearing is often progressive and can appear later during childhood
 - Preexisting maternal immunity only provides partial protection
 - Predictors of hearing loss in asymptomatic congenital CMV infection are not known
 - Pathogenesis is not well understood

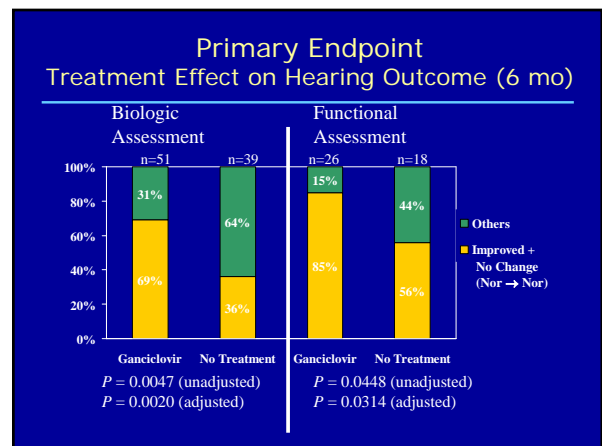
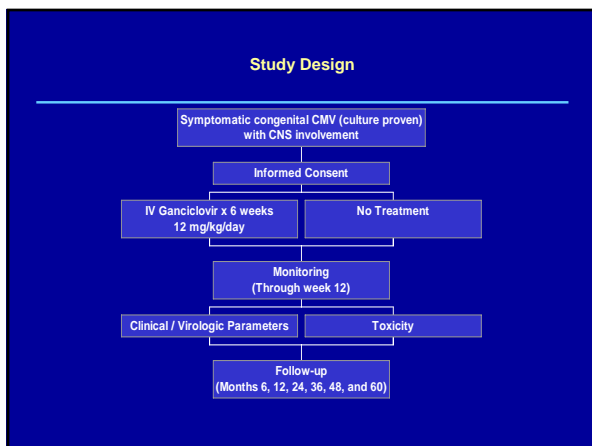
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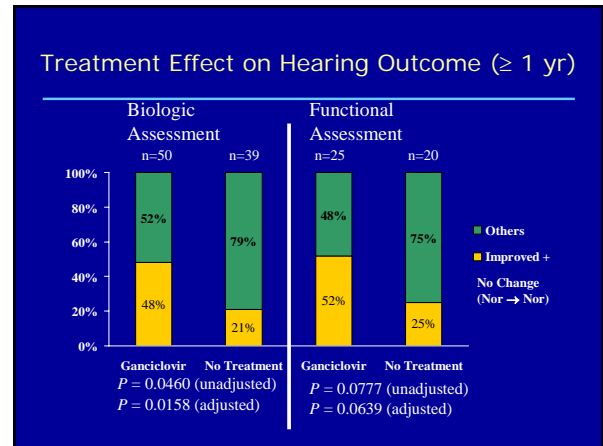
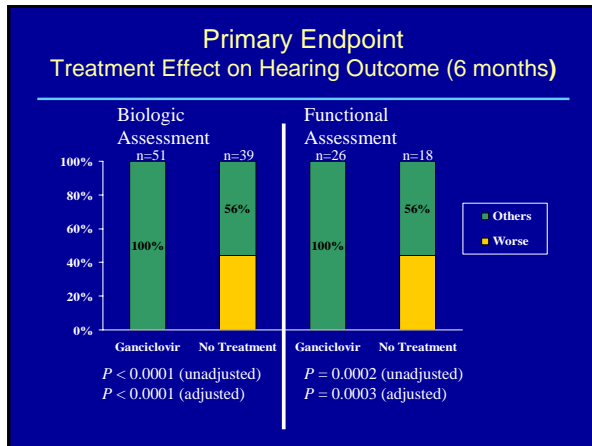
University of Alabama Birmingham Charlie Alford Bill Britt Karen Fowler Shannon Ross Zdenek Novak Nitin Arora Lisa Rivera Bob Pass Sergio Stagno Russ Bradford University of Erlangen Michael Mach	University of São Paulo Ribeirão Preto, Brazil Marisa Mussi-Pinhata Aparecida Yulie Yamamoto All India Institute of Medical Sciences New Delhi, India Lalit Dar Shobha Broor Funding Agencies NIDCD, NIAID, NICHD (Indo-US Maternal and Development and Research), ICMR
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Ganciclovir Treatment of Symptomatic Congenital Cytomegalovirus Infections: Results of a Phase III Randomized Trial

National Institute of Allergy and Infectious Diseases
Collaborative Antiviral Study Group

Kimberlin et al. *J Pediatr* 2003





- ### Conclusions
- Ganciclovir therapy both improves hearing (or maintains normal hearing) and prevents hearing deterioration at 6 months
 - Ganciclovir therapy may produce a functional effect on prevention of hearing deterioration at ≥ 1 year
 - Two-thirds of ganciclovir-treated patients developed significant neutropenia
 - Potential for bias introduced in number of non-evaluable patients