CMV genomics in congenital infections: relevance to therapeutic and vaccine design

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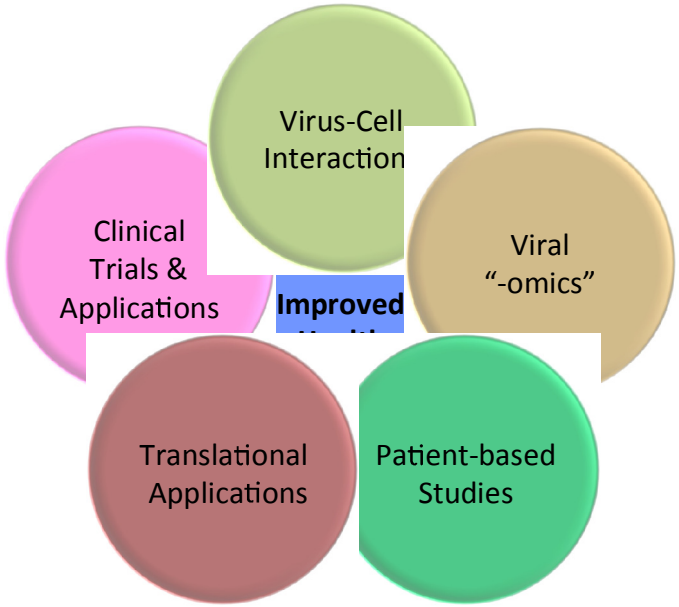
Overview

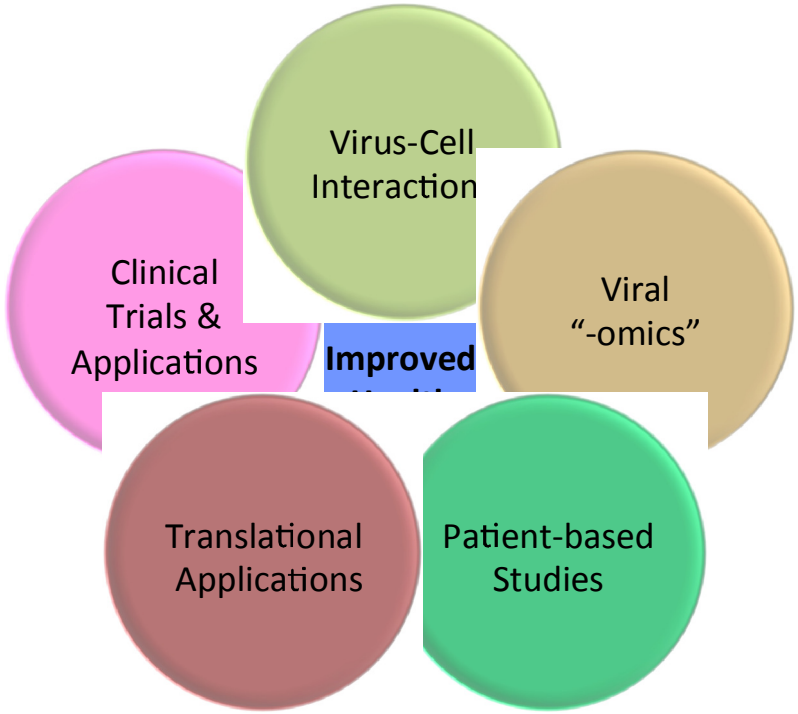
- Introduce the research approach
  - Basic to translational science and clinical trials
- Refresher on herpesviruses and CMV
- Viral genomics as a tool for discovery and translational science
- What viral evolution can teach us about congenital CMV infections

Research-Kowlab

- To use skills in molecular virology and "-omics" to uncover and understand fundamental principles in virology
- To leverage basic biology and "-omics" as platforms to develop new understandings of infectious diseases
- To leverage basic and translational studies to develop new or improved treatments and prevention strategies
- To sustain a robust research team and highly active collaborations to facilitate the transition from discovery to improved health



Research program in practice



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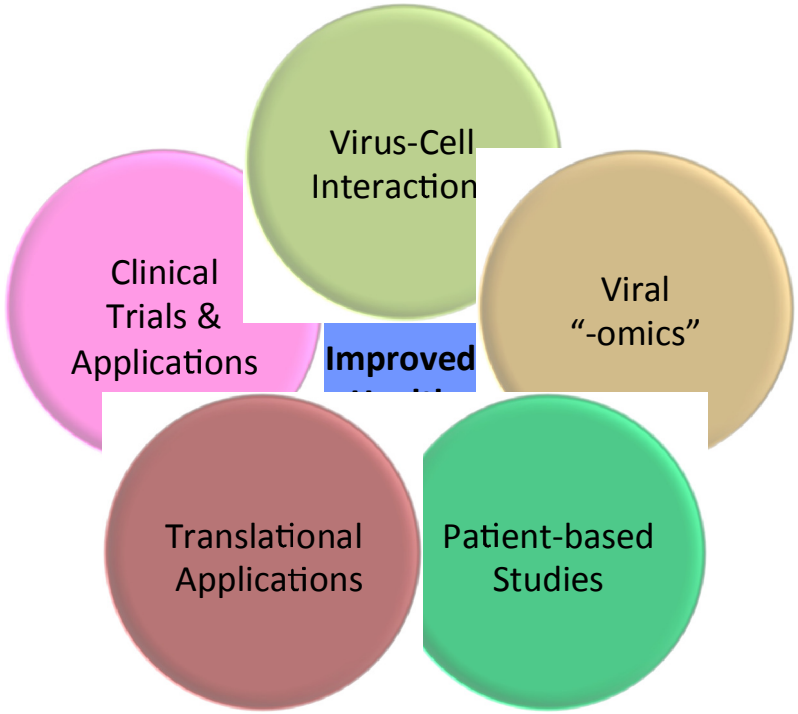

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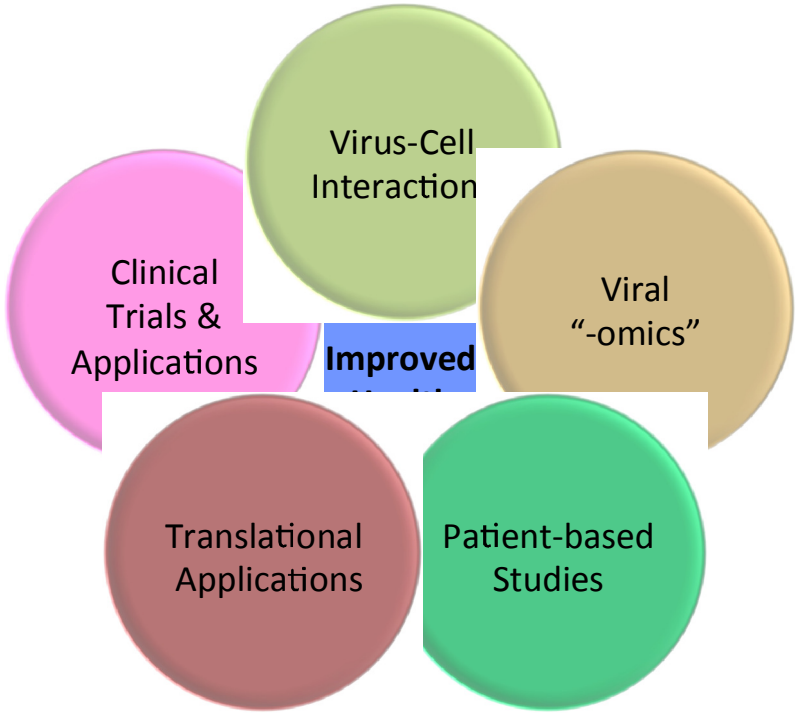
Research program in practice



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Herpesviridae

- 8 (actually 9) human herpesviruses
  - HSV-1, HSV-2, VZV
  - CMV, HHV-6A, HHV-6B, HHV-7
  - EBV, KHSV
- Features in common
  - Large, complex virions
  - Large, complex genomes
    - Encode proteins associated with viral DNA replication
    - Encode many proteins (and miRNAs) involved in immune evasion
  - Persistent and latent infections

Like diamonds, herpesviruses are forever…

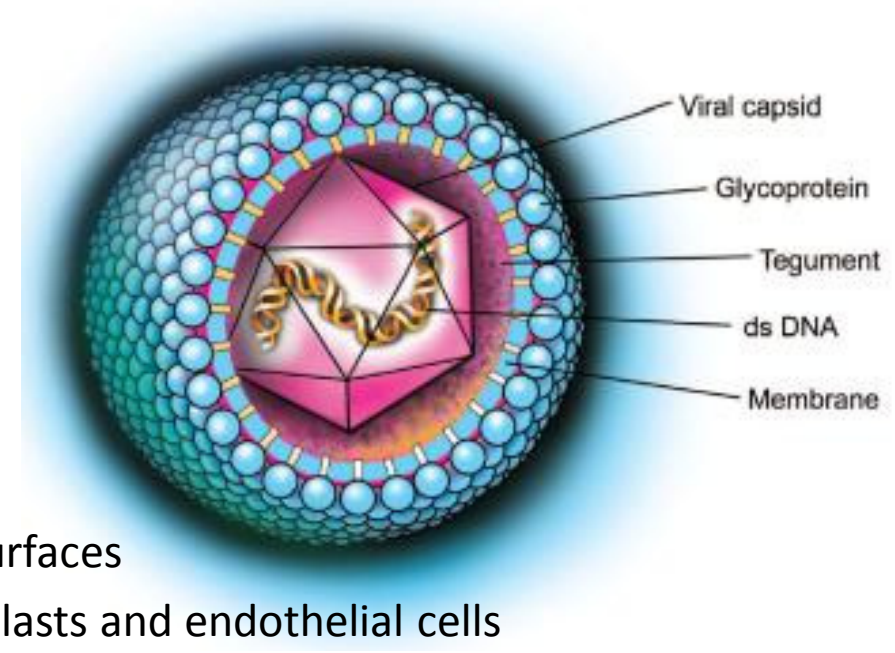
Herpesviridae cont’d

- Natural history of infections dictates early childhood infections
  - Limited impact on host

Like diamonds, you get herpesvirus infections from those close to you

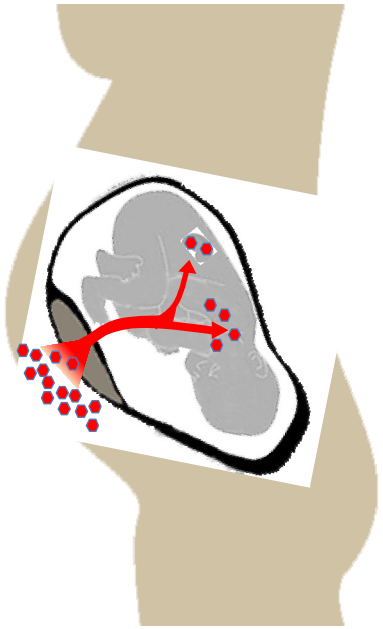
Cytomegalovirus (CMV)

- The most complex human virus
  - ~235,000 bp DNA
  - >200 open reading frames (genes)
  - Encodes miRNAs
- Infection
  - Primary infection usually via mucosal surfaces
  - Spread through epithelial cells to fibroblasts and endothelial cells
  - Monocyte/macrophage infection
    - Source of in vivo dissemination
    - Latency (CD34+ stem cells and lineage)



CMV diseases

- Largely asymptomatic in healthy individuals
  - But, CMV is found in >90% of glioblastoma multiforme tumors
  - And, may play a role in age-related immunosenescence
- Generally, disease found in immunosuppressed individuals
  - Immunosuppressive disorders
  - AIDS
  - Stem cell and solid organ transplantation
  - ***Pregnancy***



CMV is the leading cause of infection-associated birth defects

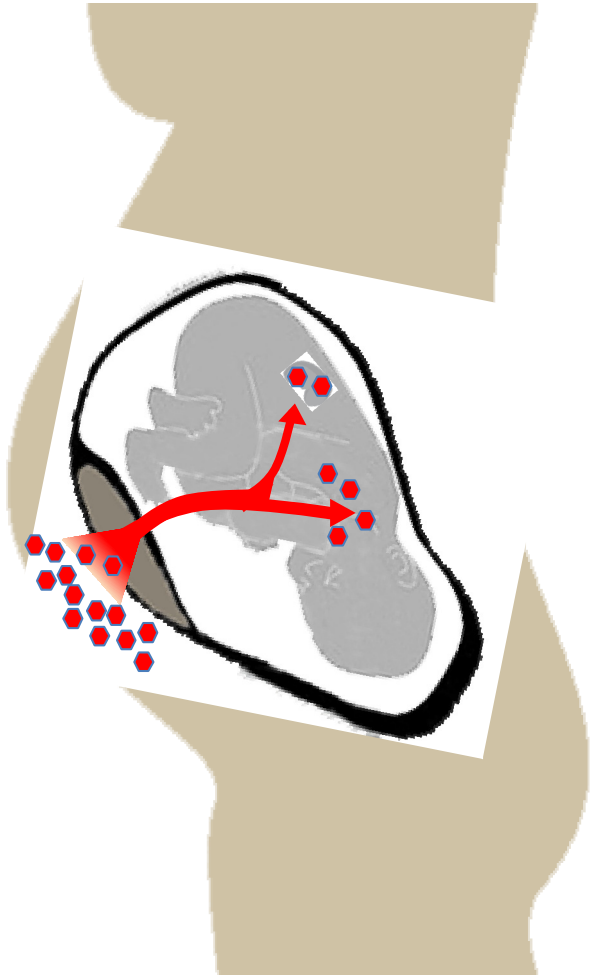
CMV is also the leading cause of nonfamilial cause of sensorineural hearing loss (SNHL)

Issues Associated with cCMV

- No screening for CMV sero-positivity in the US
  - Routine screening of pregnant women in certain European countries
- No standard of care for cCMV
  - No standard for pharmaceutical intervention
    - Off label use of ganciclovir, valganciclovir
- No standard of prenatal care/prevention strategies for cCMV
  - But see CDC links, recommendations
    - http://www.cdc.gov/cmv/index.html
    - http://www.cdc.gov/cmv/prevention.html
- No sterilizing or therapeutic vaccines

Congenital CMV Infection

- Active CMV replication during pregnancy
  - Plasma DNA
  - Antibody
- Virus crosses placenta
- CMV invades tissues throughout the fetus
  - Including CNS
  - Symptomatic & asymptomatic infections
- Sustained viral replication and shedding
  - Months to years



Genotyping CMV

- PCR amplify, sequence small regions (100's of bases) of CMV genome
  - gB gene most sequenced
    - Multiple genotypes (>4 genotypes)
    - Mixed populations
    - Genotypes change with time
    - Association with disease?

Mixed genotypes in CMV infections

Mixed Cytomegalovirus Glycoprotein B Genotypes in Immunocompromised Patients

Alain Coaquette, Alain Bourgeois, Carine Dirand, Audrey Varin, Wan Chen, and Georges Herbein
Department of Virology, Franche-Comte University School of Medicine, Besançon, France

(See the editorial commentary by Crumpacker on pages 162–4)

Association of Specific Cytomegalovirus Genotypes With Death From Myelosuppression After Marrow Transplantation

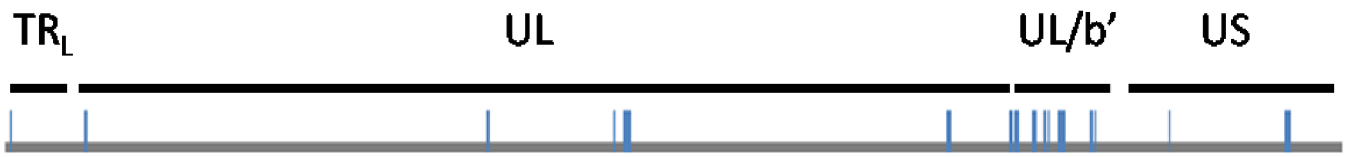
By Beverly Torok-Storb, Michael Boeckh, Cynthia Hoy, Wendy Leisenring, David Myerson, and Ted Gooley

JCM
Journals.ASM.org

Rapid Genotyping of Cytomegalovirus in Dried Blood Spots by Multiplex Real-Time PCR Assays Targeting the Envelope Glycoprotein gB and gH Genes

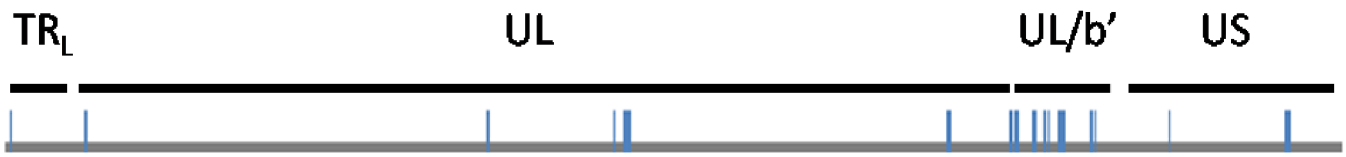
Jutte J. C. de Vries,[a] Els Wessels,[a] Anna M. H. Korver,[b] Annemiek A. van der Eijk,[c] Lisette G. Rusman,[a] Aloys C. M. Kroes,[a] and Ann C. T. M. Vossen[a]
Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands[a]; Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands[b]; and Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands[c]

Limits of genotyping



Breadth of published CMV genotypes ~5% coverage (40 papers)
Depth ~20-40 sequences/sample

Limits of genotyping



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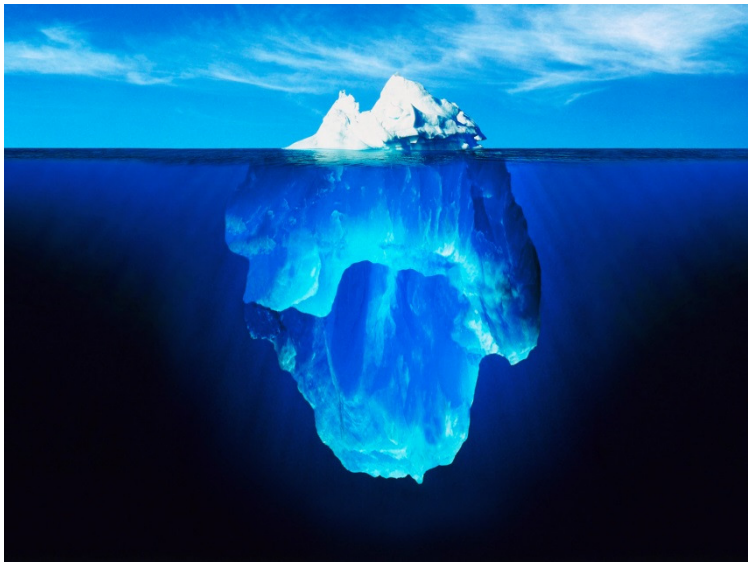
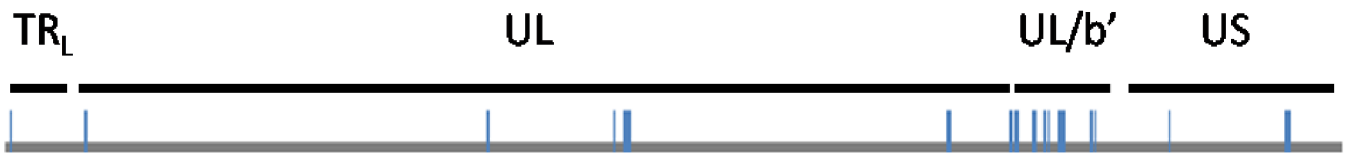


"We don't know what we don't know"—Donald Rumsfeld

Coverage using deep sequencing



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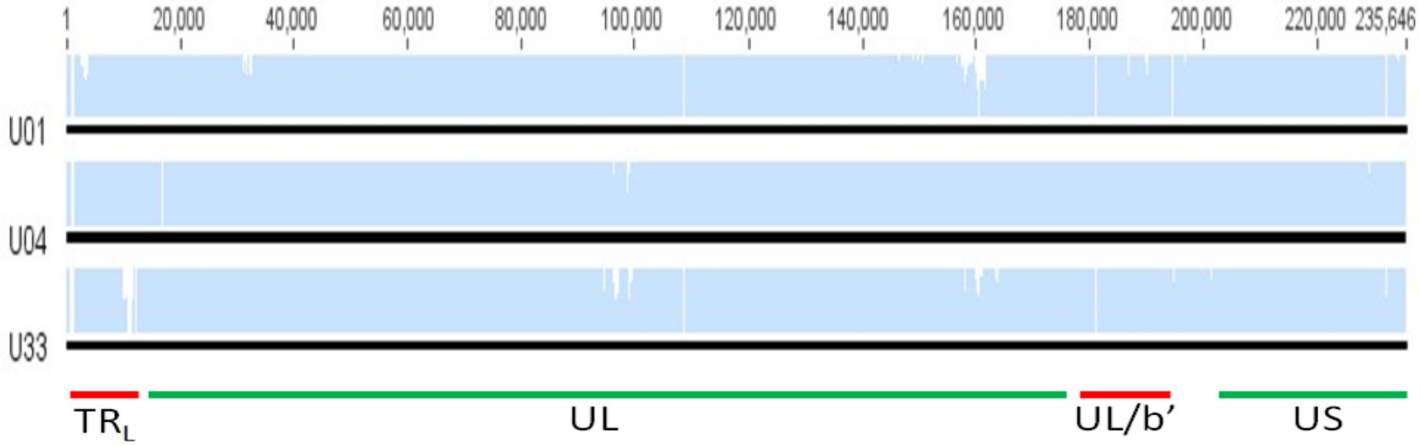
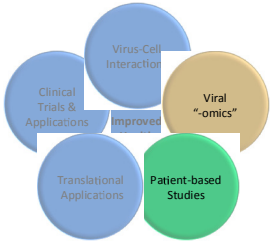




Nicholas Renzette PLoS Path 2011

Two types of data emerge from deep sequencing

- Consensus sequence
  - Sequential listing of the most common nucleotide
    - “AgggCTTgCAAAG…” (similar to Sanger sequencing)

- Sequence of the viral population
  - Compilation of short contiguous reads (36-400 bases)
  - Align to the consensus sequencing with mismatches, insertion/deletions
  - Requires error correction to “call” polymorphisms with high confidence

- Both datasets are extraordinarily useful!

Samples analyzed/in analysis

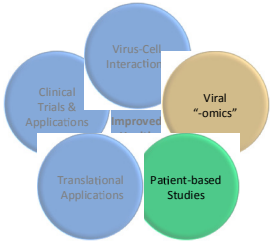
SpecimenType	Number of Specimens
Urine	32
Amniotic Fluid	1
Plasma	6
Cord Blood	2
Saliva	6
GBM	1

Patients from the US, Europe, Brazil

Sequencing summary	
Total Number of Patients	18
Total Number of Specimens	48
Total Number of HCMV Bases	41 Gb
Average coverage	91.2%
Mean Depth	3992
Total Number of SNPs Detected	840,854
Total Number of Unique SNPs	217,476
Total Number of Polymorphic Sites	150,032
Tri- or Quad-Allelic Sites (%)	10,178 (4.3%)

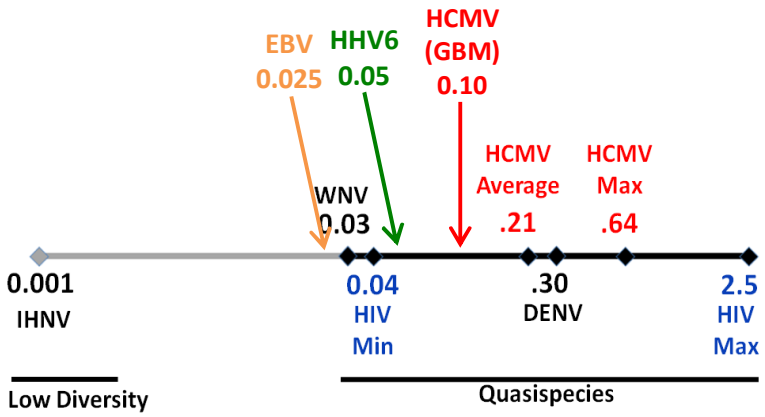
~20,000 SNPs/sample

~68% of the cCMV genome is polymorphic
-alternately-
32% of the cCMV genome is constrained



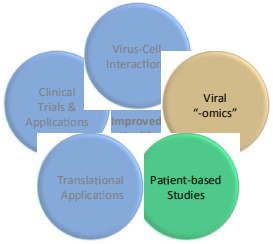
Nicholas Renzette ms in prep

Quasispecies-like structure for herpesviruses?



- Nucleotide diversity values (π) shown
- Number of SNPs = "standing variation"

<u>Nicholas Renzette:</u>
PLoS Path 2011; PLoS Gen 2013; (EBV) J. Virol (2014a)
<u>Bornali Bhattacharjee:</u>
J. Virol 2012; (HHV-6A, -6B) J. Virol, under revision

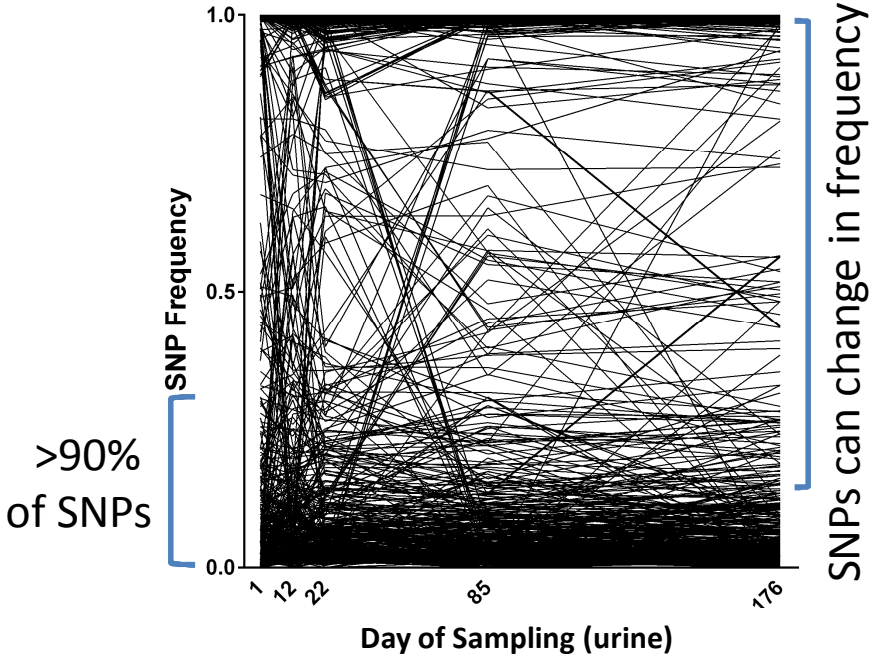


What is the source of these mutations?

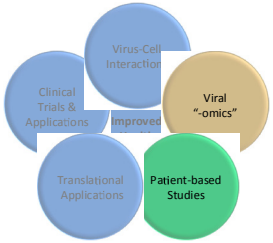
1. Standing variation

2. De novo mutations

Temporal changes in SNPs

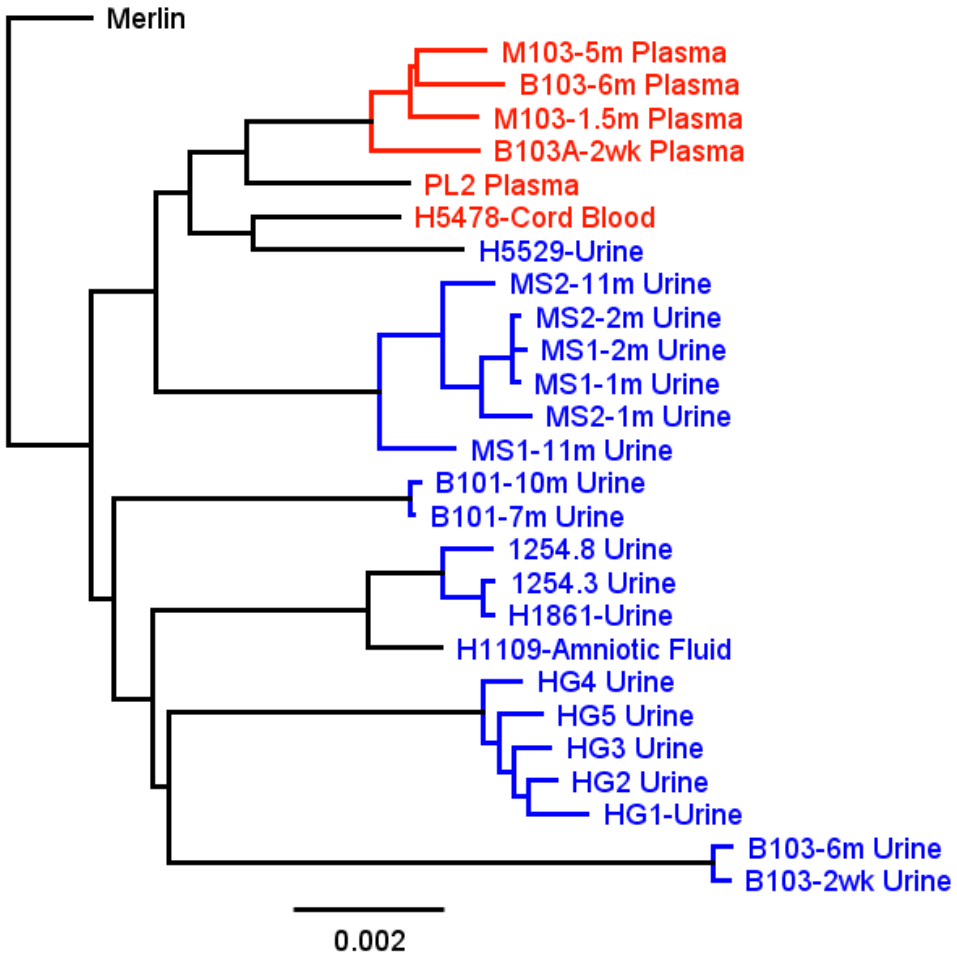


Nicholas Renzette ms in prep



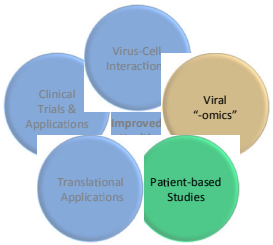
So, why ~~should~~ might you care?

cCMV sequences segregate by tissue compartment

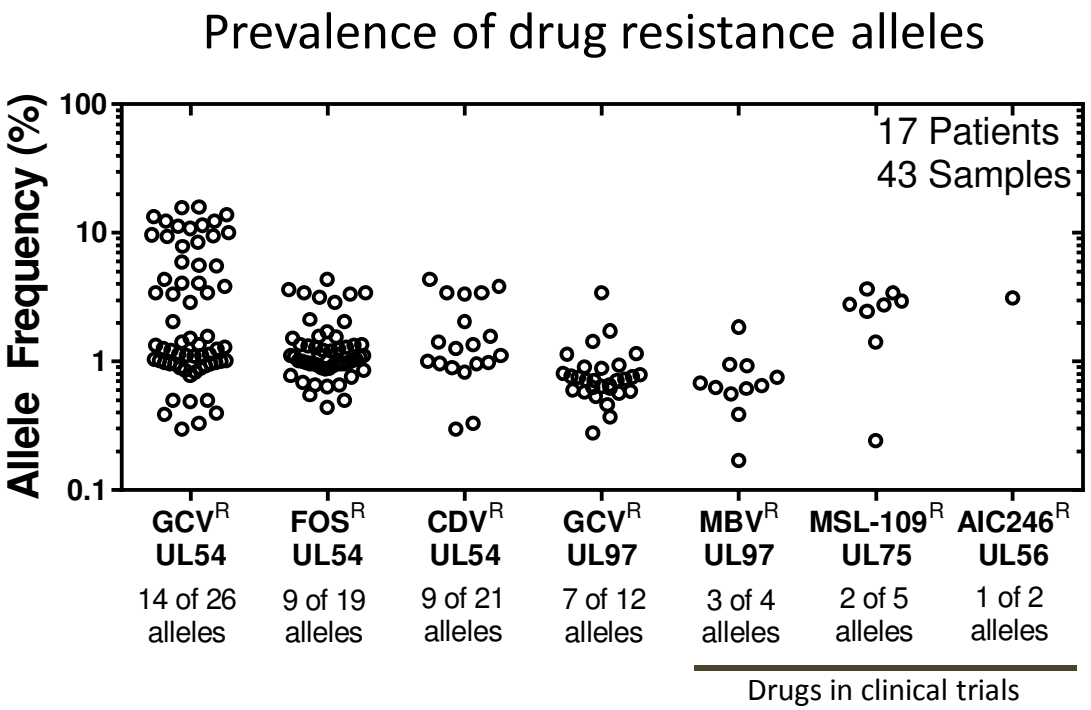


Two distinct viral populations: disease-associated and disseminating

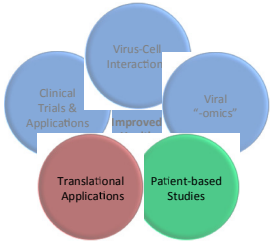
Nicholas Renzette ms in prep



Relevance to clinical infections/disease management?



Nicholas Renzette unpublished

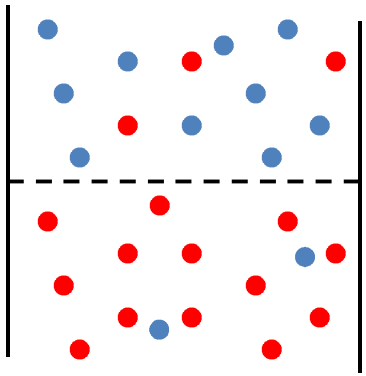


What can evolution teach us about congenital infections?

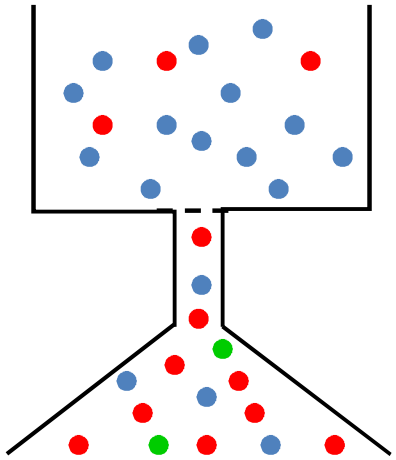
Build “best fit” models to infer major events that influenced CMV evolution

keys: standing variation
changes in variation

Selection versus Demography



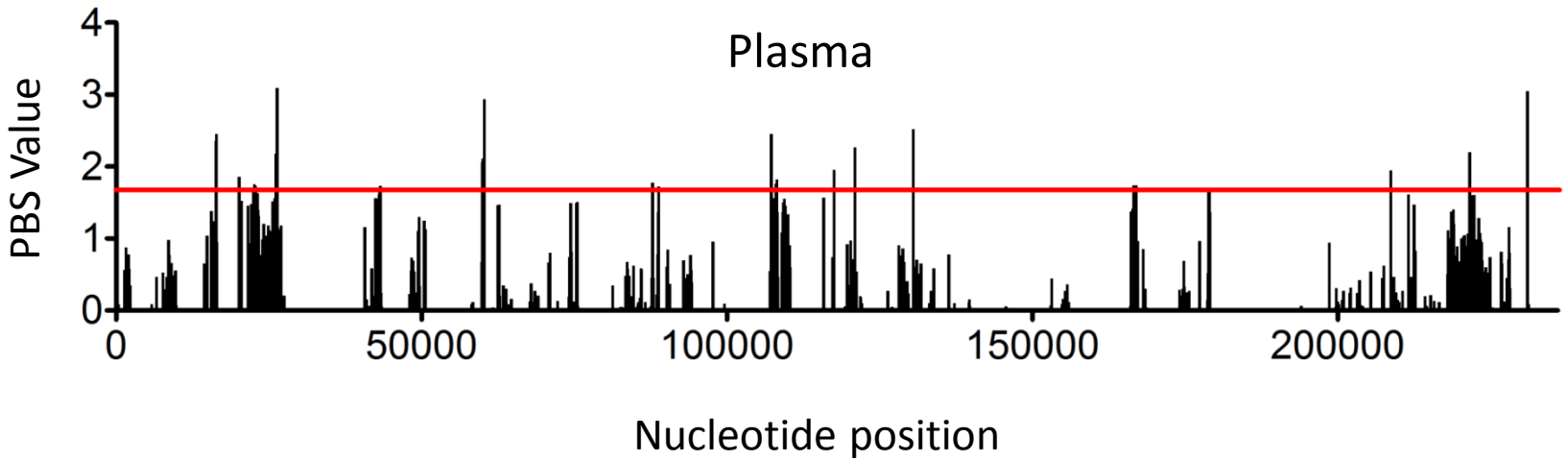
Selection



Bottleneck-Expansion

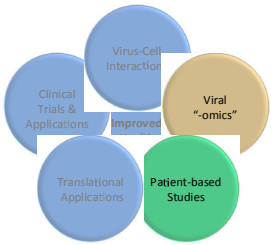
Minimal Positive Selection Over Time in Patient B103

1 week vs 6 months



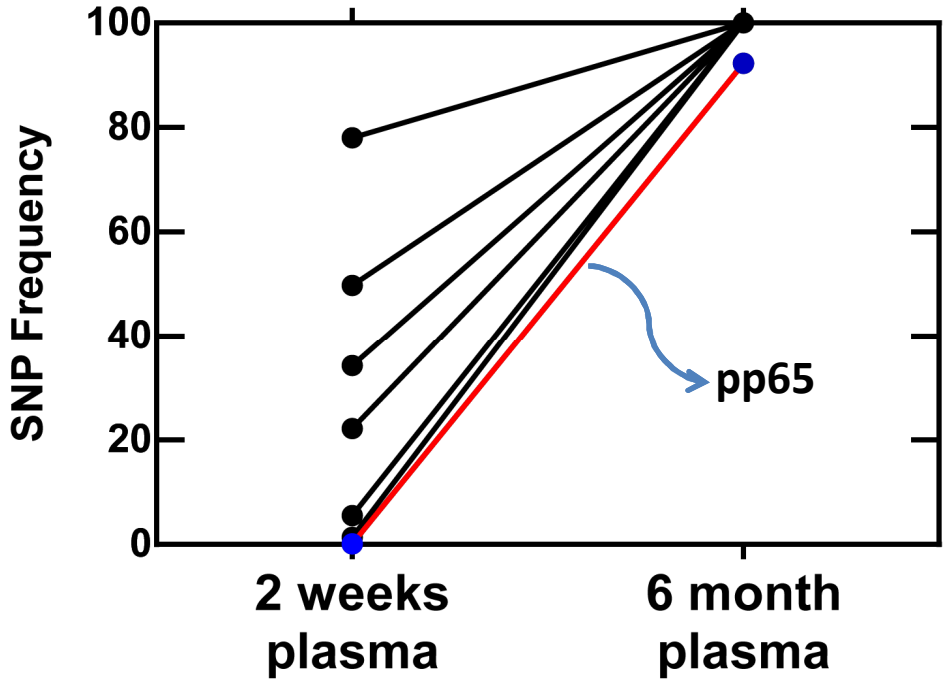
24 SNPs inferred to be under positive selection

8 SNPs are nonsynonymous (changes in amino acids)



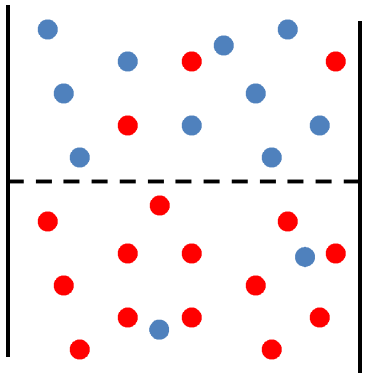
Nicholas Renzette PLoS Gen 2013

Non-synonymous SNPs within predicted CD8 T cell epitopes (plasma, patient B103)

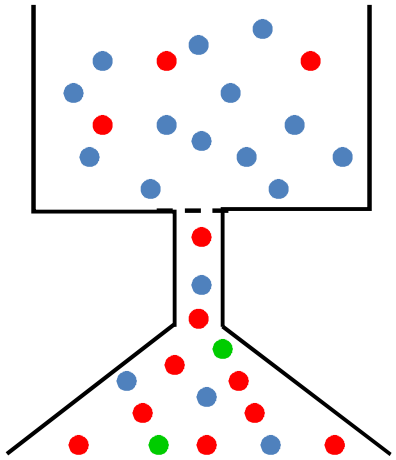


Nicholas Renzette unpublished

Selection versus Demography

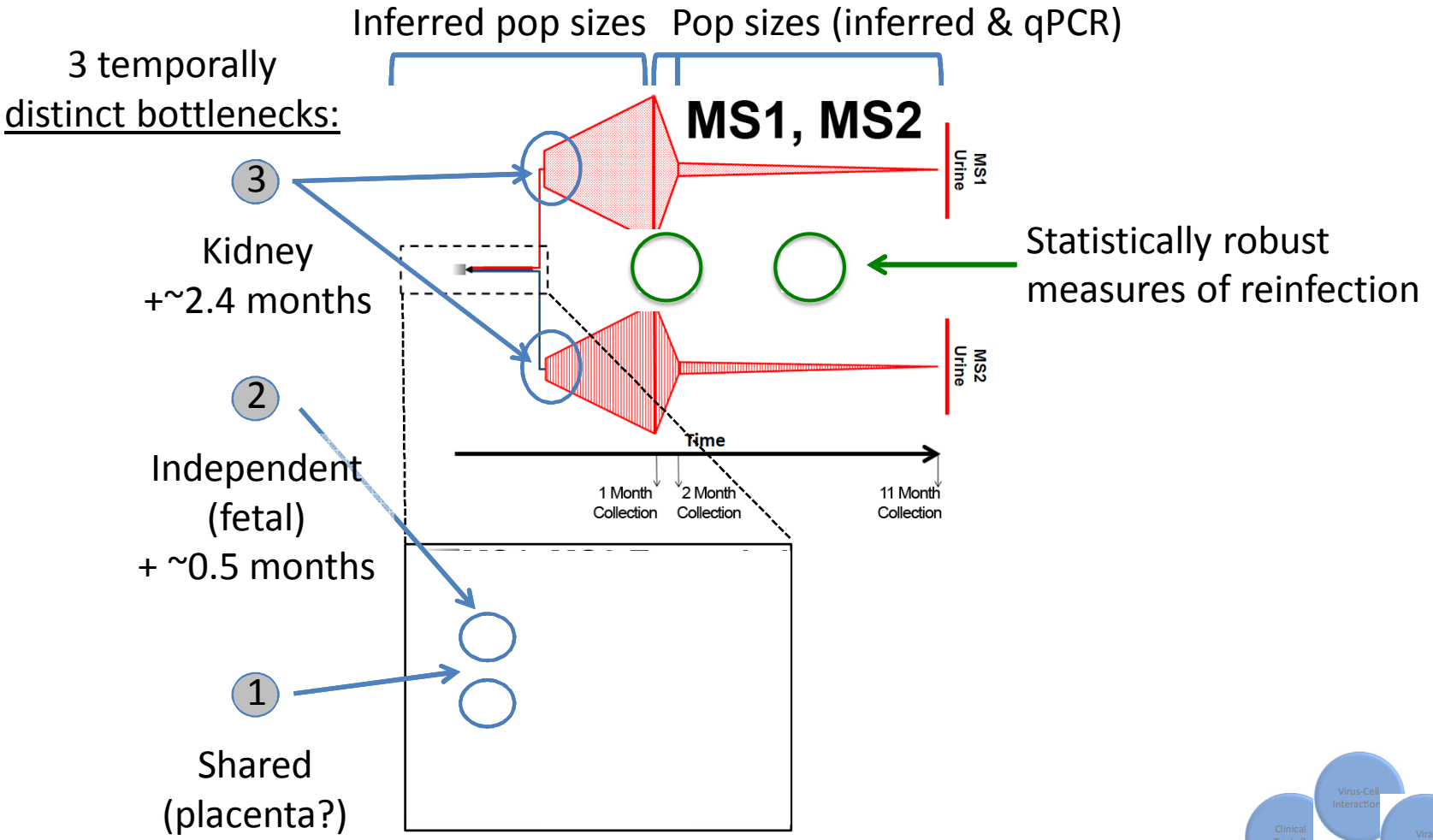


Selection



Bottleneck-Expansion

A robust demographic model of cCMV infection history [monozygotic, monochorionic twins: MS1, MS2]



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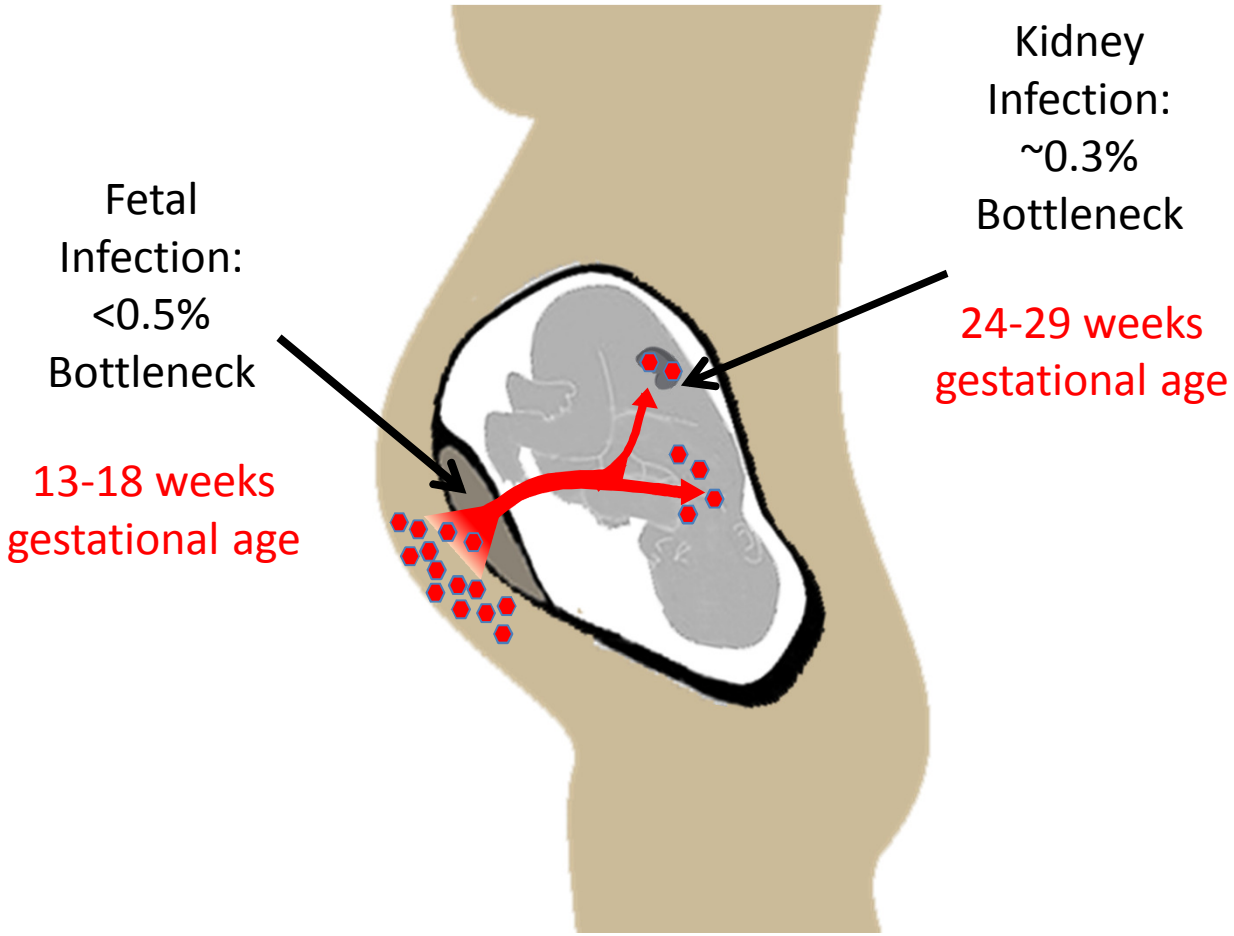
Bottlenecks associated with fetal infection & kidney colonization



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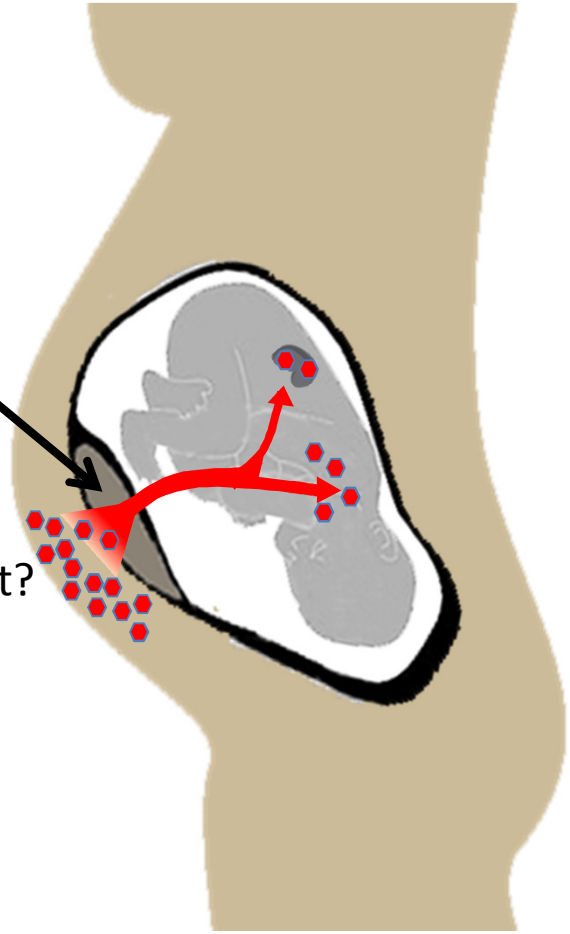
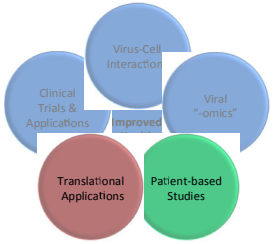
Bottlenecks associated with fetal infection & kidney colonization

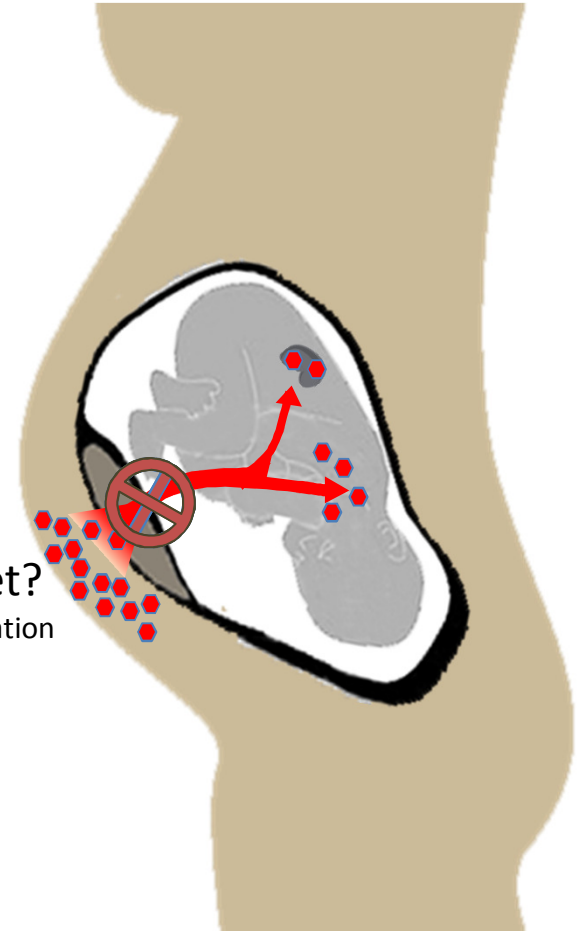
Fetal Infection: <0.5% Bottleneck



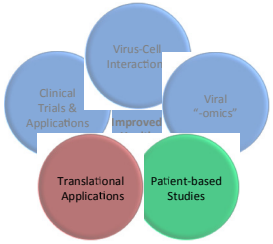
Therapeutic target?
(99.5% of maternal viral information does not cross the placenta)



Bottlenecks associated with fetal infection & kidney colonization



Therapeutic target?
(**No** maternal viral information crossing the placenta)



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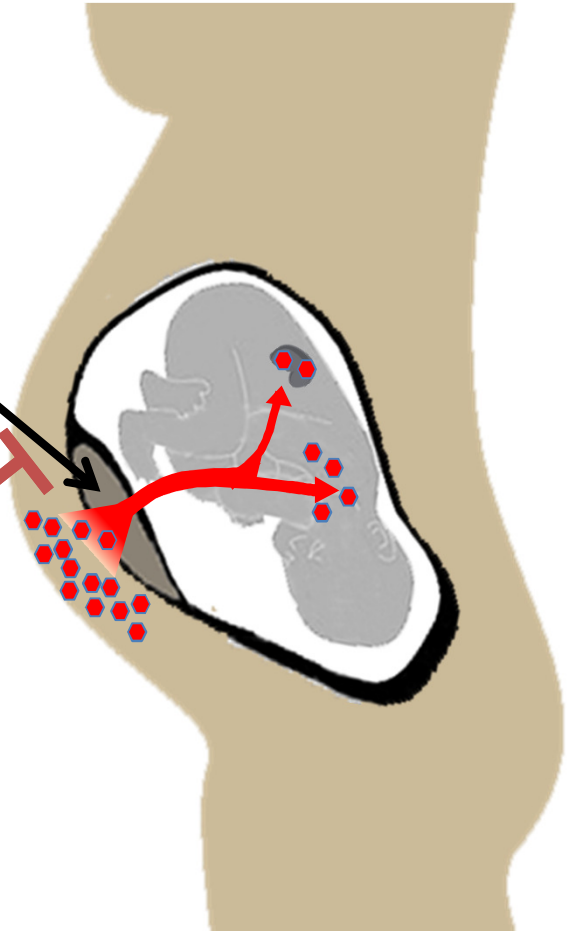
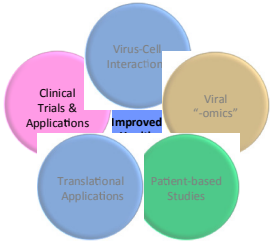


CytoGam
CSL Behring

clinicaltrials.gov NCT01376778

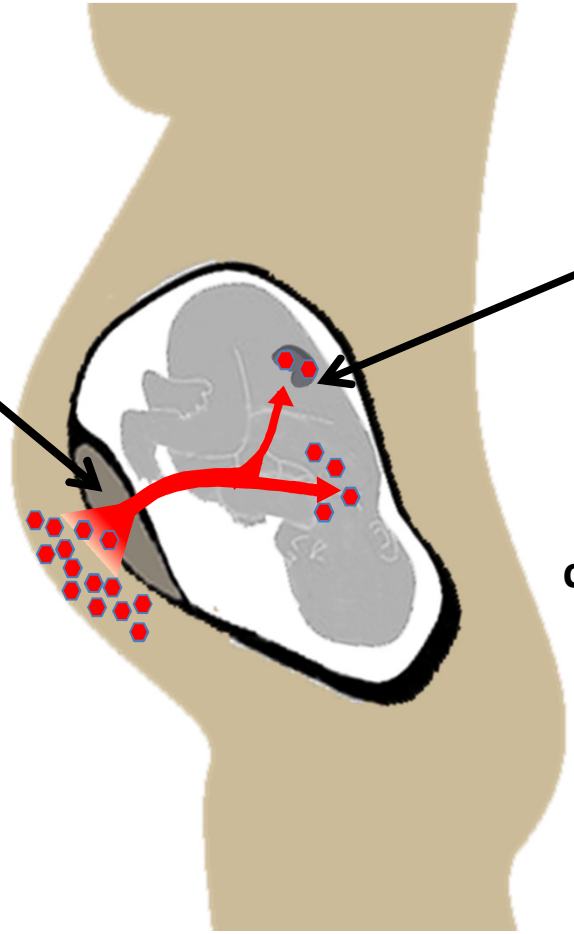
Can inferred dating of fetal infection be used to exclude false negatives?



Bottlenecks associated with fetal infection & kidney colonization

Fetal Infection: <0.5% Bottleneck

13-18 weeks gestational age

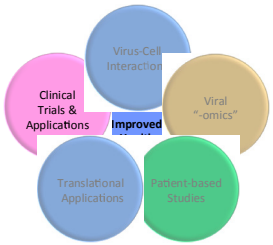


Kidney Infection: ~0.3% Bottleneck

24-29 weeks gestational age

Non-invasive method of characterizing *in utero* infections

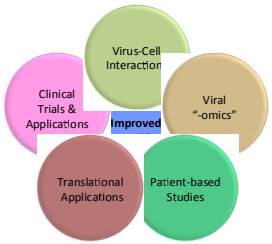
Can we use it to define differences of symptomatic vs asymptomatic infections?



Conclusions

CMV populations are variable and evolve within host compartments.
The host immune response may be a mechanism for positive selection on viral populations.

Viral genomics and population genetics tools can inform pathogenesis and the development of new therapies and vaccine designs.



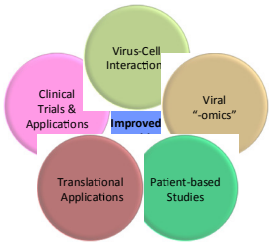
Wish list/collaborations

Longitudinal samples

Cross compartment samples (especially CNS plus another compartment)

BOTH!

Mother-infant sample sets
    symptomatic & asymptomatic sets



Acknowledgements

Kowlab members:

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Pallavi Gandhi

John Holik

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Jeff Jensen and lab members

University of Massachusetts Medical School

Laura Gibson

Richard Moser

Katherine Luzuriaga and lab members

Manual Garber and lab members

University of Minnesota Medical School

Mark Schleiss

University of Alabama School of Medicine

Bill Britt

University of São Paulo

Marisa Mussi-Pinhata

Aparecida Y. Yamamoto

University Hospital of Tuebingen

Klaus Hamprecht

Princeton University

Tom Shenk

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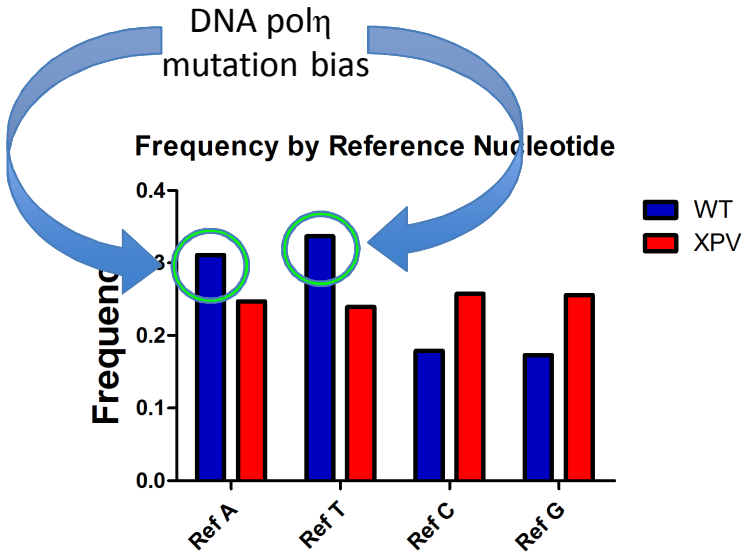
Eunice Kennedy Shriver NICHD National Institute of Child Health & Human Development

National Institute of Allergy and Infectious Diseases

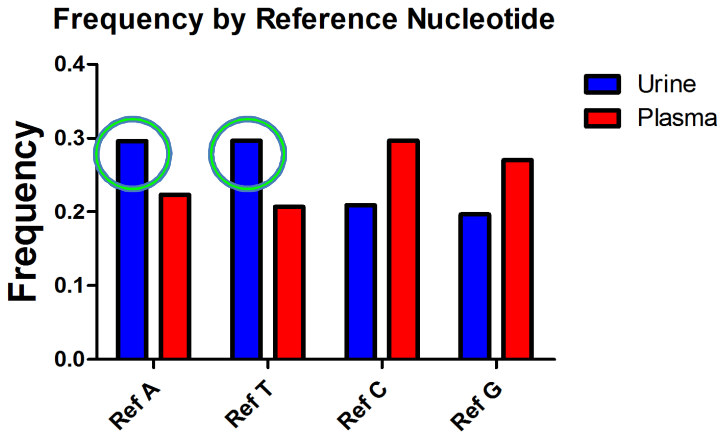
American Recovery and Reinvestment Act (ARRA)

Ruth L. Kirschstein National Research Service Award

Host cell DNA polη mutagenizes CMV DNA



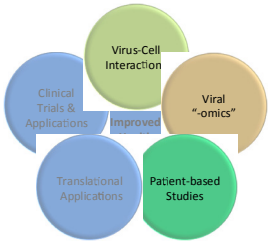
Fibroblasts *in vitro*



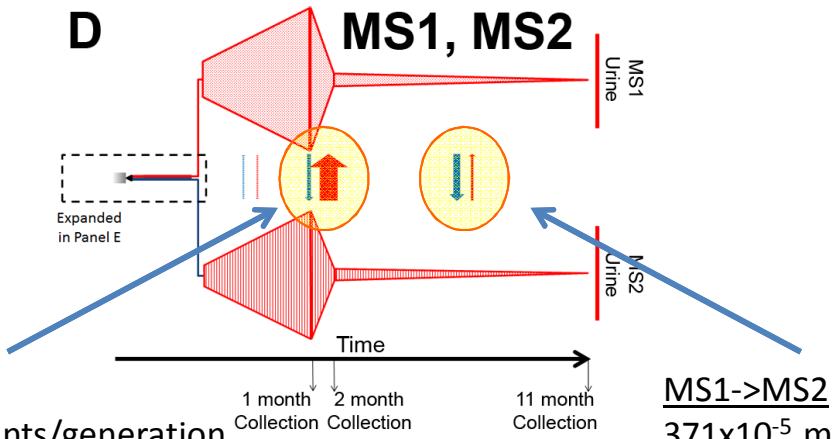
cCMV patient samples

XPV: fibroblasts from a patient with xeroderma pigmentosum complementation group V, null in *polh*

Xioafei E unpublished



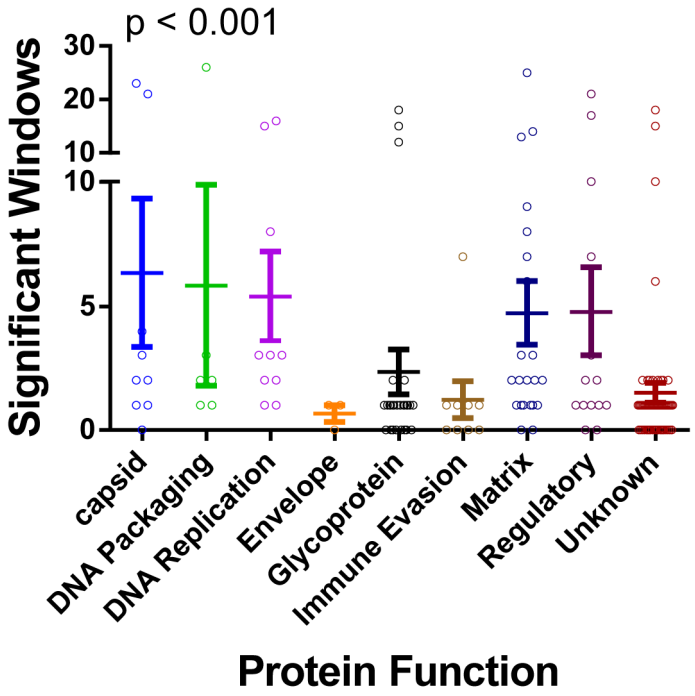
Quantitation of reinfection



MS1->MS2
92.7x10^{-5} migrants/generation
MS2->MS1
1112x10^{-5} migrants/generation

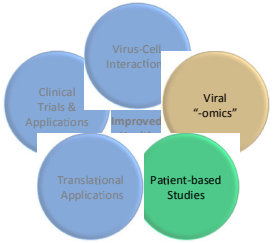
MS1->MS2
371x10^{-5} migrants/generation
MS2->MS1
21.3x10^{-5} migrants/generation

Diversity spans the CMV genome & is linked to gene function

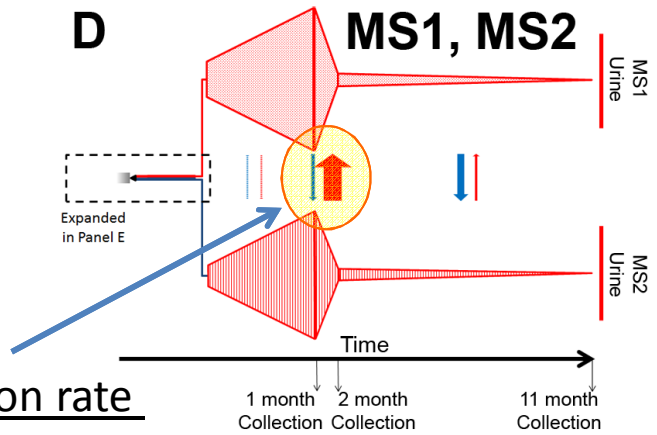


Low diversity regions are scored as sliding windows
(i.e., lower scores mean higher diversity)

Nicholas Renzette ms in prep



Migration rate context



Migration rate

~1000X>earlier rate

~3X>later rate

20X-300X>B103 intercompartment rates

Estimation of polymorphic sites in cCMV

$Loci_{MAX}$ = 161,995 (68%)

$$T_{loci} = \frac{S \times Lmax}{S + Ks} + (S \times E)$$

Where

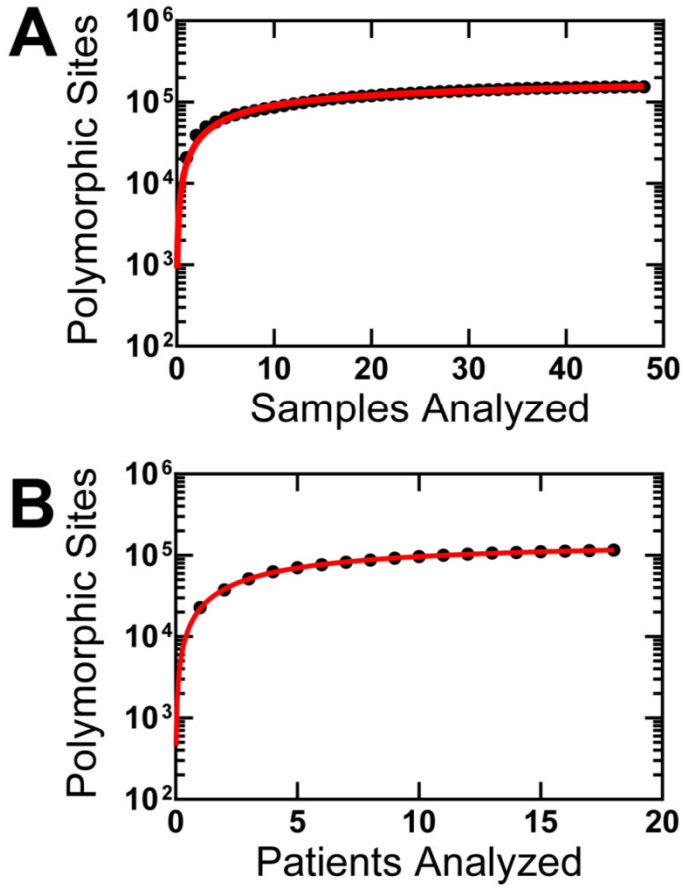
T_{loci} = Total unique polymorphic loci

S = number of samples

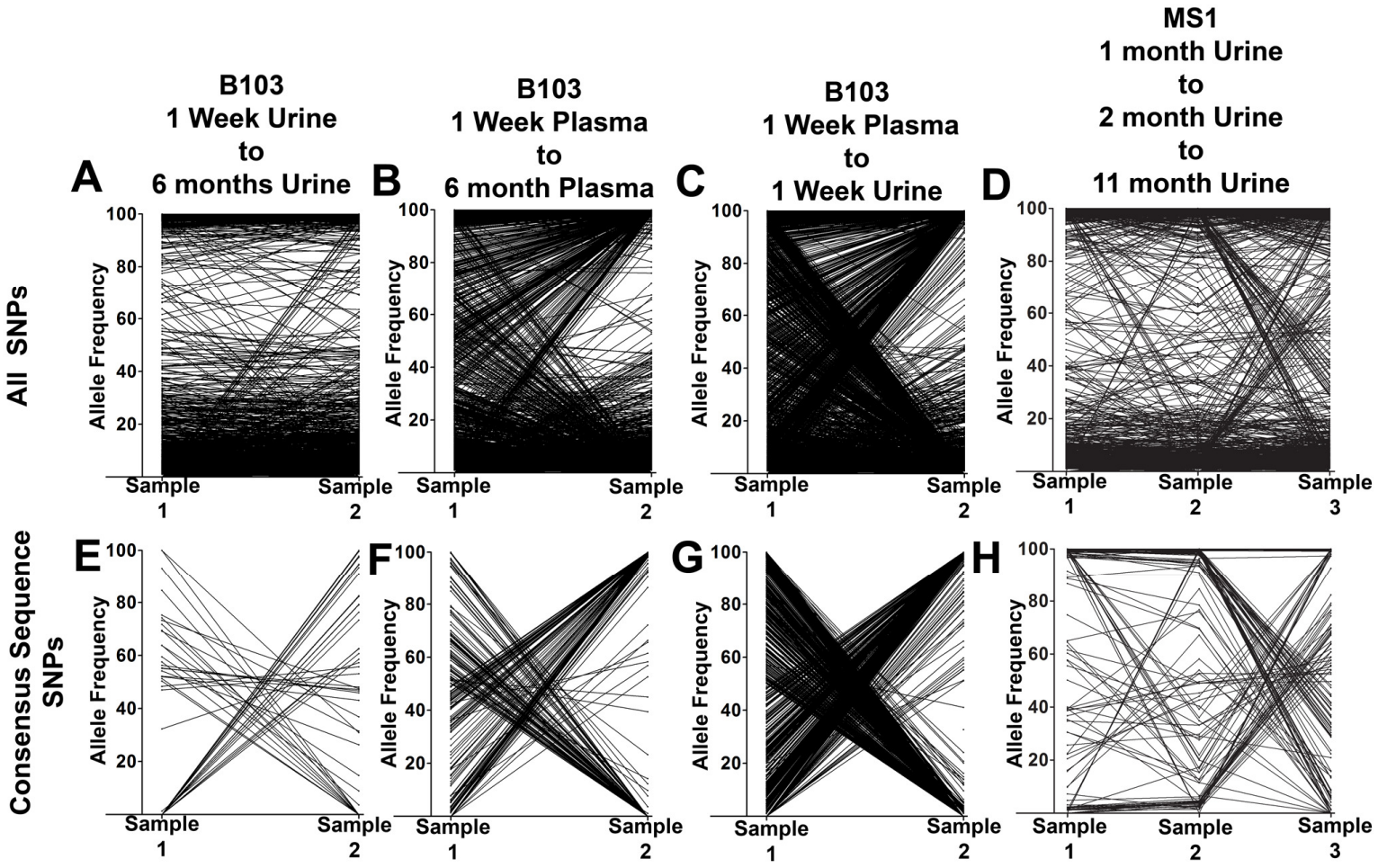
L_{max} = maximum number of polymorphic loci

E = Error rate

$Loci_{MAX}$ = 155,874 (66%)



cCMV populations evolve by time or compartment



Renzette N et al. 2013. *PLoS Genet* 9(9): e1003735, 2013

Φ = Distribution of SNP frequencies (e.g. 5 SNPs with freq=0.05, 3 with freq = 0.1, etc…)

τ = Time

$$\frac{\partial}{\partial \tau}\phi = \frac{1}{2}\sum_{i=1,2,\ldots,P}\frac{\partial^2}{\partial^2 x_i}\frac{x_i(1-x_i)}{v_i}\phi$$

$$-\sum_{i=1,2,\ldots,P}\frac{\partial}{\partial x_i}\left(\gamma_i x_i(1-x_i)+\sum_{j=1,2,\ldots,P}M_{i\leftarrow j}(x_j-x_i)\right)\phi.$$

Drift Component
ν = relative pop. size

$$\frac{\partial}{\partial \tau}\phi = \frac{1}{2}\sum_{i=1,2,\ldots,P}\frac{\partial^2}{\partial^2 x_i}\frac{x_i(1-x_i)}{\nu_i}\phi$$

$$-\sum_{i=1,2,\ldots,P}\frac{\partial}{\partial x_i}\left(\gamma_i x_i(1-x_i)+\sum_{j=1,2,\ldots,P}M_{i\leftarrow j}(x_j-x_i)\right)\phi.$$

Selection Component
(assumed = 0 in neutral datasets)

Migration Component

CMV genotyping services emerge

Cytomegalovirus (CMV) Genotype

Test Code
14980X

CPT Code(s)
87910
This test is not available for New York patient testing

Preferred Specimen(s)
1 ml plasma collected in PPT Potassium EDTA (white-top) tube

Minimum Volume
0.3 mL

Quest Diagnostics

CYTOMEGALOVIRUS (CMV) GENOTYPE

Mnemonic: CMVTYPE
Lab Discipline: Molecular Diagnostics
Maestro Care Order
Name: CYTOMEGALOVIRUS (CMV) GENOTYING
DRH Order Entry Name: CYTOMEGALOVIRUS (CMV) GENOTYPING

Institution: Duke University Health System
PDM Number: 3300246
Last Review: 6/27/2013 5:22:19 PM