

## Estimating HSV-2 superinfection using a novel custom genotyping platform

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## Disclosures

- U.S. National Institutes of Health: Grant recipient
- AiCuris GmbH, Sanofi : Principal Investigator
- Agenus, Genoecea, Vical: Co-investigator

## Why is HSV-2 superinfection relevant?

HSV-2 prophylactic vaccines are needed to control the HSV-2 epidemic

Does the natural immune response to HSV-2 protect against infection with another strain?

Will we need to create a vaccine that elicits a better or different response than natural infection?

## HSV-2 superinfection: What is known?

No standard method for HSV-2 genotyping

HSV-2 infection with more than one strain ("superinfection") has been reported in small studies

PCR based assays of variable HSV DNA repeats

HIV seronegative: 1/8 (12.5%)

HIV seropositive: 11/11 (100%)

Superinfection has been reported for other herpesviruses  
-CMV: 29% of pregnant women had 2 or more strains

Roest JID 2006  
Ross et al JID 2010

## Aims and Hypotheses

### Aim:

Determine the prevalence of and risk factors for HSV-2 superinfection.

### Hypotheses:

Prevalence of superinfection:

- Higher in women vs. men
- Higher in those with >10 sex partners vs. <10 sex partners
- Higher in HIV-infected vs. HIV-uninfected

### Approach

Phase I: Next generation sequencing to identify population prevalent SNP  
Phase II: Create genotyping platform and genotype paired samples

## HSV-2 Genomic variation

- Illumina sequencing
- Genital swab samples from 39 people
  - USA, Peru, Africa
- 2481 SNPs
- 456 prevalent SNPs evaluated for genotyping



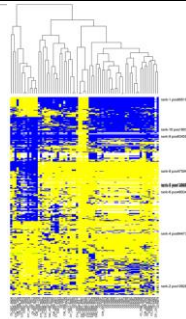
## Identification of informative SNPs

Most informative SNPs for genotyping ranked using FastTagger

With 96 SNPs:

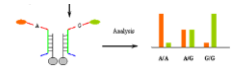
Able to determine whether samples match or do not match with >90% probability

96 SNPs best able to distinguish between specimens chosen for GoldenGate



## GoldenGate Workflow

- Bead array based platform developed for human high throughput genotyping



- Biotin label DNA hybridize to allele specific SNP oligos

- A "call" for each of 96 SNPs is generated
  - All samples with a call rate  $\geq 90\%$  SNPs considered valid

Comparison to deep sequencing:  
8 samples matched at all sites

## Methods

- Compare SNP results from paired samples
- Define superinfection  $\geq 5$  SNPs different between pairs
- Confirm mismatched pairs are from the same person
  - Deletion/insertion polymorphisms on human DNA used for forensic analysis

## Samples

- Specimen repository
  - UW-Virology Research Clinic (Washington, USA)
  - HIV Prevention Trials (HPTN039, PIP)
    - Peru, sub-Saharan Africa
- Well defined cohorts (HIV status, sexual exposure)
- Genital swabs containing  $\geq 5 \log_{10}$  copies HSV DNA/ml
- 2 samples collected from same individual over time
  - Paired samples provide ability to detect superinfection

## Results

- 1152 samples
  - 59 negative controls (4-6 per plate)
    - Median call rate: 29% (IQR: 12-45%)
  - 1093 experimental samples
    - 1004 (92%) had call rate  $\geq 90\%$
    - 960 paired samples (480 pairs)
      - 17 pairs without matching HSV sequences pending confirmation that they are from the same person
        - Excluded from this preliminary analysis
- 11 SNPs did not perform well and were excluded

## Demographics

	4,63 Pairs
Male	215 (46%)
Median Age (IQR)	34 (27, 44)
Continent	
North America (US)	274 (59%)
South America (Peru)	60 (13%)
Africa (*)	129 (28%)
Lifetime number of sexual partners median (IQR)	11 (3,34)
HIV seropositive	333 (29%)
Median months between samples (IQR)	5 (2, 11)

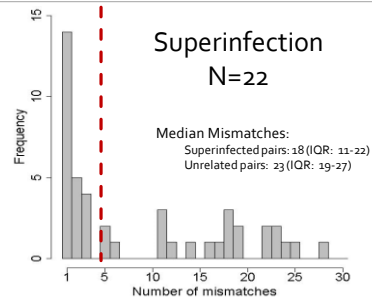
\*Botswana, Cameroon, Kenya, South Africa, Tanzania, Uganda, Zambia, Zimbabwe

## Prevalence of superinfection

Number of mismatches	Number of pairs	
	Related pairs N=463	Unrelated pairs N=1920
None	418 (90.3%)	4 (0.2%)
1-4	23 (4.9%)	11 (0.6%)
≥ 5	22 (4.8%)	1905 (99.2%)

Prevalence of superinfection (≥5 mismatches):  
22/463 (4.8%), (95% CI: 2.8%, 6.7%)

## Number of mismatches between paired specimens



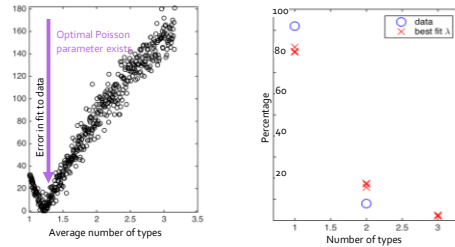
## Risk factors for superinfection

Characteristic	Univariate analysis		Multivariate analysis*	
	RR (95% CI)	p-value	RR (95% CI)	p-value
Male	0.7 (0.3, 1.5)	0.337		
Age in decades	0.8 (0.5, 1.2)	0.234		
Continent				
North America (US)	Ref	Ref	Ref	Ref
South America (Peru)	3.6 (1.0, 13.2)	0.0491	2.7 (0.7, 10.2)	0.150
Africa (*)	5.5 (2.0, 15.2)	0.001	4.0 (1.4, 11.5)	0.011
Lifetime # sex partner (each additional ten)	0.9 (0.8, 1.2)	0.592		
HIV seropositive	5.3 (2.2, 12.8)	0.0002	4.0 (1.6, 10.1)	0.0035
Samples ≥ 3 years apart	4.3 (1.5, 12.3)	0.0077		

Poisson regression  
\*No interaction between HIV status and continent

## Modeling the prevalence of superinfection

Only 2 samples tested per person  
Incorporate diversity and distribution of viral types  
Prevalence superinfection ~20%



## Strengths/Limitations

### Strengths:

- Large well characterized dataset
- Novel, robust methodology based on rationally chosen SNPs to differentiate strains

### Limitations:

- Convenience dataset
- Confirmation that additional samples are from the same person pending
- Definitions for strains are needed

## Conclusions

Naturally induced immunity at ganglion or mucosa is not sufficient to prevent reinfection  
Implications for vaccine development.

BUT, prevalence of superinfection is relatively low (~5%)  
- This is likely lowest estimate, given that 2 samples were performed per person, variable follow up

Increased risk of superinfection in persons with HIV infection  
- Lack of immunity?

Increased risk of superinfection in Africa  
- Given high seroprevalence, increased exposure?

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