# Examining the recombination landscape of Varicella-Zoster Virus through linkage disequilibrium

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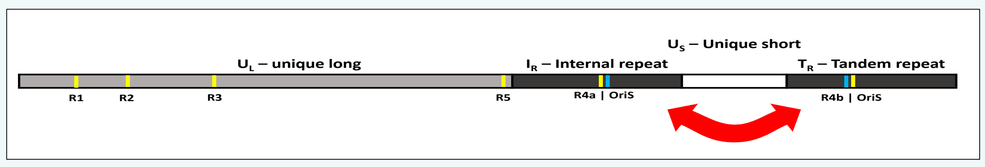
## Introduction

- Varicella-Zoster Virus (VZV), a human herpesvirus, causes varicella (chicken pox) in primary infection, and establishes life-long latency in ganglionic neurons, reactivating in a subset of individuals as zoster (shingles)
- The role of recombination in shaping the evolution of the VZV genome is not well defined using standard methods for investigating recombination
- In this study, VZV strains were collected and isolated from herpes zoster skin lesions in 1,312 patients and combined with 74 Genbank VZV sequences
- VZV recombination was evaluated using novel linkage disequilibrium approaches to:
  1. Examine recombination rate
  2. Distinguish if recombination plays a functional role in VZV evolution
  3. Identify local genomic regions in which recombination is unlikely to occur



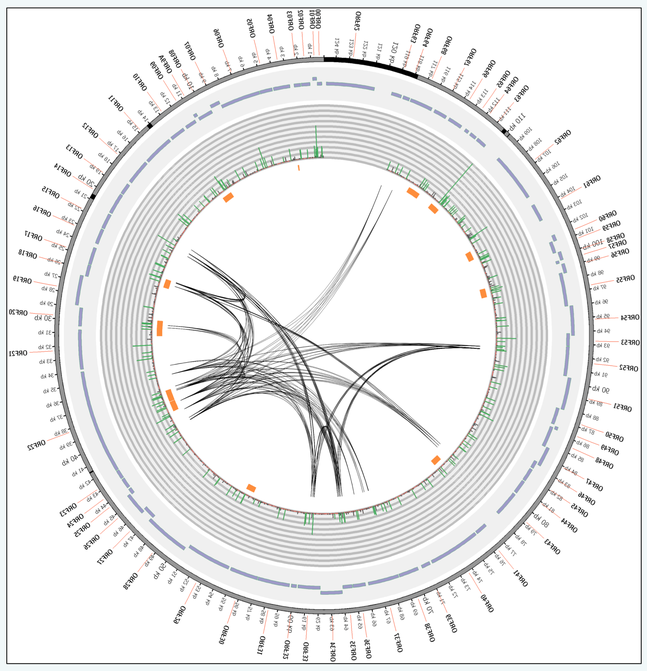

*VZV Genomic Structure. Red double-sided arrow shows identical sequence regions*

## Linkage Disequilibrium (LD) Analysis

- LD occurs when genotypes of two loci are not independent of one another and is less common as physical distance between the two loci increases, exhibiting an inverse relationship with recombination
- LD was explored in all VZV sequences:
  1. At a genome-wide scale to test LD significance between all possible bi-allelic site pairs using Fisher's exact test on the 2x2 contingency table of counts
  2. At a local scale to detect domains of LD using a sliding window scan. The $-\log10(P_{LDFisher})$ transform from a window were compared to the distribution of $-\log10(P_{LDFisher})$ for the genome-wide set of comparisons made in this range using a Mann-Whitney Wilcoxon U test

## Long-range genome wide associations

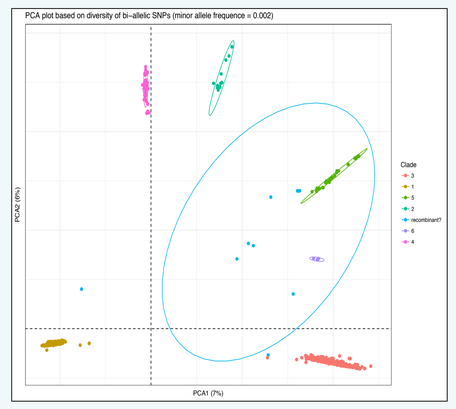
- High LD between two sites can indicate coinheritance between two loci and may distinguish areas with low recombination rates
- Linkage between 2,589 bi-allelic sites across 118 kb VZV alignments revealed **20,957** sites in significant linkage with **90% of distances less than 74 kb** (long-range)
- In circos plot: Purple bands represent ORFs. Green peaks show nucleotide diversity relative to maximum diversity (0.2) in window sizes of 50. Regions of significant linkage represented by orange blocks. Connections across genome indicates top 3% of ORFs most often linked by non-synonymous sites.



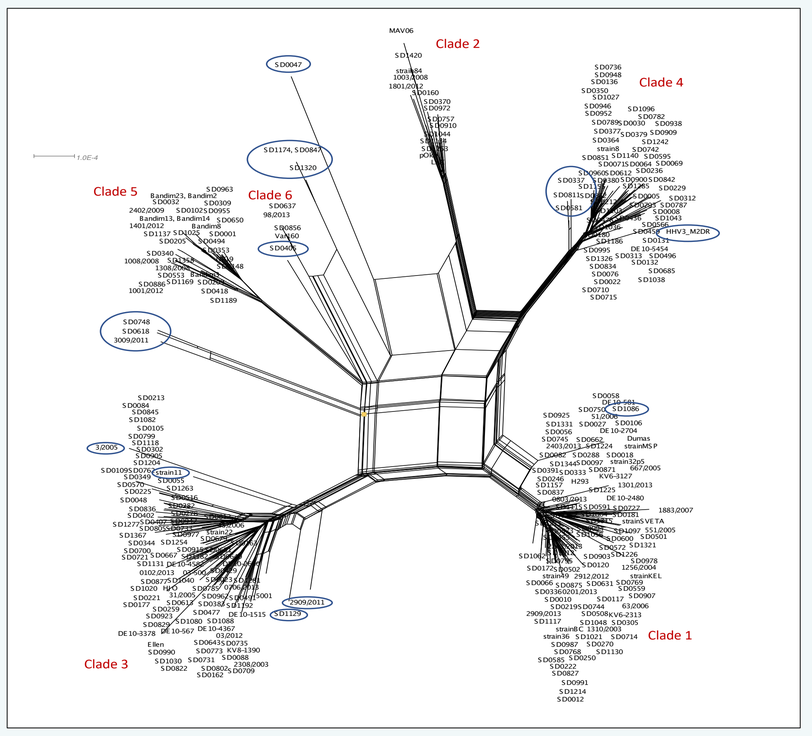
*Genome-wide and local LD*

## Recombination & Clade Structure

- Recombination has been shown to play a role in shaping the evolution of other herpesviruses (HSV-1, HSV-2, EBV, HCMV)
- Standard phylogenetic methods for evaluating recombination have been used to separate VZV strains into 6 clades and identify few inter- and intra-clade recombination events
- These methods are limited as they provide no information about recombination rate or the functional significance of recombination events



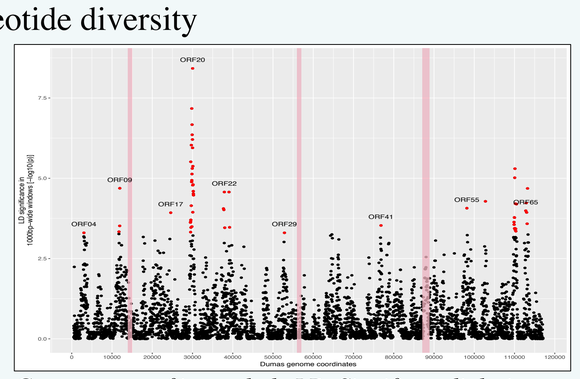

*PCA of bi-allele SNPs diversity of 1,386 VZV sequences annotated by clade*




*Phylogenetic network of 600 randomly sampled VZV strains. Distinct clades annotated in red. Potential recombinants circled in blue*

## LD scan reveals 9 local hotspots

- A sliding scale was used to test regions of 1000 bp containing 10 bi-allelic sites in 95% of the windows
- Hotspots of local linkage were identified in **9 out of 68 ORFs**
- Regions of hotspot linkage are significantly associated with areas of higher nucleotide diversity




*Genome map of inter-clade LD. Significant links in red. Pink bands indicate where bi-allelic sites < 10 per 1000 bp window*

## Conclusions

1. While VZV undergoes recombination, the frequency at which recombination occurs seems to be much lower than other herpesvirus types, which lack well-defined clade structure arising from controlled recombination
2. Lower recombination rates may indicate that recombination is not a significant driver in the functional evolution of VZV
3. High abundance of both short- and long-range linkage implies that recombination is not freely occurring throughout the genome
4. Association of linkage among hypervariable loci may identify candidate regions for selective sweeps – variation is reduced or eliminated around a fixed mutation with strong positive natural selection. Hotspot regions may lack recombination because advantageous mutations are becoming fixed across populations.

## Literature Cited

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