# VZV Molecular Clade Analysis and the Incidence of Vaccine Strain Compared to Wild-Type Virus in CNS and Non-CNS Disease

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## Background

- Varicella zoster virus (VZV) is the causative agent of varicella (chickenpox) and zoster (shingles).
- The introduction of live-attenuated vaccine has drastically reduced VZV burden in the population, but the virus continues to be a significant public health issue.
- VZV commonly causes mild disease but can result in severe complications including CNS involvement with encephalitis
- Circulation of clades 1-6 occur worldwide (**Figure 1**), with dominant clades representing each region
- The clade 2-derived vaccine strain can cause adverse reactions and has been documented to establish latency and reactivate to cause zoster
- Reported cases of vaccine-associated CNS disease are rare and the extent of vaccine-associated CNS involvement is unclear.
- We aimed to assess the frequency of vaccine-associated CNS disease and compare VZV clade distribution in CNS and non-CNS disease.

**Figure 1: Global distribution of VZV clades**

- Schmidt-Chanasit and Sauerbrei, Genetics and Evolution 11 (2011).

## Methods

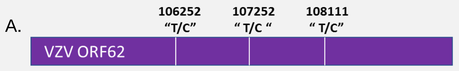
### Wild-type/Vaccine Discrimination

- Cerebrospinal fluid (CSF) from patients with encephalitis or meningitis, collected from 2004-2017, and non-CSF specimens (lesion, genital, rectal swabs), collected from 2013-2017, were reviewed for study.
- VZV-positive samples were selected for further characterization, including wild-type/vaccine discrimination and genotype analysis.
- VZV viruses were determined to be wild-type or vaccine using three separate bi-allelic TaqMan real-time PCR assays, each targeting a specific known SNP marker in ORF62 of VZV **(Figure 2A**).
- These positions discriminate between wild-type and the vOka (vaccine) strains due to a change from a thymidine in wild-type to a cytosine nucleotide in the vaccine strain.

### Genotyping

- Genotyping was performed on all wild-type VZV specimens.
- Amplification of ~500bp fragments of ORF21, 22, and/or ORF50 was performed by conventional PCR using Qiagen's HotStarTaq DNA Polymerase (Germantown, MD).
- PCR products were visualized on 1% TAE agarose gels and purified for sequencing using ExoSAP-IT™ PCR Product Cleanup Reagent (Affymetrix, Santa Clara, CA).
- Bi-directional dideoxy sequencing was performed on an ABI 3730xl DNA analyzer.
- Unique, clade-specific SNPs were determined at multiple known sites across the sequenced fragments using Geneious 9.1.7.
- Clades were identified by analysis of the SNP combinations found within the three ORFs **(Figure 2B).**
- Statistical analyses were performed using Pearson's chi-squared test in SPSS software.

A.




B.

| ORF | 21 | | 22 | | | | 50 |
|---|---|---|---|---|---|---|---|
| Nucleotide position | 33725 | 33728 | 37902 | 38055 | 38081 | 38177 | 87841 |
| Clade 1 | T | T | A | T | A | G | C |
| Clade 2 | C | C | G | C | C | A | T |
| Clade 3 | C | C | A | T | A | G | T |
| Clade 4 | C | C | A | C | C | A | T |
| Clade 5 | C | C | A | T | C | G | T |
| Clade 6 | C | C | A | T | C | A | T |

**Figure 2**: **VZV wild-type/vaccine strain discrimination and genotyping schemes**. **A**. Three separate real-time assays target known SNPs in ORF62 of the VZV genome. **B**. Clade-specific SNP combinations used for VZV genotyping.

## Results

### Wild-type/Vaccine Detection

- 277 VZV-positive CSF samples and 600 VZV-positive non-CSF samples were tested for WT/VAC determination.
- 13 vaccine strains were detected (1.48%). One vaccine strain was detected in the CSF of an 11-year-old male collected in 2010 **(Table 1**).
- Of the 12 vaccine strains detected in non-CNS disease, nine were from children less than 10, two were in the 11-20-year age group, and one patient was in the 41-50 year age group **(Table 1).**
- Vaccine status of patients is currently unknown**.**

**Table 1**: **Detection of VZV vaccine strains in CSF and non-CSF samples**

| Sample Type | VZV Positive | Vaccine strain detected (% of total) | Age of patients with vaccine strain detected (%) | | |
|---|---|---|---|---|---|
| | | | 0-10 yrs. | 11-20 yrs. | 41-50 yrs. |
| CSF | 277 | 1 (0.36) | | 1 (100) | |
| Non-CSF | 600 | 12 (2) | 9 (75) | 2 (17) | 1 (8) |

### Clade Analysis and Distribution in CNS and non-CNS Disease

- 158 CSF and 571 non-CSF samples were successfully genotyped.
- A diversity of clades were detected in both CNS and non-CNS disease. Clades 1-5 were detected in both sample sets.
- Three clade 6 viruses were detected in non-CSF specimens from three different patients in 2017.
- Clades 1 and 3 were most prominent in both CNS and non-CNS disease.
- Distribution of clades 1-5 were statistically similar between CNS and non-CNS disease.
- Gender proportions in CNS and non-CNS disease were statistically similar.
- VZV incidence between CNS and non-CNS disease was statistically similar in age groups 21-40, 41-60, and 61-100 years (p>0.05, Pearson chi-squared test). Patients in the 0-20 year age group showed a statistically higher incidence of non-CNS disease, as compared to CNS disease (p=0.03, Pearson chi-squared test).

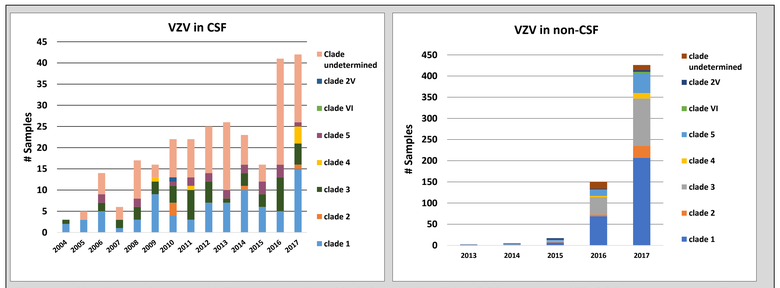



**Figure 4: Yearly distribution of VZV clades in New York State.** **A**) **VZV in CSF samples**. 277 CSF samples were VZV-positive. One vaccine strain was detected in 2010. 158 samples were successfully genotyped. **B) VZV in non-CSF samples**. 600 samples were VZV-positive. 12 vaccine strains were detected overall. 559 wild-type viruses were successfully genotyped. Clade 2V represents detection of the vaccine strain. "Clade undetermined" indicates that real-time wildtype/vaccine discrimination PCR was VZV-positive, but genotyping was unsuccessful due to low viral DNA concentrations in the sample.

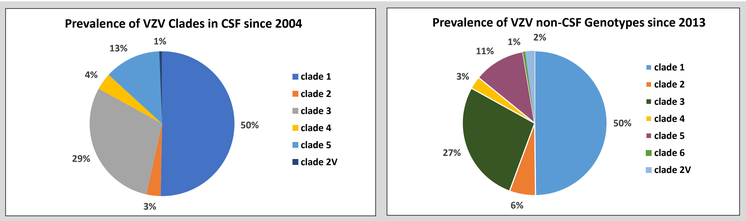
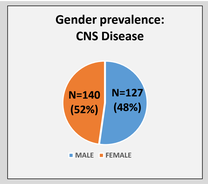



**Figure 5: Clade prevalence in CNS and non-CNS disease. A) CSF samples. B) Non-CSF samples.** Clades 1 and 3 were most prominent in both CSF and non-CSF samples. Clade 1 represented 50% of the successfully genotyped samples in both sample sets. Clade 6 was detected in three patients with non-CNS disease (1%). Statistical analyses indicated there was no significant difference between clade proportions in CNS and non-CNS disease (Clades 1-5, p>0.05, all comparisons, Pearson's chi-squared test).

## Results

A.

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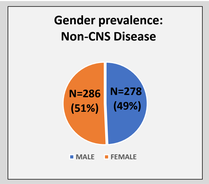
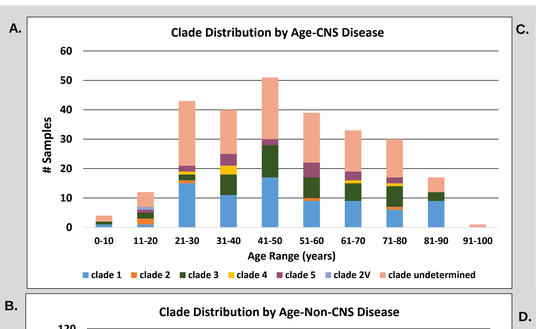
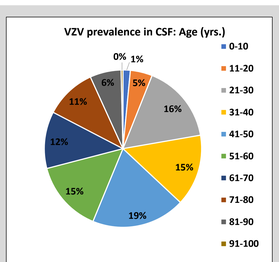



**Figure 6: Gender prevalence in CNS and non-CNS disease. A) CNS disease. B) Non-CNS disease.** No statistically significant difference exists between gender proportions in CNS and non-CNS disease (p>0.05, Pearson's chi-squared test).

A.




C.

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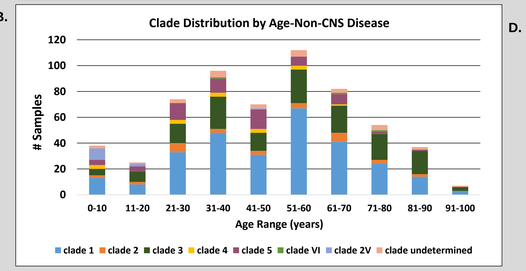


D.

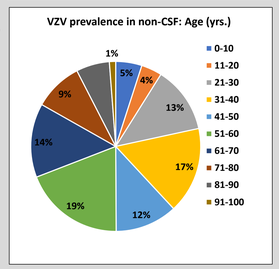


**Figure 7: VZV clade distribution and prevalence, by age, in CNS and non-CNS disease. A) Distribution in CNS disease.** The majority of samples were received from patients in the 41-50 year age group. VZV-positive CSF samples were lowest in the youngest and oldest age groups. Due to low viral titers in the CSF, a majority of VZV viruses failed to genotype**. B). Distribution in non-CNS disease**. The highest number of samples were received from patients in the 51-60 year age group. **C). Prevalence in CNS** and **D) Non-CNS disease**. Patients aged 0-20 yrs showed statistically higher incidence in non-CNS disease (p=0.03, chi-squared test). All other age ranges were statistically similar in CNS vs. non-CNS disease incidence.

## Conclusions/Discussion

- The vaccine strain of VZV can cause disease in all ages, but at a much lower rate than wild-type virus.
- Vaccine strain was detected in only one CSF sample from a patient with meningitis, suggesting that, while capable of causing CNS disease, this is a rare occurrence.
- A high diversity of VZV clades circulate and cause CNS and non-CNS disease, however, clades 1 and 3 represented the majority of VZV genotypes associated with both CNS and non-CNS disease.
- Interestingly, statistical analysis indicated that clade proportions are distributed similarly in mild and moderate VZV disease manifestations to those in more severe CNS disease.
- These findings highlight the pathogenic potential of VZV, independent of clade.

## Works Cited

- VZV global circulation paper
- Real-time paper (VZV vaccine SNP paper)-
- Genotyping paper -Lubreva

## Acknowledgements

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