

# Deep Sequencing of Varicella-Zoster Virus in Aqueous Humor From a Patient With Acute Retinal Necrosis Presenting With Acute Glaucoma

Joanna von Hofsten,<sup>1,2</sup> Johan Ringlander,<sup>3</sup> Peter Norberg,<sup>3</sup> Madeleine Zetterberg,<sup>1,4</sup> Maria Andersson,<sup>3</sup> Magnus Lindh,<sup>3</sup> and Tomas Bergström<sup>3</sup>

<sup>1</sup>Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, <sup>2</sup>Department of Ophthalmology, Halland Hospital Halmstad, Halmstad, Sweden, <sup>3</sup>Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Sweden, <sup>4</sup>Department of Ophthalmology, Sahlgrenska University Hospital, Mölndal, Gothenburg, Sweden

We report a case of acute retinal necrosis presenting with acute glaucoma preceding inflammatory signs by several days. High-throughput sequencing on aqueous humor revealed a low-level diversity in the viral genome comparable to diversity seen in cutaneous vesicles in contrast to high diversity in encephalitis.

**Keywords.** acute retinal necrosis; aqueous humor; high-throughput sequencing; varicella zoster virus.

Acute retinal necrosis (ARN) is a rare sight-threatening disease caused by reactivation of herpes simplex virus (HSV)1, HSV2, or varicella-zoster virus (VZV) [1]. Clinical signs include progressive peripheral retinal necrosis, occlusive vasculopathy, and prominent panuveitis. Incidence is approximately 1 case per 2 million people a year. Visual prognosis is poor despite antiviral treatment. The cause of the severity of ARN is unknown. Patients are often immunocompetent. Visual loss, floaters, and occasionally pain are common complaints in ARN patients. Different stages of inflammation are observed at presentation.

Deep sequencing has become an important application for detection and genetic characterization of pathogens in disease. Considering analysis of virus in intraocular fluids reports have been sparse [2, 3]. Sequences of VZV registered in GenBank derive from other body compartments.

## CASE PRESENTATION

A 65-year-old healthy woman presented with 5 days of discomfort in the left eye. Her visual acuity was 20/20 and she reported no symptoms of floaters or visual loss. Intraocular pressure (IOP) was 50 mmHg. The patient was examined by a senior ophthalmologist with slit lamp biomicroscopy at the tertiary referral hospital in the region of Halland, Sweden. The cornea was clear and flare or corneal precipitates were absent. No inflammatory reaction was found in the posterior segment. Because of the clear conditions, gonioscopy could be performed and no apparent pathology was detected in the iridocorneal angle. Pharmacological IOP-reducing treatment was initiated immediately.

Follow-up was scheduled 4 days later at a smaller hospital. At this time, inflammatory sign had evolved and visual acuity had dropped to 20/40. Corneal edema, flare with cells in the anterior chamber, and vitreous condensations were present. A retinal hemorrhage at the margin of the optic disc was noted. The patient was ordered corticosteroid topically at an hourly basis. There was significant deterioration during the following 7 days, and the patient was referred back to the regional referral hospital. The visual acuity was now hand movements and peripheral necrosis was evident. She was treated for suspected ARN because all criteria of ARN were met. Acyclovir was administered intravenously (15 mg/kg 3 times a day) and an aqueous tap was performed at the day of arrival. Aqueous humor was positive for VZV deoxyribonucleic acid (DNA) by TaqMan quantitative real-time polymerase chain reaction (qPCR) with a viral load of  $1.1 \times 10^9$  DNA copies per milliliter [4].

Despite treatment, the patient subsequently deteriorated and complications occurred with peripheral circumferential detachment of the retina and hypotony. Visual acuity remained at hand movements at follow-up.

From the aqueous humor sample, DNA was extracted with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany). No amplification by PCR or other target enrichment was done before sequencing, which was performed using the Ion Torrent/Ion S5 system (Life Technologies, Carlsbad, CA). We produced libraries of approximately 10 000 000 reads in total that were mapped to a VZV reference genome (Dumas strain; GenBank accession no. NC\_0011348.1) using CLC Genomic Workbench 11 aligner (QIAGEN, Hilden, Germany); parameters are stated in the [Supplementary Material](#). Number of reads aligned to VZV were 68,576. The complete genome was covered, with an average and a maximum coverage of 98.91 and 235 reads depth, respectively. Coverage graphs are found in the [Supplementary Material](#). The consensus sequence was retrieved using CLC

Received 27 April 2020; editorial decision 18 May 2020; accepted 22 May 2020.

Correspondence: Joanna von Hofsten, MD, Department of Ophthalmology, Halland Hospital Halmstad, S-301 85 Halmstad, Sweden ([joanna.vonhofsten@regionhalland.se](mailto:joanna.vonhofsten@regionhalland.se))

Open Forum Infectious Diseases®

© The Author(s) 2020. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)  
DOI: 10.1093/ofid/ofaa198



Heterogeneous within-host populations may be explained either by spontaneous mutations occurring after primary infection or by multiple infections with different strains. To investigate which of these scenarios that underlies the heterogeneity detected here, we created consensus sequences including all minority SNPs for respective strain. These were named Zos/Cli/SWE/aq/1908/2015(CM) and Zos/Cli/SWE/aq/2109/2015(CM) and included in the phylogenetic analyses (Figure 1). The minority consensus strains cluster adjacent to the majority strains, indicating that the SNPs are exclusive for these strains. This was further confirmed by manual comparison of each SNP to other VZV sequences available in GenBank. We thus conclude that these SNPs are results of spontaneous mutations after primary infection rather than multiple infections or recombination of different strains.

#### Patient Consent and Ethical Approval

Patient consent was obtained from both patients participating in this work. Ethical approval was granted by the Swedish Ethical Review Authority, and the tenets of the Declaration of Helsinki were followed.

## DISCUSSION

We present a case with high IOP as an initial sign of ARN. Despite meticulous clinical examination, no signs of inflammation were present at first visit. Intraocular hypertension is per se not an uncommon feature in ARN. However, the mechanism behind raised pressure is believed to be secondary to inflammation such as occlusion of the trabecular meshwork by inflammatory cells, synechiae formation blocking the aqueous circulation, or, after longstanding inflammation, synechiae in the iridocorneal angle. Virus trabeculitis has also been suggested, although it is considered a speculative term.

For ocular disease, latency of VZV after chickenpox is known to be localized in the trigeminal ganglion, which makes sense for reactivations such as herpes zoster ophthalmicus with cutaneous affection. However, in ARN and in particular in this case, regions with mainly autonomous innervation are primarily affected, suggesting possible reactivation from autonomous ganglia (such as the ciliary ganglion). Richter et al [6] analyzed autonomic ganglia from human cadavers with PCR, and these were shown to harbor VZV DNA. An alternative autonomous path for reactivation for ARN was proposed.

Varicella-zoster virus is considered to have a well conserved genome of approximately 125 000 base pairs with few spontaneous mutations and geographically separated clades.

Variations in the nucleotide sequence is described as SNPs and can be used to distinguish clades phylogenetically.

However, if there is a varying frequency of SNPs in the same host, this indicates subpopulations of virus. Depledge et al [7] investigated whether there is a difference in variability depending on body compartment and found a significantly higher variation in nonvesicle fluid (such as cerebrospinal fluid, bronchoalveolar lavage, serum) compared with vesicle fluid. Variation was also significantly higher in cerebrospinal fluid in encephalitis compared with meningitis. Samples from encephalitis patients had up to 169 SNPs in VZV genome compared with vesicle fluid with an average of less than 10 SNPs. Zos/Cli/SWE/aq/1908/2015 and Zos/Cli/SWE/aq/2109/2015 in our material had less than 10 SNPs, resembling variability in vesicle fluid. To our knowledge, the variability of VZV in ocular fluids has not previously been investigated, and we have no data on variability in other ocular clinical manifestations of VZV.

A possible explanation of higher variability is reactivation of virus from multiple ganglia or neurons in encephalitis. Low variability in ARN is therefore in keeping with presumed reactivation from a single ganglion and/or combined with a bottle-neck effect in the path from the reactivating ganglia to the eye.

Our observations confirm that deep sequencing successfully can be performed on aqueous humor and data used for antiviral resistance detection and viral genotyping. The method might also be valuable for unbiased pathogen detection in ocular infections in which no causative agent has been found when using PCR or other targeting methods.

## CONCLUSIONS

Acute glaucoma can be the first sign of ARN even in absence of overt inflammation. Emerging inflammatory signs in the aftermath of acute glaucoma should alert physicians on the possibility of ARN, requiring high doses of antiviral treatment to stop progression. Despite atypical presentation of a rare form of a serious VZV manifestation with a poor prognosis, the causative virus had a comparatively low genetic variability and clustered phylogenetically with other wild-type strains that are common in Europe. Variability was comparable to SNPs in VZV strains derived from vesicle fluid. Further investigations are needed to determine the genetic variability in viral ocular infection and its clinical relevance.

#### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

#### Acknowledgments

We thank the patients who agreed to participate in this study.

**Disclaimer.** The views expressed are those of the authors and not necessarily those of the funders of this work.

**Financial support.** This work was funded by grants from the Swedish Medical Research Council (Region Halland and ALF [to M. Z., T. B.], GLS [Göteborg Medical Society]) and D&B Dahlin Foundation.

**Potential conflicts of interest.** All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

## References

1. Takase H, Okada AA, Goto H, et al. Development and validation of new diagnostic criteria for acute retinal necrosis. *Jpn J Ophthalmol* **2015**; 59:14–20.
2. Abernathy E, Peairs RR, Chen MH, et al. Genomic characterization of a persistent rubella virus from a case of Fuch' uveitis syndrome in a 73 year old man. *J Clin Virol* **2015**; 69:104–9.
3. Heath G, Depledge DP, Brown JR, et al. Acute retinal necrosis caused by the zoster vaccine virus. *Clin Infect Dis* **2017**; 65:2122–5.
4. Persson A, Bergström T, Lindh M, et al. Varicella-zoster virus CNS disease–viral load, clinical manifestations and sequels. *J Clin Virol* **2009**; 46:249–53.
5. Huson DH. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* **1998**; 14:68–73.
6. Richter ER, Dias JK, Gilbert JE 2nd, Atherton SS. Distribution of herpes simplex virus type 1 and varicella zoster virus in ganglia of the human head and neck. *J Infect Dis* **2009**; 200:1901–6.
7. Depledge DP, Cudini J, Kundu S, et al. High viral diversity and mixed infections in cerebral spinal fluid from cases of varicella zoster virus encephalitis. *J Infect Dis* **2018**; 218:1592–601.