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# Detection of Varicella-Zoster Virus Genome in the Vitreous Humor From Two Patients With Acute Retinal Necrosis; Lacking or Having a *Pst*I Cleavage Site

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**Abstract:** Using polymerase chain reaction, we detected the varicella-zoster virus genome in the vitreous humor of two patients with clinically diagnosed acute retinal necrosis. One of the two cases was thought to be caused by infection with a varicella-zoster virus lacking a *Pst*I cleavage site. We could not find any clinical differences between the two substrains. The presence of a *Pst*I cleavage site on the varicella-zoster virus genome might not be associated with the occurrence of acute retinal necrosis. **Jpn J Ophthalmol 1998;42:208–212** © 1998 Japanese Ophthalmological Society

**Key Words:** Acute retinal necrosis, *Pst*I cleavage site, varicella-zoster virus, vitrectomy.

## Introduction

Acute retinal necrosis (ARN) was first described in 1971 by Urayama et al.[1] Varicella-zoster virus (VZV) or herpes simplex virus (HSV) type 1 or 2 were presumed to be the causative agent in most cases.[2–4] Viral culture of intraocular specimens and detection of intraocular antibodies to viral antigens have been used to diagnose viral infections.[2,5] The recently developed polymerase chain reaction (PCR), a rapid yet sensitive diagnostic technique, is now useful in the clinical confirmation of ARN cases suspected to be of viral origin.[6,7]

Hondo et al[8] reported that the mutation that eliminates the *Pst*I cleavage site is a reliable marker for the discrimination of VZV stains in Japan. A strain lacking a *Pst*I cleavage site was detected from the VZV genome found in the aqueous humor of patients diagnosed as having ARN.[9]

We first employed PCR to detect herpesvirus DNA in the vitreous specimens from patients with clinically diagnosed ARN. After identifying the VZV genome, we used the *Pst*I restriction endonuclease to differentiate the VZV strain.

## Patients and Methods

### *Case Reports*

**Case 1.** A healthy 57-year-old man with no prior history of systemic disease complained of decreased vision in his left eye on September 20, 1996. On examination in our eye clinic on September 24, corrected visual acuity was 0.7 in the right eye and 0.5 in the left; intraocular pressure was 14 mm Hg in the right eye and 40 mm Hg in the left. The fundus of the left eye was obscured by corneal edema and severe anterior chamber inflammation, with moderate mutton-fat keratic precipitates. The right eye was completely normal. We began treating the patient with a topical cycloplegic agent, a topical antiglaucoma drug, an oral carbonic anhydrase inhibitor, and a systemic corticosteroid because we suspected uveitis with secondary glaucoma. Three days later, the left eye showed marked papillitis, diffuse retinal vasculitis, necrotizing retinitis, and vitreous opacities. An extensive examination to confirm uveitis was negative; in particular, the value of angiotensin-converting enzyme was normal and serology tests for syphilis, toxoplasmosis, and rheumatoid factors were negative. No diagnostic increase was seen in serum antibody titers to VZV,

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HSV, human T-lymphotropic virus type 1 (HTLV-1), and Epstein-Barr virus. The patient showed a human leukocyte antigen phenotype of A2, A26, B61, and Cw3; the DR locus was not analyzed. Because echography showed the presence of retinal detachment, we performed a vitrectomy with silicone oil tamponade on October 24. A vitreous specimen was collected during the vitrectomy and preserved at −80°C for later examination. Postoperatively, we presumed a diagnosis of ARN and began administering oral acyclovir (1000 mg five times daily). We removed the silicone oil on December 18. The next day, the retina was again detached and the patient underwent another vitrectomy with silicone oil tamponade and phacoemulsification without an implant. Postoperatively the retina remained attached; however, we later performed a laser iridotomy on the iris bombé; which developed from persistent iritis. At the last follow-up 5 months later, his left eye had a visual acuity of 0.03 and an intraocular pressure of 13 mm Hg.

**Case 2.** On November 11, 1996, a 70-year-old man was examined at an eye clinic because of a progressive visual loss in his right eye. The patient had visual acuity of light perception in the right eye and 0.6 in the left eye. Intraocular pressure was 23 mm Hg in the right eye and 15 mm Hg in the left. The right eye showed circumlimbal ciliary injection, keratic precipitates, and massive cells and flare in the anterior chamber and mild vitreous cells. The posterior segment of the eye showed marked papillitis, diffuse occlusive vasculitis of the retinal arteries, retinal hemorrhages, and confluent areas of yellow-white retinal lesions. The left eye was completely normal. A diagnosis of ARN was made and the patient was put on acyclovir (500 mg three times daily). Ten milligram of betamethasone was started, then reduced to 20 mg of prednisolone. Daily doses of 2.5 g gammaglobulin were administered intravenously for 10 days. In addition, 162 mg of aspirin was given three times daily. Panretinal photocoagulation was performed posterior to the zones of retinitis. The visual acuity of the patient's right eye improved to 0.3.

The patient was referred to us for further therapy on December 20, 1996 because of retinal detachment. On the same day, we performed a vitrectomy with silicone oil tamponade. A vitreous specimen was collected during the vitrectomy and preserved at −80°C for later examination. We continued the systemic administration of acyclovir and prednisolone postoperatively. Serology tests for syphilis, toxoplasmosis, and rheumatoid factors were negative. No diagnostic increase was observed in serum antibody titers to VZV, HSV, and HTLV-1. We performed phacoemulsification with an intraocular lens implant and removal of the silicone oil on April 10, 1997. Two months later, the patient's right eye had a visual acuity of 0.3 and an intraocular pressure of 6 mm Hg. No retinal detachment occurred.

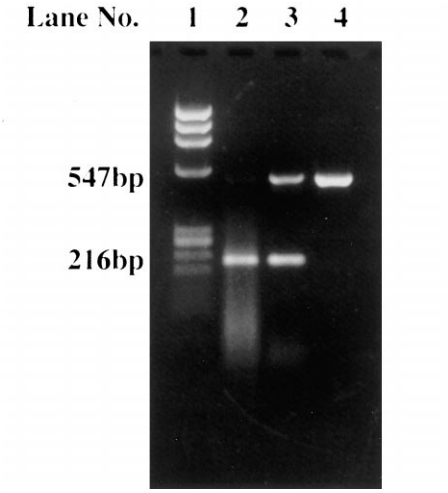


**Figure 1.** Agarose gel (3%) electrophoresis of polymerase chain reaction products for varicella-zoster virus (VZV). Lane 1: ∅×74/*Hae* III as size reference; lane 2: band of 216-bp in length (VZV primers) from vitreous humor of Case 1 with ARN; lane 3: positive control (Batoson strain of VZV); lane 4: negative control (human placenta). Band of 547-bp is internal control.

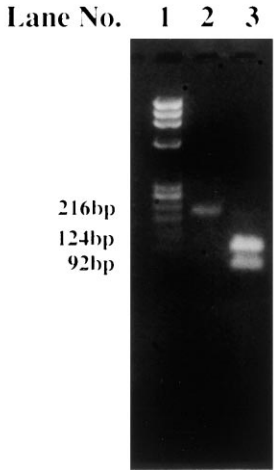


**Figure 2.** Agarose gel (3%) electrophoresis of polymerase chain reaction (PCR) products before and after digestion with *Pst*I. Lane 1: ∅×174/*Hae* III as size reference; lane 2: before being digested with *Pst*I, PCR products for varicella-zoster virus obtained from vitreous humor of Case 1 shows no *Pst*I cleavage pattern. Lane 3: Following digestion, *Pst*I cleavage pattern is seen, showing two bands, 124-bp and 92-bp.

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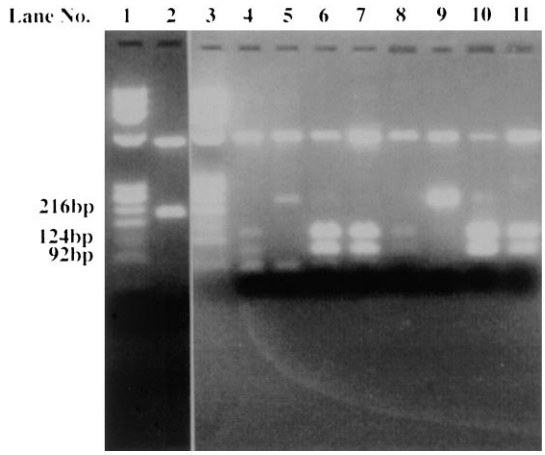


**Figure 3.** Agarose gel (3%) electrophoresis of polymerase chain reaction (PCR) products after digestion with *Pst*I. Lanes 1 and 3: ∅×174/*Hae* III as size reference; lane 2: after being digested with *Pst*I, PCR products for varicella-zoster virus obtained from vitreous humor of Case 2 shows no *Pst*I cleavage pattern. Lane 4 to 11 show results in aqueous humor samples. Lanes 4, 6, 7, 8, 10, and 11: Following digestion, *Pst*I cleavage pattern is seen, showing two bands of 124-bp and 92-bp. Lanes 5 and 9: Following digestion, *Pst*I cleavage pattern is not seen. Small DNA fragments shown under band of 92-bp in lanes 4 and 5 are primer-dimers.

### *Polymerase Chain Reaction*

The two primers specific for VZV used in PCR had the following sequences: 5′-TCACGAACCGTTGACAGGAC-3′ and 5′-CCACTACTCATTGTATCCGCG-3′.[10] These primers were used to amplify the 216-bp target sequence that was incorporated in the *Eco*RI fragment D of the VZV genome. Primers 5′-AGATGGCGAGCCACATCTC-3′ and 5′-CTCCGGATACGGTATCGTC-3′ specific for HSV were also used in PCR. For amplification of VZV or HSV DNA, 5 μL of sample was amplified in 50 μL reaction mixture containing 10 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 1 U *Taq* polymerase, and 0.5 μmol/L each of the two specific primers. PCR was performed as follows: Samples were heated to 96°C for 10 minutes to denature the DNA. Forty amplification cycles were then performed. Each cycle consisted of 1 minute at 94°C for denaturation, 1 minute at 57°C to anneal the primers, and 1 minute at 72°C for extension. To exclude any unspecified DNA amplification, controls were performed in all amplification experiments.

A 10-μL sample of each PCR product was electrophoresed in an agarose gel and stained with ethidium bromide. Photographs were taken of the gel on an ultraviolet light transilluminator.

### *Restriction Endonuclease Analysis*

The PCR products were incubated overnight with *Pst*I at 37°C in the appropriate buffer; the digested PCR products were then resolved by electrophoresis as described above.

**Table 1.** Summary of Cases

| Factors | Kumano et al[11] | Case 1 | Case 2 |
|---|---|---|---|
| Age | 65 | 57 | 70 |
| Sex | Female | Male | Male |
| Eye | OD | OS | OD |
| Onset of Symptoms | November 1993 | September 1996 | November 1996 |
| Visual Acuity | | | |
| Pretherapy | HM | 0.5 | LP |
| Posttherapy | HM | 0.03 | 0.3 |
| IOP (mm Hg) | 13 | 40 | 23 |
| Serology | | | |
| RA | − | − | − |
| Syphilis | − | − | − |
| Toxoplasmosis | − | − | − |
| Serum antibody | | | |
| HSV | 16 (NT) | <4 (CF) | 4 (CF) |
| VZV | 18.6 (IgG, EIA) | <4 (CF) | 4 (CF) |
| PCR | | | |
| HSV | − | − | − |
| VZV | + (aqueous humor) | + | + |
| *Pst*I | + | + | − |

OD: right eye. OS: left eye. HM: hand motion. LP: light perception. IOP: intraocular pressure. RA: rheumatoid factor. HSV: herpes simplex virus. NT: neutralization test. CF: complement fixation. VZV: varicella-zoster virus. IgG: immunoglobulin G. EIA: enzyme immunoassay. PCR: polymerase chain reaction.

The 216-bp target sequence specific for VZV contains one *Pst*I recognition site and thus exhibited two bands of 124-bp and 92-bp.

### *Samples of Aqueous Humor*

Samples of the aqueous humor from eight patients (male:female = 6:2; mean age, 50.5 years; age range, 25–67 years) clinically diagnosed as having anterior uveitis without retinal vasculitis and/or necrotizing retinitis were prepared for detecting the VZV genome using the same above primers. The resulting PCR products were also digested with *Pst*I and analyzed for *Pst*I cleavage sites.

## Results

Using the two VZV primers, we detected the VZV genome in the vitreous humor samples from all patients and amplified the 216-bp DNA fragment (Figure 1). The length of each amplified DNA fragment was compared with a routine molecular weight standard and was consistent with the expected length. No HSV DNA was detected in any of the vitreous humor samples.

After the PCR products for VZV obtained from Case 1 were digested with *Pst*I, a *Pst*I cleavage pattern of 124-bp and 92-bp bands was observed (Figure 2). However, after the PCR products for VZV obtained from Case 2 were digested with *Pst*I, no *Pst*I cleavage pattern was seen (Figure 3, lane 2).

Two of eight aqueous humor samples from patients without ARN contained a VZV genome that carried the mutation that eliminates the *Pst*I cleavage site (Figure 3, lanes 5 and 9). The other six samples had a *Pst*I cleavage pattern (Figure 3, lanes 4, 6–8, 10 and 11).

## Discussion

Hondo et al[8] reported that 25% of VZV strains in Japan did not have a *Pst*I cleavage site We found that 25% of the aqueous humor samples contained the VZV genome with the mutation that eliminates the *Pst*I cleavage site. It is presumed that the mutation ratio of a *Pst*I cleavage site in ocular samples is the same as that in vesicles.

Using PCR, we identified the VZV genome in both vitreous humor samples from the two patients diagnosed as having ARN. Neither sample was positive for the HSV primer and no diagnostic increases in serum antibody titers to VZV and HSV were observed in these two patients. We concluded that the two cases of ARN were caused by infection with VZV. We did not, however, challenge the intraocular antibodies with specific viral antigens to rule out false-positive results.

Several studies have shown that the VZV genome may be detected by PCR analysis only in aqueous humor samples and not in vitreous specimens.[9,11] The investigators proposed that few or no infected cells were retained in the collected vitreous sample during or after complete treatment with acyclovir.[9,11] However, the VZV genome was detected in Case 2, despite sufficient treatment with acyclovir. The total dosage of an antiviral drug may or may not affect the incidence of positive PCR results in the vitreous specimen. In the future, quantifying the amount of the virus genome detected by PCR would be useful in appropriate medical treatment.

Nishi et al[9] reported finding two strains lacking a *Pst*I cleavage site among three VZV genomes from patients diagnosed as having ARN, and suggested that the VZV genome lacking a *Pst*I site may be responsible for ARN. Kumano et al[11] reported that ARN in one patient was caused by a VZV strain with a *Pst*I site. We encountered two different substrains in the vitreous humor samples from patients with ARN, one lacking and the other having a *Pst*I cleavage site. It appears that the presence of a *Pst*I cleavage site on the VZV genome is not associated with the occurrence of ARN. However, the difference in severity of the ARN could be attributed to the presence or absence of a *Pst*I cleavage site in the VZV genome. Nishi et al[9] did not include the data of patients. We were able to compare our cases to the patient of Kumano et al[11] and could not find any clinical differences among them (Table 1). A difference in host immune response may relate to the occurrence of ARN induced by the substrains.[12,13] However, we could not investigate the immune function in our patients. The true clinical characteristics of these two substrains remain to be clarified because the number of patients studied was not large enough to draw a definite conclusion.

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