JVI Journals.ASM.org

Frequency and Abundance of Alphaherpesvirus DNA in Human Thoracic Sympathetic Ganglia

Maria A. Nagel,[a] April Rempel,[a] Jonathon Huntington,[a] Forrest Kim,[a] Alexander Choe,[a] Don Gilden[a,b]

Departments of Neurology[a] and Microbiology,[b] University of Colorado School of Medicine, Aurora, Colorado, USA

Alphaherpesvirus reactivation from thoracic sympathetic ganglia (TSG) and transaxonal spread to target organs cause human visceral disease. Yet alphaherpesvirus latency in TSG has not been well characterized. In this study, quantitative PCR detected varicella-zoster virus (VZV), herpes simplex virus 1 (HSV-1), and HSV-2 DNA in 117 fresh TSG obtained postmortem from 15 subjects. VZV DNA was found in 76 (65%) ganglia from all subjects, HSV-1 DNA was found in 5 (4%) ganglia from 3 subjects, and no HSV-2 was found.

After primary infection, varicella-zoster virus (VZV) becomes latent in cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia along the entire neuraxis (1–7), herpes simplex virus 1 (HSV-1) DNA becomes latent in cranial nerve, dorsal root, and autonomic ganglia (2, 5–10), and HSV-2 DNA becomes latent primarily in sacral ganglia as well as in cranial nerve and other dorsal root ganglia (5, 9). Alphaherpesvirus reactivation from thoracic sympathetic ganglia (TSG) and transaxonal spread to target organs cause human visceral disease. However, latency in TSG has not been well characterized because earlier studies used formalin-fixed paraffin-embedded (FFPE) tissue. To assess autonomic ganglionic infection, quantitative PCR analyzed VZV, HSV-1, and HSV-2 DNA in 117 fresh unfixed TSG.

Briefly, 117 TSG from 15 deidentified autopsied subjects were deemed exempt by the Colorado Institutional Review Board (IRB). Table 1 provides age, sex, cause of death, and postmortem interval of the subjects. Of the 15 subjects, 5 (33%) were women and 10 (67%) were men. Ages ranged from 42 to 84 years (mean, 61 ± 12 years; median, 62 years). None of the subjects died of causes related to herpesvirus infection.

Ganglia were obtained <24 h postmortem, except for subject 13, whose ganglia were obtained >24 h postmortem; ganglia were rinsed in phosphate-buffered saline, and extraneous tissue was removed. DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Valencia, CA). DNA concentration and purity were determined with a Thermo Scientific Nanodrop ND-1000 spectrophotometer. DNA was quantitated with a TaqMan real-time PCR on a model 7500 real-time PCR system (Applied Biosystems, Grand Island, NY) as described previously (11) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH), VZV, and HSV-1 DNA-specific primers and probe (12) and HSV-2 DNA-specific primers (forward, 5′-TACCACGCGTCGCTTTTG-3′; reverse, 5′-TAAACGTGCGGC CCGTAAT-3′ and probe/56-FAM/TTGCTCCCCAGAGCCTG/3IA BkFQ_3′/). Primer efficiencies for VZV, HSV-1, and HSV-2 were similar (104, 104, and 102, respectively). Amplification of serial dilutions of known concentrations of GAPDH, VZV, HSV-1, and HSV-2 DNA provided positive controls. DNA was omitted from reaction wells as a negative control. Ganglia were considered positive for viral DNA if (i) no virus amplification was detected in wells with water, (ii) GAPDH was detected in wells with ganglionic DNA, and (iii) at least two of four PCR replicates amplified target DNA.

PCR detected cellular GAPDH DNA in all 117 ganglia (data not shown), VZV DNA in 76 ganglia (65%), and HSV-1 DNA in 5 ganglia (4%); HSV-2 DNA was not found in any ganglia (Table 2). VZV DNA was detected in at least one TSG from all 15 subjects, and HSV-1 DNA was found in at least one TSG from 3 (20%) subjects. The VZV DNA load, which varied among subjects (Fig. 1 and 2), was <1,000 copies/100 ng of total DNA in all subjects except subject 14, in whom 3 TSG contained >1,000 copies of VZV DNA per 100 ng total DNA (Table 2). In TSG from subjects 1, 5, and 14 that contained HSV-1 DNA, the average load was ≤302 HSV-1 DNA copies/100 ng total DNA (Table 2).

Both fresh and FFPE autonomic ganglia have been examined by PCR for alphaherpesvirus DNA. In one study (3), VZV DNA was detected in FFPE celiac (sympathetic) ganglia in 5/12 (42%) subjects and nodose ganglia from 1/11 (9%) subjects, while

TABLE 1 Clinical features of subjects from whom TSG were analyzed

Subject	Age (yr), gender[a]	Cause(s) of death[b]	Postmortem interval (h)
1	70, M	Interstitial lung disease	17
2	47, M	Bowel ischemia	16
3	64, M	CAD	14
4	62, F	Liver disease	9
5	50, M	CAD	24
6	70, F	Breast cancer	8
7	65, M	CAD	24
8	72, F	Respiratory failure and CAD	11
9	68, F	Cancer	17
10	44, M	Cancer	5
11	84, F	CAD	16
12	59, M	Leukemia	24
13	61, M	Cancer	>24
14	52, M	Pneumonia	23
15	42, M	Brain cancer	13

[a] M, male; F, female.
[b] CAD, coronary artery disease.

Received 15 April 2014 **Accepted** 23 April 2014
Published ahead of print 30 April 2014
Editor: R. M. Sandri-Goldin
Address correspondence to Maria A. Nagel, maria.nagel@ucdenver.edu.

doi:10.1128/JVI.01070-14

TABLE 2 Presence of VZV, HSV-1, and HSV-2 DNA in TSG[a]

Subject	TSG	Copy number of alphaherpesvirus/100 ng DNA (avg ± SD)[b]		
		VZV	HSV-1	HSV-2
1	1	<100[c]	302 ± 8	0
	2	0	0	0
	3	<100[c]	0	0
	4	0	0	0
	5	<100[c]	0	0
	6	<100[c]	0	0
	7	0	0	0
	8	<100[c]	0	0
	9	<100[c]	0	0
2	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	28 ± 11	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0
	8	0	0	0
	9	0	0	0
	10	0	0	0
3	1	<10[c]	0	0
	2	18 ± 2	0	0
	3	33 ± 15	0	0
	4	7 ± 1	0	0
	5	4 ± 1	0	0
	6	21 ± 8	0	0
	7	24 ± 2	0	0
4	1	<10[c]	0	0
	2	<10[c]	0	0
	3	0	0	0
5	1	<10[c]	0	0
	2	<10[c]	0	0
	3	<10[c]	0	0
	4	<10[c]	0	0
	5	31 ± 11	0	0
	6	18 ± 11	0	0
	7	<10[c]	0	0
	8	22 ± 3	0	0
	9	12 ± 2	0	0
	10	<10[c]	0	0
	11	<10[c]	0	0
	12	<10[c]	0	0
	13	<10[c]	<100[c]	0
	14	<100[c]	<100[c]	0
	15	0	0	0
6	1	99 ± 25	0	0
	2	316 ± 5	0	0
	3	185 ± 17	0	0
	4	92 ± 7	0	0
	5	0	0	0
	6	278 ± 125	0	0
	7	0	0	0
7	1	265 ± 3	0	0
	2	0	0	0
	3	52 ± 24	0	0
8	1	0	0	0
	2	28 ± 18	0	0
	3	54 ± 31	0	0
	4	32 ± 6	0	0
	5	36 ± 37	0	0
	6	<10[c]	0	0
	7	<10[c]	0	0
	8	92 ± 47	0	0
	9	126 ± 19	0	0
	10	<10[c]	0	0
	11	<10[c]	0	0
9	1	46 ± 9	0	0
	2	23 ± 0	0	0
	3	0	0	0
	4	0	0	0
	5	62 ± 22	0	0
	6	0	0	0
	7	30 ± 9	0	0
	8	0	0	0
	9	17 ± 0	0	0
	10	<100[c]	0	0
10	1	10 ± 7	0	0
	2	2 ± 0	0	0
	3	2 ± 0	0	0
	4	9 ± 0	0	0
	5	9 ± 1	0	0
	6	<100[c]	0	0
	7	<100[c]	0	0
	8	322 ± 73	0	0
	9	<100[c]	0	0
	10	2 ± 1	0	0
	11	<100[c]	0	0
	12	0	0	0
11	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	<10[c]	0	0
	8	0	0	0
	9	0	0	0
	10	0	0	0
12	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	<100[c]	0	0
13	1	<100[c]	0	0
	2	127 ± 5	0	0
	3	0	0	0
	4	225 ± 22	0	0
	5	0	0	0
	6	0	0	0
	7	574 ± 261	0	0
14	1	1,645 ± 781	<100[c]	0
	2	1,246 ± 479	106 ± 4	0
	3	1,251 ± 425	0	0
15	1	0	0	0
	2	<100[c]	0	0
	3	4 ± 1	0	0
	4	120 ± 34	0	0

[a] Of a total of 117 TSG, 76 were VZV positive, 5 were HSV-1 positive, and none were HSV-2 positive.
[b] SD, standard deviation.
[c] Copy number was detectable but not quantifiable based on amplification of the standard curve.

HSV-1 DNA was found in nodose ganglia of 1/11 (9%) subjects but not in celiac ganglia. Subsequent PCR analysis of fresh postmortem sympathetic ganglia did not detect VZV DNA in three subjects but did reveal HSV-1 DNA in two subjects; unfortunately, the type of sympathetic ganglia obtained was not identified (10). More recent analyses of multiple fixed human autonomic ganglia revealed VZV and HSV-1 DNA, respectively, in 11/58 and 23/58 pterygopalatine ganglia, in 14/60 and 25/60 ciliary ganglia, in 8/50 and 15/50 otic ganglia, in 4/47 and 14/47 submandibular ganglia, in 10/58 and 18/58 superior cervical ganglia, and in 1/36 and 12/36 nodose ganglia (7). Our detection of VZV DNA in greater numbers of freshly isolated TSG clearly indicates that the VZV DNA is from VZV viremia in childhood; the presence of HSV-1 and HSV-2 DNA is rare to nonexistent, with HSV-1 latency mostly limited to cranial nerve ganglia and HSV-2 mostly limited to sacral ganglia, presumably after retrograde transaxonal transport of virus from the face to cranial nerve ganglia.

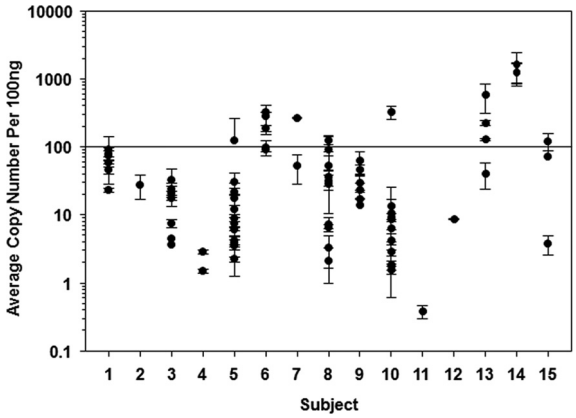

FIG 1 VZV DNA burden in human thoracic sympathetic ganglia (TSG). A total of 117 TSG from 15 subjects were obtained. DNA was extracted from each ganglion and quantitative PCR (qPCR) was performed using VZV-specific primers and probe to determine viral DNA copy numbers per 100 ng of DNA. Each point represents one ganglion. Ganglia that did not contain VZV DNA are not shown. DNA copy numbers were determined using a standard curve for known viral DNA concentrations. Based on the standard curve, some samples had copy numbers of <100 (detectable but not quantifiable). VZV DNA was found in all 15 subjects and in 76 (65%) of the 117 ganglia.

Our current detection of VZV DNA in TSG from 100% of the subjects most likely reflects enhanced PCR amplification of DNA from fresh rather than FFPE tissue. Recently, VZV DNA was found in resected segments of gastrointestinal tract from 6/6 children with a history of varicella and in 6/7 children who received varicella vaccine but not in 7 children with no history of varicella or varicella vaccination (13). Overall, VZV DNA is present not only in cranial nerve ganglia and dorsal root ganglia but also in sympathetic and parasympathetic ganglia of most humans as well as in the enteric nervous system.

Importantly, TSG supply postganglionic fibers to blood vessels, skin, heart, lung, pancreas, gastrointestinal tract, liver, spleen, adrenal glands, kidneys, ureters, and bladder and also connect to dorsal root ganglia via gray communicating rami (14, 15). Thus, virus reactivation from TSG and dorsal root ganglia followed by virus spread to target organs may provide a potential pathway for virus-induced visceral disease. This is supported by VZV-induced pancreatitis and the presence of VZV antigen, large basophilic inclusions, and Cowdry type A inclusion bodies in associated sympathetic ganglia of a patient with fatal VZV meningoradiculitis without skin involvement (16), as well as ad-

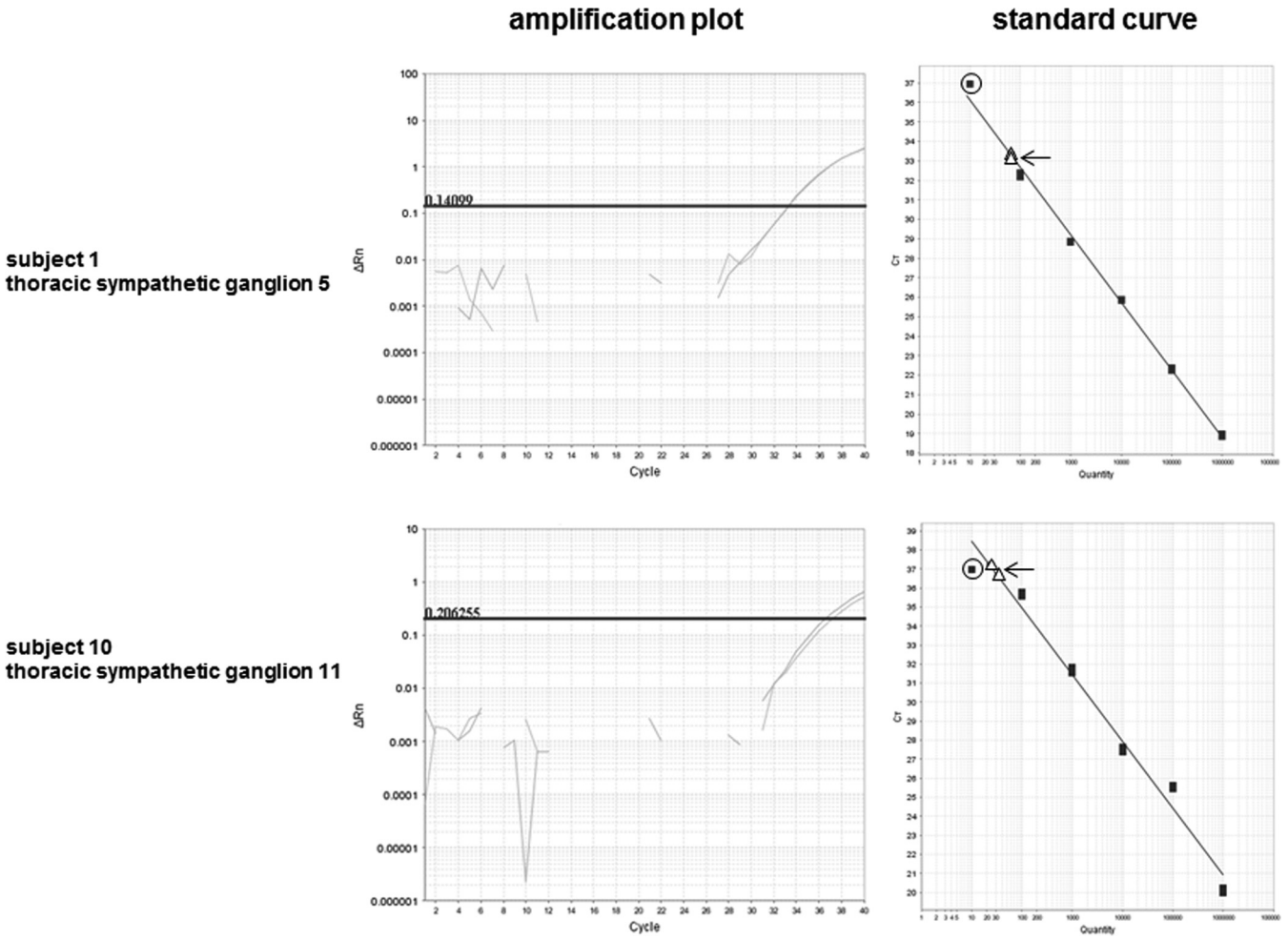

FIG 2 Amplification plot derived from two human thoracic sympathetic ganglia that contained low-abundance VZV DNA with corresponding standard curves using known concentrations of VZV DNA. Black squares (≥100 copies of VZV DNA) reflect duplicate data points; circles around black squares on the standard curves indicate single data point (10 copies of VZV DNA). Arrows indicate duplicate thoracic sympathetic ganglionic samples containing 10 to 100 copies of VZV DNA that are denoted as <100 copies in Table 2.

ditional reports of zoster-associated pancreatitis and hepatitis, and the presence of VZV DNA and VZV antigen in biopsy specimens of patients with zoster-associated gastritis (17–23).

VZV is well known to reactivate in the absence of rash (24). Two reports describe VZV-verified hepatitis and gastritis without rash in bone marrow transplant recipients. The first report was of a 74-year-old patient with common variable immunodeficiency of uncertain origin who died of fulminant hepatic failure, and VZV DNA was found in the blood and liver (25); serum of the second patient contained anti-VZV IgM antibody, and the patient was successfully treated with intravenous acyclovir (19). Finally, there is a remarkable report of VZV reactivation from multiple ganglia in a patient who developed right thoracic zoster sine herpete, followed 6 days later by left thoracic-distribution zoster associated with pancreatitis, cholecystitis, and gastric ulcerations that contained VZV DNA (21).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AG006127 (D.G.), AG032958 (D.G.), and NS 067070 (M.A.N.) from the National Institutes of Health.

We thank Marina Hoffman for editorial review and Lori DePriest for manuscript preparation.

We report no conflicts of interest.

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