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Recurrent herpes zoster in the Shingles Prevention Study: Are second episodes caused by the same varicella-zoster virus strain?

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ABSTRACT

Herpes zoster (HZ) is caused by reactivation of varicella zoster virus (VZV) that established latency in sensory and autonomic neurons during primary infection. In the Shingles Prevention Study (SPS), a large efficacy trial of live attenuated Oka/Merck zoster vaccine (ZVL), PCR-confirmed second episodes of HZ occurred in two of 660 placebo and one of 321 ZVL recipients with documented HZ during a mean follow-up of 3.13 years. An additional two ZVL recipients experienced a second episode of HZ in the Long-Term Persistence Substudy. All episodes of HZ were caused by wild-type VZV. The first and second episodes of HZ occurred in different dermatomes in each of these five participants, with contralateral recurrences in two. Time between first and second episodes ranged from 12 to 28 months.

One of the five participants, who was immunocompetent on study enrollment, was immunocompromised at the onset of his first and second episodes of HZ.

VZV DNA isolated from rash lesions from the first and second episodes of HZ was used to sequence the full-length VZV genomes. For the unique-sequence regions of the VZV genome, we employed target enrichment of VZV DNA, followed by deep sequencing. For the reiteration regions, we used PCR amplification and Sanger sequencing. Our analysis and comparison of the VZV genomes from the first and second episodes of HZ in each of the five participants indicate that both episodes were caused by the same VZV strain. This is consistent with the extraordinary stability of VZV during the replication phase of varicella and the subsequent establishment of latency in sensory ganglia throughout the body. Our observations also indicate that VZV is stable during the persistence of latency and the subsequent reactivation and replication that results in HZ.

1. Introduction

Varicella-zoster virus (VZV) is a human herpesvirus that causes varicella (chickenpox) on primary infection, during which the virus establishes life-long latency in sensory and autonomic neurons, and the host develops immunity to VZV. As this immunity wanes, latent VZV may reactivate years or decades after primary infection to cause herpes zoster (HZ). HZ is associated with a spectrum of complications, including postherpetic neuralgia (PHN), a debilitating syndrome of persistent neuropathic pain. An episode of HZ boosts host immunity to VZV, thereby protecting against recurrences [1–3]. Consequently, recurrent episodes of HZ are relatively infrequent, with reported recurrence rates ranging from < 1% to 6.4% [1,4–13].

A variety of factors affect the reported rate of HZ recurrence, including study design, case definition, accuracy of diagnosis, length of follow-up and confounders, such as immunosuppression.

The Shingles Prevention Study (SPS), carried out between November 1998 and April 2004, was a randomized, double-blind efficacy trial, in which 38,546 immunocompetent participants ≥60 years of age were enrolled and actively followed for a mean of 3.13 years after receiving live attenuated Oka/Merck zoster

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vaccine (ZVL) or placebo [14]. The diagnosis of HZ was established by PCR assay in >93% of the cases and by an expert adjudication committee in the remainder [14,15]. In the SPS, PCR-confirmed second episodes of HZ occurred in two of 660 placebo and one of 321 ZVL recipients with confirmed HZ [14]. To determine the duration of vaccine efficacy, two follow-up studies were carried out with subsets of SPS participants: a Short-Term Persistence Substudy (STPS [16]), in which 14,270 ZVL and placebo recipients were followed for a mean of 1.81 years between December 2004 and March 2006, and a Long-Term Persistence Substudy (LTPS [17]), in which 6867 ZVL recipients were followed for a mean of 3.74 years between March 2006 and December 2010. No second episodes of HZ occurred among 179 cases of HZ in the STPS. Two PCR-confirmed second episodes occurred among 263 study participants who experienced an episode of HZ in the LTPS (Fig. 1).

VZV is a double-stranded DNA virus with a genome that is approximately 125,000 base pairs in length [18]. The VZV genome comprises at least 71 unique open reading frames (ORFs), with 64 in the unique long (U_L) region [18–20]. The U_L is flanked by short inverted repeats: terminal repeat long (TR_L) and internal repeat long (IR_L). The unique short (U_S) region, which contains four ORFs, is flanked by inverted repeats: internal repeat short (IR_S) and terminal repeat short (TR_S). Three ORFs (ORFs 62–64) and the origin of replication (Ori_S) are located in the IR_S; these are duplicated in the TR_S as ORFs 69–71 and Ori_S. [18,19]. The VZV genome is highly conserved, with 99.8% percent identity between the two most distantly related clades [19,21]. The five reiteration regions (R1–R5), which consist of variable numbers of short direct sequence repeats, exhibit more genetic variability [22–27]. R1, R2 and R3 are located within the coding regions of ORF11, ORF14 and ORF22, respectively. R4 is situated in a non-coding region near the Ori_S in the IR_S and is duplicated in the TR_S. R5 is located in a non-coding region between ORF60 and ORF61. While the VZV unique sequence regions have been reported to be genetically stable, the reiteration regions have been found to be variable, both between different strains of VZV and within individual isolates [26]. The functions of the individual reiteration regions remain unclear.

VZV strains isolated from the same patient during varicella and a subsequent episode of HZ were shown to be identical by restriction enzyme analysis [28,29]. Nevertheless, reinfection [30] and subsequent reactivation of different VZV genotypes may occur [31,32]. It was reported previously that two episodes of HZ in an immunocompetent person were caused by two different strains of VZV, representing two different VZV clades [31].

Here, we describe the clinical characteristics of the first and second episodes of HZ in five participants from the SPS and LTPS. In addition, we present the complete genome sequences of all 10 viruses causing these cases of HZ, and compare each pair of viruses from the first and second episodes of HZ to determine whether they were distinct or identical.

2. Methods

Clinical data and specimens: Rash lesion specimens were obtained from study participants with suspected HZ during the SPS [14] and LTPS [17] with written informed consent; all samples were de-identified as a precondition for use in this study. The diagnosis in each case of HZ was confirmed by detecting wild-type VZV DNA in skin lesions by real-time PCR assay [15].

Clinical information on HZ-associated pain, rash characteristics, HZ-related complications, and other HZ-related information was collected repeatedly over a period of ≥182 days following HZ rash onset during each episode of HZ. HZ-associated pain severity was measured repeatedly using the validated Zoster Brief Pain Inventory, which included a 0 (“no pain”) to 10 (“worst pain imaginable”) Likert scale [14,33]. The HZ severity-of-illness score was defined as the area under the curve (AUC) of HZ-associated pain severity plotted against time during the 182-day period after HZ rash onset. The HZ severity-of-illness score corresponds closely with HZ-related decrements in health-related quality of life and ability to perform activities of daily living [34].

DNA sequencing and analysis: Total DNA was extracted from each HZ rash lesion specimen using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer’s instructions. Sequencing libraries were made from 200 ng of DNA, enriched for VZV DNA using SureSelect™ target enrichment as previously described [35,36] and subsequently deep sequenced on an Illumina NextSeq 550. A consensus genome sequence for each of the 10 VZV strains was generated by optimized reference-guided assembly against VZV reference strain Dumas (NC_001348) and subsequently aligned using MAFFT v7 [37] and visualized using MEGA7 [38] and SplitsTree4 [39].

The reiteration regions R1, R2, R3, R5 and the duplicated R4 region (here referred to as R4A and R4B) were PCR-amplified with primers that used the VZV Dumas strain as a reference. Amplicons were sequenced using conventional Sanger sequencing on a 3730xl DNA Analyzer (Applied Biosystems). Samples with insufficient DNA underwent an additional round of PCR amplification before sequencing. Sequences were analyzed using Sequencher v5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA). Repeat elements within the individual reiteration regions were designated according to

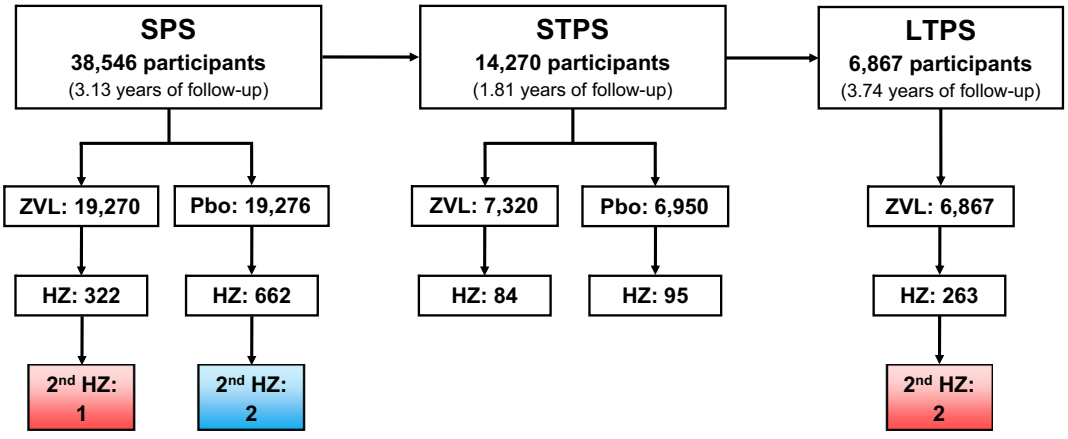

Fig. 1. Distribution of cases of herpes zoster (HZ) in the Shingles Prevention Study (SPS) and its Persistence Substudies. There was one HZ recurrence in a zoster vaccine (ZVL) recipient and two recurrences in placebo (Pbo) recipients in the SPS; two further HZ recurrences occurred in vaccine recipients during the Long-Term Persistence Substudy (LTPS). No second episodes of HZ were observed during the Short-Term Persistence Substudy (STPS).

the nomenclature suggested by Jensen et al. [in preparation] (Fig. 3A).

The use of de-identified DNA extracted from HZ rash lesions and associated clinical and laboratory data was approved by the VA San Diego Healthcare System Institutional Review Board (IRB). The CDC IRB determined that CDC's participation was human subjects research, CDC not engaged.

3. Results

3.1. Clinical characteristics

The clinical characteristics of first and second episodes of HZ in each of the five participants are summarized in Table 1. Three episodes of recurrent HZ were documented in the SPS, one in a ZVL recipient and two in placebo recipients. Two ZVL recipients experienced their first and second episode of HZ during the LTPS.

The first and second episodes of HZ occurred in different dermatomes in each of the five participants, with contralateral recurrences in two. The time interval between the two episodes of HZ ranged from 12.2 to 28.5 months. Four of the five participants, all female, were immunocompetent when both episodes of HZ occurred. Participant 4, the only male, was immunocompetent on SPS enrollment but was immunocompromised as a consequence of mantle cell lymphoma and its therapy at the onset of both episodes of HZ.

During the first episode of HZ in the three SPS participants, the time until the severity of HZ-associated pain declined to <3 (scores <3 are not associated with significant decrements in quality of life or ability to carry out activities of daily living [33]) was 10 days in both placebo recipients (participants 1 and 2) and 42 days in the ZVL recipient (participant 3). During the first episode of HZ in the two LTPS participants, the time until the severity of HZ-associated pain declined to <3 was 34 days in participant 4 and 32 days in participant 5. During the second episodes of HZ, the time until the severity of HZ-associated pain declined to <3 ranged from 3 to 70 days (Table 1).

HZ severity-of-illness scores ranged from 0 (first episode in participant 2, a placebo recipient) to 464.5 (second episode in participant 3, a vaccine recipient). Except for participant 5, all participants had a higher HZ severity-of-illness score during their second episode of HZ than during their first (Table 1). None of the episodes of HZ were complicated by PHN (defined as HZ-associated pain and discomfort rated as ≥3 persisting or appearing more than 90 days after rash onset [14]).

With three second episodes among 981 SPS participants with a first episode of HZ, the incidence of recurrence was 2.02 cases per 1000 person years, based on a mean surveillance time of 1.52 years after onset of the first episode of HZ in these 981 participants. The incidence of recurrence for placebo recipients with HZ (2/660) was 1.96 cases per 1000 person years, and for ZVL recipients (1/321) 2.15 cases per 1000 person years. Thus, the incidence of recurrence was less than one-fifth of the incidence of first episodes of HZ among placebo recipients in the SPS (11.12 cases per 1000 person years) [14].

3.2. VZV unique sequence regions

Target enrichment and deep sequencing of the VZV unique sequence regions were successful for all 10 viruses, with ≥20× coverage of ≥99.98% in nine (Table 2). Coverage of sample SD1361 was lower (≥20× coverage of 96.90%) but yielded sufficient information to be included in the analysis.

Illumina deep sequencing showed that the viruses causing all 10 episodes of HZ were wild-type VZV, and that there was no indication of recombination between the wild-type virus and the Oka vaccine strain in those who had received ZVL. The unique sequence regions of the viruses causing both episodes of HZ in each of the five participants were identical, except for the virus from the first episode of HZ in participant 1 (sample SD1137), which was polymorphic (T/C, versus C) at non-coding position 105,012 at the boundary between the IR_L and IR_S.

In each of the five participants, the VZV genotypes of the viruses causing the two episodes of HZ clustered together within the same clade (Fig. 2), Clade 1 from both episodes of HZ in three participants, Clade 4 from both episodes in one participant, and Clade 5 from both episodes in one participant.

3.3. VZV reiteration regions

The nucleotide sequences and copy numbers of the individual repeat elements in the reiteration regions [26] of the viruses from the two episodes of HZ in the five participants are displayed in Fig. 3B.

Table 1
Clinical characteristics of first and second episodes of herpes zoster.

Study participant	Study	Treatment	Sex[1]	Race[1]	Age at onset of first episode of HZ (years)	Time from first to second episode of HZ (months)	HZ episode	Dermatome	Antiviral therapy	Time to pain score <3 (days)[2]	HZ severity-of-illness score[3,4]
1	SPS	Placebo	F	W	68	22.8	1	Right L3	Fam	10	55.0
							2	Right T5	Fam	17	71.5
2	SPS	Placebo	F	W	77	25.0	1	Left S1	Fam	10	0.0
							2	Left T7	Fam	14	48.5
3	SPS	Vaccine	F	W	66	12.2	1	Left T5	A	42	252.5
							2	Right T9	Fam	70	464.5
4[5]	LTPS	Vaccine	M	W	68	25.3	1	Left C3/C4	Val	34	108.0
							2	Left T2	Fam	67	224.0
5	LTPS	Vaccine	F	W	78	28.5	1	Right T5	Fam	32	80.0
							2	Left T6	Fam	3	19.5

A: Acyclovir; **C**: cervical; **F**: female; **Fam**: Famciclovir; **HZ**: herpes zoster; **L**: lumbar; **LTPS**: Long-Term Persistence Substudy; **S**: sacral; **SPS**: Shingles Prevention Study; **T**: thoracic; **Val**: Valacyclovir; **W**: white, Caucasian/European.

[1] Self-reported.

[2] In laboratory confirmed episodes of HZ in the SPS, the mean time to pain score <3 was 57.8 days in placebo recipients and 40.9 days in vaccine recipients. In laboratory confirmed episodes of HZ in the LTPS, the mean time to pain <3 was 43.1 days.

[3] In laboratory confirmed episodes of HZ in the SPS, the mean HZ severity-of-illness score was 176.4 in placebo recipients and 154.3 in vaccine recipients. In laboratory confirmed episodes of HZ in the LTPS, the mean HZ severity-of-illness score was 169.3.

[4] No case of HZ was complicated by postherpetic neuralgia.

[5] This participant, who was immunocompetent on SPS enrollment, was immunocompromised during both episodes of HZ.

Table 2
Illumina NextSeq sequencing coverage for VZV DNA from first and second episodes of herpes zoster (HZ).

Study participant	HZ episode	Sample ID	Number of total read pairs	% Coverage		
				≥20×	≥50×	≥100×
1	1	SD1137	320,740	99.99	99.97	99.90
	2	SD1161	208,960	99.99	99.97	99.77
2	1	SD0979	221,746	100.00	100.00	99.75
	2	SD1361	42,367	96.90	2.76	0.00
3	1	SD1218	252,286	100.00	100.00	99.98
	2	SD0782	300,040	100.00	100.00	99.97
4	1	SD0705	176,010	99.98	99.96	99.44
	2	SD0821	244,962	99.98	99.96	99.73
5	1	SD0908	191,286	100.00	100.00	99.69
	2	SD0825	209,012	100.00	100.00	99.79

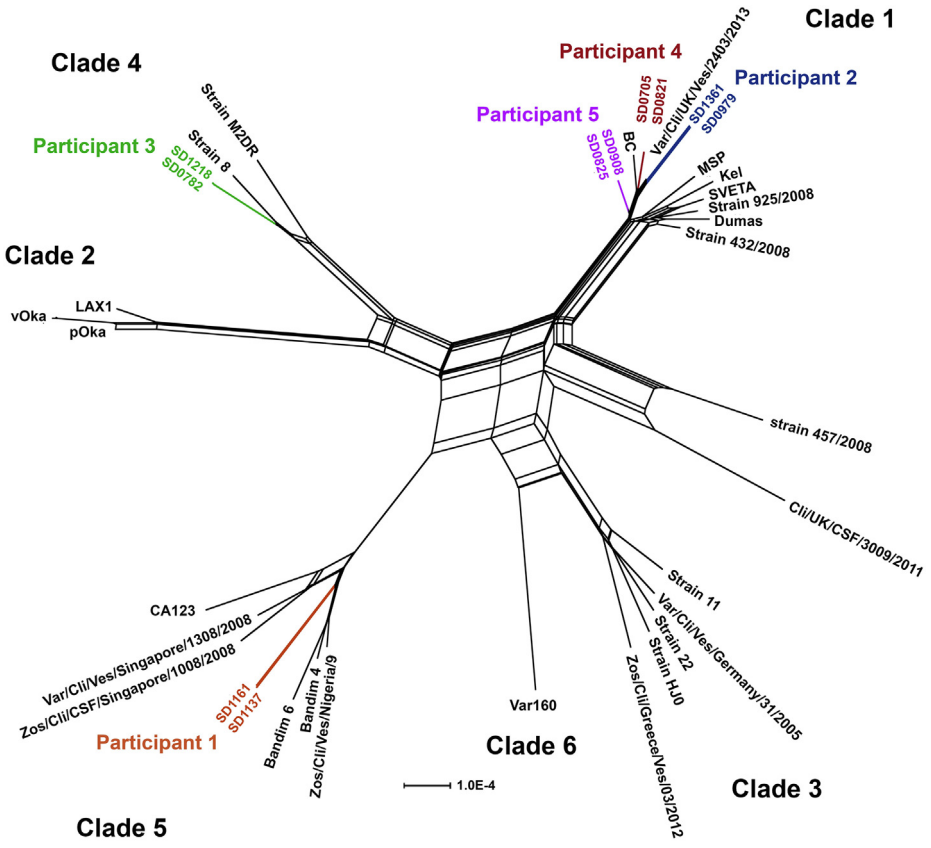

Fig. 2. Phylogenetic network showing the six major VZV clades, based on whole genome sequences of reference strains, together with the 10 VZV strains from this study. The live attenuated Oka vaccine strain (vOka) and the wild-type Oka parent strain (pOka) belong to Clade 2.

3.3.1. R1

The R1 reiteration region consists of varying copy numbers of 15 base pair (bp) and 18 bp elements [26]. In addition, a 6 bp sequence that has been observed in most Clade 5 viruses [27] was present as a single copy in the Clade 5 viruses from both episodes of HZ in participant 1. All R1 regions terminate with a 3 bp element [18,26]. The individual R1 repeat elements were identical in the viruses from the first and second episodes of HZ in each of the five participants (Fig. 3B).

3.3.2. R2

R2 regions comprise varying copy numbers of 42 bp elements and terminate in a single copy of a partial element identical to the 5′ 32 bp of the 42 bp element [26]. The R2 sequences were identical in the viruses from the two episodes of HZ in each of the five participants.

3.3.3. R3

R3 regions consist of varying copy numbers of 9 bp elements and terminate in a single 4 bp element (GCCC) that appears to be invariant. R3 is reported to be more variable than the other reiteration regions, with a wide range of copy numbers of the 9 bp elements, both within individual VZV isolates and among different VZV isolates [26]. The R3 regions of the VZV strains causing seven of the 10 episodes of HZ consisted of mixtures with different copy numbers of the 9 bp elements. In these seven, only the variants with the strongest histogram signals are displayed in Fig. 3B. Length disparities of R3 between the first and second episodes of HZ were observed in participants 1 and 2. The R3 region of the virus from the second episode of HZ in participant 1 was more than twice as long as the R3 region of the virus from the first episode (16 copies of the 9 bp elements [148 bp] versus 7 copies [67 bp]). Since the R3 region from the first episode of HZ consisted of a mixture, it

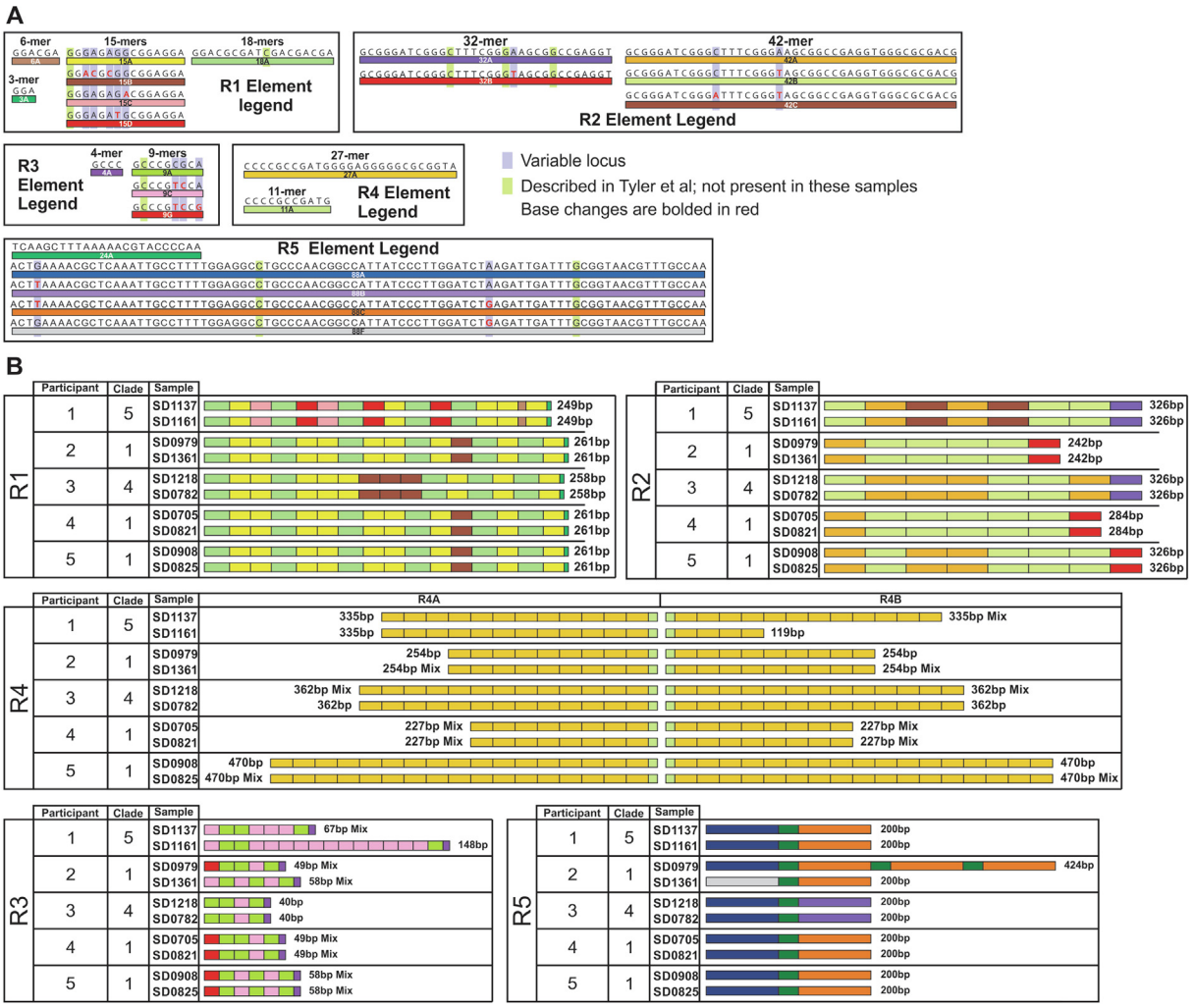

Fig. 3. Map of the variable repeat elements within the reiteration (R) regions of viruses from participants' first and second episode of HZ. (A) Legend for R region elements present in one or more study isolates. Elements are color-coded for ease of identification in the R region maps. The colors in 3B correspond to those in 3A. Elements are displayed to show relative length within each set of R region elements. Variable loci are identified with a vertical bar. (B) R region maps for the 10 viruses analyzed in this study. Maps indicate both the number and type of elements present in each R region. Where mixtures of copy numbers of the repeat elements within an R region were observed, this is indicated with "Mix". In mixtures, the variant with the strongest histogram signal was selected for display. Elements are displayed for each R region, with relative length proportionate to their actual length in base pairs.

is possible that the longer region observed in the second episode was present as a minority variant in the VZV strain from the first episode of HZ.

3.3.4. R4

The duplicated reiteration regions R4A (IR_S) and R4B (TR_S) contain variable numbers of a 27 bp element and terminate with a partial 11 bp element identical to the first 11 bp at the 5′ end of the 27 bp element [26]. Only a single 27 bp element (27A) was observed, and the 11 bp element was also invariant. However, as reported elsewhere [26,40,41], the number of 27 bp elements is variable. As described for the R3 region, mixtures of R4 regions of variable length were observed within the same VZV strains (five of ten R4A regions and six of ten R4B regions). Only the R4 sequences with the strongest histogram signals are displayed in Fig. 3B. The predominant number of 27A elements present in the R4A and R4B regions were identical in the viruses from 9 of the 10 episodes of HZ; in one episode of HZ (participant 1, second episode), the R4B region contained four copies of 27A, whereas the R4A region contained 12 copies.

3.3.5. R5

The R5 region consists of combinations of a variable 88 bp element and an invariant 24 bp element [26] in the following configuration: an initial 88 bp element followed by variable numbers of 24–88 bp element pairs. The R5 regions of the paired viruses causing the first and second episodes of HZ in four of the five participants were identical, both in length and in element content (participants 1, 3, 4 and 5; Fig. 3B), and all R5 regions of the viruses from participants 1, 4 and 5 were identical.

The R5 regions from the two episodes of HZ in participant 2 differed both in length and in the sequence of the initial 88 bp element. The virus from the first episode of HZ in participant 2 provides the first instance of an R5 region with an initial 88 bp element followed by three 24–88 bp element pairs, and is the longest yet reported, with a total length of 424 bp. In addition, although the R5 region of the virus from the second episode of HZ in participant 2 displayed the more conventional 88–24–88 bp element pattern, it had an 88F element in the first position, versus the 88A first position element present in the viruses from the first episode and from both episodes of HZ in all of the other study participants. The 88F differs from the 88A element by one base pair in

position 61, and has been thus far reported in four other VZV strains (GenBank Acc# JN704700, JN704702, JN704703 [42]).

All amplifications of R3 utilized a semi-nested PCR protocol. Three DNA samples failed to yield sufficient product and required an additional round of PCR: The sample from the second episode of HZ in participant 1 required an additional round of PCR to obtain the reverse R3 sequence, and the sequence of this product aligned perfectly with the standard PCR protocol product obtained using the forward primer. An additional round of PCR was also needed for the R3 region of the virus from the second episode of HZ in participant 2, as well as for the R4A region of the virus from the second episode of HZ in participant 1.

4. Discussion

4.1. HZ recurrence rates

Studies of HZ recurrence have reported recurrence rates ranging from 0.5% during 10 years of follow-up [5] to 6.4% during 6.5 years of follow-up [11]. The rates of HZ recurrence observed in the SPS and LTPS were significantly lower than those reported in many other studies [1,4,6,7,10–13], which likely reflects differences in case ascertainment. HZ cases in most previous studies were identified retrospectively based on medical records. Reliance on physician diagnosis and medical codes alone can result in overestimation of the incidence of HZ [11,14,43]. Laboratory confirmation is important to rule out other diagnoses, such as contact dermatitis and zosteriform herpes simplex [44–46]. The SPS and its Persistence Substudies were prospective studies that only enrolled immunocompetent persons, actively followed all participants for HZ, and set a very low threshold for evaluating possible cases. More than 95% of the participants completed the study and a close-out interview that indicated that every episode of HZ was likely detected and evaluated during the studies. More than 93% of HZ cases were diagnosed by PCR. These study features avoided most potential confounders, such as misdiagnosis of HZ and age- or gender-related difference in healthcare seeking behavior.

Within the SPS, the HZ recurrence rates in both placebo (2/660; 0.30%) and ZVL recipients (1/321; 0.31%) were 0.1% per year, with an overall incidence of HZ recurrence of 2.02 cases per 1000 person years. This is more than five-times lower than the incidence of initial cases of HZ among SPS placebo recipients (11.12 per 1000 person years). The rate of recurrence in the LTPS, which followed only ZVL recipients for a mean of 3.74 years, was 0.76% (2 of 263 participants).

4.2. HZ recurrence and sex

The risk of HZ recurrence has been generally reported to be greater in females than males [4,7,11,12,48] and, while not statistically significant, the results of this study are consistent with these reports. In addition, while all four females in our study were immunocompetent throughout, the only male with a recurrence was immunocompromised when he experienced both episodes of HZ.

4.3. HZ-associated pain and recurrence

Nakamura et al. [47] reported that in participants 50–79 years of age, HZ-associated pain was significantly less severe in second than in first episodes. However, in our five participants, the HZ severity-of-illness scores were higher in all but one of the second episodes of HZ (Table 1), suggesting that a first episode of HZ may not mitigate the pain associated with a second episode.

4.4. HZ recurrence and zoster vaccine

An episode of HZ boosts VZV-specific cell-mediated immunity (VZV-CMI) [3], and similar T-cell responses have also been observed in ZVL recipients [49]. Although our numbers are small, ZVL does not appear to protect against HZ recurrence; in the SPS, HZ recurrence rates were the same in vaccine and placebo recipients. This is not surprising since any residual difference in VZV-CMI between ZVL and placebo recipients would likely have been eliminated by the VZV-CMI response to their first episode of HZ. A cohort study in immunocompetent adults $\geq$60 years of age with a recent episode of HZ that compared the incidence of recurrent HZ in vaccinated and unvaccinated persons, also failed to observe an impact of ZVL on the risk of recurrence [9]. While the temporal decline of ZVL effectiveness [16,17,50,51] may have contributed to the risk of HZ recurrence, a unique susceptibility to reactivation of latent VZV and recurrence of HZ seems likely to contribute to the increased risk of recurrent HZ in particular individuals [52]. Participant 4 was immunocompromised at the onset of both episodes of HZ, which likely increased his susceptibility to HZ recurrence.

All 10 episodes of HZ were caused by wild-type VZV. No case of HZ during the SPS and its substudies was caused by ZVL, and only one case of HZ caused by Oka vaccine VZV in an immunocompetent Zostavax® recipient has been reported to date [53]. This suggests that establishment of latency by the Oka vaccine strain of VZV is rare in immunocompetent older individuals who are already latently infected with wild-type VZV when vaccinated.

4.5. VZV genotypes and HZ recurrence

Analysis of the unique sequence regions of the VZV strains from all 10 episodes of HZ indicated that each second episode of HZ was caused by the same VZV genotype as the first episode, confirming that VZV is extremely stable during latency and reactivation. Except for one SNP in non-coding position 105,012, no sequence changes were observed within the unique VZV genome regions of any of the pairs of viruses from recurrent episodes of HZ in the five participants. Position 105,012 is located at the junction of the IR_L and the IR_S, a region that has been previously reported to exhibit strain-independent heterogeneity [26].

These findings document the extraordinary stability of VZV during latency, in keeping with the prediction of a mathematical model of ZVL that VZV does not replicate during latency [54]. Whole-genome sequencing of VZV from an outbreak of varicella has also shown that VZV is extremely stable during transmission between household contacts [27].

4.6. Variability in the reiteration regions

Others have previously described variability in the VZV reiteration regions, both within a single clinical isolate and in different tissue culture passages of the same virus, with R3 and R4 being most variable [23,26,55,56]. In a study of circulating VZV genotypes during an outbreak of varicella, R1, R4 and R5 appeared to be conserved during transmission between household contacts, with more variability in R2 [27]. While most reiteration regions exhibited the same pattern of repeat elements in the VZV strains from both episodes of HZ in each of the five participants, we did observe some variability between the viruses from the paired episodes of HZ. The variations observed in R3 in participants 1 and 2, and in R4B in participant 1 may be explained by establishment of neuronal latency by otherwise clonal viruses with mixtures in those reiteration regions, and reactivation of different variants causing the first and second episodes of HZ.

The R5 regions in the viruses from the first and second episodes in participant 2 differed, not only in length, but in the sequence of

one of the 88 bp elements: In the virus from the first episode of HZ, R5 begins with the 88A element, which is shared with every other VZV strain in the study – independent of clade, whereas the R5 region of the virus from the second episode of HZ had an 88F element in the first position. Nine variable loci have been identified in the 88 bp element of R5 (NJJ and DSS, personal communication), and the 88F element differs from the 88A element at one of them. R5 regions have not been reported to be present as mixtures in individual VZV strains, even after repeated passage in tissue culture; thus, the possibility that the R5 variants seen in the VZV strains from the first and second episode of HZ in participant 2 were present in both samples at different concentrations seems unlikely. R5 regions were subjected to only a single round of PCR and, while we used a high-fidelity polymerase, point mutation at low level at one of the variable loci specifically associated with the 88 bp element could have resulted from PCR. However, truncation by deletion of two paired sets of 24–88 motifs caused by PCR amplification seems unlikely.

R5 regions with 2 tandemly duplicated 24–88 bp element pairs 3′ to the initial 88 bp element (312 bp total) have been observed previously, primarily in Clade 2 viruses [26,57], and rarely also in Clade 1, Clade 4 and Clade 9 viruses (NJJ and DSS, personal communication). However, the R5 region of the Clade 1 VZV strain responsible for the first episode of HZ in participant 2 is the only example reported of an R5 region with three tandemly duplicated 24–88 bp element pairs 3′ to the initial 88 bp element (424 bp total).

Taha et al. [31], using targeted VZV SNP analysis, determined that the VZV strains obtained from a first and a second episode of HZ in an immunocompetent patient were genetically distinct, belonging to different VZV clades (Clade 1, first episode; Clade 3, second episode). In 2009, Quinlivan et al. [32] evaluated a case of varicella in a 19-month-old child and found that the patient was co-infected with two distinct strains of VZV (Clade 3 and Clade 4). In addition, a number of studies of complete genome sequences of VZV have provided evidence that the currently circulating VZV clades arose, at least in part, through inter-strain recombination, which requires co-infection of cells with two different VZV strains [32,58,59].

While the number is too small to determine the relative frequency with which two episodes of HZ in an individual are caused by identical versus different strains of VZV, the cases reported here represent five of only six cases of recurrent HZ in which the viruses causing both episodes have been subjected to molecular analysis. The unique regions of all five pairs of viruses in this study were identical, and thus they were all clonal viruses. Identity was also observed in most of the R regions, with several exceptions. R3 and R4 are well-documented to exhibit variability in copy number within the same VZV isolate, possibly representing R region variants that pre-existed in the original infecting VZV strain. The R5 regions in the VZV strains from the first and second episodes of HZ in participant 2 are an anomaly, since that region has not previously been reported to occur as a mixture within the same VZV strain. Thus, it seems reasonable to anticipate that in most immunocompetent persons, first and second episode HZ will be caused by the same VZV strain.

This study has limitations: The STPS and LTPS were only carried out at 12 of the 22 SPS study sites, and enrollment was not randomized or blinded to study drug assignment (ZVL or placebo). Furthermore, during the STPS the population of placebo recipients was shrinking as SPS placebo recipients received ZVL. Consequently, there were no SPS placebo recipients enrolled in the LTPS. Because of these differences in study populations, we cannot combine the data to calculate an overall rate of HZ recurrences throughout the SPS and its Persistence Substudies. In addition, the sample of five participants with recurrent HZ is small, although it includes all participants who developed two episodes of HZ during the SPS, as well as all who developed two episodes of HZ during the STPS (none) and the LTPS.

Our report indicates that VZV is extremely stable during the replication phase of varicella and the subsequent establishment of latency in sensory ganglia throughout the body, and it demonstrates the stability of VZV during latency, reactivation, and the subsequent replication that results in HZ.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Diseases Control and Prevention (CDC) or the Department of Veterans Affairs.

Potential conflicts of interest

Please see individual Declaration of Competing Interest forms.

Authors' contributions

RH, NJJ, DPD, GRJ, DSS, JB, and MNO contributed to the conception and the design of the study; RH and MEA prepared the samples; NJJ and DPD performed the sequencing; GRJ prepared the clinical data; RH, NJJ, DPD and MEA conducted the analysis; RH drafted the manuscript. All of the authors revised and critically reviewed the manuscript.

Each author has approved the final article

All authors attest they meet the ICMJE criteria for authorship.

References

[1] Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. Proc R Soc Med 1965;58:9–20.
[2] Oxman MN. Immunization to reduce the frequency and severity of herpes zoster and its complications. Neurology 1995;45:S41–6.
[3] Weinberg A, Zhang JH, Oxman MN, Johnson GR, Hayward AR, Caulfield MJ, et al. Varicella-zoster virus-specific immune responses to herpes zoster in elderly participants in a trial of a clinically effective zoster vaccine. J Infect Dis 2009;200:1068–77.
[4] Ragozzino MW, Melton 3rd LJ, Kurland LT, Chu CP, Perry HO. Population-based study of herpes zoster and its sequelae. Medicine (Baltimore) 1982;61:310–6.
[5] Han ES, Choi HR, Seo SJ, Hong CK, Ro BI. A clinical study of herpes zoster during the last 10 years. Korean J Dermatol 1994;32:286–93.
[6] Donahue JG, Choo PW, Manson JE, Platt R. The incidence of herpes zoster. Arch Intern Med 1995;155:1605–9.
[7] Yawn BP, Wollan PC, Kurland MJ, St Sauver JL, Saddier P. Herpes zoster recurrences more frequent than previously reported. Mayo Clin Proc 2011;86:88–93.
[8] Jeong SK, Kim IH. Recurrence rate of herpes zoster during the previous decade. Korean J Dermatol 2012;50:287–9.
[9] Tseng HF, Chi M, Smith N, Marcy SM, Sy LS, Jacobsen SJ. Herpes zoster vaccine and the incidence of recurrent herpes zoster in an immunocompetent elderly population. J Infect Dis 2012;206:190–6.
[10] Kawai K, Gebremeskel BG, Acosta CJ. Systematic review of incidence and complications of herpes zoster: towards a global perspective. BMJ Open 2014;4:e004833.
[11] Shiraki K, Toyama N, Daikoku T, Yajima M, Society MD. Herpes zoster and recurrent herpes zoster. Open Forum Infect Dis 2017;4:ofx007.
[12] Ha JW, Lee JY, Her Y, Kim CW, Kim SS. Frequency of herpes zoster recurrence in central district of Korea. Ann Dermatol 2017;29:602–7.
[13] Kim YJ, Lee CN, Lee MS, Lee JH, Lee JY, Han K, et al. Recurrence rate of herpes zoster and its risk factors: a population-based cohort study. J Korean Med Sci. 2019;34:e1.
[14] Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med 2005;352:2271–84.
[15] Harbecke R, Oxman MN, Arnold BA, Ip C, Johnson GR, Levin MJ, et al. A real-time PCR assay to identify and discriminate among wild-type and vaccine strains of varicella-zoster virus and herpes simplex virus in clinical specimens, and comparison with the clinical diagnoses. J Med Virol 2009;81:1310–22.
[16] Schmader KE, Oxman MN, Levin MJ, Johnson G, Zhang JH, Betts R, et al. Persistence of the efficacy of zoster vaccine in the shingles prevention study and the short-term persistence substudy. Clin Infect Dis 2012;55:1320–8.
[17] Morrison VA, Johnson GR, Schmader KE, Levin MJ, Zhang JH, Looney DJ, et al. Long-term persistence of zoster vaccine efficacy. Clin Infect Dis 2015;60:900–9.
[18] Davison AJ, Scott JE. The complete DNA sequence of varicella-zoster virus. J Gen Virol. 1986;67(Pt 9):1759–816.
[19] Arvin AM, Gilden D. Varicella-zoster virus. In: Knipe DM, Howley PM, editors. Fields Virology. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. p. 2015–57.
[20] Depledge DP, Ouwendijk WJD, Sadaoka T, Braspenning SE, Mori Y, Cohrs RJ, et al. A spliced latency-associated VZV transcript maps antisense to the viral transactivator gene 61. Nat Commun 2018;9:1167.
[21] Norberg P, Liljeqvist JA, Bergstrom T, Sammons S, Schmid DS, Loparev VN. Complete-genome phylogenetic approach to varicella-zoster virus evolution: genetic divergence and evidence for recombination. J Virol 2006;80:9569–76.
[22] Hondo R, Yogo Y. Strain variation of R5 direct repeats in the right-hand portion of the long unique segment of varicella-zoster virus DNA. J Virol 1988;62:2916–21.
[23] Kinoshita H, Hondo R, Taguchi F, Yogo Y. Variation of R1 repeated sequence present in open reading frame 11 of varicella-zoster virus strains. J Virol 1988;62:1097–100.
[24] Yoshida M, Tamura T, Miyasaka K, Shimizu A, Ohashi N, Itoh M. Analysis of numbers of repeated units in R2 region among varicella-zoster virus strains. J Dermatol Sci 2003;31:129–33.
[25] Sauerbrei A, Zell R, Wutzler P. Analysis of repeat units in the R2 region among different Oka varicella-zoster virus vaccine strains and wild-type strains in Germany. Intervirology 2007;50:40–4.
[26] Tyler SD, Peters GA, Grose C, Severini A, Gray MJ, Upton C, et al. Genomic cartography of varicella-zoster virus: a complete genome-based analysis of strain variability with implications for attenuation and phenotypic differences. Virology 2007;359:447–58.
[27] Depledge DP, Gray ER, Kundu S, Cooray S, Poulsen A, Aaby P, et al. Evolution of cocirculating varicella-zoster virus genotypes during a chickenpox outbreak in Guinea-Bissau. J Virol 2014;88:13936–46.
[28] Straus SE, Reinhold W, Smith HA, Ruyechan WT, Henderson DK, Blaese RM, et al. Endonuclease analysis of viral DNA from varicella and subsequent zoster infections in the same patient. N Engl J Med 1984;311:1362–4.
[29] Hayakawa Y, Yamamoto T, Yamanishi K, Takahashi M. Analysis of varicella-zoster virus DNAs of clinical isolates by endonuclease HpaI. J Gen Virol 1986;67(Pt 9):1817–29.
[30] Arvin AM, Koropchak CM, Wittek AE. Immunologic evidence of reinfection with varicella-zoster virus. J Infect Dis 1983;148:200–5.
[31] Taha Y, Scott FT, Parker SP, Syndercombe Court D, Quinlivan ML, Breuer J. Reactivation of 2 genetically distinct varicella-zoster viruses in the same individual. Clin Infect Dis 2006;43:1301–3.
[32] Quinlivan M, Sengupta N, Breuer J. A case of varicella caused by co-infection with two different genotypes of varicella-zoster virus. J Clin Virol 2009;44:66–9.
[33] Coplan PM, Schmader K, Nikas A, Chan ISF, Choo P, Levin MJ, et al. Development of a measure of the burden of pain due to herpes zoster and postherpetic neuralgia for prevention trials: adaptation of the brief pain inventory. J Pain 2004;5:344–56.
[34] Schmader KE, Sloane R, Pieper C, Coplan PM, Nikas A, Saddier P, et al. The impact of acute herpes zoster pain and discomfort on functional status and quality of life in older adults. Clin J Pain 2007;23:490–6.
[35] Depledge DP, Palser AL, Watson SJ, Lai IY, Gray ER, Grant P, et al. Specific capture and whole-genome sequencing of viruses from clinical samples. PLoS ONE 2011;6:e27805.
[36] Depledge DP, Kundu S, Jensen NJ, Gray ER, Jones M, Steinberg S, et al. Deep sequencing of viral genomes provides insight into the evolution and pathogenesis of varicella zoster virus and its vaccine in humans. Mol Biol Evol 2014;31:397–409.
[37] Katoh K, Misawa K, Kuma K, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30:3059–66.
[38] Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.
[39] Huson DH. SplitsTree: analyzing and visualizing evolutionary data. Bioinformatics 1998;14:68–73.
[40] Takada M, Suzutani T, Yoshida I, Matoba M, Azuma M. Identification of varicella-zoster virus strains by PCR analysis of three repeat elements and a PstI-site-less region. J Clin Microbiol 1995;33:658–60.
[41] Ida M, Kageyama S, Sato H, Kamiyama T, Toyomoto T, Ozaki T, et al. Characterization of acyclovir susceptibility and genetic stability of varicella-zoster viruses isolated during acyclovir therapy. J Dermatol Sci 2000;23:63–72.
[42] Zell R, Taudien S, Pfaff F, Wutzler P, Platzer M, Sauerbrei A. Sequencing of 21 varicella-zoster virus genomes reveals two novel genotypes and evidence of recombination. J Virol 2012;86:1608–22.
[43] Scott FT, Leedham-Green ME, Barrett-Muir WY, Hawrami K, Gallagher WJ, Johnson R, et al. A study of shingles and the development of postherpetic neuralgia in East London. J Med Virol 2003;70(Suppl 1):S24–30.
[44] Slavin HB, Ferguson Jr JJ. Zoster-like eruptions caused by the virus of herpes simplex. Am J Med 1950;8:456–67.
[45] Heskel NS, Hanifin JM. "Recurrent herpes zoster": an unproved entity?. J Am Acad Dermatol 1984;10:486–90.
[46] Kalman CM, Laskin OL. Herpes zoster and zosteriform herpes simplex virus infections in immunocompetent adults. Am J Med 1986;81:775–8.
[47] Nakamura Y, Miyagawa F, Okazaki A, Okuno Y, Mori Y, Iso H, et al. Clinical and immunologic features of recurrent herpes zoster (HZ). J Am Acad Dermatol. 2016;75(950–6):e1.
[48] Molin L. Aspects of the natural history of herpes zoster. A follow-up investigation of outpatient material. Acta Derm Venereol 1969;49:569–83.
[49] Levin MJ, Oxman MN, Zhang JH, Johnson GR, Stanley H, Hayward AR, et al. Varicella-zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. J Infect Dis 2008;197:825–35.
[50] Tseng HF, Harpaz R, Luo Y, Hales CM, Sy LS, Tartof SY, et al. Declining effectiveness of herpes zoster vaccine in adults aged >/=60 years. J Infect Dis 2016;213:1872–5.
[51] Izurieta HS, Wernecke M, Kelman J, Wong S, Forshee R, Pratt D, et al. Effectiveness and duration of protection provided by the live-attenuated herpes zoster vaccine in the Medicare population ages 65 years and older. Clin Infect Dis 2017;64:785–93.
[52] Suo L, Lu L, Li J, Sun M, Wang H, Peng X, et al. A case control study on family history as a risk factor for herpes zoster and associated outcomes, Beijing. China. BMC Infect Dis 2017;17:334.
[53] Tseng HF, Schmid DS, Harpaz R, LaRussa P, Jensen NJ, Rivailler P, et al. Herpes zoster caused by vaccine-strain varicella zoster virus in an immunocompetent recipient of zoster vaccine. Clin Infect Dis 2014;58:1125–8.
[54] Weinert LA, Depledge DP, Kundu S, Gershon AA, Nichols RA, Balloux F, et al. Rates of vaccine evolution show strong effects of latency: implications for varicella zoster virus epidemiology. Mol Biol Evol 2015;32:1020–8.
[55] Breuer J. VZV molecular epidemiology. Curr Top Microbiol Immunol 2010;342:15–42.
[56] Won YH, Kim JI, Kim YY, Lee CH. Characterization of the repeat sequences of varicella-zoster virus. J Bacteriol Virol 2014;44:326–35.
[57] Yoshida M, Tamura T. An analytical method for R5 repeated structure in varicella-zoster virus DNA by polymerase chain reaction. J Virol Methods 1999;80:213–5.
[58] McGeoch DJ. Lineages of varicella-zoster virus. J Gen Virol 2009;90:963–9.
[59] Norberg P, Depledge DP, Kundu S, Atkinson C, Brown J, Haque T, et al. Recombination of globally circulating varicella-zoster virus. J Virol 2015;89:7133–46.