Infect Dis Ther (2023) 12:2621–2630
https://doi.org/10.1007/s40121-023-00884-0

CASE REPORT

Mutations in Coding and Non-Coding Regions in Varicella-Zoster Virus Causing Fatal Hemorrhagic Fever Without Rash in an Immunocompetent Patient: Case Report

Juan Camacho · Anabel Negredo · Bartolomé Carrilero · Manuel Segovia · Antonio Moreno · Francisco Pozo · Juan-Emilio Echevarría · José-Manuel Echevarría · M. Paz Sánchez-Seco · David Tarragó

Received: June 15, 2023 / Accepted: October 3, 2023 / Published online: October 23, 2023

ABSTRACT

Introduction: We report the case of a fatal hemorrhagic varicella primary infection in an immunocompetent man and whole-genome characterization of the virus for the investigation of biomarkers of virulence.

Case: A 38-year-old patient born in Nigeria presented to the emergency department with abdominal pain and subsequently developed fatal hemorrhagic disease without skin rash. Extensive laboratory tests including serology and PCR for arenaviruses, bunyaviruses and ebolaviruses were negative. Varicella-zoster virus (VZV) PCR of sera, liver and spleen tissue samples from autopsy revealed the presence of VZV DNA. Primary infection by varicella-zoster virus with hemorrhagic manifestations was diagnosed after virological testing. The VZV genome was sequenced using a mWGS approach. Bioinformatic analysis showed 53 mutations across the genome, 33 of them producing non-synonymous variants affecting up to 14 genes. Some of them, such as ORF11 and ORF 62, encoded for essential functions related to skin or neurotropism. To our knowledge, the mutations reported here have never been described in a VZV causing such a devastating outcome.

Discussion: In immunocompetent patients, viral factors should be considered in patients with uncommon symptoms or severe diseases. Some relevant mutations revealed by using whole genome sequencing (WGS) directly from clinical samples may be involved in this case and deserves further investigation.

Conclusion: Differential diagnosis of varicella-zoster virus in immunocompetent adults should be considered among patients with suspected VHF, even if the expected vesicular rash is not present at admission and does not arise thereafter. Whole genome sequencing of strains causing uncommon symptoms and/or mortality is needed for epidemiological surveillance and further characterization of putative markers of virulence. Additionally, this report highlights the recommendation for a VZV vaccination policy in non-immunized migrants from developing countries.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40121-023-00884-0.

J. Camacho · A. Negredo · F. Pozo · J.-E. Echevarría · J.-M. Echevarría · M. P. Sánchez-Seco · D. Tarragó (✉)
National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain
e-mail: davtarrago@isciii.es

B. Carrilero · M. Segovia · A. Moreno
Virgen de la Arrixaca Hospital, Murcia, Spain

F. Pozo · J.-E. Echevarría · D. Tarragó
CIBER Epidemiología y Salud Pública, Madrid, Spain

A. Negredo · M. P. Sánchez-Seco
CIBER Enfermedades Infecciosas, Madrid, Spain

Keywords: Hemorrhagic fever; Varicella-zoster virus; Metagenomic whole genome sequencing (mWGS); Varicella-zoster virus clades; Varicella-zoster virus virulence; Varicella-zoster virus mutations; Varicella-zoster virus cell tropism; Single-nucleotide polymorphism (SNP)

Key Summary Points

We report an uncommon case of fatal hemorrhagic fever caused by varicella-zoster virus without rash in an inmunocompetent adult.

Varicella zoster virus was fully characterized by whole genome sequencing (WGS) and several relevant mutations affecting essential genes related to viral replication in skin as well as non-coding regions significant in regulating the virus-host interaction were found.

Further studies are needed to relate these mutations to severe disease.

INTRODUCTION

Varicella-zoster virus (VZV) is a common human infectious agent worldwide. Acute primary VZV infection causes chickenpox, which is common in unvaccinated childhood populations. VZV disease without rash occurs frequently and is not always neurological. Herpes zoster is due to reactivation and is usually symptomatic with a classical clinical appearance and affects mainly adults [1, 2]. Primary infection in adulthood often causes more severe disease. In Nigeria, primary infection with VZV tends to occur at later stages of life (i.e., adulthood), resulting in a larger population of susceptible adults and potentially a higher proportion of severe cases [3]. Serious complications of chickenpox include pneumonia, encephalitis and hepatitis among adults and children, respectively.

VZV is genetically stable, although up to nine clades with prevailing geographical distributions have been described. Classification is based on seven established clades (1–6 and 9) and two putative clades (VII and VIII) [4, 5]. Genotypes of European origin included Clade 1 and Clade 3; Clade 2 Asian origin (Japanese), Clade 4 (Asia and the Americas), Clade 5 (Asia, Africa, South America) and Mosaic (Clade VI, VII). Clade 5 mostly comprises viruses from Africa and regions with emigrants from Africa [6]. In a study with 327 children with confirmed varicella or zoster, European origin clades were associated with more severe disease [7]. Furthermore, VZV viruses within each clade carry multiple SNPs, and some of them may cause varicella or zoster cases of sufficient severity that the patients seek medical care, mainly immunocompromised or critical patients. However, the consequences of most SNPs on severity and pathogenesis remain unknown. Mutations in ORF68 (gpE) have been associated with a higher virulence of strains [8, 9]. A single nucleotide change within codon 150, GAC to AAC, which in turn led to an amino acid change from aspartic acid to asparagine [10], produced the loss of a B-cell epitope crucial in the immune response to the virus. Furthermore, increased severity of human cases of infection with gE mutant viruses correlates with the data from the severe combine immunodeficient (SCID) mouse model of VZV infection [11].

Recently, the use of human tissue xenografts in murine models of infection demonstrated that deletions and/or mutations in genes such as ORF62 (transcriptional regulator ICP4, IE62 is major viral transactivator; IFN inhibition), ORF63 and 70 (IE63 phosphoprotein; represses IE62; transactivates EF-1α) as well as ORF11 (tegument protein; required for normal levels of IE4, IE62, IE63 and gE) cause impaired virus replication in the human skin [12].

Our study reports a fatal hemorrhagic varicella case in an immunocompetent adult without cutaneous rash and the genome characterization of this VZV strain by mWGS for the investigation of mutations which may confer virulence. All methods were carried out in accordance with relevant guidelines and UE regulations. Written informed consent for the publication of the data included in this case report was obtained from the patient's wife.

Ethical approval was given by Ethics Committee of the "Instituto de Salud Carlos III" (CEI PI 68_2017-v2).

CASE PRESENTATION AND CLINICAL SETTING

Here we present a fatal case of VZV infection with hemorrhagic fever in an inmunocompetent adult diagnosed by real-time PCR and subsequent whole-genome sequencing to characterize a putative virulent VZV strain.

A 38-year-old man born in Nigeria was admitted to the emergency unit of Virgen de la Arrixaca Hospital (Murcia, Spain) on January 29, 2012. The patient displayed abdominal pain without fever, regional lymph node enlargement or other significant symptoms. He had been living in Spain for 12 years and had not traveled abroad during the last 6 years but had consumed food brought from his country by his relatives at home. Anamnesis recorded discal herniation at L5–S1, diagnosed in 2008 and under current treatment with non-steroidal anti-inflammatory drugs and analgesics. No recent corticosteroid treatment was noticed at that time. Leukocytosis (12,230 cells/mm^3, 66% neutrophils) and abnormal liver enzyme levels (GOT: 382 UI/l; GPT: 364; FA: 39; GGT: 55; LDH: 873) were found, but liver looked normal under CT examination and ascites was absent. Neurological exploration was also normal. The patient was discharged to the Gastroenterology Unit of the hospital a few hours later. Leucocytosis persisted, and platelet count was under the lower limit (120,000/mm^3). Cardiac echography performed the next day revealed myopericarditis. Lung endoscopy was planned, but the patient's condition deteriorated quickly, and he was transferred to the Intensive Care Unit. High fever and hemorrhagic features developing in conjunctiva, ears and nose emerged during the next 48 h. VHF was suspected, and he was moved into an isolation room. Multiorgan failure presented on January 31, and the patient died on February 1. Investigation of risk factors for severe VZV primary infection was begun after the laboratory findings and suggested data previously unnoticed: Due to recent lumbar lesions, the patient had self-prescribed corticosteroid treatment at the time of virus exposure. Disseminated VZV infection with hemorrhagic presentation resulting in death without rash was suspected.

Virological Testing

Serum markers of hepatitis B and C viruses and HIV infections, and PCR for ebolaviruses RNA, were analyzed on a serum sample taken at presentation. Because of the lack of significant findings, other pathogens were tested by PCR: *Plasmodium*, *Leptospira*, *Rickettsia*, Crimea-Congo hemorrhagic fever virus, dengue viruses, enteroviruses, herpes simplex virus 1 and 2, VZV, human cytomegalovirus, Epstein-Barr virus and human herpesvirus 6, human herpesvirus 7 and human herpesvirus 8. Only VZV tested positive, and VZV DNA was found in serum samples and in liver and spleen tissue samples from autopsy as well. The presence of VZV DNA was examined by using a Multiplex real-time PCR, which included enterovirus, HSV and VZV. In addition, both serum samples available tested negative for VZV-specific IgG and IgM antibody.

Shotgun Metagenomic WGS

Whole-genome sequencing was performed using a metagenomic approach. Briefly, total nucleic acid from a blood sample was extracted using QiAmp Mini Elute Virus Spin Kit (Qiagen) without RNA carrier. Libraries were constructed using NEBNext Ultra II Directional RNA Library Prep kit (New England Biolabs). To enrich VZV genomes, a Twist Capture Panel V2 comprising a wide set of probes against viral pathogens including VZV was used. The sequencing was performed on a Miseq sequencer using MiSeq Reagent Micro Kit, v2 (Illumina).

Sequencing samples were analyzed for viral consensus genome reconstruction and de novo assembly using Viralrecon v2.5 pipeline (https://github.com/nf-core/viralrecon) [13] written in Nextflow (https://www.nextflow.io/) in collaboration between nf-core (https://nf-co.re/) community and the Bioinformatics Unit of

the Institute of Health Carlos III (BU-ISCIII) (https://github.com/BU-ISCIII). Trimmed reads were mapped with bowtie2 v.2.4.4 [14] against the reference genome DQ479954.1. Variant calling was carried out using ivar variant v.1.3.1 [15], which calls for low- and high-frequency variants from which variants with an allele frequency > 75% were kept for inclusion in the consensus genome sequence. Finally, Bedtools v2.30.0 [16] was used to obtain the viral genome consensus with the filtered variants and mask genomic regions with coverage values < 10×.

Host reads were removed prior to de novo assembly performing kmer-based mapping of the trimmed reads against the GRCh38 NCBI human genome with Kraken2 v.2.1.2 [17]. For the de novo assembly analysis, the remaining reads after host removal were assembled using SPADES v3.15.4 [18]. A fully ordered genome sequence was generated using ABACAS v1.3.1 [19] based on the Kel strain DQ479954.1 genome because it is a clinical strain of clade 1.

The annotated complete genome was uploaded to GenBank (NCBI) with accession number OQ871571. Previous analysis of seven relevant SNPs from ORF22, ORF 21 and ORF 50 was confirmed by WGS and showed that strain OQ87571 belongs to clade 1, which is predominant in Spain [20].

In addition to Viralrecon v2.5 pipeline, sequencing data were also analyzed on IDseq v3.1 pipeline (https://czid.org/) for de novo assembly and consensus reconstruction against three sequence references. Whole-genome sequencing yielded a 95/95.2/95.5% coverage breadth and 90.7/91.1/91.1% of genome called against clade 1 clinical strain DQ479954.1, Refseq MH709361.1 and Dumas strain NC_001348.1, respectively (Table 1).

Compared to clinical clade 1, Kel strain DQ479954.1 up to 33 genome variants or mutations were found producing changes in amino acid composition (Table 2). These

Table 1 A. Quality of viral consensus genome: Assembly metrics and coverage stats. B. Metagenomics shotgun result

(A) Taxon	Mapped reads	%GC	SNPs	%id	Info. nucleotid	% Genome called	NCBI ref.	Ref. length	Cov. depth	Cov. breadth (%)
HHV3	344139	44.6%	45	100	113972	91.1%	MH709361.1	125127	390.2x	95.2
HHV3	342544	44.7%	38	100	113660	90.7%	DQ479954.1	125374	389.6x	95.0
HHV3	343408	44.7	43	100	113729	91.1%	NC_001348.1	124884	391.4x	95.5

(B) Taxon	rPM	*r*	Contig	Contig r	%id	*L*	*E* value
HHV3	577,954.6	415,339	10	411,117	99.9	30,034.3	10–303

Mapped reads: number of reads aligning to the reference accession; GC content: percentage of bases that are either guanine (G) or cytosine (C); %id: percentage of nucleotides of the consensus genome that are identical to those in the reference accession; Informative nucleotides: number of nucleotides that are A, T, C or G. Nucleotides were only called if 10 or more reads aligned; % Genome called: percentage of the genome meeting thresholds for calling consensus bases; Reference length: length in the base pairs of the reference; Coverage depth: average read depth of aligned contigs and reads over the length of the accession; Coverage breadth: percentage of the accession covered by at least one real or contig

rPM: number of reads aligning to the taxon in the NCBI NR/NT database, per million reads sequenced; *r*: Number of reads aligning to the taxon in the NCBI NT/NR database; *contig:* number of assembled contigs aligning to the taxon; *contig r:* total number of reads across all assembled contigs; *%id:* average percent-identity of alignments to NCBI NT/NR; *L:* average length of the local alignment for all contigs and reads assigned to this taxon; *E value:* average expected value (*e*-value) of alignments to NCBI NT/NR

Table 2 Mutations found on OQ871571 VZV against reference DQ479954.1

POS	REF	ALT	GENE	ORF	EFFECT	HGVS_C	HGVS_P
5342	G	T	HHV3gp07	6	Missense_variant	c.3236C > A	p.Thr1079Asn
13870	T	C	HHV3gp13	11	Missense_variant	c.281 T > C	p.Phe94Ser
14144	C[14N]G	C	HHV3gp13	11	Disruptive_if_del	c.575_589delG[13N]G	p.Gly192_Glu196del
14192	C[14N]G	C	HHV3gp13	11	Disruptive_if_del	c.623_637delG[13N]G	p.Gly208_Glu212del
14207	G	T	HHV3gp13	11	Missense_variant	c.618G > T	p.Glu206Asp
14255	G	T	HHV3gp13	11	Missense_variant	c.666G > T	p.Glu222Asp
19725	T	G	HHV3gp16	14	Missense_variant	c.1407A > C	p.Lys469Asn
20897	A	T	HHV3gp16	14	Missense_variant	c.235 T > A	p.Ser79Thr
41470	G	A	HHV3gp24	22	Missense_variant	c.7370G > A	p.Arg2457Gln
41485	CG	TC	HHV3gp24	22	Missense_variant	c.7385_7386delCGinsTC	p.Ala2462Val
41494	TC	CG	HHV3gp24	22	Missense_variant	c.7394_7395delTCinsCG	p.Val2465Ala
41517	A	C	HHV3gp24	22	Missense_variant	c.7417A > C	p.Thr2473Pro
41524	G	A	HHV3gp24	22	Missense_variant	c.7424G > A	p.Arg2475Gln
42409	GAAA	G	HHV3gp28	26	ups_gene_variant	c.-2115_-2113delAAA	Non-coding
54554	T	C	HHV3gp30	28	ups_gene_variant	c.-3899A > G	Non-coding
78152	CTTT	C	HHV3gp45	43	ups_gene_variant	c.-38_-36delTTT	Non-coding
78792	GG	AA	HHV3gp45	43	Missense_variant	c.602_603delGGinsAA	p.Gly201Glu
78794	G	A	HHV3gp45	43	Missense_variant	c.604G > A	p.Asp202Asn
84831	T	G	HHV3gp49	48	Missense_variant	c.144 T > G	p.His48Gln
85581	G	T	HHV3gp49	48	Missense_variant	c.894G > T	p.Glu298Asp
92899	C	T	HHV3gp54	53	Missense_variant	c.973G > A	p.Asp325Asn
105031	GC	G	HHV3gp59	58	ups_gene_variant	c.-4739delG	Non-coding
105083	C	CG	HHV3gp59	58	ups_gene_variant	c.-4791_-4790insC	Non-coding
109266	GCC	G	HHV3gp62	61	ups_gene_variant	c.-4762_-4761delGG	Non-coding
110460	CTA	C	HHV3gp63	62	ups_gene_variant	c.-1308_-1307delTA	Non-coding
110611	TAA	T	HHV3gp63	62	ups_gene_variant	c.-1459_-1458delTT	Non-coding
112379	GAAAA	G	HHV3gp63	62	ups_gene_variant	c.-3229_-3226delTTTT	Non-coding
113039	G	A	HHV3gp63	62	ups_gene_variant	c.-3885C > T	Non-coding
118015	ATTTT	A	HHV3gp72	62	ups_gene_variant	c.-3239_-3236delTTTT	Non-coding
119787	GTT	G	HHV3gp70	64	ups_gene_variant	c.-1197_-1196delAA	Non-coding
119928	CTA	C	HHV3gp70	64	ups_gene_variant	c.-1338_-1337delTA	Non-coding
121132	AGG	A	HHV3gp70	64	ups_gene_variant	c.-2542_-2541delCC	Non-coding

Table 2 continued

POS	REF	ALT	GENE	ORF	EFFECT	HGVS_C	HGVS_P
125316	A	AC	HHV3gp72	62	downs_gene_variant	c.*129_*130insC	Non-coding

The nucleotide positions are those of the Kel strain genome (GenBank accession no. DQ479954.1)
POS position of the variant; *REF* reference sequence; *ALT* altered sequence; *GENE* gene name in annotation file; *ORF* open reading frame; *EFFECT* effect of the variant; *HGVS_C* position annotation at CDS level; *HGVS_P* position annotation at protein level

variants affected 14 relevant proteins of VZV (Table 3).

DISCUSSION AND CONCLUSIONS

Overall, the delicate balance between VZV immune evasion and host immune responses enables the virus to replicate and spread to naive individuals in a controlled manner. In its severe form, VZV infection can be fatal, especially in immunocompromised patients. However, a fatal outcome has also been described in immunocompetent individuals [21, 22]. Despite skin involvement, the symptoms of disseminated varicella also include multiple hemorrhages (including intracranial hemorrhage, hemorrhagic gastritis, hemorrhagic pulmonary edema, splenic rupture, adrenal hemorrhage, cystorrhagia and hyphema), encephalitis, pneumonia and abdominal pain [23]. Intense abdominal pain is often an early symptom of dissemination, which reveals that multi-system organs are involved, such as the stomach, bowel and spleen. Thrombocytopenia was observed in this case, which may have led to the occurrence of gastrorrhagia, cystorrhagia and eventually hemorrhagic shock.

Cortisol therapy modified the main events of the infection in some patients [24–27]. Corticosteroid treatment at the time of virus exposure may have led to hemorrhagic manifestations, acute liver failure and eventually death in the present case.

However, viral genetic features related to virulence could also be involved. Mutations in viral glycoprotein E impairing specific B-cell recognition were involved in some of such fatal outcomes [10] but they were not found in this case. Noticeably, analysis of whole genome revealed several non-synonymous mutations affecting essential genes as well as non-coding regions which recently have been described as significant in regulating the virus-host interaction. These variants included substitutions, insertions, duplications, in-frame deletions and disruptive deletions and insertions. Whether these mutations were related to the uncommon clinical presentation or not needs further study.

Some of these genes have been demonstrated to impair replication of virus in human skin xenografts in the SCID-hu mouse model of infection but have no effect on T cells when they have been deleted (ORF 62) or had mutation of phosphorylation sites (ORF63 and ORF70) [12]. Several mutations were found in gp13 (ORF11) in the present case that may have a high impact on protein functionality if they cause protein truncation, loss of function or triggering nonsense mediated decay. Gp13 is a tegument protein required for normal levels of IE4, IE62, IE63 and gE. Truncation of ORF11 causes impaired replication in skin in animal models of infection [12]. This finding might be associated with VZV disease without rash observed in this patient. Some other mutations are located in non-coding regions of ORF26, ORF28, ORF43, ORF58, ORF61, ORF62 and ORF64. These regions often contain sncRNAs and miRNAs, and some may regulate infection of host cells [28].

When VZV infection is suspected, viral DNA is usually detected by PCR. We applied whole-genome sequencing to further characterize the

Table 3 VZV protein variants in VZV accession number OQ871571

Annotated gP Kel strain	Function or note
HHV3gp07	"Helicase-primase primase subunit." Component of DNA helicase-primase complex"/"**ORF6**; similar to HHV-1 UL52"
HHV3gp13	rpt_family = "R1direct tandem reiteration **ORF11**"; Tegument protein; required for normal levels of IE4, IE62, IE63 and gE; RNA-binding domain (amino acids 1–22); binding to ORF9 required for efficient virion assembly
HHV3gp16	"Cell attachment"/note = "type 1 membrane protein; contains a signal peptide; binds cell surface heparan sulphate; binds complement C3b to block neutralization" "role in cell entry"/"**ORF14**; similar to HHV-1 UL44"
HHV3gp24	"Capsid transport"/note = "complexed with tegument protein UL37; ubiquitin-specific protease (N-terminal region)"/codon_start = 1/product = "large tegument protein""tegument protein"/ "**ORF22**; similar to HHV-1 UL36
HHV3gp28	"DNA encapsidation"/function = "possibly capsid transport"/product = "DNA packaging protein UL32DNA packaging protein"/"**ORF26**; similar to HHV-1 UL32"
HHV3gp30	"DNA replication"/product = "DNA polymerase catalytic subunit" **ORF28**; similar to HHV-1 UL30
HHV3gp45	"DNA encapsidation"/function = "capsid transport"/note = "capsid-associated" product = "DNA packaging tegument protein UL17" tegument protein"/"**ORF43**; similar to HHV-1 UL17
HHV3gp49	"DNA processing"/product = "deoxyribonuclease" role in maturation of DNA"/"**ORF48**; similar to HHV-1 UL12"
HHV3gp54	"Virion morphogenesis"/product = "tegument protein UL7", tegument protein"/"**ORF53**; similar to HHV-1 UL7"
HHV3gp59	Note = "alpha gene; **ORF58**; similar to HHV-1 UL3"
HHV3gp62	"Gene regulation"/function = "cellular protein degradation"/function = "latency"/note = "contains a RING finger; disrupts ND10;proteasome-dependent degradation of several cellular proteins"role in modulating cell state and gene expression"/"**ORF61**; similar to HHV-1 RL2"
HHV3gp63*	"Gene regulation"/product = "transcriptional regulator ICP4; "**ORF62**; similar to HHV-1 RS1
HHV3gp72*	"Gene regulation"/product = "transcriptional regulator ICP4"; **ORF62**; similar to HHV-1 RS1
HHV3gp70	"Unknown"/product = "virion protein US10" tegument protein"/note = "**ORF64**; similar to HHV-1 US10

*Genes that encode ORF62 protein are duplicated

full genome of the strain. Metagenomic WGS could also be very useful for unbiassed detection and identification of unexpected etiological agents of disease when patients have no skin involvement, which may lead to misdiagnosis and poses diagnostic and therapeutic challenges, especially when some other disease manifestations exist. Testing consecutive sets of viruses by PCR can be time consuming, and, in cases of severe VZV disease, antiviral therapies, which may be effective in reducing disease severity, should be initiated along with supportive treatment as soon as possible to reduce mortality.

ACKNOWLEDGEMENTS

We are grateful to Michael McConnell and Juan Antonio Real Velázquez for their kind help in preparing this manuscript.

Author Contribution. Juan Camacho carried out the NGS experiments. Anabel Negredo, Francisco Pozo and M. Paz Sánchez-Seco performed PCR experiments. Bartolomé Carrilero, Manuel Segovia and Antonio Moreno reported patient's data and submitted clinical samples for testing. Juan-Emilio Echevarría and José-Manuel Echevarría conceived the study. David Tarragó designed the study, performed NGS analysis and wrote the manuscript. All authors reviewed the article before submission not only for spelling and grammar but also for its intellectual content.

Funding. This work was supported by a grant from Instituto de Salud Carlos III. Project code MPY1372/12. The journal's Rapid Service fee was paid by Consorcio Centro de Investigación en Red (CIBER). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability. The annotated complete genome is available at GenBank (NCBI) with accession number OQ871571.

Declarations

Conflict of Interest. All named authors declare no conflicts of interest.

Ethical Approval. All methods were carried out in accordance with relevant guidelines and UE regulations. Written informed consent for the publication of the data included in this case report was obtained from the patient's wife. Ethical approval was given by Ethics Committee of the "Instituto de Salud Carlos III" (CEI PI 68_2017-v2).

REFERENCES

1. Mueller NH, Gilden D, Cohrs RJ, Mahalingam R, Nagel MA. Varicella zoster virus infection: clinical features, molecular pathogenesis of disease, and latency. Neurol Clin. 2008;26:675–96.

2. Gilden D, Cohrs RJ, Mahalingam R, Nagel MA. Neurological disease produced by varicella zoster virus reactivation without rash. Cur Top Microbiol Immunol. 2010;342:243–53.

3. Hussey H, Abdullahi L, Collins J, Muloiwa R, Hussey G, Kagina B. Varicella zoster virus-associated morbidity and mortality in Africa - a systematic review. BMC Infect Dis. 2017;17(1):717. https://doi.org/10.1186/s12879-017-2815-9.

4. Breuer J, Grose C, Norberg P, Tipples G, Schmid DS. A proposal for a common nomenclature for viral clades that form the species varicella-zoster virus: summary of VZV Nomenclature Meeting 2008, Barts and the London School of Medicine and Dentistry, 24–25 July 2008. J Gen Virol. 2010;91(Pt 4):821–8. https://doi.org/10.1099/vir.0.017814-0.

5. Jensen NJ, Rivailler P, Tseng HF, Quinlivan ML, Radford K, Folster J, Harpaz R, LaRussa P, Jacobsen S, Scott SD. Revisiting the genotyping scheme for varicella-zoster viruses based on whole-genome comparisons. J Gen Virol. 2017;98(6):1434–8. https://doi.org/10.1099/jgv.0.000772.

6. Breuer J. Molecular genetic insights into Varicella Zoster virus (VZV), the vOka vaccine strain, and the pathogenesis of latency and reactivation. J Infect Dis. 2018;218(suppl_2):S75–80. https://doi.org/10.1093/infdis/jiy279.

7. Marshall HS, Clarke M, Heath C, Quinn H, Richmond PC, Crawford N, Elliott E, Toi C, Kynaston A, Booy R, Macartney K. Severe and Complicated varicella and associated genotypes 10 years after introduction of a one-dose Varicella vaccine program. J Infect Dis. 2019;219(3):391–9. https://doi.org/10.1093/infdis/jiy518.

8. Grose C, et al. Complete DNA sequence analyses of the first two varicella-zoster virus glycoprotein E (D150N) mutant viruses found in North America: evolution of genotypes with an accelerated cell spread phenotype. J Virol. 2004;78:6799–807.

9. Wirgart BZ, Estrada V, Jackson W, Linde A, Grose C. A novel varicella-zoster virus gE mutation discovered in two Swedish isolates. J Clin Virol. 2006;37:134–6.

10. Santos RA, et al. Varicella-zoster virus gE escape mutant VZV-MSP exhibits an accelerated cell-to-cell spread phenotype in both infected cell cultures and SCID-hu mice. Virology. 2000;275:306–17.

11. Moffat JF, et al. Attenuation of the vaccine Oka strain of varicella-zoster virus and role of glycoprotein C in alphaherpesvirus virulence demonstrated in the SCID-hu mouse. J Virol. 1998;72:965–74.

12. Zerboni L, Nandini Sen N, Oliver SL, Arvin AM. Molecular mechanisms of varicella zoster virus pathogenesis. Nat Rev Microbiol. 2014;12(3):197–210. https://doi.org/10.1038/nrmicro3215.

13. Patel H, Varona S, Monzón S, Espinosa-Carrasco J, Heuer ML, Underwood A, Gabernet G, Bot NC, Ewels P, MiguelJulia KS, Wilson S, Sameith K, Garcia MU, Jcurado MK. Nf-core/viralrecon: nf-core/viralrecon v2.4.1-plastered magnesium marmoset. 2022. Zenodo. https://doi.org/10.5281/zenodo.6320980.

14. Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.

15. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, De Jesus JG, Main BJ, Tan AL, Paul LM, Brackney DE, Grewal S, Gurfield N, Van Rompay KKA, Isern S, Michael SF, Coffey LL, Loman NJ, Andersen KG. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biol. 2019;20(1):8. https://doi.org/10.1186/s13059-018-1618-7.

16. Quinlan AR, Ira M. Hall, BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010;26(6):841–2. https://doi.org/10.1093/bioinformatics/btq033.

17. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019;20:257. https://doi.org/10.1186/s13059-019-1891-0.

18. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes de novo assembler. Curr Protoc Bioinform. 2020;70: e102. https://doi.org/10.1002/cpbi.102.

19. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. ABACAS: algorithm based automatic contiguation of assembled sequences. Bioinformatics. 2009;25:1968–9. https://doi.org/10.1093/bioinformatics/btp347.

20. González I, Molina-Ortega A, Pérez-Romero P, Echevarría JE, He L, Tarragó D. Varicella-zoster virus clades circulating in Spain over two decades. J Clin Virol. 2019;110:17–21. https://doi.org/10.1016/j.jcv.2018.11.008.

21. Matsuo Y, Igarashi Y, Aoyama N, Satta Y, Oikawa R, Noguchi Y, Takahashi H, Matsumoto N, Yamamoto H, Itoh F. Visceral disseminated varicella-zoster virus infection in an immunocompetent host. Clin J Gastroenterol. 2022;15(3):568–74. https://doi.org/10.1007/s12328-022-01607-7.

22. Defaux BA, Brabant S, Chatellier D, Bourgoin A, Robert R, Ruckes T, Agius G. Disseminated varicella with multiorgan failure in an immunocompetent adult. J Med Virol. 2009;81:747–9. https://doi.org/10.1002/jmv.21447.

23. Pannu AK, Manikandan G. Disseminated Varicella Infection. N Engl J Med. 2019;381(11): e21. https://doi.org/10.1056/NEJMicm1814626.

24. Abreu-Vélez AM, Smoller BR, Gao W, Grossniklaus HE, Jiao Z, Arias LF, Dudley SC, Howard MS. Varicella-zoster virus (VZV) and alpha 1antitrypsin: a

fatal outcome in a patient affected by endemic pemphigus foliaceus. Int J Dermatol. 2012;51:809–16.

25. Leung J, Siegel S, Jones JF, Schulte C, Bloq D, Scott Schmid C, Bialek SR, Marin M. Fatal varicella due to the vaccine strain of varicella-zoster virus. Hum Vaccin Immunother. 2014;10:146–9.

26. Ma C, Walters B, Fedorak RN. Varicella-zoster meningitis complicating combined anti-tumor necrosis factor and corticosteroid therapy in Crohn's disease. World J Gastroenterol. 2013;19:3347–51.

27. Ben-Chetrit E, Lachish T, Nesher G. Fatal haemorrhagic varicella in a patient with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Clin Exp Rheumatol. 2013;31:S95.

28. Markus A, Golani L, Ojha NK, Borodiansky-Shteinberg T, Kinchington PR, Goldstein RS. Varicella-zoster virus expresses multiple small noncoding RNAs. J Virol. 2017;91(24):e01710-e1717. https://doi.org/10.1128/JVI.01710-17.

Publisher's Note