Mutations in coding and non-coding regions in varicella-zoster virus causing fatal hemorrhagic fever without rash in an immunocompetent patient: Case report

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Table 1 Supplementary Material. Mutations found on OQ871571 VZV against reference Dumas strain NC_001348.1.

POS	REF	ALT	ORF	EFFECT	HGVS_C	HGVS_P
14068	GA	AC	ORF11	missense_variant	c.479_480delGAinsAC	p.Gly160Asp
14071	A	C	ORF11	missense_variant	c.482A>C	p.Glu161Ala
14116	GA	AC	ORF11	missense_variant	c.527_528delGAinsAC	p.Gly176Asp
14119	A	C	ORF11	missense_variant	c.530A>C	p.Glu177Ala
14237	G	T	ORF11	missense_variant	c.648G>T	p.Glu216Asp
20795	A	T	ORF14	missense_variant	c.319T>A	p.Ser107Thr
20879	A	T	ORF14	missense_variant	c.235T>A	p.Ser79Thr
20921	A	T	ORF14	missense_variant	c.193T>A	p.Ser65Thr
20963	A	T	ORF14	missense_variant	c.151T>A	p.Ser51Thr
41452	G	A	ORF22	missense_variant	c.7370G>A	p.Arg2457Gln
41467	CG	TC	ORF22	missense_variant	c.7385_7386delCGinsTC	p.Ala2462Val
41485	CG	TC	ORF22	missense_variant	c.7403_7404delCGinsTC	p.Ala2468Val
41499	A	C	ORF22	missense_variant	c.7417A>C	p.Thr2473Pro

42391	GAA	G	ORF26	ups_gene_variant	c.-2114_-2113delAA	Non-coding
51920	T	C	ORF29	missense_variant	c.1064T>C	p.Met355Thr
54535	T	C	ORF28	ups_gene_variant	c.-3899A>G	Non-coding
54564	T	C	ORF28	ups_gene_variant	c.-3928A>G	Non-coding
60266	CA	C	ORF36	ups_gene_variant	c.-4540delA	Non-coding
65669	C	T	ORF36	missense_variant	c.863C>T	p.Ser288Leu
66879	C	T	ORF37	missense_variant	c.806C>T	p.Pro269Leu
78771	GG	AA	ORF43	missense_variant	c.602_603delGGinsAA	p.Gly201Glu
78773	G	A	ORF43	missense_variant	c.604G>A	p.Asp202Asn
90202	G	T	ORF51	missense_variant	c.2322G>T	p.Gln774His
102203	A	G	ORF57	ups_gene_variant	c.-2577T>C	Non-coding
102403	A	C	ORF57	ups_gene_variant	c.-2777T>G	Non-coding
104898	A	G	ORF58	ups_gene_variant	c.-4626T>C	Non-coding
105010	C	G	ORF58	ups_gene_variant	c.-4738G>C	Non-coding
105012	T	C	ORF58	ups_gene_variant	c.-4740A>G	Non-coding
105015	T	C	ORF58	ups_gene_variant	c.-4743A>G	Non-coding
105045	CG	C	ORF58	ups_gene_variant	c.-4774delC	Non-coding
105062	C	CG	ORF58	ups_gene_variant	c.-4791_-4790insC	Non-coding

110049	AG	A	ORF62	ups_gene_variant	c.-917delC	Non-coding
110050	G	A	ORF62	ups_gene_variant	c.-917C>T	Non-coding
110212	T16N	T	ORF62	ups_gene_variant	c.-1095-1080delCTATATATATATATAT	Non-coding
110228	G	T	ORF62	ups_gene_variant	c.-1095C>A	Non-coding
110360	TA	T	ORF62	ups_gene_variant	c.-1228delT	Non-coding
112198	G	A	ORF62	ups_gene_variant	c.-3065C>T	Non-coding
112783	G	A	ORF62	ups_gene_variant	c.-3650C>T	Non-coding
117699	C	T	ORF62	ups_gene_variant	c.-3065C>T	Non-coding
119527	GT	G	ORF64	ups_gene_variant	c.-1196delA	Non-coding
119667	C16N	C	ORF64	ups_gene_variant	c.-1351_-1336delTATATATATATATAGA	Non-coding
119669	C	A	ORF64	ups_gene_variant	c.-1337G>T	Non-coding
119838	AC	A	ORF64	ups_gene_variant	c.-1507delG	Non-coding
124825	A	AC	ORF62	downs_gene_variant	c.*129_*130insC	Non-coding
124842	AC	A	ORF62	downs_gene_variant	c.*147delC	Non-coding

The nucleotide positions are those of the Dumas strain genome (GenBank accession no. NC_001348.1); 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

Table 2 Supplementary material. Mutations found on OQ871571 VZV against reference KEL strain 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.281T>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.235T>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.144T>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
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.

Table 3. Primers and probes used for detecting VZV in this study.

Primer name	Sequence (5´-3´)
VZV	Forward: ATCGATCCATCAGCGGTCC
	Reserve: CCCCGCAAGACGTTTGG
	Probe: *VIC*-CGATCCGAGGATTCGTA-*MGB*
EV	Forward: ACAIGGTGYGAAGAGYCTATTGAGC
	Reverse: TGCTCCRIRGTTRGGATTAGC
	Probe: Texas red-CCTCCGGCCCCTGAATGCG-BHQ2
HSV1	Forward: GCGGTAGGCACAAAATTCGG
	Reverse: CCCCCATTGGGCTGTTG
HSV2	Forward: AGCGGTATGCGCAAAATTCG
	Reverse: CCCATCGGGCTGCTGG
	Probe HSV: *FAM*-CGACAGTCGATAATC-*MGB*
	Forward: CAGATTAGCAATTGGTGCGAA

Internal Control	Reverse: GTGGGCAAATCCGAGGAA
	Probe: *IRD-700*-AATGATTGGGCCACGTCACG-BHQ3

Multiplex real-time PCR: Runs were performed on a CFX ThermoFisher thermocycler. Real- time PCR was performed using Quantitect Multiplex RT-PCR kit (Qiagen) following manufacturer instructions and 10 µl of nucleic acid extract in a final volume of 50 µl. Thermocycler was programmed for 50ºC for 20 minutes, cycling of 6x touchdown 0,5ºC: 94ºC for 20", 61ºC 20", 72ºC 20" and cycling x40 94ºC 30" and 58ºC for 90 seconds.