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Molecular Analysis of Varicella-Zoster Virus Strains Circulating in Tanzania Demonstrating the Presence of Genotype M1▽

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Based on analysis of 16,392 bp encompassing the complete open reading frames (ORFs) 1, 5, 31, 36, 37, 47, 60, 62, 67, and 68 of the genome of genotype M1 varicella-zoster virus (VZV) was found in swab samples originating from eight Tanzanian zoster patients. Moreover, sequence analysis suggests recombination events between different VZV genotypes within ORFs 1, 31, 60, and 67.

Varicella-zoster virus (VZV; also known as *Human herpesvirus 3*), belonging to the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*, is the cause of chickenpox (varicella) and may reactivate from latency, causing shingles (zoster). Until the beginning of the 21st century, the double-stranded DNA genome of VZV was believed to be highly conserved. Therefore, efforts to analyze entire genome sequences of VZV strains were very rare, and only the entire genome of the VZV prototype strain Dumas was known for decades (2). The VZV genotypes demonstrate a specific geographical distribution. Genotypes E1 and E2 have been detected mainly in Europe and the Americas, whereas genotype J strains are dominant in Japan. Genotype M2 strains probably arose through recombination of genotype E1 and E2 strains with genotype J strains and currently are distributed in countries where the European/American genotypes may have recombined with the Japanese genotype (12). In contrast, genotype M1 strains seem to be associated with nonwhite patients (3, 4, 8). The genotype M1 prototype strain CA123 was isolated in 1990 in California (4).

Based upon the results of partial and full-genome sequence analyses of several VZV strains, an out-of-Africa model for VZV evolution, suggesting that VZV coevolves with humankind and diversified from ancestral VZV genotypes into Japanese (J) and European/American (E1 and E2) genotypes (7, 12), was proposed. Another model suggests that VZV evolution is driven by climatic factors and that VZV strain distribution is associated with temperate and tropical climate conditions (1, 3, 8). Recombination analysis suggests that genotype M2 strains and genotype M1 strains are recombinant strains that originated from strains of the European/American (E1/E2) and Japanese (J) genotypes, respectively (6).

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Several VZV genotyping schemes based on partial VZV genome sequences were proposed for genotype classification (3, 5, 12). Full-genome sequence analysis allowed the development of two classification schemes, resulting in different VZV genotype nomenclatures (4, 6, 7). Recently, we described a simple and reliable VZV genotyping scheme based on analysis of a 1,990-bp region originating from open reading frames (ORFs) 51 to 58. This procedure allows the typing of VZV wild-type strains by high-throughput procedures directly from clinical samples without intermediate virus propagation. Genotyping by this novel procedure and that based on full-genome phylogenetic analysis resulted in the same classification of all strains analyzed (9, 10).

As there is limited sequence information available for African VZV wild-type strains, our objectives were (i) to identify the genotype of circulating VZV wild-type strains in Tanzania and (ii) to reconstruct their evolutionary history.

Vesicle fluid samples were obtained in 2007 from four male and four female nonwhite adults who were zoster patients that were referred to the Ikonda hospital, Makete district, Iringa region, Tanzania. DNA was isolated from the samples by using the RTP DNA/RNA virus mini kit (Invitek, Berlin, Germany) according to the manufacturer's instructions. DNA amplification reactions were carried out with 25-μl volumes with 5 μl of extract or control DNA, 12.5 μl *Taq* PCR master mix (Qiagen, Hilden, Germany), corresponding to 0.6 U *Taq* polymerase, 1 μl of each forward and reverse primer (for detailed information, see Table 1), corresponding to 0.5 μM, and 5.5 μl water. Thermal cycling comprised an initial hot start at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at primer-pair-specific temperatures (Table 1) for 30 s, and polymerization at 72°C for 2 min. Finally, an extension step at 72°C for 10 min was carried out. The PCR products were visualized with a UV transilluminator following separation on 1.5% agarose gels containing ethidium bromide. Purification and sequencing of PCR products, as well as VZV sequence and phylogenetic analyses, were performed as described earlier (9, 10).

Phylogenetic analysis based on stretches of 16,392 and

TABLE 1. Primers used to amplify and sequence genes in VZV strains

Primer	Sequence (5′–3′)	Nucleotide positions[a]	ORF no.[b]	Amplicon size (bp)[b]	T_m (°C)[c]
ORF1F	ATATTTTTGGGATCCGCA	933–916	1	362	51.5
ORF1R	TCCGGAAGGGGAAGGTA	571–587			54.7
ORF5F	TGCCATCTTCCACGGGTC	5292–5275	5	1,058	58.7
ORF5R	GCGTGTTTAGTCACATGA	4234–4251			47.2
ORF31F	GCGTTTTCATAACCTCCGTTACG	56952–56974	31	965	60.6
ORF31R	CCGTTCGTTTTGGCTTCCAG	57917–57895			59.4
ORF31F1	GAACCTGCAGCGCGGAAC	57845–57862	31	875	60.5
ORF31R1	CTCGTTATCTGTTCCAAGCTGGC	58720–58698			62.4
ORF31F2	CTACGCGTTGTTATAGCCGTCC	58632–58653	31	1,038	62.1
ORF31R2	CCTGTGATGCGTAATGGAGACAC	59692–59670			62.4
ORF36F	CGTTTGTCTACAATAAAC	64789–64806	36	1,064	40.4
ORF36R	ATACGTAAATACTAGGTATA	65853–65834			35.8
ORF37F	GCGGTGATATTGTAGCGCAAG	66042–66062	37	1,042	59.8
ORF37R	GCGGTAATCCAAACTCTCTTCGG	67084–67062			62.4
ORF37F1	GTTTATTTACTTGGACGTGGGTTGG	66992–67016	37	1,084	61.3
ORF37R1	CTGTGTCCCTGGACAGCTGG	68076–68057			63.5
ORF37F2	ACTCCACACCCATGTACCAGAAG	67981–68003	37	740	62.4
ORF37R2	TTTGGTAGAGTGCACCAAACACC	68721–68699			60.6
ORF47F	GACGAAGCGTTACTTACACA	83148–83167	47	1,570	51.0
ORF47R	TTTTGGCTGGCTGGGGGC	84718–84701			65.3
ORF60F	AGGGAAAACACAAGCGTC	101667–101650	60	518	53.5
ORF60R	TTTGAATCCGATAGTTTCA	101149–101167			46.5
ORF62F	GGCGCTCACGAGAAAAGGAG	105172–105191	62	1,204	61.4
ORF62R	CTGTCGACCCGAGACCTGG	106376–106358			63.1
ORF62F1	CAAAGCGGGTCCATCCCTG	106245–106263	62	1,249	61
ORF62R1	CCAAGCTGACCGGTGTCAACTC	107494–107473			64
ORF62F2	CGGAACGGGAGACGCTACG	107388–107406	62	1,299	63.1
ORF62R3	GGAAACGGGCAGAGGTACGAC	108687–108667			63.7
ORF62F4	ACCGCTGGTCTTCCCGTTG	108578–108596	62	622	61
ORF62R4	TCGCAATCCTTTGAAGGCTG	109200–109181			57.3
ORF67F	GCGCCTCATTTAATCGCG	114478–114496	67	1,103	58.2
ORF67R	TAAAATCCGGGATAATTAG	115581–115563			45.1
ORF68F	ATTCCGAGGGTCGCCTGTAA	115787–115806	68	1,910	60.1
ORF68R	GTTGCCCCGGTTCGGTGA	117697–117680			63.9

[a] The nucleotide positions are given according to the numbering in the Dumas reference strain (accession number NC001348).
[b] The values refer to both the forward and reverse primers.
[c] T_m, melting temperature.

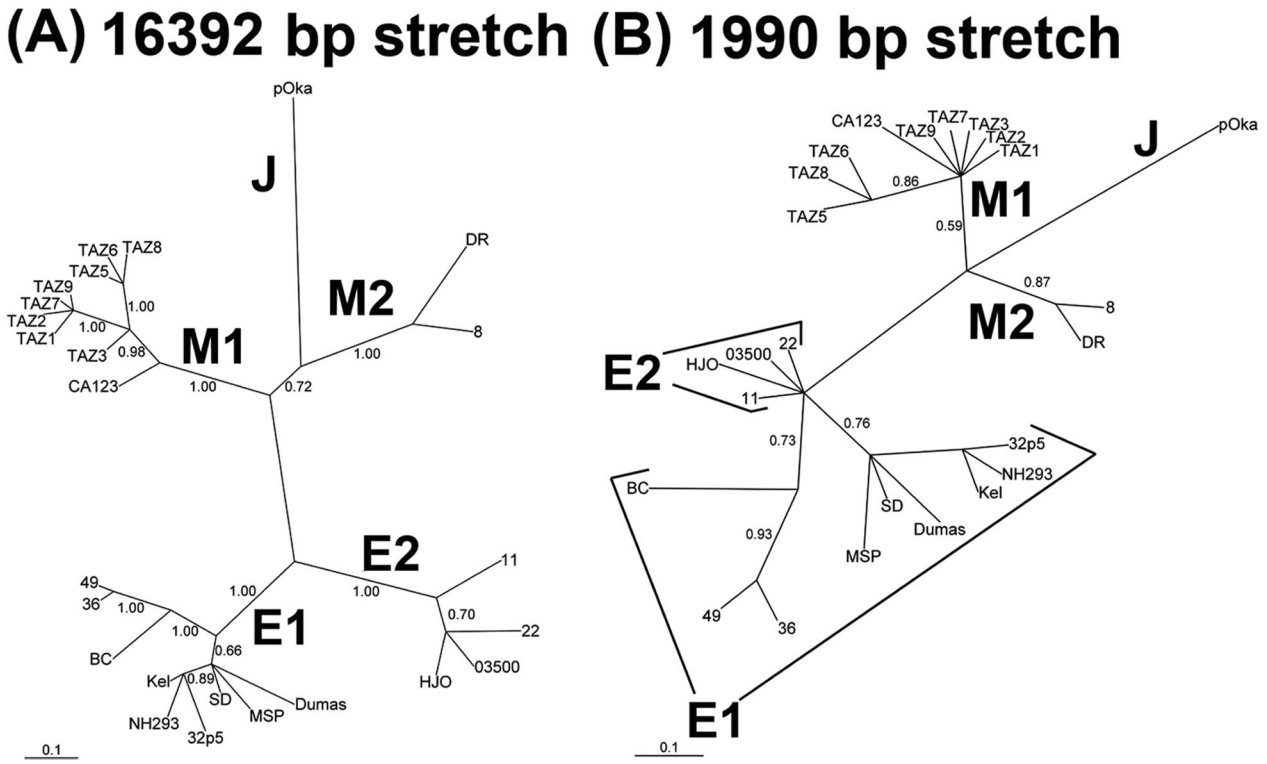

FIG. 1. Phylogenetic trees of the novel Tanzanian VZV strains and the previously described genotype E1, E2, M1, M2, and J VZV strains. Trees based on the 16,392-bp stretch (A) and based on the 1,990-bp stretch (B) that are used in the genotyping scheme described recently (9). Posterior probabilities are shown on each branch.

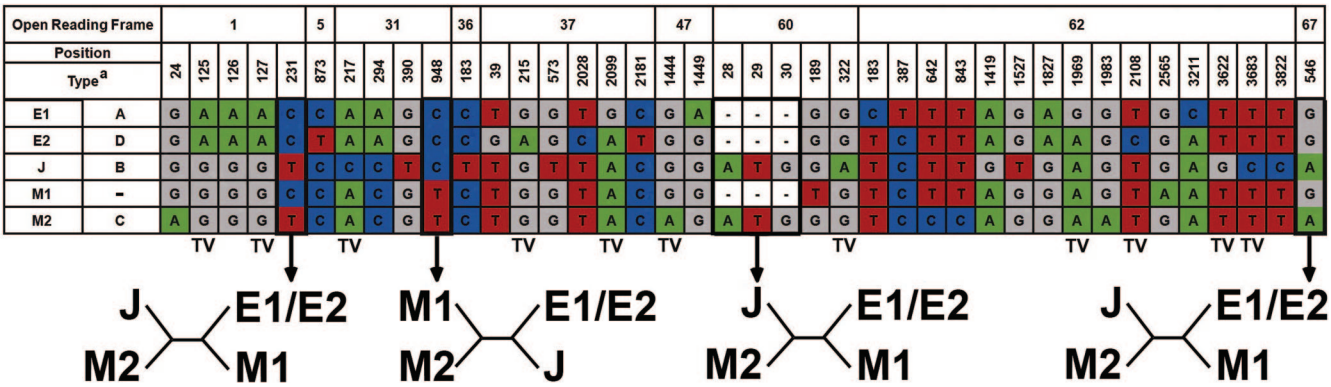

FIG. 2. Schematic representation of a recombination analysis of a 16,392-bp stretch of VZV, including only phylogenetically informative sites ($n = 40$) that are found in all strains of the respective genotypes. Genotype E1 includes nine strains: Dumas (NC001348), from The Netherlands; BC (AY548171), from British Columbia, Canada; 36 (DQ479958), from New Brunswick, Canada; 49 (DQ479959), from New Brunswick, Canada; MSP (AY548170), from Minnesota; 32p5 (DQ479961), from Texas; Kel (DQ479954), from Iowa; SD (DQ479953), from South Dakota; and NH293 (DQ674250), from the United States. Genotype E2 includes four strains: 11 (DQ47995), from New Brunswick, Canada; 22 (DQ479956), from New Brunswick, Canada; 03-500 (DQ479957), from Alberta, Canada; and HJO (AJ871403), from Germany. Genotype J includes one strain, pOka (AB097933), from Japan. Genotype M1 includes the prototype CA123 strain (DQ457052), from California, and the eight novel Tanzanian strains. Genotype M2 includes two strains: 8 (DQ479960), from New Brunswick, Canada; and DR (DQ452050), from the United States. The four putative sites that are informative for recombination events are indicated in with arrows and bold text, and the possible tree topologies of the four sites are shown below the table. TV, transversions. The genotype designations are based on those used in references 4 and 12. Dashes indicate nucleotide deletions.

1,990 bp of the genotype M1 strains from Tanzanian zoster patients demonstrated a close relationship to the recently described genotype M1 prototype strain CA123 (Fig. 1A and B). Analysis of the genotype M1 strains from Tanzanian patients revealed genotype M1-specific single-nucleotide polymorphisms in ORF 60 (G189T) and ORF 62 (G2565A) (Fig. 2) and within the ORF 51 genotyping area (G1947A) (data not shown). Moreover, the amplification and sequence analysis of more than 16 kb of the VZV genome revealed putative sites within ORFs 1, 31, 60, and 67, which show that viral recombination events may have occurred between genotype M1 or M2 and E1 or E2 (Fig. 2). In comparison to a previous study (6), two out of four sites (ORF 31 and ORF 67, nucleotides [nt] 948 and 546, respectively) were confirmed in this study, whereas the remaining sites (ORF1 and ORF60, nt 231 and 28 to 30, respectively) were found exclusively in this study (Fig. 2).

Interestingly, two of the most conserved herpesvirus genes (ORF 31 and ORF 67), coding for the envelope glycoproteins B and I (12), were obviously included in the potential recombination event. Furthermore, the in-frame translation initiation ATG insertion within the envelope glycoprotein L gene (ORF 60) linked the African genotype M1 strains to the European/American genotype E1/E2 strains (Fig. 2) and may again suggest recombination between these genotypes. Moreover a unique 9-nt deletion (3733 to 3742) resulting in an amino acid deletion of two glycines and one asparagine within the ORF 62 protein in TAZ5 was observed (data not shown). As ORF 62 codes for the VZV immediate-early regulatory gene *IE62*, polymorphisms have been considered to be a potential reason for attenuation and linked to transversions at nt 1882, 2872, and 3778 (11). Interestingly, the deletion observed in the ORF 62 of TAZ5 is located closely to position 3778.

In conclusion, genotype M1 VZV wild-type strains circulating in Tanzania seem to be recombinant strains and associated with African origins. There is an urgent need to analyze more VZV wild-type strains from different regions of Africa to explain why the recombinant-like genotype M1 is common in a region from which *Homo sapiens* dispersed out of Africa.

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