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Genotyping of clinical varicella-zoster virus isolates collected from Yunnan in Southwestern China

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Abstract. Varicella-zoster virus (VZV) belongs to the α-herpesvirus family. Genetically, it is stable and is divided into several genotypes based upon the genetic variations. The genotypes of VZV are rarely studied in the Southwestern region of China. In the present study, the common genetic variations in the VZV genes were examined in 42 VZV isolates collected from the patients with herpes zoster in the Yunnan province (Southwestern China). The restriction fragment length polymorphism analysis of open reading frames (ORFs) 38, 54 and 62 in the VZV genes showed that all the collected VZV isolates were *Pst*I, *Bgl*I and *Sma*I positive. The R5 variable-repeat region in these isolates was variable (R5A: 46.4%; R5B: 53.6%). The sequencing data of ORFs 1, 21, 22 and 54 indicated that 41 of the 42 collected VZV isolates could be grouped into genotype J or J1. Only one VZV isolate was identified as genotype A1 or M2. No new substitutions in the sequenced fragments were found in the collected VZV isolates. The results of the present study provided a preliminary genetic characterization of the VZV strains in the Yunnan province of Southwestern China.

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Key words: varicella-zoster virus, genotype, genomic variations, zoster, open reading frames

Introduction

Varicella-zoster virus (VZV) belongs to the α-herpesvirus family (1,2). VZV can infect humans and vertebrates (including chimpanzees and gorillas). The primary VZV infection in humans results in chickenpox. Following the recovery from chickenpox, the VZV becomes dormant in the sensory ganglia-like dorsal root ganglia (3,4). In certain conditions, VZV can reactivate and cause herpes zoster. There is a difference in onset time of primary VZV infection in different geographic regions (4). Generally, the infection time in people who have lived in temperate regions is earlier than those who have lived in tropical regions. In temperate regions, the first infection of VZV is mostly found in children, and in the tropical regions, the first infection is delayed until adulthood.

The genome of VZV is a linear duplex DNA molecule (5). The genome of VZV contained ~25,000 base pairs, including ≥70 open reading frames (ORFs). Genetic variations have been identified in the genome of VZV strains (6-13). Based on the specific single-nucleotide polymorphisms (SNPs) in ORFs 1, 21, 50 and 54, VZV strains have been divided into the following genotypes: A (Africa/Asia), B and C (Europe and North America), and J (Japanese) (6). By analyzing a short region of nucleotides [447 base pairs (bp)] in ORF 22, VZV strains could be divided into genotype E (Europe), genotype isolates J (Japanese) and genotype M (Mosaic) (13). The genotype M strains can be further divided into genotypes of M1, M2, M3 and M4 (13-15). The R5 variable regions (R5A and R5B) between ORF60 and ORF61 are also used to distinguish the genotypes of VZV strains (10,11). By phylogenetic analysis of the complete VZV genomes, the VZV strains could be broadly grouped into 5 clades (clades 1 and 3: European/North American strains; clade 2: Asian strains, particularly for the strains from Japan; clade 4: Certain strains from Europe; and clade 5: Indian strains) (16).

Until now, the VZV strains in China were not extensively studied. In the study by Loparev *et al* (13), 6 VZV samples in China were analyzed. The 3 samples in South China were all type M2. The other 3 samples in North China were all type E. Liu *et al* (17) collected 19 VZV samples from the patients with zoster or varicella in Hefei city of Anhui province, which is located in the middle Eastern part of China (18). The collected

Table I. Primers used for amplifying the fragments in the VZV genes.

Gene	Primer sequences (5'→3')	Size, bp	Note	(Refs.)
ORF1	F: TCAGCTGGCTTTTCTAAGAATTCG	506	PCR; sequencing	(9)
	R: TATTTTTGGGATCCGCAATGTC		PCR; Sequencing	
ORF21	F: TGGCGCGGTTTAAATGAATTGA	503	PCR; sequencing	(9)
	R: CACGTGTAGCTCCAAAAACCTAGG		PCR; sequencing	
ORF22	F: GGGTTTTGTATGAGCGTTGG	447	PCR; sequencing	(9)
	R: CCCCCGAGGTTCGTAATATC		PCR; sequencing	
ORF38	F: AAGTTTCAGCCAACGTGCCAATAAA	647	PCR; RFLP analysis (*Pst*I)	(9)
	R: AGACGCGCTTAACGGAAGTAACG		PCR; RFLP analysis (*Pst*I)	
ORF54	F: CGTAATGCATAACAGGCCAACAC	497	PCR; RFLP analysis (*Bgl*I); sequencing	(9)
	R: AAACCTGGCGTCAAACATTACA		PCR; RFLP analysis (*Bgl*I); sequencing	
R5	F: GGCAAATACTTAGACCGTTTT	359 (R5A)	PCR	(10)
	R: TAATGGACTTTTAATGGATTG	471 (R5B)	PCR	
ORF62	F: TTCCCACCGCGGCACAAACA	268	PCR; RFLP analysis (*Sma*I)	(12)
	R: GGTTGCTGGTGTTGGACGCG		PCR; RFLP analysis (*Sma*I)	

RFLP, restriction fragment length polymorphism; R5A, R5 variable region type A; R5B, R5 variable region type B; ORF, open reading frame; VZV, varicella-zoster virus; PCR, polymerase chain reaction; F, forward; R, reverse.

VZV isolates were grouped into genotype J or J1. The genotype of R5A and R5B were found in these isolates by analyzing the R5 variable region. In the present study, 42 VZV isolates were collected from Yunnan province of Southwestern China. Using the described genotyping method of Liu *et al* (17), a genetic characterization of the VZV strains was made in Southwestern China.

Materials and methods

Patients and clinical samples. The VZV isolates were collected from the 42 ambulatory and hospitalized patients with herpes zoster in The First People's Hospital of Yunnan Province (The Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan, China) between August 2013 and December 2014. Vesicle fluid was collected from skin lesions, and the collected samples were stored at -20˚C. The patients were all Han Chinese in the Yunnan province of Southwestern China. The study was approved by the Institutional Ethics Committee of the First People's Hospital of Yunnan Province. The written informed consent was signed and obtained from all the patients who participated. The study was performed according to the principles of the Declaration of Helsinki. Genomic DNA of VZV was extracted from vesicle fluid from skin lesions using a viral DNA extraction kit (Da An Gene Co., Ltd., of Sun Yat-Sen University, Guangzhou, China). The collected isolates were first detected by nested polymerase chain reaction (PCR) and quantitative PCR, as described by Weidmann *et al* (19). The results of the nested PCR and quantitative PCR showed that all the collected isolates exhibited the VZV gene.

PCR and sequencing. PCR was performed in a 25-μl reaction mixture containing 10X LA PCR Buffer II (Mg^{2+} Plus), 2.5 units of TaKaRa LA Taq (Takara Bio, Inc., Dalian, China), 50 μM of each deoxyribonucleotide, 0.2 μM of each primer and 50 ng DNA. The primers for amplifying and sequencing the fragments of the VZV genes referred to the previous studies by Liu *et al* (17), Barrett-Muir *et al* (6), Hawrami and Breuer (10) and Loparev *et al* (13), and are shown in Table I. The conditions for PCR amplification was as follows: A denaturation cycle at 94˚C for 5 min, followed by 35 cycles at 94˚C for 30 sec, annealing at 55˚C for 30 sec and extension at 72˚C for 40 sec, and ending with a final extension at 72˚C for 7 min.

The PCR products of ORFs 1, 21, 22 and 54 in the VZV genes were purified using Genomic DNA Purification kits (Tiangen Biotech, Beijing, China) and were sequenced using the forward and reverse primers and the Big Dye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) on an ABI PRISM 3730 DNA sequencer (Applied Biosystems).

Restriction enzyme reactions. Restriction endonuclease digestion was performed in a 20 μl mixture containing 7 μl PCR products, 1 μl endonuclease (*Bgl*I, *Pst*I or *Sma*I; Takara Bio Inc.), 2 μl of the accompanying 10X endonuclease buffer and 13 μl sterile water. The reaction mixture for *Bgl*I and *Pst*I was incubated at 37˚C for 3 h. The reaction mixture for *Sma*I was incubated at 30˚C for 3 h. The digested products of *Bgl*I, *Pst*I and *Sma*I were resolved by 6% polyacrylamide gels and were silver stained prior to visualization. The genotyping data were further validated by sequencing 2 randomly selected VZV samples.

Results

Restriction fragment length polymorphism (RFLP) analysis of ORF 38, 54 and 62. The specific SNPs in the VZV strains that

Table II. Genotype of the VZV strains in the present study and other studies.

VZV strain	ORF38 (*Pst*I)	ORF54 (*Bgl*I)	ORF62 (*Sma*I)	R5 type (%)	SNP in ORF22	SNP in ORFs 1, 21 and 54
MLS (17)	+	+	-	R5A	M1	A1
v-Oka (17)	-	+	+	R5B	J	J2
p-Oka (17)	-	+	-	R5B	J	J1
VZV isolates from Anhui city of China (18)	+	+	-	R5A (47.4) R5B (52.6)	J	J1
VZV isolates from Yunnan province (present study)	+	+	+	R5A (46.4) R5B (53.6)	J (41/42) M2 (1/42)	J1 (41/42) A1 (1/42)

Varicella-zoster virus (VZV) genotyping scheme using single-nucleotide polymorphism (SNP) in open reading frame (ORF) 22 referred to the description by Loparev *et al* (13). The genotyping scheme by using SNP in ORFs 1, 21 and 54 referred to the description by Barrett-Muir *et al* (6) and Quinlivan *et al* (23). R5A, R5 variable region type A; R5B, R5 variable region type B.

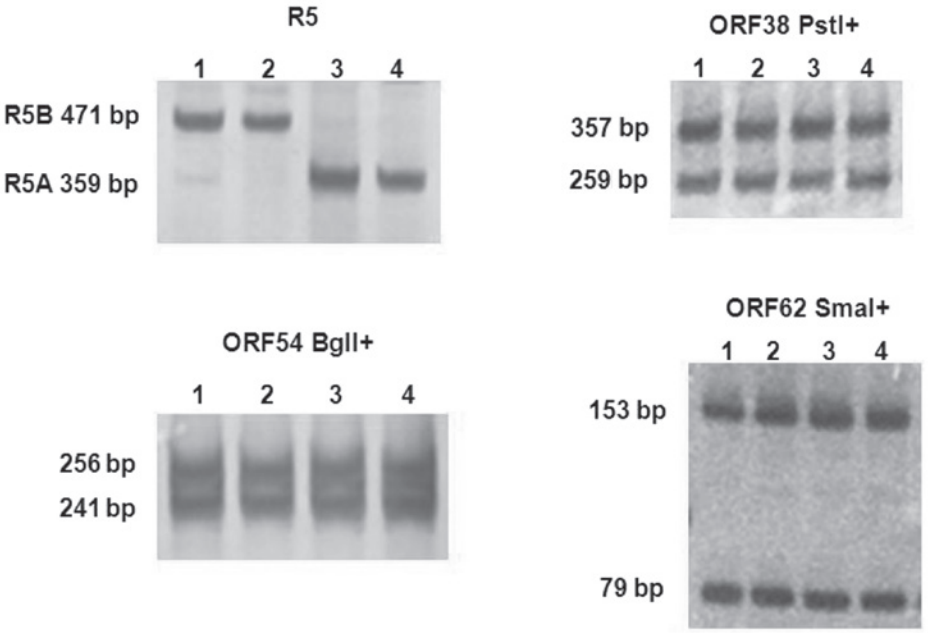

Figure 1. Representative results of the R5 variable region and restriction fragment length polymorphism analysis of open reading frame (ORF) 38, 54 and 62.

are located in ORFs 38, 54 and 62 have been used as genetic markers in the study of VZV epidemiology. The PCR products of ORFs 38, 54 and 62 in the VZV genes were amplified and they were 647, 497 and 268 bp, respectively. RFLP analysis was used to determine the genotype of ORF38 (*Pst*I), ORF54 (*Bgl*I) and ORF62 (*Sma*I) in the collected VZV isolates, as shown in Fig. 1. The PCR products of ORF38 were digested by *Pst*I, and produced 2 fragments of 357 and 290 bp. The digestion of PCR products of ORF54 by *Bgl*I yielded 2 fragments of 256 and 241 bp. Similarly, the PCR products of ORF62 were digested by *Sma*I, and produced 3 fragments of 153, 79 and 36 bp. These data indicated that the VZV isolates collected in the study contained the cleaving site of *Pst*I, *Bgl*I and *Sma*I, and that the collected isolates were all *Bgl*I positive (*Bgl*I$^+$), *Pst*I$^+$ and *Sma*I$^+$ (Table II).

Analysis of the R5 variable region. The R5 variable region has been shown to be vary among different VZV strains. The R5 variable region was amplified by PCR and analyzed by electrophoresis to determine its distribution in the collected VZV isolates. The representative results are shown in Fig. 1. The PCR products of R5A were 359 bp, while the PCR products of R5B were 471 bp. In the collected VZV isolates, R5A and R5B were observed. The percentages of R5A and R5B were 46.4 and 53.6%, respectively, in the collected isolates.

Nucleotide sequence analysis of ORFs 1, 21, 22 and 54. The sequence of ORFs 1, 21, 22 and 54 in VZV genes have been sequenced and analyzed in certain areas of the world, such as the United Kingdom, Brazil and Anhui city of China. Therefore, a nucleotide sequence analysis was performed on ORFs 1, 21, 22 and 54 in the collected VZV isolates. The informative polymorphic markers on the regions are shown in Fig. 2. Based on the genotyping scheme of Barret-Muir *et al* (6), the VZV isolates in the study could be grouped into genotype J1 (41/42) or A1 (1/42), as shown in

A

Gene	ORF1														ORF21						ORF54								
Nucleotide Position	560	561	685	703	750	763	766	780	781	789	790	791	829	892	33608	33646	33647	33722	33725	33728	95108	95118	95150	95241	95262	95300	95333	95339	Genotype
Dumas	T	G	G	T	G	T	A	G	T	T	T	T	T	C	C	G	A	T	T	T	C	G	T	T	G	C	T	C	C
MLS [17]	T	G	G	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	T	T	C	A1
vOka [17]	C	G	A	C	G	C	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J2
pOka [17]	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
1	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
2	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
3	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
4	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
5	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
6	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
7	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
8	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
9	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
10	T	G	G	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	A1
11	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
12	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
13	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
14	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
15	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
16	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
17	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
18	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
19	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
20	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
21	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
22	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
23	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
24	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
25	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
26	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
27	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
28	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
29	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
30	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
31	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
32	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
33	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
34	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
35	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
36	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
37	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
38	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
39	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
40	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
41	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1
42	T	G	A	T	G	T	A	G	T	C	C	C	T	C	C	G	A	C	C	C	C	G	T	C	G	C	T	C	J1

Figure 2. (A) Genomic variations on open reading frames (ORFs) 1, 21 and 54 of 42 varicella-zoster virus (VZV) isolates collected from Yunnan province of Southwestern China. The sequence of the Dumas, MLS, vOka and pOka VZV strains referred to the published data. The sequence positions referred to the sequence of Dumas VZV strain (genebank accession no. NC_001348). Grey cells represents genotype C markers. Light grey cells represents genotype J markers. Dark grey cells represent various genetic variations (or mutations).

Fig. 2A. The VZV isolates could be grouped into genotype J (41/42) or M2 (1/42) according to the genotyping scheme of Loparev *et al* (13), as shown in Fig. 2B.

Discussion

VZV is highly infectious for those with no VZV infection history, and becomes dormant in the sensory ganglia following the first infection. The genome of VZV is highly conserved compared to other pathological viruses, such as human papilloma virus. This may be due to the low reproduction of VZV in the infected hosts. This feature limits the frequency of the introduction of new mutations in VZV genes. As the advancement of VZV molecular epidemiology occurs, numerous genomic variations have been identified in VZV strains. Based on these genomic variations, the VZV strains have been classified into different genotypes. The VZV genotypes in China are rarely studied. The present study made a preliminary study on the genotypes of VZV isolates collected from Yunnan province of Southwestern China. To the best of

B

Gene	ORF22							
Nucleotide Position	37902	38036	38055	38059	38081	38177	38229	Genotype
Dumas	A	T	T	C	A	G	A	E
MLS [17]	A	T	T	C	C	G	A	M1
vOka [17]	G	C	C	C	C	A	A	J
pOka [17]	G	T	C	C	C	A	A	J
1	G	T	C	C	C	A	A	J
2	G	T	C	C	C	A	A	J
3	G	T	C	C	C	A	A	J
4	G	T	C	C	C	A	A	J
5	G	T	C	C	C	A	A	J
6	G	T	C	C	C	A	A	J
7	G	T	C	C	C	A	A	J
8	G	T	C	C	C	A	A	J
9	G	T	C	C	C	A	A	J
10	A	T	C	C	C	A	A	M2
11	G	T	C	C	C	A	A	J
12	G	T	C	C	C	A	A	J
13	G	T	C	C	C	A	A	J
14	G	T	C	C	C	A	A	J
15	G	T	C	C	C	A	A	J
16	G	T	C	C	C	A	A	J
17	G	T	C	C	C	A	A	J
18	G	T	C	C	C	A	A	J
19	G	T	C	C	C	A	A	J
20	G	T	C	C	C	A	A	J
21	G	T	C	C	C	A	A	J
22	G	T	C	C	C	A	A	J
23	G	T	C	C	C	A	A	J
24	G	T	C	C	C	A	A	J
25	G	T	C	C	C	A	A	J
26	G	T	C	C	C	A	A	J
27	G	T	C	C	C	A	A	J
28	G	T	C	C	C	A	A	J
29	G	T	C	C	C	A	A	J
30	G	T	C	C	C	A	A	J
31	G	T	C	C	C	A	A	J
32	G	T	C	C	C	A	A	J
33	G	T	C	C	C	A	A	J
34	G	T	C	C	C	A	A	J
35	G	T	C	C	C	A	A	J
36	G	T	C	C	C	A	A	J
37	G	T	C	C	C	A	A	J
38	G	T	C	C	C	A	A	J
39	G	T	C	C	C	A	A	J
40	G	T	C	C	C	A	A	J
41	G	T	C	C	C	A	A	J
42	G	T	C	C	C	A	A	J

Figure 2. Continued. (B) Genomic variations of open reading frame (ORF) 22 of 42 varicella-zoster virus (VZV) isolates collected from Yunnan province of Southwestern China. The sequence of the Dumas, MLS, vOka and pOka VZV strains referred to the published data. The sequence positions referred to the sequence of Dumas VZV strain (genebank accession no. NC_001348). Grey cells represents genotype C markers. Light grey cells represents genotype J markers. Dark grey cells represents various genetic variations (or mutations).

our knowledge, this is the first investigation on VZV genotype in this region of China.

The genotype of VZV strains is associated with climate. In temperate regions, the VZV strains are often *Pst*I$^+$ and *Bgl*I negative (*Bgl*I$^-$) in ORF38 and ORF54, while the majority of VZV strains in tropical regions are *Pst*I$^+$ and *Bgl*I$^+$ (13,20-22). In the present study, the VZV isolates collected from the Yunnan province of Southwestern China were all *Pst*I$^+$ and *Bgl*I$^+$ in ORF38 and ORF54 (Fig. 1A and Table II). The present results are consistent with the findings that the VZV strains in the tropical regions are often *Pst*I$^+$ and *Bgl*I$^-$ in ORF38 and ORF54, as the Yunnan province of China is located in the tropical regions.

ORF38 (*Pst*I), ORF54 (*Bgl*I) and ORF62 (*Sma*I) in VZV genes are molecular genetic markers for the genotyping of VZV strains (18,20,21). The VZV isolates in the present study were all *Pst*I$^+$, *Bgl*I$^+$ and *Sma*I$^+$ (Fig. 1 and Table II). The VZV isolates collected from Anhui city of China and the strain MLS (6) were *Pst*I$^+$, *Bgl*I$^+$ and *Sma*I$^-$ (wild-type VZV). The strain p-Oka was *Pst*I$^-$, *Bgl*I$^+$ and *Sma*I$^-$ (17). The strain v-Oka was *Pst*I$^-$, *Bgl*I$^+$ and *Sma*I$^+$ (17). The results indicated that the VZV isolates collected from the Yunnan province of Southwestern China were different from that of Anhui city of middle eastern China. The results supported the conclusion that the genotype of VZV strains may vary in different regions of China. Further studies are required to find out the VZV distributions in China.

The R5 variable region in the VZV genes has been shown to be geographically related (10,23). The type of R5A (359 bp) is mainly found in Europe and North America. The type of R5B (471 bp) is a major type in Japan. In the present study, R5A and R5B were observed in the collected VZV isolates (Fig. 1). The genotype frequency of R5A and R5B are nearly identical in these samples. The results are consistent with the findings by Liu *et al* (17). It is possible that either R5A or R5B is equally distributed in Chinese VZV strains.

Until now, there was no gold standard VZV genotyping scheme, although several genotyping schemes have been proposed in recent years. The genetic variations were identified in VZV ORFs 1, 21, 22, 50 and 54 (17,19,24,25). By referring to the SNPs in VZV ORFs 1, 21, 50 and 54, the VZV strains could be grouped into 4 genotypes: A, B, C and J. By referring to the VZV ORF22, 7 VZV genotypes are identified, which were E1, E2, J, M1, M2, M3 and M4. The VZV strains of genotype J are most common in Asia, particular in Japan. In the present study, the above genotyping scheme was used to analyze the VZV samples collected from the Yunnan province of Southwestern China (Fig. 2). The collected VZV isolates are mainly genotype J or J1, which is a major genotype in Asia. The results are consistent with the finding by Liu *et al* (17), who collected the VZV isolates from Anhui city of middle eastern China, which were genotyped as J or J1. In addition to the previously reported genetic variations in the VZV genes, no new genetic variations were identified in the collected VZV isolates by sequencing the ORFs 1, 21, 22 and 54 in VZV genes. The results indicated that the VZV strains in Yunnan province may be highly conserved, as several reports have shown new genetic variations in these sequenced fragments. Of note, one sample in the collected isolates was classified as genotype A1 or M2. This may be due to the population migrations.

In conclusion, a preliminary study was performed on VZV genotypes in the Yunnan province of Southwestern China. The results of the present study will aid in the understanding of the genetics of VZV in China. The limitation of the study is that the sample size is small, although it is larger than the previous

studies in China. Further studies with a larger sample size are required to understand the VZV genotype distributions in this region and China.

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