Original Article

Characterization and Comparison of Genetic Variation in Clinical Varicella-Zoster Virus Isolates Collected from Shanghai and Urumqi, China

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SUMMARY: Varicella-zoster virus (VZV) is a ubiquitous human herpesvirus that causes chickenpox and zoster. Considering that VZV is a relatively and genetically stable virus, its global surveillance clades provide essential information for VZV evolution, immigration, and importation of different viral strains and recombination events. Eighty-eight VZV isolates from China (Shanghai and Urumqi) were genotyped using a scattered single-nucleotide polymorphism method in this prospective study. Our results were based on sequencing the open reading frames 1, 6, 12, 16, 17, 21, 22, 35, 37, 38, 50, 54, 55, 56, 60, and 66. We found that the majority of these 88 strains (81.8%) belonged to Clade 2 with significantly high homogeneity from Shanghai. However, in the Urumqi area, some strains were grouped to Clade 5, and some could not be attributed to any of the established VZV clades, although the majority of Urumqi strains belonged to Clade 2. Our results illustrated that due to geographical location, VZV could undergo genetic recombination, suggesting that VZV diversity is more complicated in certain areas and geographical separation contributes to VZV complexity.

INTRODUCTION

Varicella-zoster virus (VZV) is a member of the *Herpesviridae* family that causes varicella (chickenpox) and zoster (shingles). The severity of the disease depends on the individuals' age upon acquiring infection, and the significantly young and old vulnerable individuals are associated with an increased risk of experiencing the disease (1,2).

The VZV genome is considered as the most conserved genome and contains approximately 70 open reading frames (ORFs). The genomic variation within the genome is approximately 0.1% (3,4). Based on single-nucleotide polymorphism (SNP) analysis, several geographical distinct genotypes have been reported previously (5). Comparison of the deoxyribonucleic acid (DNA) sequences of the Oka vaccine strain (vOka) and the parental Oka strain (pOka) showed that there were only 42 nucleotide differences along the whole genome, and 15 were located in the transactivator ORF 62 (6,7).

The varicella vaccine has been used in China for more than 30 years, and the vaccine strains from VZV-infected individuals are distributed epidemiologically

(8). Considering the advancements in molecular genotyping methods, the molecular epidemiology of VZV in different geographical regions can be monitored. However, VZV isolates or samples from China have rare genotypes; specifically, the detailed geographical differences across the country have not been investigated. In the current study, we aimed to compare and analyze the genetic variations and sequences in Shanghai (eastern China) and Urumqi (western China, close to mid-Asia). Our results demonstrated that the 41 Shanghai strains were significantly highly homogenous, and the 41 Urumqi strains were highly variable from the wild-type strains.

MATERIALS AND METHODS

Patient and clinical enrollment: The study cohort included 88 patients clinically diagnosed with varicella and zoster infection. A total of 41 samples were collected in Tongren Hospital (Shanghai, China) and 47 from Karamay People's Hospital (Karamay, China). Samples were collected as swabs of vesicle fluid in viral transport medium from skin lesions and transported to the laboratory within 4 h on wet ice (9). Virus was recovered by the inoculation of fluid from a single vesicle on a human embryonic lung fibroblast (HELFL) monolayer and was confirmed by immunofluorescence with an anti-VZV gE monoclonal antibody (Biodesign International, ME, USA) (10). The study was approved by the Research Ethics Committee of The Tongren Hospital, Shanghai Jiao Tong University School of Medicine, and all patients provided written informed consent for inclusion in the study.

VZV DNA extraction and polymerase chain

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Geographical Separation and VZV Complexity

Table 1. Primes sequences used in this study

Gene	Sequence
ORF1-forward	5'-TCAGCTGGCTTTTCTAAGAATTCG-3'
ORF1-reverse	5'-CCAATCCCAAATCCCTATGC-3'
ORF6A-forward	5'-CCTCCAGTGTAATGCGTCCAG-3'
ORF6A-reverse	5'-CCAATCCCAAATCCCTATGC-3'
ORF6B-forward	5'-ATATCTCGTAGTGAAGCGGCAACAA-3'
ORF6B-reverse	5'-CCACTATTCCCTCAAATCCACCC-3'
ORF12-forward	5'-AATAGACAGCGGACTTCTC-3'
ORF12-reverse	5'-AAATACAAACGACCACTTGA-3'
ORF17-forward	5'-CCGATATTTGTATTGGAACG-3'
ORF17-reverse	5'-GGATGGCATTGTAGAGGTAA-3'
ORF21-forward	5'-TGGCGCGGTTTAAATGAATTGA-3'
ORF21-reverse	5'-CACGTGTAGCTCCAAAACCTAGG-3'
ORF22A-forward	5'-CATGGTACGGTTAGGCTTG-3'
ORF22A-reverse	5'-TGCCGATAGTGTAGTTTCTTCC-3'
ORF35-forward	5'-ACAGCCCTTTGAACATCCAC-3'
ORF35-reverse	5'-CAGGCGAAACGTCTCCATTA-3'
ORF37-forward	5'-TGTCGGCAATACCAACACG-3'
ORF37-reverse	5'-CCCCCGTGGTTAGATACCTAA-3'
ORF38-forward	5'-AAGTTTCAGCCAACGTGCCAATAAA-3'
ORF38-reverse	5'-AGACGCGCTTAACGGAAGTAACG-3'
ORF50-forward	5'-CGCACCCAAAGTGAACATCAT-3'
ORF50-reverse	5'-TCTCGGATGTCAAATATGTTACGA-3'
ORF54-forward	5'-CGTAATGCATAACAGGCCAACAC-3'
ORF54-reverse	5'-AAACCTGGCGTCAAACATTACA-3'
ORF55-forward	5'-GGGGCTGTTATCTTTGGCATT-3'
ORF55-reverse	5'-GGGGCTGTTATCTTTGGCATT-3'
ORF56-forward	5'-TAAACCCTCTACGGGAACGA-3'
ORF56-reverse	5'-CAGAACCGACCCAGGACATA-3'
ORF60-forward	5'-CCGATAGTTTCATTTCATTGG-3'
ORF60-reverse	5'-GTCGTAGTGAAGGGAAAACACA-3'
ORF66-forward	5'-CGGCATAGAATCTGAGGATG-3'
ORF66-reverse	5'-CTGTTTCGGTAACGTGGTAAT-3'

reaction (PCR): DNA was extracted using the PureLink Genomic DNA Mini kit (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. All patients were referred to the dermatology department and were enrolled in the local hospitals. HELFs infected with the vOka, pOka, and an American wild-type strain (macrolide-lincosamide-streptogramin) served as the control. The VZV gene ORFs 1, 6, 12, 16, 17, 21, 22, 35, 37, 38, 50, 54, 55, 56, 60, and 66 were amplified by PCR (11). Primer sequences are listed in Table 1.

DNA sequencing and phylogenetic analysis: The electrophoresis products were purified using an agarose purification kit (Takara Biotechnology, Shiga, Japan) and were sequenced using a sequencing kit (Applied

Biosystems, Foster city, CA, USA). An ABI Prism 7700 DNA Analyzer (Perkin Elmer Applied Biosystems, Foster city, CA, USA) was used to perform all the sequencing (12). The sequencing was compared with the published strains from PubMed using MEGA 4.02. SNP profiles were established to construct the phylogenetic tree. Evolutionary analysis was conducted using MEGA7 (13).

RESULTS

VZV Oka stain SNP analysis: ORF38 (*Pst* I, SNP69349), ORF54 (*Bgl* I, SNP95241), and ORF62 (*Sma* I, SNP106262) were the SNPs that were initially

Table 2. Distribution of VZV Clades in Shanghai and Urumqi

Location	Number	<i>Pst</i> I-	<i>Bgl</i> I+	<i>Sma</i> I+	Clade 2	Clade 5	Unclassified
Shanghai	41	0	41	0	41	0	0
Urumqi	47	0	47	0	31	11	3
Total	88	0	88	0	72	11	3

used to distinguish between strains. VZV strains including *Pst* I- *Bgl* I+ *Sma* I- and *Pst* I- *Bgl* I+ *Sma* I+ were previously reported in China. Here, we amplified ORF38, ORF54, and ORF62 and found that all the strains in the study were *Pst* I+ *Bgl* I+ *Sma* I- (Table 2).

VZV clade distribution in Shanghai and Urumqi:

We used the VZV clade analysis (Fig. 1) to compare the sequence of ORFs 1, 6, 12, 16, 17, 21, 22, 35, 37, 38, 50, 54, 55, 56, 60, and 66 in all 88 samples (Table 2) (14). The majority of all samples (81.8%, 72/88) belonged to Clade 2. All Shanghai samples belonged to Clade 2, and 70.2% (33/47) of Urumqi samples belonged to Clade 2 (Fig. 2). We found a consecutive nucleotide (CGG) insertion in ORF1, which was previously reported in Chinese strains, confirming the existence of the consecutive nucleotide insertion in Clade 2 ORF60. Clade 5 comprised 23.4% (11/47) of all the samples. Interestingly, a total of 3 Urumqi samples (6.4%) could not be categorized in the existing clades (Fig. 2). Considering the geographical location; hence, the prevalence of European (Clade 1), mid-Asian (Clade 5), and eastern Asian (Clade 2) clades caused high-frequency gene recombination.

A phylogenetic tree demonstrating VZV subclades,

		CLADE							
ORF	SNP	1	2	3	4	5	VI	VII	
1	508	C	C	C	C	C	T	ND	
1	685	G	A	G	G	A	G	ND	
1	790	T	C	T	C	C	T	ND	
6	5827	C	C	A	C	C	C	ND	
6	6850	G	G	G	A	A	G	ND	
12	17834	C	T	C	C	T	T	ND	
12	18082	C	T	C	C	C	C	ND	
16	23294	A	G	G	G	A	G	ND	
17	24533	A	A	A	C	A	A	ND	
17	24578	A	G	G	G	A	G	ND	
21	33725	T	C	C	C	C	C	ND	
22	37902	A	G	A	A	A	A	G	
22	38019	G	G	G	G	A	G	G	
22	38055	T	C	T	T	C	T	T	
22	38081	A	C	A	C	C	C	C	
22	38177	G	A	G	G	A	A	G	
22	39394	G	A	A	A	G	A	ND	
35	64703	G	G	G	A	A	G	ND	
37	68101	T	T	C	T	T	T	ND	
37	68172	G	A	A	A	A	A	ND	
37	68254	C	C	T	C	C	T	ND	
50	87841	C	T	T	T	T	T	ND	
54	95241	T	C	T	C	C	T	ND	
55	98437	T	C	T	T	C	C	ND	
56	98825	T	T	T	C	C	C	ND	
60	101464	C	C	C	A	C	C	ND	
66	113243	A	A	C	A	C	C	ND	

Fig. 1. VZV clades analysis that was utilized in the study.

variants, and unclassified strains is illustrated in Fig. 3. The phylogenetic analysis illustrated that all the 41 Shanghai strains were highly homologous. Isolated

	ORF1										ORF6	ORF12	16	ORF17	21	ORF22					35	ORF37	50	54	55	56	ORF60					66	Clade						
Strain	508	685	780	781	790	5827	6850	17834	18054	18082	21294	24533	24578	33725	37902	38019	38055	38081	38177	39394	64703	68101	68172	68254	87841	95241	98437	98825	101464	101623	101624	113243							
Dumas	C	G	C	.	.	C	T	C	G	C	T	C	A	A	T	A	G	T	A	G	A	G	T	G	C	C	T	T	T	C	.	.	T	A	1				
p-Oka	C	A	C	.	.	C	C	C	G	T	T	T	G	A	G	C	G	C	C	A	G	G	T	A	C	T	C	C	T	C	C	A	T	C	A	2			
v-Oka	C	A	C	.	.	C	C	C	G	T	T	T	G	A	G	C	G	C	C	A	G	G	T	A	C	T	C	C	T	C	C	A	T	C	A	2			
VZV11	C	G	C	.	.	C	T	A	G	C	C	C	G	A	G	C	A	G	T	A	G	G	C	A	T	T	T	T	T	C	.	.	T	C	3				
DR	C	A	C	.	.	C	C	C	A	T	T	C	A	A	A	C	A	G	C	C	A	A	T	A	C	T	C	C	C	C	C	A	T	C	T	A	4		
CA123	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	C	A	C	.	.	T	C	5		
MLS	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	C	A	C	.	.	T	C	5		
S-all	C	A	C	.	.	C	C	C	G	T	T	C	G	A	G	C	G	C	C	A	G	G	T	A	C	T	C	C	T	C	C	A	T	C	T	A	2		
U-others	C	A	C	.	.	C	C	C	G	T	T	C	G	A	G	C	G	C	C	A	G	G	T	A	C	T	C	C	T	C	C	A	T	C	T	A	2		
U-Z-3	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	A	C	.	.	T	C	5		
U-Z-11	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-18	C	G	C	.	.	C	T	C	G	C	T	C	G	A	G	C	A	G	T	A	G	A	G	T	A	C	T	T	T	T	T	C	.	.	T	A	?		
U-Z-25	C	G	C	.	.	C	T	C	G	C	T	C	G	A	G	C	A	G	T	A	G	A	G	T	A	C	T	T	T	T	T	C	.	.	T	A	?		
U-Z-38	C	G	C	.	.	C	T	C	G	C	T	C	G	A	G	C	A	G	T	A	G	A	G	T	A	C	T	T	T	T	T	C	.	.	T	A	?		
U-Z-12	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-22	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-24	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-28	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-31	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-V-32	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-35	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-Z-39	C	G	C	.	.	C	C	C	A	C	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5
U-V-42	C	G	C	.	.	C	C	C	A	C	C	C	G	C	G	C	A	G	T	C	G	G	A	T	A	C	T	C	T	T	T	A	C	.	.	T	C	5	
U-V-13	C	A	C	C	G	G	C	C	C	G	T	T	C	G	A	G	C	G	C	C	A	G	G	T	A	C	T	C	C	T	C	C	A	T	C	T	A	2	
U=Urumqi; S=Shanghai; Z=zoster; V=varicella																																							
										Clade 1		Clade 2		Clade 3		Clade 4		Clade 5																					

Fig. 2. (Color online) Analysis of the strain-associated SNPs of the Shanghai and Urumqi samples. S-all represented SNPs in Shanghai samples. Variations existed in Urumqi samples but not identified in Shanghai samples. U-others presented SNPs in Clades 2 that were not included.

Fig. 3. Phylogenetic analysis using Maximum Likelihood method (MLM). The evolutionary history was inferred by using MLM based on the Tamura-Nei model. The tree with the highest log likelihood (-14470.65) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood approach, using the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. The final dataset included a total of 8917 positions. MLS, macrolide-lincosamide-streptogramin.

variations were observed in Urumqi strains.

DISCUSSION

VZV is a highly infectious disease, causing significant outbreaks of varicella in various populations. The present study described the largest study on VZV genotyping in China, and the major finding of the study was that the geographical region contributes significantly to VZV genomic variations.

Novel CGG insertions were present in the VZV isolates. Additionally, new nucleotide substitutions were observed in Chinese VZV isolates. According to the worldwide distribution of VZV clades, Clades 1 and 3 dominate in European and American countries, whereas Clades 4 and 5 are mainly observed in the immigrants of African origins. Consistent with the result of a previous study, we found that Clade 2 is the dominant clade in Chinese populations (15). Clade 5 in Urumqi strains indicated the genetic recombination events during VZV evolution from the history of immigration (Fig. 2) (10).

Phylogenetic analysis showed that all the 41 Shanghai strains included in this study were highly homologous (Fig. 3) (16). This was not surprising because the strains were isolated in Shanghai, where 99.9% of the residents are native Chinese. These results were consistent with the results of the previous observations (17). However, it was slightly different from the predominant genotype in suburban Shanghai in the previous report (18).

Genetic variations are observed in the strains from Urumqi, where the populations are mixed with

immigrants, including Caucasian and Mongolian. These results confirmed that VZV strain distribution is associated with immigration patterns (16,19). On the contrary, phylogenetic analysis using the full nucleotide sequences identified five distinct clades, consistent with the result of the previous findings in other countries (20). Concatenated amino acid sequences and coding nucleotide sequences can be utilized in future phylogenetic analysis. The undetermined clades in Urumqi suggest that new clade or subclade could be extended to western China, which is geographically close to Europe and mid-Asia.

Phylogenetic analyses using the sequences of the individual ORFs suggested that the 12 ORFs might be important in distinguishing vaccine strains from clinical strains. ORF0, also known as ORFS/L, is considered to be essential for VZV growth and encodes a membrane protein with 129 amino acid residues, which is essential for vesicular trafficking and altering cell adhesion molecules in infected cells (21). Genetic variations observed in the current study will serve as the molecular markers in future epidemiological studies. Four or five clusters are differentiated using the SNPs and insertions, which are within the residing local geographical areas.

In conclusion, the genetic variations in VZV strains significantly highlight the ongoing status of VZV infection, and the genetic variations in VZV strains during continuing disease epidemics should be highly considered for future vaccine strategies and clinical management.

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Conflict of interest None to declare.

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