JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2008, p. 325–327 Vol. 46, No. 1
0095-1137/08/$08.00+0 doi:10.1128/JCM.01735-07

Novel Varicella-Zoster Virus Glycoprotein E Gene Mutations Associated with Genotypes A and D▽

Jonas Schmidt-Chanasit,[1,2]* Karoline Bleymehl,[1] Susanne G. Schäd,[3] Gerd Gross,[3] Rainer G. Ulrich,[4] and Hans Wilhelm Doerr[1]

Institute of Medical Virology, Hospital of the Johann Wolfgang Goethe University, D-60596 Frankfurt am Main,[1] Bernhard-Nocht-Institute for Tropical Medicine, Clinical Virology Laboratory, D-20359 Hamburg,[2] Department of Dermatology and Venereology, University of Rostock, D-18055 Rostock,[3] and Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, D-17493 Greifswald,[4] Germany

Received 31 August 2007/Returned for modification 7 November 2007/Accepted 9 November 2007

Here, we describe the association of certain varicella-zoster virus (VZV) genotypes with unique glycoprotein E (gE) gene mutations. Within 45 analyzed VZV wild-type strains of genotypes A and D, five novel gE mutations were discovered. A statistically significant ($P < 0.0001$) association of certain gE mutations with VZV genotype D was found.

Varicella-zoster virus (VZV), human herpesvirus 3, is a member of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae*. VZV is the etiologic agent of two distinct clinical syndromes in humans: chicken pox (varicella), occurring during the primary infection, and shingles (zoster), appearing after reactivation from latency. The VZV genome is a 125-kb linear double-stranded DNA molecule and is predicted to code for at least 71 proteins (1). The glycoprotein E (gE) contains 623 amino acid residues and is highly immunogenic; i.e., it elicits a strong immune response following both primary VZV infection and reactivation (10).

Previously, two VZV gE mutant strains were identified in the United States and Canada (4, 8). These mutants contain a nonsynonymous G→A mutation in codon 150 (G150A) of the gE gene, resulting in an aspartic acid-to-asparagine exchange at position 150 (D150N). This amino acid exchange causes the loss of a B-cell epitope for the murine monoclonal antibody 3B3 (amino acid residues 149 to 161) and a phenotype of faster cell-to-cell spread (4, 5). Partial gE sequence analysis of 15 VZV isolates from the United States revealed additional sequence variations, i.e., a synonymous mutation (C513T) and a nonsynonymous mutation (T955C, corresponding to amino acid exchange W319R) that are located outside the coding regions of the epitopes e1 and c1 (7). Recently, two novel VZV gE mutant strains harboring nonsynonymous mutations were discovered in Italy in a patient with fatal hepatitis (D161G) and in Sweden (R152S) (3, 12). Both mutations affect the gE ectodomain and the B-cell epitope targeted by monoclonal antibody 3B3 and may indicate an important functional significance of these regions (9).

As associations between defined VZV genotypes, gE gene mutations, and immunocompromising factors have not been considerably investigated, the objective of this study was to reveal these possible associations.

Vesicle fluid samples were obtained between 2003 and 2007 from 45 patients that were referred to the hospital of the Johann Wolfgang Goethe University, Frankfurt am Main, Germany, or to the Clinic of Dermatology and Venereology, University of Rostock, Rostock, Germany. Detailed patient information is summarized in Table 1. DNA was isolated from the samples by using the RTP DNA/RNA virus mini kit according to the instructions of the manufacturer (Invitek, Berlin, Germany). The VZV gE gene amplification reactions were carried out in 50-μl volumes with 10 μl of viral DNA from vesicle fluid samples or control DNA; 0.5 μl of proofreading *Pfu* polymerase (Fermentas, St. Leon-Rot, Germany), corresponding to 1.25 U; 5 μl of 10× *Pfu* buffer containing 25 mM $MgSO_4$; 1 μl of the forward primer (ORF68-F; 5′-ATTCCGAGGGTCGC CTGTAA-3′) and the associated reverse primer (ORF68-R; 5′-GTTGCCCCGGTTCGGTGA-3′), corresponding to 1 μM; 1 μl of a deoxynucleoside triphosphate mix at a final concentration of each deoxynucleoside triphosphate of 200 μM; and 31.5 μl of water. Thermal cycling comprised an initial hot start at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at primer pair-specific temperatures for 30 s, and polymerization at 72°C for 4 min. Finally, an extension step at 72°C for 10 min was carried out. Purification and sequencing of PCR products as well as VZV genotyping were performed as described recently (6). The genotypes of VZV wild-type strains Ger1 to Ger89 were determined in a previous study (6). The statistical analyses consisting of Fisher's exact test were carried out using the SPSS version 11 (SPSS Inc., Chicago, IL) statistical program.

Recently, we described a practical and simple VZV genotyping scheme based on analysis of a region of 1,990 bp of open reading frames (ORFs) 51 to 58 that allows the typing of VZV wild-type strains by high-throughput procedures directly from clinical samples (from 21 patients) without intermediate virus propagation (6). Samples from 24 additional patients were investigated by this novel genotyping scheme. The complete

* Corresponding author. Mailing address: Bernhard-Nocht-Institute for Tropical Medicine, Clinical Virology Laboratory, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany. Phone: 49-40-42818-205. Fax: 49-40-42818-400. E-mail: jonassi@gmx.de.

▽ Published ahead of print on 21 November 2007.

TABLE 1. VZV genotypes and associated glycoprotein E mutations[a]

VZV strain	Genotype	gE gene mutation(s)[b]	Age of patient (yrs)	Sex[c]	Varicella or zoster[d]	Immunocompromising factor
MSP	A	*G448A*	5	M	V	Cancer
BC	A	*G448A*	75	M	Z	
Kel	A				Z	
36	A				V	
11	D	*C1606A*			Z	
22	D	*C119T*, T660C, *C1606A*			Z	
03-500	D	*C119T*, T660C, *C1606A*				
HJO	D	*C119T*, T660C, *C1606A*			Z	
8	C	*C119T*			Z	
pOka	B	*C119T*		M	V	
Ger1*	A		41	F	Z	AIDS
Ger4*	A		72	F	Z	
Ger8*	A		52	M	Z	Cancer
Ger53*	A	*C119T*, T660C, *C1606A*	50	F	Z	AIDS
Ger54*	A	*C119T*, **C393A**, T660C, *C1606A*, **C1626T**	40	M	Z	AIDS
Ger70*	A		78	F	Z	
Ger80*	A		55	F	Z	
Ger81*	A	C513T	61	F	Z	Bone marrow transplantation
Ger84*	A		81	M	Z	
Ger97	A		63	M	Z	Cancer
Ger99	A		86	F	Z	Cancer
Ger100	A		19	F	Z	
Ger102	A		78	F	Z	Apoplexia
Ger103	A		66	F	Z	
Ger107	A	**G1284A**	78	M	Z	
Ger110	A		42	M	Z	
Ger111	A		57	F	Z	
Ger112	A		77	M	Z	
Ger113	A		39	M	Z	
Ger114	A		30	M	Z	Atopic eczema
Ger117	A		67	M	Z	
Ger118	A		63	M	Z	
Ger119	A		84	F	Z	Cancer
Ger120	A		55	F	Z	
Ger121	A		65	M	Z	
Ger29*	D		39	M	Z	Bone marrow transplantation
Ger31*	D	*C119T*, T660C, *C1606A*	48	M	Z	Bone marrow transplantation
Ger33*	D		1	M	V	
Ger45*	D	*C119T*, T660C, *C1606A*	61	M	Z	Cancer
Ger47*	D	*C119T*, T660C, *C1606A*	62	F	Z	
Ger55*	D	*C119T*, T660C, *C1606A*	70	F	Z	
Ger66*	D	*C119T*, T660C, *C1606A*	60	M	Z	
Ger69*	D		54	F	Z	
Ger78*	D	*C119T*, T660C, *C1606A*	74	F	Z	
Ger82*	D	*C119T*, T660C, *C1606A*	69	F	Z	
Ger88*	D	*C119T*, T660C, *C1606A*	53	M	Z	
Ger89*	D	*C119T*, T660C, *C1606A*, **C1752T**	11	F	V	
Ger94	D	*C119T*, T660C, *C1606A*	64	M	Z	Atopic eczema
Ger95	D	*C119T*, T660C, *C1606A*, **T1683G**	81	F	Z	
Ger101	D	*C119T*, T660C, *C1606A*	77	M	Z	Diabetes mellitus
Ger104	D	*C119T*, T660C, *C1606A*	53	M	Z	Wegener's granulomatosis
Ger105	D	*C119T*, T660C, *C1606A*	75	M	Z	Multiple sclerosis
Ger108	D	*C119T*, T660C, *C1606A*	63	F	Z	Hyperthyreosis
Ger115	D	*C119T*, T660C, *C1606A*, **T1683G**	56	F	Z	
Ger116	D	*C119T*, T660C, *C1606A*	78	F	Z	Myelodysplastic syndrome

[a] Nucleotide positions refer to the European strain Dumas. Only changes from the Dumas sequence are shown. Reference strains from previous studies were included. Accession numbers and origins are as follows: MSP, Minnesota (AY548170); BC, British Columbia, Canada (AY548171); Kel, Iowa (DQ479954); 36, New Brunswick, Canada (DQ479958); 11, New Brunswick, Canada (DQ47995); 22, New Brunswick, Canada (DQ479956); 03-500, Alberta, Canada (DQ479957); HJO, Germany (AJ871403); 8, New Brunswick, Canada (DQ479960); and pOka, Japan (AB097933). An asterisk indicates that the genotype was identified in a previous study (6).The e1 epitope has previously been determined to be localized within amino acids 109 to 123, and the c1 epitope has been found to be localized within amino acids 160 to 316 (7).

[b] Nonsynonymous SNPs are indicated in italics, and novel SNPs are indicated in bold.

[c] F, female; M, male.

[d] V, varicella; Z, zoster.

ORF of the gE gene was also amplified from samples from all 45 patients and sequenced. In analyses of the total nucleotide sequences of the gE ORF, five novel synonymous gE mutations were found (Table 1). Interestingly, two of the novel gE mutations were detected in a sample from a genotype A (strain Ger54)-infected AIDS patient undergoing combined antiretroviral therapy. Another novel gE mutation (T1683G in strains Ger95 and Ger115) was found in samples from two elderly female patients. All novel mutations were located outside the coding regions of the epitopes e1 and c1, demonstrating that these epitopes were highly conserved in the investigated VZV wild-type strains. Therefore, VZV glycoproteins with conserved epitopes may be suitable vaccine candidates.

The analysis of five VZV genotype D strains originating from Thailand, Iceland, and the United States showed a unique gE single-nucleotide polymorphism (SNP) profile that is represented by a synonymous T660C mutation and the two nonsynonymous mutations C119T and C1606A, corresponding to amino acid exchanges T40I and L536I, respectively (2, 11). In line with these results, we have found 17 genotype D strains that contain this SNP profile (Table 1). The association of this gE SNP profile with VZV genotype D was found to be statistically significant ($P < 0.0001$). However, our investigations suggest that this gE SNP profile is not restricted to genotype D, as it was detected also in the genotype A strains Ger53 and Ger54. In addition, genotype D strains Ger29, Ger33, and Ger69 and the genotype D reference strain 11 did not show this SNP profile (Table 1). No statistically significant association of gE mutations with age, sex, or immunocompromising factors was observed.

This unique association of genotype D VZV wild-type strains with a distinct SNP profile of the gE gene and the gE sequence variations will be studied by our group for a potential role in VZV pathogenesis or autoimmune diseases.

We acknowledge Holger Felix Rabenau, Martin Stürmer, Regina Allwinn, and Annemarie Berger for helpful discussions and Marhild Kortenbusch and Margarethe Podporski for technical assistance.

The work was supported by the Hospital of the Johann Wolfgang Goethe University and Paul and Ursula Klein-Stiftung.

REFERENCES

1. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.
2. **Faga, B., W. Maury, D. A. Bruckner, and C. Grose.** 2001. Identification and mapping of single nucleotide polymorphisms in the varicella-zoster virus genome. Virology **280:**1–6.
3. **Natoli, S., M. Ciotti, P. Paba, G. P. Testore, G. Palmieri, A. Orlandi, A. F. Sabato, and F. Leonardis.** 2006. A novel mutation of varicella-zoster virus associated to fatal hepatitis. J. Clin. Virol. **37:**72–74.
4. **Santos, R. A., J. A. Padilla, C. Hatfield, and C. Grose.** 1998. Antigenic variation of varicella zoster virus Fc receptor gE: loss of a major B cell epitope in the ectodomain. Virology **249:**21–31.
5. **Santos, R. A., C. C. Hatfield, N. L. Cole, J. A. Padilla, J. F. Moffat, A. M. Arvin, W. T. Ruyechan, J. Hay, and C. Grose.** 2000. 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 **275:**306–317.
6. **Schmidt-Chanasit, J., M. Stürmer, A. Hahn, S. G. Schäd, G. Gross, R. G. Ulrich, G. Heckel, and H. W. Doerr.** 2007. Novel approach for genotyping varicella-zoster virus strains from Germany. J. Clin. Microbiol. **45:**3540–3545.
7. **Shankar, V., S. Fisher, B. Forghani, and A. Vafai.** 2001. Nucleotide sequence analysis of varicella-zoster virus glycoprotein E epitope coding regions. Vaccine **19:**3830–3833.
8. **Tipples, G. A., G. M. Stephens, C. Sherlock, M. Bowler, B. Hoy, D. Cook, and C. Grose.** 2002. New variant of varicella-zoster virus. Emerg. Infect. Dis. **8:**1504–1505.
9. **Tyler, S. D., G. A. Peters, C. Grose, A. Severini, M. J. Gray, C. Upton, and G. A. Tipples.** 2007. Genomic cartography of varicella-zoster virus: a complete genome-based analysis of strain variability with implications for attenuation and phenotypic differences. Virology **359:**447–458.
10. **Vafai, A., B. Forghani, D. Kilpatrick, J. Ling, and V. Shankar.** 2000. Stability of a varicella-zoster virus glycoprotein E epitope. Arch. Virol. **145:**85–97.
11. **Wagenaar, T. R., V. T. Chow, C. Buranathai, P. Thawatsupha, and C. Grose.** 2003. The out of Africa model of varicella-zoster virus evolution: single nucleotide polymorphisms and private alleles distinguish Asian clades from European/North American clades. Vaccine **21:**1072–1081.
12. **Wirgart, B. Z., V. Estrada, W. Jackson, A. Linde, and C. Grose.** 2006. A novel varicella-zoster virus gE mutation discovered in two Swedish isolates. J. Clin. Virol. **37:**134–136.