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A 12-year molecular survey of clinical herpes simplex virus type 2 isolates demonstrates the circulation of clade A and B strains in Germany

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ABSTRACT

Background: Recently two different herpes simplex virus type 2 (HSV-2) clades (A and B) were described on DNA sequence data of the glycoprotein E (gE), G (gG) and I (gI) genes.
Objective: To type the circulating HSV-2 wild-type strains in Germany by a novel approach and to monitor potential changes in the molecular epidemiology between 1997 and 2008.
Study design: A total of 64 clinical HSV-2 isolates were analyzed by a novel approach using the DNA sequences of the complete open reading frames of glycoprotein B (gB) and gG. Recombination analysis of the gB and gG gene sequences was performed to reveal intragenic recombinants.
Results: Based on the phylogenetic analysis of the gB coding DNA sequence 8 of 64 (12%) isolates were classified as clade A strains and 56 of 64 (88%) isolates were classified as clade B strains. Analysis of the gG coding DNA sequence classified 4 (6%) isolates as clade A strains and 60 (94%) isolates as clade B strains. In comparison, the 8 isolates classified as clade A strains using the gB sequence data were classified as clade B strains when using the gG coding DNA sequence, suggesting intergenic recombination events. Intragenic recombination events were not detected.
Conclusion: The first molecular survey of clinical HSV-2 isolates from Germany demonstrated the circulation of clade A and B strains and of intergenic recombinants over a period of 12 years.

1. Background

Herpes simplex virus type 2 (HSV-2, *Human Herpesvirus 2*) is a human representative of the subfamily *Alphaherpesvirinae* within the family *Herpesviridae*. After primary infection HSV-2 typically establishes latency in the sacral ganglia. HSV-2 reactivation from latency is the main cause of genital herpes worldwide and responsible for an increased risk of human immunodeficiency virus (HIV) acquisition in HIV-negative individuals.[8] The HSV-2 genome is a 155 kb linear, double-stranded DNA molecule and is predicted to code for at least 74 proteins.[4] The first HSV-2 DNA sequence-based molecular epidemiological study including 27 clinical isolates from Tanzania, 10 isolates from Norway and 10 isolates from Sweden demonstrated the presence of two different HSV-2 clades designated A and B.[16] Clade A strains were found to circulate exclusively in Tanzania, whereas clade B strains were detected in Tanzania, Sweden and Norway.[16] Recombination networks and bootscan analysis performed with DNA sequence data of the glycoprotein G (gG), I (gI) and E (gE) genes revealed intergenic and intragenic recombination events.[16] In contrast to the high seroprevalence to other human herpesviruses such as herpes simplex virus type 1 (HSV-1), the seroprevalence for HSV-2 is rather low. Thus, in Germany, the overall HSV-2 seroprevalence is 13%,[9] whereas it was reported to be slightly lower in neighboring countries such as The Netherlands (9%) or Czech Republic (6%).[18] However, in Germany prevalences are significantly higher in risk groups such as prostitutes (78%) and HIV-positive individuals (61%).[3]

2. Objective

In contrast to the large number of seroepidemiological studies performed in Central Europe, no molecular epidemiological study on HSV-2 has been performed so far in Central Europe and in Germany in particular. Therefore, the objectives of our study in Germany were (i) to type the circulating HSV-2 wild-type strains by a novel approach and (ii) to monitor potential changes in the molecular epidemiology between 1997 and 2008.

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3. Study design

Swabs were obtained between 1997 and 2008 from 64 herpes patients that were referred to the Hospital of the Johann Wolfgang Goethe University, Frankfurt am Main, Germany. Virus isolation was performed in Vero cells (ATCC CRL-1587, Rockville, MD) using the shell vial assay and subsequent staining with HSV-2-specific monoclonal antibodies (Argene/Biosoft, Varilhes, France) as described recently.[3] If several isolates were available from a single patient only the first isolate was included in the analysis. Viral DNA was isolated from cell culture supernatant using the RTP DNA/RNA Virus Mini Kit (Invitek, Berlin, Germany) according to the manufacturer's instructions. For extensive phylogenetic analysis the complete open reading frames of gB (2715 nt, size refers to HSV-2 reference strain HG52 GenBank accession number Z86099) and gG (2100 nt) were amplified by PCR, using the primers UL27F (5′-CCCGTTAGCACATGTCTGCATTTG-3′), UL27R (5′-TCGTGGTTTACGACCGAGACGTTT-3′), US4F (5′-GGCCCTCGGGCTTTGGTGT-3′) and US4R (5′-CGTCCTTCATCGTTTCTCGCCC-3′). DNA amplification reactions were carried out in 25 μl volumes containing 5 μl of extracted 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 corresponding to 0.5 μM, and 5.5 μl nuclease-free

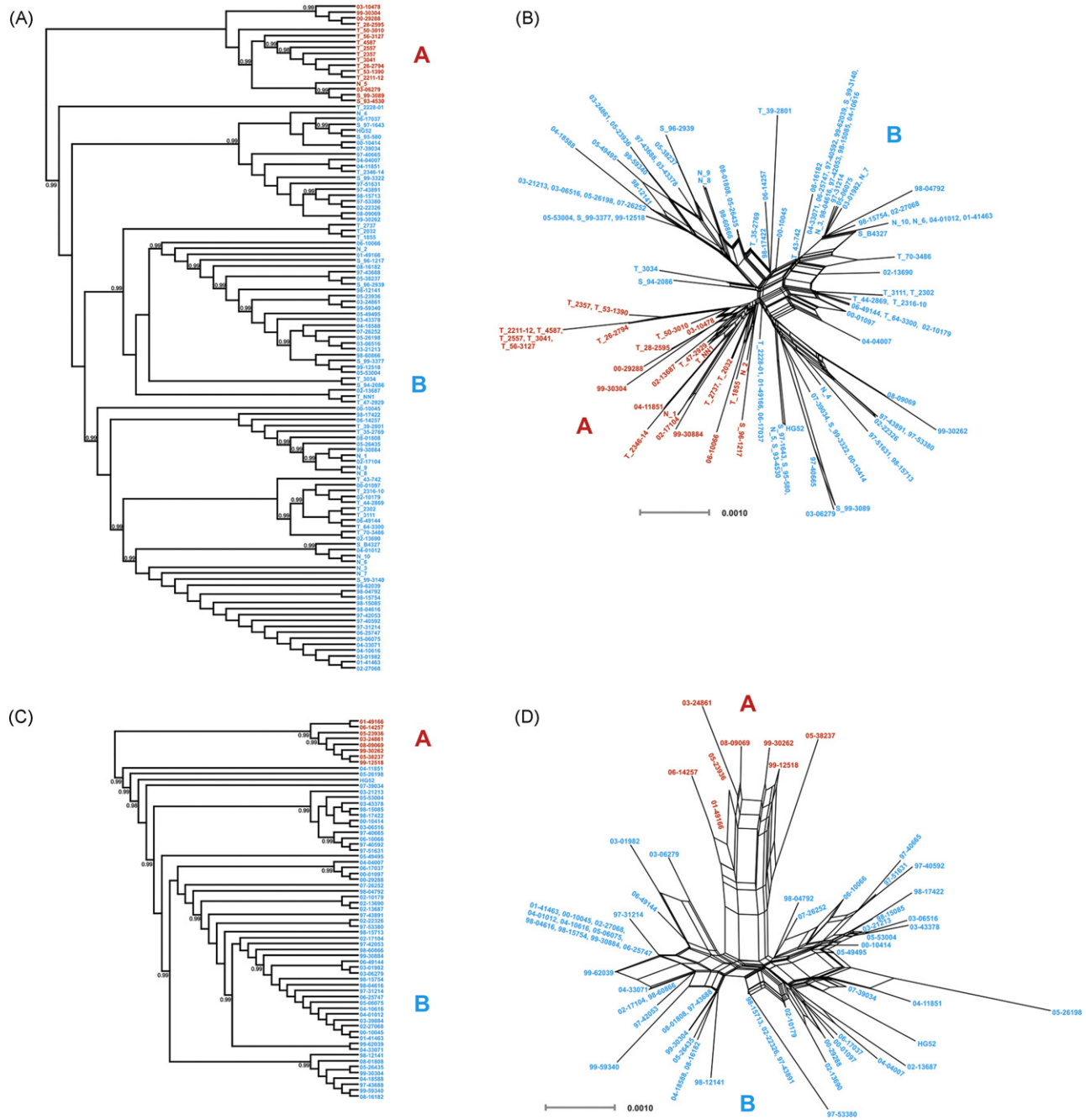

Fig. 1. Maximum-likelihood (ML) phylogenetic trees (A and C) and corresponding Neighbor-net networks (B and D) of 64 novel (GenBank accession numbers HM011303–HM011430) and 48 previously described (GenBank accession numbers EU106374, EU106421 and Z86099) gG (A and B) and gB (C and D) gene sequences of clade A (red) and B (blue) HSV-2 strains. The HSV-2 strain HG52 (Z86099) was used as reference for clade B. Chi^2-based probabilities are shown on each node of the ML trees if values were higher than 0.98.