

# Evolution of human alpha-herpesviruses

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## ABSTRACT

*Herpesviridae* is a large virus family with more than 100 members, which are highly disseminated among animals. Three sub-families have been classified; alpha-herpesviruses, beta-herpesviruses and gamma-herpesviruses. Eight herpesviruses have hitherto been identified in humans of which three belong to the alpha-herpesviruses; (i) herpes simplex virus type 1 (HSV-1), which is a ubiquitous pathogen causing mainly oral or genital lesions, (ii) herpes simplex virus type 2 (HSV-2), which is closely related to HSV-1, and is the most common sexually transmitted virus globally, causing mainly genital lesions, and (iii) Varicella zoster virus (VZV), which is the cause of chicken pox and shingles. All alpha-herpesviruses give lifelong infections and establish latency in the sensory ganglia. In the present work, the genetic variability of clinical HSV-1, HSV-2 and VZV isolates was investigated.

Twenty-eight clinical HSV-1 isolates were collected from patients suffering from oral or genital lesions or encephalitis and compared with the laboratory strains F, KOS321 and 17. Phylogenetic analyses based on the genes US4, US7 and US8 divided the isolates into three genogroups, arbitrarily designated as A, B and C, differing in DNA sequences by approximately 2%. In addition, seven clinical isolates as well as strain 17 were classified as recombinants. To facilitate further genotyping of clinical isolates an assay was developed based on restriction enzyme cleavage of PCR-products. Furthermore, a polymorphic tandem repeat (TR) region was detected in US7. The region encodes the amino acids serine, threonine and proline, which are targets for O-linked glycosylation. Using a synthetic peptide, containing two of the repeated blocks, it was shown that the described TR-region is a substrate for massive O-linked glycosylation, and hence codes for a mucin region. Mucin regions have not been described previously within herpesvirus-encoded proteins.

The corresponding genes were sequenced and investigated for 45 clinical HSV-2 isolates collected in Sweden, Norway and Tanzania. Phylogenetic analysis revealed a divergence of the isolates in one Tanzanian and one European genogroup, arbitrarily designated as A and E, differing by approximately 0.4%. In addition, analyses using recombination networks, the BootsScan method and the phi-test, suggested that most HSV-2 isolates are mosaic recombinants.

The complete genome was sequenced for two VZV isolates and compared with the laboratory strains MSP, Dumas, BR, p-Oka and the vaccine strain v-Oka. The results show a division of VZV into four genogroups, designated as E, J, M1 and M2, of which M1 and M2 were suggested to be recombinants derived from ancient recombination events between viruses from the E and J genogroups.

In conclusion, the results presented here demonstrate that clinical isolates, for all three investigated human alpha-herpesviruses, can be divided into different genogroups. Estimations of evolutionary timescales suggest that the divergence of the three HSV-1 genogroups may have occurred approximately 500,000 Myears BP, i.e. prior to the emergence of *Homo sapiens*. Furthermore, it is evident that intrastrain recombination is a prominent feature of the evolutionary history of these viruses. Thus, homologous recombination is suggested to be a powerful evolutionary mechanism for human alpha-herpesviruses to exchange genetic segments between different viral strains, as well as to create variability of TR-regions.

*Keywords:* HSV-1, HSV-2, VZV, evolution, recombination, phylogenetic analysis.

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- I. **Norberg, P., Bergström, T., Rekabdar, E., Lindh, M. & Liljeqvist, J-Å.** Phylogenetic analysis of clinical herpes simplex virus type 1 isolates identified three genetic groups and recombinant viruses. *J Virol* 2004; 78: 10755-10764.
- II. **Norberg, P., Bergström, T. & Liljeqvist, J-Å.** Genotyping of clinical herpes simplex virus type 1 isolates by use of restriction enzymes. *J Clin Microbiol* 2006; 44: 4511-4514
- III. **Norberg, P., Olofsson, S., Agervig Tarp, M., Clausen, H., Bergström, T. & Liljeqvist J-Å.** Glycoprotein I of herpes simplex virus type 1 contains a unique polymorphic tandem repeated mucin region. Submitted for publication.
- IV. **Norberg, P., Kasubi, M.J., Haarr, L., Bergström, T. & Liljeqvist, J-Å.** Evolution of herpes simplex virus type 2 –Identification of two genogroups and multiple recombinants. In manuscript.
- V. **Norberg P., Liljeqvist, J-Å., Bergström, T., Sammons, S., Schmid, D.S. & Loparev V.N.** Complete-genome phylogenetic approach to varicella-zoster virus evolution: genetic divergence and evidence for recombination. *J Virol* 2006; 80: 9569-9576.