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Molecular Epidemiology of Varicella Zoster Virus

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Summary

**Varicella zoster virus has highly conserved genome 125,000 base pairs.
The different molecular genetic methods of analyzing VZV genome are discussed, as well as their results with regards to the virus phylogenesis, geographic distributions, possible recombination and virulence of different VZV strains.**

Key words: varicella zoster virus – DNA genome – genotyping – single nucleotide polymorphism (SNP)

Souhrn

Boštíková V., Boštík P., Chlíbek R., Schmid D. S., Salavec M., Smetana J., Špliňo M.: Molekulární epidemiologie viru Varicella zoster

**Varicela zoster virus (VZV) se vyznačuje vysoce konzervativním genomem, obsahujícím přibližně 125 000 nukleotidových párů bází.
Článek přehledně shrnuje vývoj molekulárně genetických přístupů zkoumání VZV v průběhu posledních deseti let. Autoři se zabývají výstupy těchto metod, jako jsou fylogenetické vztahy mezi jednotlivými kmeny VZV, jejich virulencí, geografickou distribucí, možnými rekombinacemi.**

Klíčová slova : varicela zoster virus – DNA genom – genotypová analýza – single nuclotide polymorphism (SNP).

Varicella zoster virus (VZV) is a member of the family *herpesviridae*. The virus has icosahedral symmetry and contains centrally located double-stranded DNA genome with a surrounding envelope. The VZV genome contains 125,000 base pairs and encodes around 75 proteins [1].

Humans are the only known reservoirs for this highly contagious pathogen. Primary VZV infection in humans results in chickenpox, which has characteristic clinical manifestation (febrile vesicular rash, itchy, crops, low-grade fever, malaise, and anorexia). In most immunocompetent and healthy children, chickenpox is a self-limiting but highly infectious disease with household secondary attack rate > 80 %. However, complications such as secondary bacterial super-infection of lesions can occur. In adults, complications are more frequent in pregnant women, with negative consequences for the fetus and the newborns, and in persons with cellular immune deficiencies, with an increased risk of mortality. VZV infections can lead to life-threatening complications including pneumonitis, hepatitis, and meningoencephalitis [2]. Localized bacterial secondary infection by *Staphylococci* and *Streptococci* rarely results in septicemia, bacterial pneumonia, otitis media, or necrotizing fasciitis [3].

Secondary VZV infection manifests as herpes zoster (HZ), and is due to the reactivation of dormant virus in the dorsal root ganglia following primary VZV infection [4]. Herpes zoster occurs frequently in adults, but is not uncommon in immuno-compromised and even normal children. HZ often presents as painful vesicular eruptions; the rash is often unilateral and limited to a single dermatome.

The epidemiology of primary VZV infection varies geographically [19]. Human VZV infections occur at a much earlier age in temperate climates whereas in warm tropical areas they tend to occur

in older individuals [5]. Thus in European Union and United States as many as 60 % of the preschool children demonstrate IgG antibodies to VZV. In contrast, the mean age of onset of chickenpox is higher in tropical countries and more cases occur among adults suggesting absence of prior antibodies [6]. It is unclear whether the strain distribution is actually driven by climate or/and other factors such as immigration patterns.

Thankful to modern medicine the survival rate of immunocompromised patients has been increasing. In these patients infections with various viruses have often much more dangerous course compared to the immunocompetent hosts. Therefore the development and improvement methods of virus detection and identification presence are extremely important. In this regard, especially semiquantitative and quantitative sensitive detection methods based on PCR have perspective. The quantitative levels of virus in organism usually well correlate well with the clinical picture of disease. Due to the continuous development of advanced therapeutic approaches, design of new discrimination technologies which enable identification of individual VZV strains becomes an issue of extreme importance.

The VZV genome is highly conserved, carrying one SNP in about every 1000 bp [7]. Frequent recombination events between different VZV strains are possible [8]. Both of these factors complicate strategies for genotypic analysis of VZV and for strain surveillance. Nonetheless, continuous investigations of VZV genetic variability have led to discrimination of several VZV genotypes.

DNA restriction fragment length polymorphism (RFLP) methods have been used to confirm the identity of the specific VZV strain that leads to primary varicella and later reactivates to cause zoster. Relatively consistent restriction enzyme digestion profiles for different VZV strains were observed, providing the first evidence that VZV has a highly conserved genome. Subsequently, intra-strain variations in restriction enzyme fragment profiles among wild-type VZV isolates were observed. However, the most prominent differences were linked to variations in the number and composition of VZV genome repeat elements [7].

Several RFLP strategies are used to distinguish VZV isolates from different geographic regions and also to differentiate between the live-attenuated Oka vaccine strain and wild-type viruses [1, 7, and 8], using SNP located in ORF 38 (PstI site in many wild-type strains) and ORF 54 (BglI site in Oka) [9, 10]. It was also determined that wild type VZV strains isolated in the US and Japan has distinctive PstI and BglI RFLP profiles. Japanese isolates are PstI+, BglI+ or PstI-, BglI+, while most isolates from the US, Europe, and eastern Australia are PstI+, BglI- [11, 12]. BglI+ strains, apart from those isolate in Japan, are common in tropical regions such as equatorial Africa, India, Bangladesh, China, Central America and northern Australia [7, 12]. An unusual PstI-, BglI- VZV strain was reported in Australia, a strain that could represent recombination between the dominant genotypes or point mutation at the ORF 38 locus. SNP at position 106262 (SmaI restriction site) in ORF 62 discriminate between wild-type and Oka vaccine virus, using real-time FRET (fluorescent resonance energy transfer)-based PCR. Additional testing for vaccine-associated SNP at position 107252 was performed. Real-time quantitative PCR analysis was performed to determine VZV viral load using real-time PCR with the Minor Groove Binding (MGB) Eclipse probe System for Allelic Discrimination (Epoch Biosciences, Inc.) [6, 7, 13, 14, 20].

The most recent studies utilizing genotyping of VZV based on sequence variations in open reading frame ORF 21, ORF 22, ORF 50 and other discrimination methods using the markers in ORF 38, ORF 54 and ORF 62 led to a categorization of VZV into distinct genotypes named European 1 (E1), European 2 (E2), Japanese (J) and Mosaic as M (M1, M2, M3 and M4), means combined type [7, 12, 13, 14].

The E genotype is the most prevalent genotype in the United States, Canada, Europe and eastern Australia. The number of reference mutations in each particular genotype was found to be relatively constant in Europe despite the fact that its population has history of excessive traveling and migration. The data was surprising because a number of recombination can be expected between two major E1 and E2 genotypes that dominate in European countries, and with M1, M2 and M4 genotype strains circulating in this continent, leaving several intermediate mosaic variants as sub-genotypes. However predicted intermediates were not eminent, but mostly random strain specific mutations were represented in limited amount. There is strong phylogenetic evidence for a close relationship between the European genotypes E1 and E2, as well as with M4. It is well known that in Europe there is significant proportion 90% of VZV strains which were originally described as European strains and only recently further classified into genotypes E1 (65%) or E2 (25%) [13]. Interestingly, E2 strains were identified for the first time as dominant genotype in Iceland (88%), Finland (64%), Czech Republic (53%), Germany (41%) [13, 18].These data justify presence of at least two distinct European genotypes. Taking in account that these viruses

were significantly prevalent in senior patients with zoster, those findings support the hypothesis that E1 and E2 genotypes are native in Europe, supporting their classification and geographic distribution. Strikingly, E1 and E2 strains currently circulating in 19 countries of Europe are practically identical to the strains found in North America, Australia and New Zealand [14]. It remains a possibility however, given very limited number of genetic changes involved, that these variant strains could have simply arisen through mutation.

Surprisingly significant amount of M4 strains were discovered in Southern European countries-Italy, Spain and France. M4 strains have close relationship to E1 and especially to E2 strains. These data allow us to speculate that clade E2 and M4 strains may have arisen more than a century ago in remote European colonies such as Australia, New Zealand, and North America through recombination events between E1 and tropical clade M strains and effectively competed with the original local strains and imported E1 strains, establishing them in the population. Alternatively, E2 and M4 strains could have arisen in Europe in those countries having E1 and M strains in circulation, and spread afterwards to colonized countries and continents. Alternatively recombinant variants of E2 could have been selected by temperate climates to which increasing numbers of people migrated from regions with warmer climates [13, 14, and 16].

The prevalence of other M strains: Mosaic 1 (M1) in Africa and Mosaic 2 (M2) in France, Germany, Iceland, and North America. The additional Mosaic 3 (M3) is unique in the US [13, 17].

The J strains of VZV are dominant in Japan, and M1 strains dominated in tropical Africa. It is unclear whether different genotypes have been actually distributed by climate or/and other factors such as introduction of strains from different geographical region due immigration or/and travel. The J genotype is typically found in Japan; some unpublished data suggest that it is also prevalent in South Korea, Taiwan and Mongolia. An important recent finding confirms an absence of Japanese strains of VZV in active circulation in Europe [13]. These findings outline relationships between VZV genotypes in Europe in situation when mandatory vaccination in continent was not approved and will help future understanding of events, which could potentially take place during active vaccination with VZV vaccine strain which belongs to J genotype [13].

Whole genome screening for SNPs using a heteroduplex mobility assay was used to identify variants of VZV circulating in the UK and elsewhere [15]. This approach relied on the evaluation of selected SNP in ORF1, 21, 50 and 54. Attempts to refine this method by broadening the panel of SNP evaluated produced similar results to the original method by broadening the panel of SNP evaluated produced similar results to the original method. This technique distinguished at least three major clades (A, B, and C) of VZV isolates collected worldwide, and the distribution of genotypes was associated with the geographical region in which infection was acquired. Thus, clade A strains formed an African-Asian grouping, whereas clade B and C strains were found primarily in European populations. Subsequently, clade J was added to this genotyping scheme to accommodate Japanese strains. Recombination between A and C viruses was hypothesized, leading to the appearance of clade B viruses as well as unclassified A/C recombinants. Another group used the same approach to examine the genetic variability of clinical VZV isolates collected in Ireland, detecting all four clades.

A different genotyping strategy, based on complete DNA sequences for five glycoprotein genes (gH, gI, gL, gB and gE) and the IE62 major transactivator gene, was used to classify VZV strains into three genotypes (also designated A,B and C). This study revealed that VZV strains circulating in Japan, Iceland and the Netherlands had uniform genotypes (A, B, andC, respectively), whereas genotypes circulating in different regions of the US or even within the same US state were diverse. This study was recently modified and extended to include viruses from Singapore and Thailand. Based on this approach viral isolates from the US/Europe and Singapore/Japan were segregated into four distinct clades arbitrarily designated A,B,C and D. Viral isolates from Singapore and Japan were from clades B and C, and isolates from Western Europe and the US were from clades A and D. Finally, in a study using information available from a public database, VZV was segregated into 4 genotypes: A, B, C, D. It is important to note that these similar clade designations do not correlate among the studies described above by several independent groups.

As mentioned above, the known VZV genotypes and sub genotypes could be reliably discriminated using only four single nucleotide polymorphisms (SNP) present in a 447 bp amplicon in ORF 22 (region 1) and the E1 and E2 genotypes could be resolved with the additional of SNP located in short amplicons from ORF 21, ORF 22 (region 2) or ORF 50 [13, 14].

VZV genotypes should help in the monitoring of vaccine impact, because vaccination might be expected to increase the frequency of VZV superinfection in individuals and possibly lead to an increase in recombination events.

Careful genotypic analysis could lead to the identification of virulence factors and hence to an improved understanding of VZV biology. Another important issue is a possibility to use this methodology for elucidating phylogenetic relationships between the VZV strains responsible for recent outbreaks and their evolutionary and/or transmission history.

While DNA sequencing remains the gold standard for analyzing DNA variations, novel alternative strategies are currently under development for the detection of multiple , diverse VZV allelic heterogeneity and should foster studies of VZV evolution and global transmission. The use of DNA microarray technology is one of the most promising of these new approaches. DNA-microarrays containing specific probes immobilized on a solid surface facilitate the simultaneous analysis of multiple genetic markers and/or the processing of several samples on a single array [8].

The availability of technically practical, reliable methods for genotyping VZV strains will serve a critical functional in countries with broad varicella vaccination policies, since tracking individual strains and identifying probable sources of infection is essential to effectively monitor vaccine impact. In additional, the study of global VZV genotype patterns will likely lead to a better understanding of global transmission patterns both before and after vaccination, and of the evolutionary trends of this nearly ubiquitous virus.

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