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POLIOVIRUS O.P.V STRAINS – CORRELATION BETWEEN RECOMBINATION AND RNA SECONDARY STRUCTURES

ABSTRACT

In the first chapter, a study was conducted in order to identify recombination junctions and mutations in VP1 region of 15 poliovirus clinical isolates and assess their effects in virus fitness providing some insights into the patterns of natural evolution of polioviruses. In conclusion, some of the Sabin strains investigated in the present study displayed a different pattern of evolution in VP1 region. Non-synonymous mutations with a direct phenotypic impact to the virus fitness that will result in amino acid substitutions in structural elements of VP1 (N-Ags, canyon), and mutations associated with reversion to neurovirulence are selected, in spite of synonymous mutations with no phenotypic impact to the virus fitness. Thus, virus can replicate and evolve for a longer period of time in the host, escaping the host's immune response.

Full genomic sequencing of two isolates which present extraordinary interest, was conducted. Extensive genomic sequencing of the first strain, isolate 7/b/97, revealed 1.87% VP1 sequence divergence and a recombination event between a Sabin 1 strain and a strain belonging to the group HEV-C, in the 2A genomic region. The genomic features of isolate 7/b/97 may place this strain in the cVDPV category. The 1,87% divergence in VP1 revealed that isolate 7/b/97, was circulated in a region free from poliovirus for approximately 2 years. In the past, cVDPV strains were responsible for polio epidemics, due to their increased neurovirulence and their increased ability for transmission.

Full genomic sequencing of the second strain (K/2002) revealed a Sabin 3/Sabin 2 recombination junction in VP1 genomic region. Intertypic capsid recombination is a very rare event. In our study the age of isolate K/2002, was approximately 11 months, while the age of the patient was 4 months. This means that isolate K/2002 has been circulated in the community, posing questions about the safety of OPV live vaccine.

In order to determine the correlation between the increased frequency of appearance of recombination junction of types Sabin 3/Sabin x (x: Sabin 2 or Sabin 1)

and Sabin 2/Sabin x (x: Sabin 1 or Sabin 3) in 2C and 3D regions respectively, we constructed the secondary structure models of positive and negative strand of Sabin 1, Sabin 2 and Sabin 3 strains. Recombination junctions which identified in clinical strains of this study, as well as in clinical strains of previous studies, were superimposed on RNA secondary structure models of 2C and 3D regions. Furthermore an in vitro model for the production of recombinant Sabin strains was created. We aimed to the comparison between the results which refer to the correlation of recombination junctions and RNA secondary structures in clinical strains, with the respective results of the in vitro model.

In conclusion, in vitro model results are in consensus with the results which ensued from the superimposition of recombination junctions of clinical strains on RNA secondary structures. The majority of recombination junctions are correlated with RNA secondary structure elements, which are identical between Sabin strains that participate in the recombination, and also are consisted of base pairs with high probabilities. Furthermore, many of these elements are also included in the minimum free energy structure. As a result, the positive prognostic value of the RNA secondary structure elements where recombination junctions are located, is very high. Finally, we can deduce that the distribution of recombination junctions in Sabin strains is not accidental, and is correlated with RNA secondary structure elements identical to both recombination partners.