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RECOMBINANT POLIOVIRUS STRAINS- SITES OF GENETIC RECOMBINATION

ABSTRACT

This thesis describes the analysis of recombinant poliovirus strains obtained from clinical and environmental samples.

Five polio/Sabin recombinant strains isolated from healthy vaccinees or from VAPP patients after OPV administration, were analysed. RT-PCR, followed by Restriction Fragment Length Polymorphism (RFLP) screening analysis were applied in four distant genomic regions (5' UTR, VP1, 2C and 3C-3D) in order to detect any putative recombinant. The detected recombinants were sequenced from 2C to the end of the genome (3' UTR) and the exact recombination sites were determined with computational analysis. Two of the recombinants were found in the 2C genomic region, isolates EP16:S3/S2, EP23:S3/S1, two in 3D genomic region isolates EP6:S2/S1, EP12:S2/S1 and one in 3A genomic region isolate EP9:S2/Sl. Point mutations were found in strains EP3, EP6, EP9 and EP12.

Five more clinical Sabin intertypic recombinant strainswere investigated. Strains EPA, EPB and EPC were found to be bi-recombinant Sabin3/Sabin2/Sabin3 (S3/S2/S3), one strain was characterized as a probable S3/S2- CAV18 or CAV21-S2/S1 multi-recombinant (EDP11) and one was identified as a tripartite one S3/S2/S1 (EDP12). Samples EPA, EPB and EPC presented a common recombination junction in the 2C genomic region. Moreover, strains EPA and EPB shared also the second recombination site in the 3D genomic region, whereas the second recombination of EPC was also determined in 3D but in a different nucleotide position. Strains EDP11 and EDP12 presented both identical recombination motifs and recombination sites. The first was detected in the 2C genomic region and the second in the 3D region. Strain EDP11 presented an interesting feature since a sequence of 120 nucleotides seems to have derived from a member of human

enteroviruses species C (CAV18 or CAV21). This finding is of great importance, considering that this strain (EDP11) was isolated from an area and time period, where no Coxsackie A virus or poliovirus epidemics occurred.

Polioviruses (PVs) Sabin strains were also isolated from sewage treatment plants from Metamorphosis, Athens, Greece during the time period from May to October 1996, and from two other sites located at Nicosia and Limassol in Cyprus between April and December 2003 were investigated for the detection of recombinant PVs. Three PVs (LK3, LK 6, LK 10) isolates were found as tripartite recombinants, S3/S2/S1 in the 2C genomic viral region. The first recombination site S3/S2 was located close to the 5' end of 2C while the second recombination site S2/S1 was located towards the 3' end of 2C. Such recombination is a rare event producing a tripartite hybrid 2C protein. Three more PVs isolates (ENP 5, ENP 6 and ENP8) were characterized as bipartite S2/S1 recombinants and one ENP 7 as S2/S3 bipartite recombinant in different parts of the 3D genomic region.

Last but not least a vaccine-derived multirecombinant poliovirus strain which was isolated from a 5-month-old child with vaccine-associated paralytic poliomyelitis after oral poliovirus vaccine administration is analysed. The isolate had an S2/S1/S2/S1 primary genomic structure as revealed by restriction fragment length polymorphism and sequencing analysis. Recombination of the middle S1/S2 region is extremely rare and one of the few characterized types of recombination with Sabin type 1 as a 5' partner. An evolutionary analysis of the contributing sequences was performed using the identified mutations in comparison with the original Sabin sequences.

Conclusively in this thesis recombination specific types and sites re-occurrence along with point mutations were discussed concerning the polioviruses evolution. As well as the role of specific positions and motifs of the poliovirus genomic sequences that involves recombination events with other non –polio enteroviruses as Coxsackie A viruses that might be considered as possible counterparts of the recombination. Furthermore the detection of recombinant circulating vaccine-derived PVs (cVDPVs) is also crucial, since increased transmissibility over that of the parental Sabin strains has been proposed to be the result of recombination

events. Importation of recombinant cVDPVs evolved derivatives pose a serious threat to public health and environmental surveillance should be implemented during and after PVs eradication.