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GENETIC DIVERSITY AND MOLECULAR ANALYSIS OF HUMAN PARAINFLUENZA VIRUS TYPES 1 AND 3 IN SAINT PETERSBURG, RUSSIA

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Abstract

Human parainfluenza viruses (hPIVs) are important pathogens, responsible for acute respiratory tract diseases especially in young children. Due to the limited information of hPIV1 and hPIV3 circulation and their diversity pattern in Russia, the aim of this study was to perform a molecular and genetic characterization of these viruses in Saint Petersburg, Russia.

Human parainfluenza viruses (hPIVs) are important leading causes of upper and lower respiratory tract infections (RTIs), responsible for a wide spectrum of clinical illnesses, ranging from common cold, to severe lower respiratory tract diseases, such as pneumonia and bronchiolitis [1, 2]. The most vulnerable groups to these viruses are infants, the elderly, and immuno-compromised individuals [3]. hPIVs are members of the family *Paramyxoviridae*. They have non-segmented, negative sense, single stranded RNA genomes. Based on genetic and antigenic criteria, they have been classified into two genera, among which hPIV1 and hPIV3 belong to the genus *Respirovirus*, whereas hPIV2 and hPIV4 are classified as members of the genus *Rubulavirus* [1].

In Russia, the epidemiology and economic impact of hPIV1 and hPIV3 infections are undetermined. As comprehensive information of molecular and genetic characterization is missing, the present study was designed to elucidate the genetic diversity and phylogenetic analysis of hPIV1 and hPIV3, circulating in Saint Petersburg, Russia, in a period of eight years (2017–2025) through the whole genome-based genetic and phylogenetic analysis of these pathogens.

For both hPIV1 and hPIV3, two sets of 18 primer pairs each were designed to achieve full genome coverage. These primer designs were based on consensus sequence genomes obtained from multiple alignments of complete 227 hPIV1 and 419 hPIV3 genomes available in NCBI. Each primer pair covers approximately 1 kb of the genome with about 200 bp overlap of amplicons. The research was conducted at the laboratory of molecular virology of Smorodintsev Research Institute of Influenza (St. Petersburg, Russia). Detection of respiratory pathogens was carried out by real-time PCR using commercial kits "AmpliSens® ARVI-screen-FL" assay (Central Research Institute of Epidemiology, Moscow, Russia), in accordance with the manufacturer's instructions. Positive samples with Ct < 25 were selected for further whole genome amplification, using the developed primer panels. For each sample, two separate multiplex RT-PCR reactions (pools 1 and 2) were performed using the Biolabmix BioMaster RT-PCR–Premium (2×) kit, according to the manufacturer's recommendations. PCR products were subsequently sequenced on both Illumina NextSeq 2000 platform and MGI DNBSEQ G-400 platform. Nucleotide acid and deduced amino acid sequences of hPIV1 and hPIV3 were analyzed and aligned with MAFFT software. Phylogenetic trees were constructed with the maximum likelihood (ML, GTR+G) method using RAxML and TreeSub programs. The generated phylogenetic tree was visualized using the FigTree tool v1.4.4.

As a result of this study, 109 hPIV1 and 303 hPIV3 sequences were first obtained and documented in Russia. The whole-genome-based ML phylogenetic tree of hPIV1 sequences identified four distinct clades: A, B, C, and D. The Russian strains were distributed across clades B, C, and D, with clade C containing the majority of them. Similarly, the phylogenetic tree of hPIV3 strains delineated four genetic clusters A, B, C and D, emphasizing the emergence of a novel cluster D for the first time. Russian hPIV3 strains were primarily grouped into clusters C and D; further dividing into subclusters C5a, C5b, C3b, C3e and C3a. Most of the isolates were assigned to subcluster C3a, followed by C5b.

These findings shed light on the dynamic nature of both hPIV1 and hPIV3 and strongly underscore the critical need for further global surveillance and molecular analyses for better understanding of the viruses' circulation patterns and genetics.

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