РАЗДЕЛ IV

ВИРУСОЛОГИЯ

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DEVELOPMENT OF PRIMER PANEL FOR AMPLICON SEQUENCING OF HUMAN PARAINFLUENZA VIRUS TYPE 3

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Abstract

Human parainfluenza viruses (hPIVs) are prominent etiological causes of acute respiratory tract infections. Genomic surveillance of hPIVs is not carried out in Russia. To date, there are no whole sequence data available that would allow for detailed epidemiological and phylogenetic analysis of circulating strains. This research aimed to develop primer panels for sequencing the whole viral genome of hPIV3 and describe the genetic diversity of these viruses.

Respiratory tract illnesses are significant global health concerns, impacting millions of people annually. Human parainfluenza viruses (hPIVs) are key contributors to these respiratory illnesses. Seasonal hPIV epidemics impose a significant disease burden, especially in children, accounting for 40 % of pediatric hospitalizations for lower respiratory tract illnesses (LRTIs) second only to respiratory syncytial virus (RSV) and 75 % of croup cases. In immunocompromised or elderly adults, hPIV infections can progress to severe and life-threatening pneumonia [1]. Based on genetic, antigenic and morphological criteria, human parainfluenza viruses have been divided into four distinct serotypes 1–4, with hPIV1 and hPIV3 belonging to the Respirovirus genus and hPIV2 and hPIV4 belonging to the Rubulavirus genus [2]. The viral genome encodes six structural proteins, with the hemagglutinin-neuraminidase (HN) surface glycoprotein being of particular interest. The HN protein plays a critical role in pathogenesis, including host cell recognition, fusion, and viral release [3, 4].

In Russia, genomic surveillance of human parainfluenza viruses is not conducted. As a result, comprehensive molecular and genetic characterization data are lacking. Viral sequence information is needed to understand their genetic diversity, evolution, and potential recombination events, which is vital for thorough monitoring, prevention, and control of these pathogens. The present study attempted to design primer panels facilitating the amplification of the whole genome of human parainfluenza virus type 3, and perform a phylogenetic analysis based on HN gene sequences circulating of Russian strains.

A Set of 23 primer pairs was designed using the PrimalScheme tool (https://primalscheme.com) to cover the entire viral genome. The primer design was based on the consensus sequence obtained from a multiple alignment of 735 complete hPIV3 genomes available at NCBI. Each primer pair covers approximately 1 kb of the genome with an amplicon overlap of approximately 200 bp (Fig. 1). Two primer pools were prepared where each of them contained 12 and 11 primer pairs, with even and odd numbers.

Positive samples with high viral load (Ct < 22) were selected for whole genome amplification. Using the developed primer panel, 47 hPIV3 samples were amplified. For each parainfluenza 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 instructions. RT-PCR products were used for further sample preparation of libraries for sequencing. Whole genome sequencing of amplicons was performed using Illumina Nextseq platform. Multiple alignment of nucleotide sequences was performed using MAFFT software. Phylogenetic trees were constructed using the maximum likelihood (ML) method using the RAxML and TreeSub. FigTree v1.4.4 tool was used to visualize phylogenetic trees.

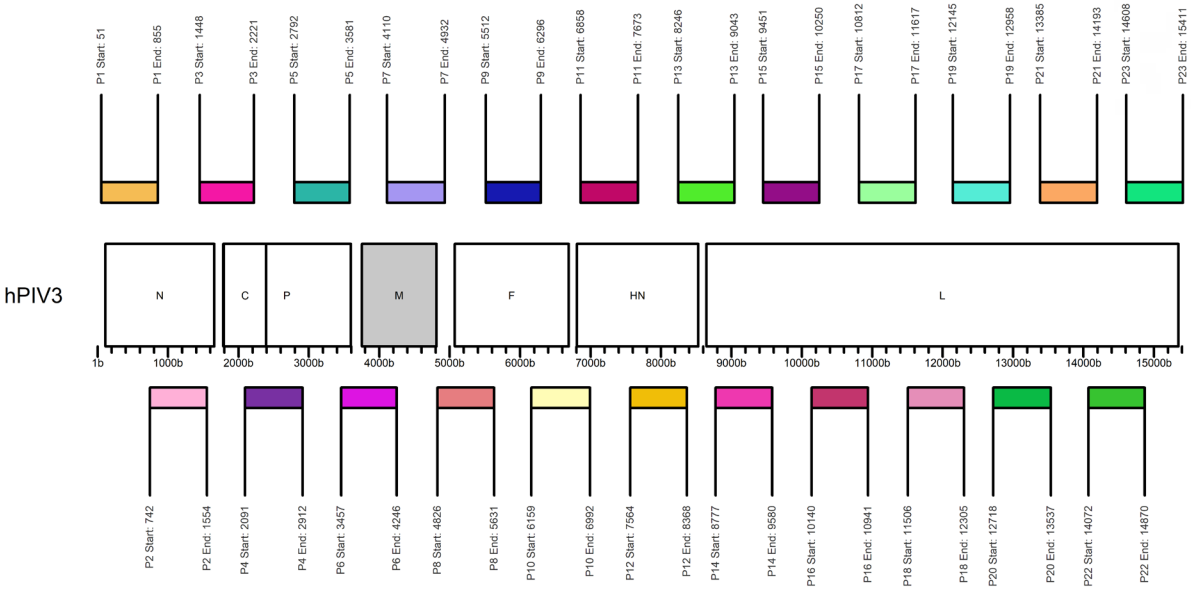

Fig. 1. Scheme of primer arrangement on the genome of parainfluenza virus type 3

With a data volume of 300 thousands reads per sample, the developed panel demonstrated median coverage, allowing for the establishment of a consensus genome sequence (Fig. 2). The proportion of the genome with coverage of more than 20x was around 95 % for most sequences. As a result of this study, 15 full-genome sequences of human parainfluenza virus type 3 from Russia were obtained for the first time. The developed primer panels can be used in the framework of genomic surveillance of ARVI pathogens.

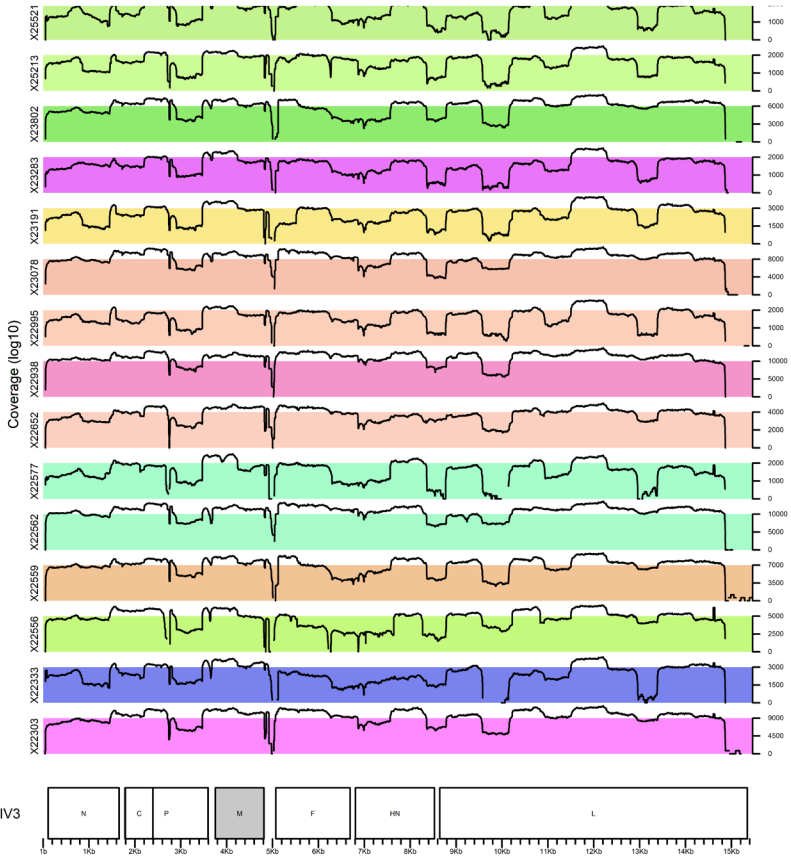

Fig. 2. Coverage plot of hPIV3 sequences. The coverage plot was made using VizCov R script (https://github.com/LMV-NIC-St-Petersburg/VizCoV)

The genetic analysis conducted during the study showed heterogeneity in the composition of circulating populations of human parainfluenza virus type 3. Phylogenetic analysis of the HN gene showed the presence of various phylogenetic groups among the viruses studied. It was established that HN sequences belong to cluster C, but within it are distributed among different subclusters.

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