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Molecular and genetic characteristics of group A rotaviruses detected in Moscow in 2015–2020

Olga A. Petrusha[1]✉, Ekaterina R. Korchevaya[1], Ramil R. Mintaev[1], Alexandra A. Nikonova[1], Igor Yu. Isakov[1], Elena R. Meskina[1,2], Anna Yu. Ushakova[2], Marima K. Khadisova[2], Vitaly V. Zverev[3], Evgeny B. Faizuloev[1]

[1]I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia;
[2]M. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Russia;
[3]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

Abstract

The **aim** of the study was to analyze genetic characteristics of strains belonging to group A rotaviruses (RVA) circulating in Moscow in 2015–2020, including rare strains non-typeable by polymerase chain reaction (PCR).

Materials and methods. A total of 289 stool samples were tested; the samples were collected from children aged 1 month to 17 years, hospitalized with acute gastroenteritis. Immunochromatography and real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) assays were used for detection of rotaviruses in the samples. The rotavirus genome sequencing was performed using the Sanger technique and nanopore sequencing.

Results and discussion. RVA RNA was detected in 131 clinical samples, and the *G/[P]* genotype was identified in 125 samples. The general profile showed prevalence of *RVA* strains with the *G9P[8]I1* genotype (37%) followed by *G3P[8]I2*, *G4P[8]I1*, *G2P[4]I2*, *G1P[8]I1,* and *G3P[8]I1* variants (18, 15, 11, 5, and 2%, respectively). Seven (5%) isolates were identified as *GxP[8]*. In 2015–2020, the region reported a decline in *G4P[8]I1* genotype prevalence (from 39% to 9%) and an increase in the proportion of the *G9P[8]I1* genotype (from 6% to 37%) as compared to 2009–2014. In 2018–2020, a large number of cases with the previously unknown DS-1-like reassortant strain with the *G3P[8]I2* genotype were reported; the above strain has become widely common worldwide in the recent years. Nanopore sequencing was performed to analyze the genome of the *G3P[8]I2* strain and the rare *G4P[6]I1* strain. It was found that the *G4P[6]I1* strain was phylogenetically related to porcine rotaviruses.

Conclusion. In the recent years, the genetic diversity of RVA circulating in the Moscow Region has changed significantly. The obtained results prove the importance of continuous monitoring of rotavirus infection and selective sequencing of RVA genes to fine-tune data of the type-specific real-time RT-PCR. The ever-changing genetic composition of the circulating RVA strains calls for regular optimization of RVA genotyping systems based on real-time RT-PCR.

Keywords: *group A rotaviruses, acute gastroenteritis, G/[P]-genotyping, nanopore sequencing*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the I. Mechnikov Research Institute of Vaccines and Sera (Protocol No. 6, September 24.2021).

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Научная статья
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Молекулярно-генетические особенности ротавирусов группы А, выявленных в Москве в 2015–2020 гг.

Петруша О.А.[1]✉, Корчевая Е.Р.[1], Минтаев Р.Р.[1], Исаков И.Ю.[1], Никонова А.А.[1], Мескина Е.Р.[1,2], Ушакова А.Ю.[2], Хадисова М.К.[2], Зверев В.В.[3], Файзулоев Е.Б.[1]

[1]Научно-исследовательский институт вакцин и сывороток имени И.И. Мечникова, Москва, Россия;
[2]Московский областной научно-исследовательский клинический институт имени М.Ф. Владимирского, Москва, Россия;
[3]Первый Московский государственный медицинский университет имени И.М. Сеченова (Сеченовский Университет), Москва, Россия

Аннотация

Цель работы — анализ генетических характеристик штаммов ротавирусов группы А (РВА), циркулировавших в Москве в 2015–2020 гг., включая редкие штаммы, нетипируемые методом полимеразной цепной реакции (ПЦР).

Материалы и методы. Исследовали 289 фекальных образцов от детей в возрасте от 1 мес до 17 лет, госпитализированных с острым гастроэнтеритом. Выявление ротавирусов в образцах проводили методами иммунохроматографии и обратной транскрипции (ОТ) с ПЦР в реальном времени (ОТ-ПЦР-РВ). Секвенирование ротавирусного генома проводили по Сэнгеру и методом нанопорового секвенирования.

Результаты и обсуждение. В 131 клиническом образце была выявлена РНК РВА, в 125 случаях из них был установлен *G/[P]*-генотип. В общей структуре преобладали штаммы *PBA* с генотипом *G9P[8]I1* (37%), за ними следовали варианты *G3P[8]I2*, *G4P[8]I1*, *G2P[4]I2*, *G1P[8]I1* и *G3P[8]I1* (18, 15, 11, 5 и 2% соответственно). Семь (5%) изолятов были идентифицированы как *GxP[8]*. В 2015–2020 гг. в регионе снизилась частота встречаемости генотипа *G4P[8]I1* (с 39 до 9%) и выросла доля генотипа *G9P[8]I1* (с 6 до 37%) по сравнению с 2009–2014 гг. В 2018–2020 гг. выявлена высокая доля не встречавшегося ранее DS-1-подобного реассортантного штамма *G3P[8]I2*, широко распространившегося в мире в последние годы, Методом нанопорового секвенирования проведён анализ генома штамма *G3P[8]I2* и редкого штамма *G4P[6]I1*. Для штамма *G4P[6]I1* установлена тесная филогенетическая связь с ротавирусами свиней.

Заключение. За последние годы в генетической структуре РВА, циркулирующих на территории московского региона, произошли существенные изменения. Полученные результаты свидетельствуют о необходимости постоянного мониторинга ротавирусной инфекции и выборочного секвенирования генов РВА для уточнения данных типоспецифической ОТ-ПЦР-РВ. Из-за постоянных изменений генетического состава циркулирующих штаммов РВА требуется периодическая оптимизация систем генотипирования РВА на основе ОТ-ПЦР-РВ.

Ключевые слова: *ротавирусы группы А, острый гастроэнтерит, G/[P]-генотипирование, нанопоровое секвенирование*

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Group A rotaviruses (RVA) are a major cause of acute gastroenteritis hospitalizations of children aged under 5 years in countries characterized by a low rotavirus vaccination coverage. In 2016, rotavirus infection (RVI) was responsible for 258 million episodes of diarrhea and 128,500 deaths among children younger than 5 years worldwide [1].

In 2020, vaccination against RVA was included in the National Immunization Programs in 107 countries[1]. In Russia, immunization against RVI is included in the National Vaccination Schedule as required for epidemic reasons. Currently, four rotavirus vaccines have been

[1] URL: https://preventrotavirus.org/vaccine-introduction/global-introduction-status

recommended by WHO and approved for application: pentavalent vaccines — RotaTeq (Merck & Co., Inc.), ROTASIIL (Serum institute of India PVT. Ltd.); and monovalent vaccines — Rotarix (GlaxoSmithKline), ROTAVAC (Bharat Biotech). Mass vaccination against RVA decreases incidence and acute gastroenteritis hospitalization rates in all age groups, especially among infants and people over 65 years [2].

RVAs belong to species *Rotavirus A*, genus *Rotavirus*, family *Reoviridae*. The rotavirus genome consists of 11 segments of double-stranded RNA, which encode 12 proteins [3]. The present-day system of rotavirus classification offers genotyping for all 11 genes (*Gx–P[x]–Ix–Rx–Cx–Mx–Ax–Nx–Tx–Ex–Hx*). Most of the circulating human RVAs belong to 3 evolutionary lines different in genome constellations: *Wa*-like strains (*G1–P[8]–I1–R1–C1–M1–A1–N1–T1–E1–H1*), *DS-1*-like (*G2–P[4]–I2–R2–C2–M2–A2–N2–T2–E2–H2*), and *AU-1*-like strains (*G3–P[3]–I3–R3–C3–M3–A3–N3–T3–E3–H3*) [3]. The previously used binary system of typing addressed only VP7 (*G*) and VP4 (*P*) proteins. *G/[P]*-genotyping of RVA strains is performed by an reverse transcription followed by multiplex polymerase chain reaction (RT-PCR) [4] or sequencing of the respective genes.

At present, *G1P[8]*, *G2P[4]*, *G3P[8]*, *G4P[8]*, *G12P[8],* and *G9P[8]* genotypes, homotypic or partially heterotypic towards the licensed vaccines (RotaTeq and Rotarix), prevail among circulating RVA strains worldwide [5]. There have been described cases of interspecies transmission of RVA from animals to humans as well as cases when humans were infected with strains resulting from reassortment of animal and human rotavirus strains [6–8].

The evolutionary changes of RVA strains over time [9], territorial differences in distribution of circulating strains, their increased diversity after implementation of immunization in some regions, changes in the genotypic structure both in countries with and without scheduled vaccination require continuous epidemiological monitoring of RVI [10].

The **aim** of the study is the analysis of genetic characteristics of the RVA strains circulating in Moscow in 2015–2020, including rare strains, which are non-typeable by PCR tests.

Materials and methods

Clinical samples

During 5 years (2015–2020), a total of 289 stool samples were collected from children aged 1 month to 17 years, displaying symptoms of acute gastroenteritis and hospitalized to the Vladimirsky Moscow Regional Research Clinical Institute. Children vaccinated against RVI were not included in the study. The informed consent was received from the parents or legal guardians of all tested children.

A total of 45 samples were identified as positive for the RVA antigen using immunochromatography (the RIDA Quick Rotavirus reagent kit, R-Biopharm AG). Stool samples were collected from patients with diarrhea (not later than 72 hours after the onset of symptoms) into sterile containers; before they were shipped to the laboratory, samples were stored at –20ºC, then were shipped to the Mechnikov Research Institute for Vaccines and Sera for genetic analysis of rotaviruses.

RNA extraction

To isolate RNA, we used 10% fecal extracts diluted in sterile saline and cleared through centrifugation (5,000 rpm, 5 min). To isolate RNA from the extracts, we used an AmpliSens® MAGNO-sorb kit (InterLabService) in accordance with the manual. The RNA samples were stored at 80ºC.

Detection of rotavirus RNA

To detect RVA in clinical samples by real-time RT-PCR, we used single-tube reaction with primers and probes described earlier [11]. Genotyping RVA using two-stage multiplex real-time PCR for VP7 proteins (*G1*, *G2*, *G3*, *G4*, *G9*), VP4 (*P[4]*, *P[6]*, *P[8]*), and VP6 (*I1*, *I2*) was performed in accordance with the procedure requirements [12]. The analysis of RVA genotyping based on multiplex RT-PCR as well as the subsequent analysis of PCR products using agarose gel electrophoresis were used as reference methods and performed in accordance with the WHO recommendations [4].

Amplification and sequencing of VP7 and VP4 genes

For sequencing of VP7 and VP4 genes of *G3P[8] I2* strains, we performed amplification using the previously described VP7F/VP7R [13] and VP4F/VP4R [14] primers generating amplicons 881 and 663 base pairs long, respectively. Aliquots of the extracted RNA (10 µl) were mixed with 3 pmol VP7F or VP4F primers, incubated at 95ºC for 1 min and cooled down for 2–3 min to room temperature. The RT test was performed in the 25 µl reaction mixture containing an RT primer, 25 units of MMLV reverse transcriptase (Syntol, Russia). The RT stage included incubation for cDNA synthesis at 45ºC for 30 min and inactivation of the MMLV reverse transcriptase at 95ºC for 5 min. The temperature and time parameters for the real-time PCR were as follows: 95ºC — 120 sec; 95ºC — 60 sec, 52ºC — 40 sec, 72ºC — 40 sec (45 cycles); 72ºC — 40 sec. PCR-amplicons of each gene were purified with a Cleanup Standard kit (Eurogen); the sequencing of both DNA strands was performed using a NANOPHORE®05 genetic analyzer (Syntol) and a reagent kit from the Syntol Company.

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Nanopore sequencing of the rotavirus genome and bioinformatic analysis

The full-genome cDNA of rotavirus gene segments was produced using a mix of unRAf1, unRAf2, unRAf3, unRAr1, unRAr2, and unRAr3 primers (**Table 1**) for the RT test. For concurrent amplification of all segments of the RVA gene, we used universal primer Up [15] (Table 1). The amplification was performed in the following reaction mixture: TaKaRa LA Taq polymerase (2.5 units), LA PCR Buffer ll (Mg^{2+}plus), 25 mM $MgCl_2$ (final concentration Mg^{2+} 5 mM) (TaKaRa), dNTP 25 mM (Syntol), 40 pmol Up primer. The amplification parameters: 95ºC — 2 min, then 40 cycles at 95ºC, each for 30 sec, 65ºC — 30 sec, 68ºC — 3.5 min. PCR amplicons were purified using phenol/chloroform extraction. The concentration and purity of amplicons were measured by spectrophotometry (A260/230, 260/280).

The DNA library for nanopore sequencing (NPS) was constructed using a Rapid Sequencing reagent kit with a portable MinION sequencer and standard flowcell R9 (Oxford Nanopore Technologies). We developed a pipeline for accurate classification of NPS data. The Python programming language-based pipeline identified the received reads, actuating the BLAST tool-based analysis for the database of reference sequences of rotavirus genome segments[2].

Then, the reads were mapped to the reference sequence using the Minimap2 program [16]; the consensus sequence was created using the script[3] written in the Python language. In the consensus sequence, we selected a nucleotide with the highest frequency at the alignment position. In rare cases, when two nucleotides were found at the same position in the equal quantity, the second nucleotide was ignored. The RVA genotype was identified by the nucleotide sequence of gene segments using the Rotavirus A Genotype Determination online service[4] based on the software from Dan Katzel [17].

The method of nanopore sequencing of the rotavirus genome is described in detail in the article by Faizuloev et al. [18].

GenBank accession numbers (NCBI)

The nucleotide sequences corresponding to 10 segments of the genome of *Moscow-40/2020* (*G3P[8]I2*) and *Moscow-1P/2015* (*G4P[6]I1*) isolates have been deposited to GenBank under numbers MW558493–MW558502 and MT876633–MT876642, respectively. The partial sequences of three other strains with the *G3P[8]I2* genotype have been deposited under numbers MT648671, MT648671, MT648671 for VP7 and MT814324, MT814326 for VP4.

Table 1. Primers for amplification of full length rotavirus gene segments

Primer	Primer sequence 5'–3'
unRAf1	GCCGGAGCTCTGCAGAATTCGGCTWTWAAA
unRAf2	GCCGGAGCTCTGCAGAATTCGGCTTTTTTT
unRAf3	GCCGGAGCTCTGCAGAATTCGGCTTTTAAT
unRAr1	GCCGGAGCTCTGCAGAATTCGGTCAYATC
unRAr2	GCCGGAGCTCTGCAGAATTCGGTCACAWA
unRAr3	GCCGGAGCTCTGCAGAATTCAGCCACATG
Up	GCCGGAGCTCTGCAGAATTC

Phylogenetic analysis

Phylogenetic trees were built using the MEGA-X program [19] and the maximum likelihood method (the Kimura two-parameter model [20]). The bootstrap test included 1,000 replications. The RVA strains recommended by the Rotavirus Classification Working Group [3] and the strains having, based on the BLAST data, at least 99.5% similarity to the studied strains were used as reference strains.

Results

A total of 289 stool samples were tested for presence of the RNA or RVA antigen; all of them were collected from children hospitalized with symptoms of acute gastroenteritis. RNA was detected in 131 (45%) samples and was further used for *G*/[*P*]-genotyping by real-time RT-PCR and/or sequencing. The RVA *G*/[*P*]-genotype was detected in 125 samples (95.4%), and in 7 (5.3%) samples. RVA was defined only by the *P* gene, while no genotype was identified for 6 (4.6%) samples.

The genetic composition of the studied RVA strains is presented in **Fig. 1**. In 2015–2020, RVA strains with the *G9P[8]I1* genotype prevailed in the overall profile (37%), the second place was taken by *G3P[8]I2* (a new DS-1-like strain) - 18%, which was followed by *G4P[8]I1* (15%), *G2P[4]I2* (11%), *G1P[8]I1* (5%), and *G3P[8]I1* (2%). Seven (5%) strains defined only by the *P* gene belonged to the *P* gene variant *[8]*. We have also found a single case of co-infection with two genotypes (*G9P[8]I1* and *G2P[4]I2*) and a rare strain with the *G4P[6]I1* genotype.

Fig. 2 shows the year-to-year distribution of the RVA genotypes identified in the Moscow Region. In the last years, the proportion of the *G9P[8]I1* genotype has increased significantly, ranging from 36% to 41% during 2015-2020. At the same time, the prevalence of *G4P[8]I1* genotypes decreased from 38% to 9%, while the prevalence of the *G2P[4]I2* genotype increased to 14%.

[2] URL: https://github.com/lioj/bioinformatics/blob/master/py/classificationStat.py
[3] URL: https://github.com/lioj/bioinformatics/blob/master/py/bam2consensus.py
[4] URL: https://www.viprbrc.org/brc/rvaGenotyper.spg?method=ShowCleanInputPage&decorator=reo

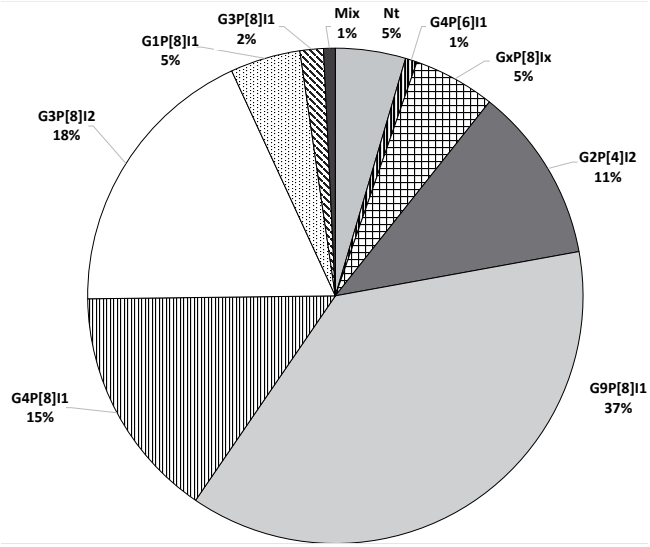

Fig. 1. Distribution of RVA *G*/[*P*]-genotypes isolated from 131 clinical samples in 2015–2020.

GxP[8]Ix — partially typed PVA; Mix — mixed genotypes; Nt — non-typed samples.

Note that the partial sequencing of VP7 (G) and VP4 (P) genes was required for G/[P]-genotyping of some strains with the GxP[8]I2 genotype, as the real-time RT-PCR-based laboratory assay was not able to identify their G/[P]-genotype [12]. The sequencing and phylogenetic analysis of RVA G and P genes (the GenBank numbers: MT648671, MT648671, MT648671 for the G gene and MT814324, MT814326 for the P gene) in the sample with detected GxP[8]I2 showed that they belong to the G3P[8]I2 genotype (Fig. 3). It was found that the absence of a signal during real-time RT-PCR genotyping was caused by the mismatch of nucleotides between the VP7 gene with the G3P[8]I2 genotype and the respective probe. The non-typeable samples with the GxP[8]I2 genotype were tested with mono-specific PCR with primers to G3 and electrophoretic detection, which identified amplicons with the expected mobility in all samples (the data are not provided), thus confirming that they belonged to the G3P[8]I2 genotype.

The BLAST search and the phylogenetic analysis demonstrated the similarity of the sequenced *VP7* and *VP4* genes to the same RVA genes of the new G3-DS-1-like constellation detected worldwide. The similar G3-DS-1-like strains were detected in Australia (2013) [21], Spain (2014–2015) [22], Hungary (2016) [23], Brazil (2016) [24], Indonesia (2016) [25], Russia (2019) [28], and other countries (Fig. 3).

In addition, the nanopore sequencing of the Moscow-40/2020 isolate with the *G3P[8]I2* genotype was performed (**Table 2**). It helped identify gene variants of 10 genome segments (the GenBank numbers MW558493–MW558502) and demonstrated a high degree (91.0–99.8%) of similarity between the consensus sequence and the reference strain RVA/Human-wt/THA/SKT-281/2013/G3P[8] (the GenBank numbers LC086714–LC086724). The rare *Moscow-1P/2015* strain with the *G4P[6]I1* genotype was detected in the clinical sample collected from an 8-year-old patient in 2015. Real-time PCR was able to identify only the *P[6]* gene variant. Sanger sequencing and NPS helped identify the genotype of this strain by 10 genes: *G4-P6-I1-R1-C1-M1-A1-N1-T1-Ex-H1* (the GenBank numbers

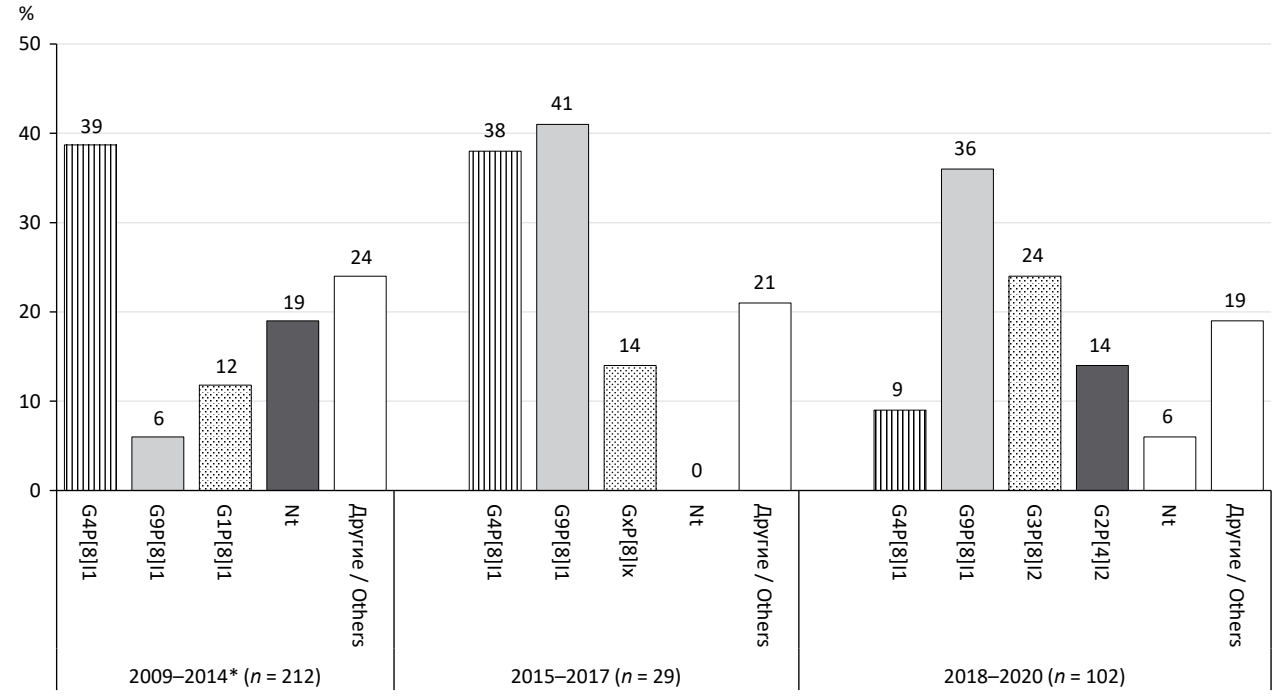

Fig. 2. Prevalence of *G*/[*P*]-genotypes detected in Moscow during the 10-year monitoring period (2009–2020) [12].

Nt — non-typed samples.

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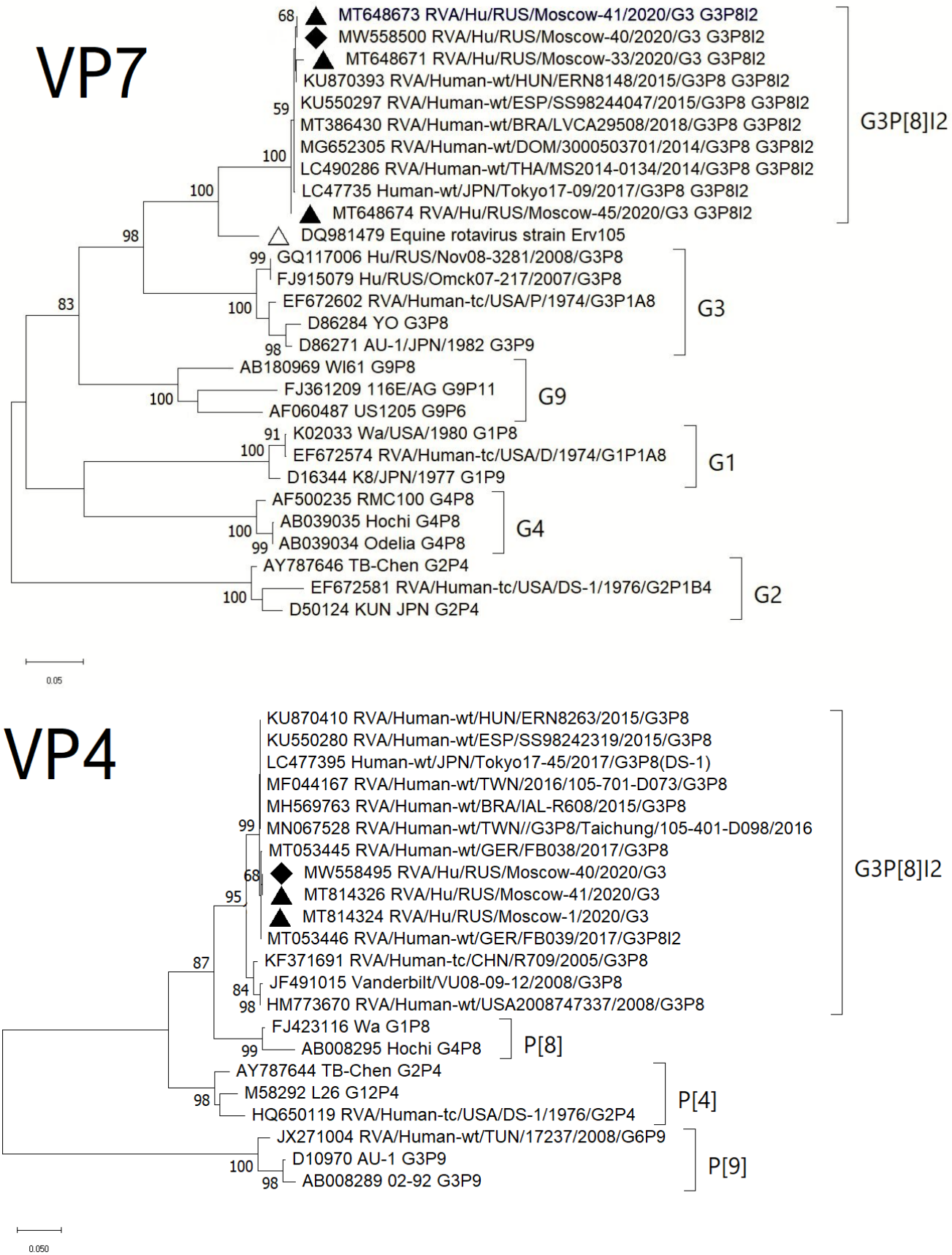

Fig. 3. Phylogenetic trees based on the sequenced genes of the VP4 and VP7 proteins of the new *G3P[8]I2* strain.

▲ — segments sequenced using the Sanger technique; ♦ — the gene sequenced using the NPS technique; Δ — the equine RVA VP7 gene. The respective GenBank accession number, name of strains and *G*/[*P*]-genotype were used for designation of strains.

Table 2. Results of nanopore sequencing of the genome of RVA strains with *G3P[8]I2* and *G4P[6]I1* genotypes

Segment	Viral protein	*G4P[6]I1*			*G3P[8]I2*		
		number of reads	segment coverage, %	genotype	number of reads	segment coverage, %	genotype
1	VP1	1,372	100	R1	12,792	100	R2
2	VP2	3,795	100	C1	11,193	100	C2
3	VP3	1,995	100	M1	–	–	Mx
4	VP4	633	20	P[6]	2,785	100	P[8]
5	NSP1	546	100	A1	53,841	100	A2
6	VP6	15,187	100	I1	16,479	100	I2
7	NSP3	14,566	100	T1	80,554	100	T2
8	NSP2	175	36	N1	40,022	100	N2
9	VP7	1,465	100	G4	25,059	100	G3
10	NSP4	–	–	Ex	10,222	100	E2
11	NSP5/6	77	100	H1	11,351	100	H2

MT876633–MT876642, MG271938), accounting for 81.7% of the genome (Table 2).

The phylogenetic analysis of genes *VP7, VP6,* and *VP4* strain *Moscow-1P/2015* (**Fig. 4**) demonstrates a high degree of similarity of the analyzed sample to genes of porcine RVA (the GenBank numbers: *VP4* — KX363402, MK227950, KX363435, MK227948; *VP6* — MK227391, MK227402, KX363414, MG066585, KJ126830; *VP7* — JX498957, JX498956, MK227392, MN133419, MN133444) or RVA strains (strain *RVA/Human-wt/CHN/R1954/2013/G4P[6]* having the GenBank numbers: KF726066–KF726076, KF726056) and isolated from human feces, though having the confirmed origin from porcine RVA [7]. The BLAST analysis of the other 7 genes also demonstrates a high degree of similarity (92–98%) of the nucleotide sequence to porcine RVA strains. Thus, the phylogenetic analysis indicates that the *G4P[6]* strain is of porcine RVA origin.

Discussion

The obtained results are indicative of significant changes, which took place in the "genetic landscape" of RVAs circulating in the Moscow Region. Based on the data from the previous studies [12, 26], before 2015, most of the genotyped RVA belonged to genotype *G4P[8]*. Our data indicate a gradual decrease in the prevalence of this genotype, from 38% to 9% in 2017–2018. At the same time, the proportion of the *G9P[8]* genotype increased to 36–41% in the Moscow Region. These data are consistent with the data obtained during the independent studies that were performed in Moscow [27, 28], Nizhny Novgorod [29, 30], and Orenburg [31].

Our study was focused on clinical samples collected from children hospitalized with acute gastroenteritis. The actual "genetic landscape" and the distribution of RVA genotypes circulating in Moscow can differ from the obtained data, since we did not include patients with mild or moderate gastroenteritis, not requiring hospitalization, in our study. Previously, in the study by E.R. Meskina, it was pointed out that severe rotavirus gastroenteritis could be associated with specific RVA genotypes [32].

The strain with genotype *G3P[8]I2*, which was discovered by us in 2018, is of special interest. It accounted for 18% in the RVA group, coming second only to the *G9P[8]I1* genotype (Fig. 1). Based on the sequencing and phylogenetic analysis (Fig. 3), we can assume that *G3P[8]I2* is related to the reassortant strain, which was first detected in Thailand in 2013 [25] and has become common in Europe, Asia, and Australia in the last years [7, 22, 33, 34]. This strain has a *DS-1*-like constellation of genes (*G3–P[8]–I2–R2–C2–M2–A2–N2–T2–E2–H2*), except for the gene of the *VP7* protein, which in the cases typical of this constellation is represented by the *G2* genovariant, while the *Wa*-like constellation is more common for the *G3* genovariant [35]. Some researchers [21, 22, 25] assume that the *G3P[8] I2* strain owes its emergence to reassortment of the human *DS-1*-like strain and the equine *Erv105* strain (the GenBank number: DQ981479.1), as the gene of the *VP7* protein of this strain demonstrates the highest similarity. During this study, we, like some other research groups [22], ran into a problem, finding it impossible to genotype a new virus strain using type-specific PCR. Both our own assay and the primers recommended by WHO [4] failed to detect the gene of the *VP7* protein of the *G3P[8]I2* strain. At the same time, the Sanger sequencing and the BLAST analysis were more efficient and made it possible to identify the genovariant as *G3*. Point mutations in genes *VP4* and *VP7* in many cases prevent type-specific PCR primers from identifying their variant [17]. Hence, the importance of sequencing methods in RVI epidemiological monitoring has increased.

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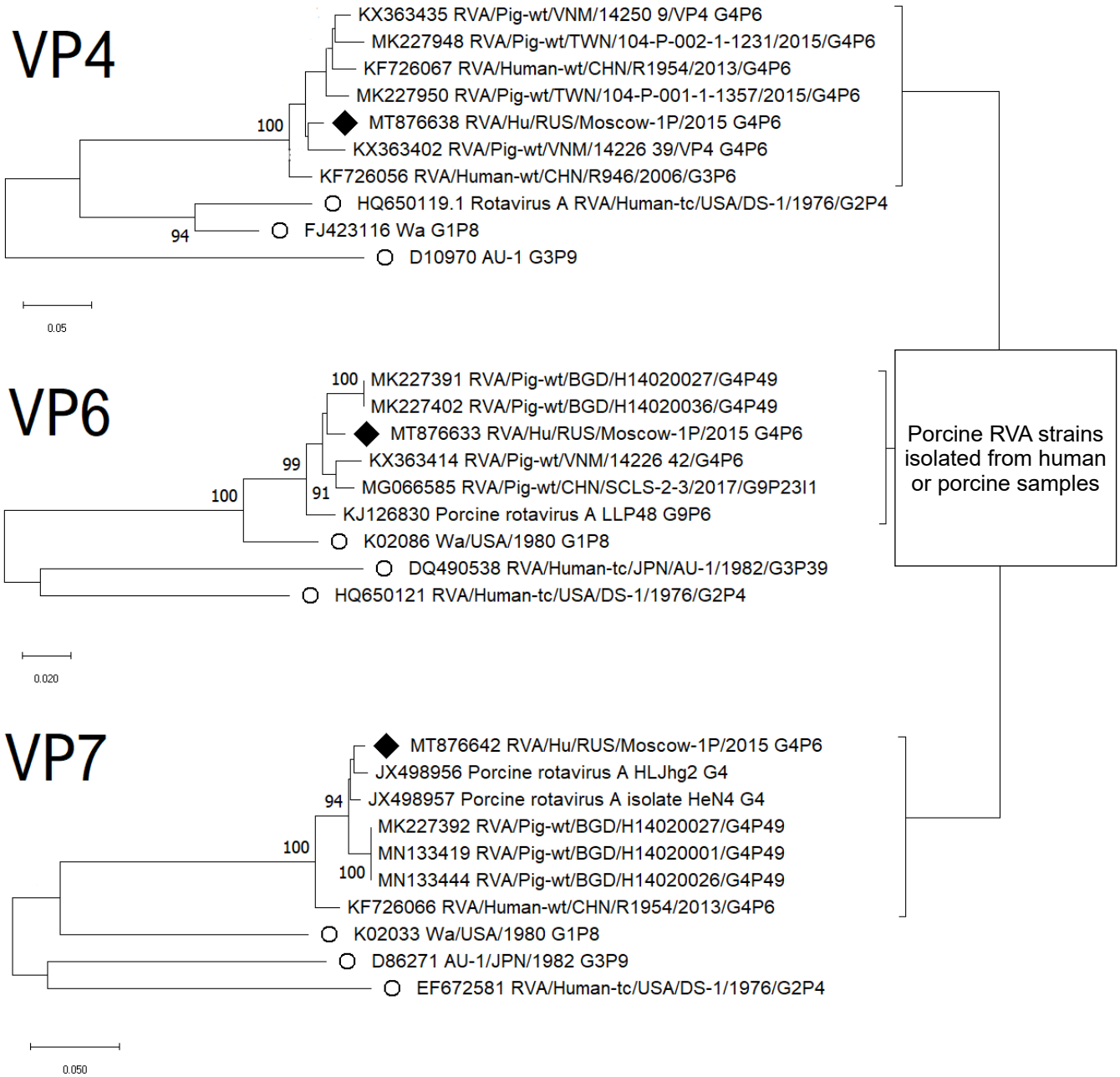

Fig. 4. Phylogenetic trees based on sequenced genes of RVA VP4, VP7, and VP6 proteins, strain *G4P[6]I1* (marked by ♦), reference strains (*Wa*, *AU-1*, *DS-1*) representatives of three evolutionary lines *RVA* (marked by ○) and RVA genes of porcine origin, phylogenetically most closely related to genes of the *Moscow-1P/2015* strain based on the BLAST analysis.

The respective GenBank number, name of the strain and *G*/[*P*]-genotype were used for designation of strains.

Another atypical strain — *Moscow-1P/2015* with the *G4P[6]I1* genotype, which was detected only in one case, may have also resulted from the reassortment of the human and animal RVA or may have the animal origin. In this particular case, it is not clear whether the infection with this strain has a human-to-human transmission route, or the patient was infected with the virus from animals. Based on the literature data, the proportion of RVA with the *G4P[6]I1* genotype is not high in the total diversity of RVA strains detected in humans **[??????].** It may be caused by some factor limiting the *G4P[6]I1* spread in the human population, for example, a species barrier, if we assume that this strain is of animal origin. On the other hand, it should be remembered that most of the related studies tend to focus on children hospitalized with rotavirus enteritis, while mild cases are generally left out of studies. Therefore, the available data do not give any reliable information about the actual prevalence of any of the RVA strains.

Porcine RVA strains are phylogenetically related to strains of human rotaviruses [34]; therefore, it is difficult to find out whether the studied strain resulted from

a direct transmission or reassortment [37–39]. Cases of reassortment or direct interspecies transmission of RVA with the *G4P[6]I1* genotype have been reported and described both in Asia [6, 25, 40] and in Europe [36].

Conclusion

In 2015–2020, the genetic profile of RVA circulating in the Moscow Region changed significantly: The detection frequency of the *G4P[8]I1* genotype, which was the most prevalent in the previous years, decreased; at the same time, the number of hospitalizations with RVI caused by the *G9P[8]I1* strain increased. In addition, the above period is characterized by emergence of RVA strains, presumably of animal origin, both in few numbers (*G4P[6]I1*) and in significant numbers (reassortant *G3P[8]I2*), thus suggesting an important role of interspecies transmission in the evolution of RVA pathogenic to humans. Our study demonstrates the importance of continuous monitoring of RVI. Epidemiological monitoring of RVI provides an effective tool for timely detection of new animal and human reassortant RVAs, which may escape post-vaccinal immunity. The RVA stains that we and other researchers have identified and that are not typeable by real-time RT-PCR (genotypes *G3P[8]I2* and *G4P[6]I1*) [6, 22] prove the significance of selective sequencing of RVA genes and the need to optimize sequences of type-specific primers for real-time RT-PCR.

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Information about the authors

Olga A. Petrusha✉ — junior researcher, Laboratory of molecular virology, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, petrusha.olga@gmail.com, https://orcid.org/0000-0002-5022-7962

Ekaterina R. Korchevaya — junior researcher, Laboratory of molecular virology, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0002-6417-3301

Ramil R. Mintaev — junior researcher, Laboratory of genetics of RNA viruses, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0001-5398-3627

Alexandra A. Nikonova — Cand. Sci. (Biol.), Head, Laboratory of molecular biotechnology, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0001-9610-0935

Igor Yu. Isakov — junior researcher, Laboratory of molecular biotechnology, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0002-5742-6550

Elena R. Meskina — D. Sci. (Med.), Professor, Department of children's infections, Therapy department, M. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Russia, https://orcid.org/0000-0002-1960-6868

Anna Yu. Ushakova — Cand. Sci. (Med.), assistant, Department of children's infections, M. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Russia, https://orcid.org/0000-0001-84387609

Marima K. Khadisova — Cand. Sci. (Med.), Department of children's infections, Therapy department, M. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Russia, https://orcid.org/0000-0001-8293-6643

Vitaly V. Zverev — D. Sci. (Med.), Professor, Academician of the Russian Academy of Sciences, Head, Department of microbiology, virology and immunology, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, https://orcid.org/0000-0002-0017-1892

Evgeny B. Faizuloev — Cand. Sci. (Biol.), Head, Laboratory of molecular virology, I. Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0001-7385-5083

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Информация об авторах

Петруша Ольга Александровна✉ — м.н.с. лаб. молекулярной вирусологии НИИВС им. И.И. Мечникова, Москва, Россия, petrusha.olga@gmail.com, https://orcid.org/0000-0002-5022-7962

Корчевая Екатерина Романовна — м.н.с. лаб. молекулярной вирусологии НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0002-6417-3301

Минтаев Рамиль Рафаилович — м.н.с. лаб. генетики РНК-содержащих вирусов НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0001-5398-3627

Никонова Александра Александровна — к.б.н., зав. лаб. молекулярной биотехнологии НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0001-9610-0935

Исаков Игорь Юрьевич — м.н.с. лаб. молекулярной биотехнологии НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0002-5742-6550

Мескина Елена Руслановна — д.м.н., зав. отд. детских инфекций отдела терапии МОНИКИ им. М.Ф. Владимирского, Москва, Россия, https://orcid.org/0000-0002-1960-6868

Ушакова Анна Юрьевна — к.м.н., ассистент кафедры хирургии МОНИКИ им. М.Ф. Владимирского, Москва, Россия, https://orcid.org/0000-0001-84387609

Хадисова Марима Касумовна — к.м.н., н.с. отд. детских инфекций, отдел терапии МОНИКИ им. М.Ф. Владимирского, Москва, Россия. https://orcid.org/0000-0001-8293-6643

Зверев Виталий Васильевич — д.м.н., профессор, академик РАН, зав. кафедрой микробиологии, вирусологии, иммунологии ПМГМУ им. И.М. Сеченова, Москва, Россия, https://orcid.org/0000-0002-0017-1892

Файзулоев Евгений Бахтиерович — к.б.н., зав. лаб. молекулярной вирусологии НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0001-7385-5083

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