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Estimates the Evolution of G8P[8] Rotaviruses in Thailand during 2012 - 2014

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Abstract

Point mutation and abrupt changes of segmented genomes of rotavirus group A (RVAs) from mixed infection are critical factors for generating genetically divergent strains. Our preceding study of rotavirus infections in 2012 - 2014 in Thailand indicated the high prevalence of G8P[8] strains. This study aimed to identify the origin of each segment of the genome of these G8P[8] RVA strains by using phylogenetic analysis. All gene segments from the 5 selected G8P[8] were sequenced partially and aligned with locally circulating rotavirus strains to estimate the phylogenic relationship. Based on the genome's constellation of the genome, these 5 strains' genetic backgrounds possessed genotype constellation 2 or called the DS-1-like genotype. Ten genes, except for VP6, were clustered within the same phylogenic tree of the cognate genes of Thai circulating G8P[8]strains isolated between 2013 - 2014. Their 5 genes VP2, VP3, NSP1, NSP3, and NSP5, also exhibited high nucleotide similarities with the first unusual Japanese DS-1-like strains G1P[8] NT004. Besides, the VP4 shared a high nucleotide sequence similarity with the cognate gene of circulating Thai human G1P[8] Wa-like and G1P[8] NT004. However, the remaining 4 gene segments (VP1, VP7, NSP2, and NSP4) were clustered with other human or animal RVA strains within the same monophyletic. All of these G8P[8] strains shown to be an intragenogroup reassortment. Thus, characterization of the genomes is crucial to monitor the evolutionary dynamics and relationships of co-circulating RVAs in predicting the effectiveness of current vaccines and future vaccine development strategies.

Keywords: Rotaviruses, Reassortment, Phylogenetic analyses, Genome characterization, Intra-genogroups

Introduction

Rotavirus group A (RVAs) is a significant cause of severe gastroenteritis, resulting in substantial morbidity and mortality in young children and animals - especially in developing countries [1]. The rotavirus genome composed of 11 segments of double-stranded (ds) RNA, encoding for 6 structural proteins (VP1-4, VP 6 and VP7) and 6 non-structural proteins (NSP 1-6) [2,3] assembled to form a triple-layered particle.

The error-prone nature of RNA polymerases during viral replication generates RVA genomic diversity by accumulating point mutations. In addition, the segmented nature of the RVA genomes enables abrupt changes in the RVA genome during mixed infection of different circulating strains, thus generating reassortant virus strains [4]. A dual classification based on nucleotide sequence variability of 2 outer capsid proteins, VP7 and VP4, defines at least the 36 G and 51 P genotypes, respectively [3,5,6]. The various G and P types tend to segregate according to species-specific patterns across the different animal species [7]. The significant genotypes responsible for 90 % of human infected cases are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] [8]. Bovine or bovine-like RVA strains have been detected in VP7 G6, G8, and G10 in conjunction with a variety of P types, P[1], P[2], P[4], P[6], P[8], P[9], P[11] and P[14] [9].

However, analyses focusing on VP7 and VP4 encoding genes were inadequate to obtain convincing evidence on the overall genomic diversity or true origin of RVAs. Nucleotide sequences of the full-genome in which VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genotypes are identified enable a comprehensive characterization of rotavirus strains [10-12]. Based on the constellation of segments composing of the genome, 3 "genogroups," the prototypes of, Wa-1-like (genotype

constellation 1), DS-1-like (genotype constellation 2), and AU-1 (genotype constellation 3) have been represented [13]. The Wa-like strains tended to possess G/P genotypes G1P[8], G3P[8], G4P[8], G9P[8], G12P[6], and G12P[8]. On the other hand, DS-1-like strains were inclined to possess G/P genotype G2P[4]. Nevertheless, the novel human intergenogroup reassortant strains, DS-1-like non-G/P genotype constellation (I2-R2-C2-M2-A2-N2-T2-E2-H2) together with G/P genotypes G1P[8], G2P[8], G3P[8], and G8P[8]; G1/2/3/8-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2 were also reported in Asia and Europe[14-21]. This genogrouping system has provided important information on the complex origin of strains and explored inter-and intra-genogroup reassortment events between human and animal strains.

Our preceding study of rotavirus infections in children hospitalized at Bamrasnaradura Infectious Diseases Institute throughout 2012 - 2014 indicated the high prevalence of G8P[8] strains of up to 21.92 % [22]. The G8 rotavirus genotype is typically derived from strains infecting bovine and other animal host species. Preliminary sequence analysis of the partial VP7 gene amid these strains revealed their close genetic relatedness to the Indian bovine strains. This study aimed to identify the origin of each segment of the genome of these G8P[8] RVA strains by using phylogenetic analysis. Analyses of all gene segments of the viruses gain a better insight into their evolutionary relationships of the G8P[8] strains circulating in Thailand during 2012 - 2014.

Materials and methods

Stool preparation

In 2012 - 2014, a surveillance study for rotavirus infections in 73 stool specimens was collected anonymously from children admitted at Bamrasnaradura Infectious Diseases Institute, Nonthaburi Province, Thailand. The genotypes were confirmed with partial sequencing of the VP8* and VP7 encoding segments. Five samples from 16 samples were randomly selected and identified as G8P8 strains in the present study, including ASH-B1007, ASH-B1025, ASH-B1032, ASH-B1038, and ASH-B2046, for entire genome analyses. The sample were store at –80 ℃ until used.

Viral dsRNA extraction and the initial strand cDNA synthesis

According to the manufacturer's instructions, total RNAs were extracted from selected stool samples utilizing the Viral Nucleic Acid Extraction Kit II (Geneaid). First-strand cDNAs were synthesized with MDEVP1F_1, MDEVP1R_1, MDEVP2F_1, MDEVP2R_2, MDEVP3F_2, MDEVP3R_1, VP6-F_1, VP6-R_1, MDENSP1F_1, MDENSP1R_1, MDENSP2F_1, MDENSP2R_1, MDENSP3F_1, MDENSP3R_2, NSP4_F_JRG30_1, NSP4_R_JRG31_1, MDENSP5F and MDENSP5R_1 specific primer for VP1-3, VP6 and NSP1-5 gene segments, respectively (**Table 1**) [23,24]. The cDNA Synthesis Kit (BR0400403) (Biotechrabbit, Germany) was applied accordingly. RT-PCR reaction contained components in the total of 20 µL (1 mM dNTP mix, 20 U RNAse inhibitor,1.25 µM random Hexamer primer, 1× cDNA synthesis buffer, 100 ng dsRNA template, and 200 U ReverseUP II reverse transcriptase). The reaction was carried out at 50 °C for an hour and subsequently ceased incubation at 99 °C for 5 min. Single-stranded cDNA products were kept at −20 °C until used.

Table 1 List of primer and PCR product size of VP1-3, VP6 and NSP1-5 gene segments [23,24].

Gene	Primer name	Primer sequence	Annealing temperature (°C)	Product size (bp)
VP1	MDEVP1F_1	ATTCACAATCTGCAGTTCAGA	50.5	337
	MDEVP1R_1	AGTGAATCAGTGTACTCTTCT		
VP2	MDEVP2F_1	CTGATAAGGTACTTTCGAA	45.9	378
	MDEVP2R_2	TCACATCATAGTCTCCGTCTGG		
VP3	MDEVP3F_2	GCAAGACTTTCAAATCGCGTA	52.3	325
	MDEVP3R_1	AATACGAGGGTGCTGATCC		
VP6	VP6-F_1	GACGGAGCGACTACATGGT	50.2	379
	VP6-R_1	GTCCAATTCATACCTGGTG		
NSP1	MDENSP1F_1	GAGACCTTCTACTCTAACAAA	45.9	344
	MDENSP1R_1	ACTGTAGTGTAATTGGCAT		
NSP2	MDENSP2F_1	GCTTGCTTTTGTTATCCC	45.5	327
	MDENSP2R_1	ATTTTCTAGATGTCTTACAG		
NSP3	MDENSP3F_1	GCAACTTCTACATTAGAA	42.0	396
	MDENSP3R_2	TGCATCATCCACTTCGACTT		
NSP4	NSP4_F_JRG30_1	GTGCGGAAAGATGGAAAAGC	46.6	708
	NSP4_R_JRG31_1	ACCGTTCCTTCCATTAAC		
NSP5	MDENSP5F	AGCGCTACAGTGATGTCTCT	42.6	337
	MDENSP5R_1	CCATTTGATCGTATCCA		

PCR amplification

Nine 5 G8P[8] RVA strains gene segments were further amplified via consensus primers for structural genes (VP1, VP2, VP3, VP6) and non-structural genes (NSP1, NSP2, NSP3, NSP4, NSP5) (**Table 1**). PCR reaction was carried out in a final volume of 25 µL containing 1x KAPA HiFi buffer, 0.3 mM dNTP mix, 0.3 µM each of gene-specific forward and reverse primer, 12 ng cDNA template, and 0.5 U DNA polymerase (KAPA HiFi HotStart PCR Kit (KK2501) (Kapa Biosystems)). The Thermocycler reaction was initially denatured at 95 °C for 3 min. Then, 35 amplification cycle conditions took place as follows: Denaturation at 98 °C for 30 s, annealing for 30 s (annealing temperature corresponding to each primer are shown in **Table 1**). Additionally, an extension at 72 °C for 45 s, and subsequently through a final extension at 72 °C for 3 min. PCR products were analyzed amid 1 % gel agarose gel electrophoresis. According to the manufacturer's instructions, DNA bands exhibiting the expected size were excised and purified from agarose gel utilizing PCR Clean-Up & Gel Extraction Kit (PureDireX, Taiwan).

Sequence and phylogenetic analysis

Purified PCR products were sequenced with 5 µM forward specific primer via the Sanger sequencing method at Macrogen Inc., Soul, South Korea. The nucleotide sequences of the RVA gene segment sequenced in this study are available on the GenBank database as accession No. MN101727-MN101751 and MT265003-MT265022. Partial genome sequences of the G8P[8] rotavirus strains sequenced in this study and VP4, VP7 gene from previous study [22] were compared among each other and individually compared to locally circulating DS-1-like G2P[4] (BD-20, NP-M51, SKT-138, SSKT-133) and Wa-like G1P[8]strains (PCB118 and SKT98) as well as other rotavirus strains available through the NCBI GenBank database using BLAST (www.blast.ncbi.nlm.gov). Their homology sequences were aligned via the CLUSTALW algorithm. The best-fitted DNA evolution model was applied to estimate the phylogenic relationship via the MEGA7 program. The phylogenic trees were subsequently constructed using the MEGA7 program utilizing the algorithm Maximum likelihood method. Models used in this study were T92(NSP1), T92+G(NSP2), T92+G(NSP3), T92+G+I(NSP4), T92+G(NSP5), T92+G+I(VP1), TN93+G+I(VP2), T92+G+I(VP3), T92+I(VP4), T92+G(VP6) and T92+G+I(VP7).

Results and discussion

Genome sequencing and sequence analysis of 5 selected G8P[8] RVA strains

The partial nucleotide sequence of 11 genes amid ASH-B1007, ASH-B1025, ASH-B1032, ASH-B1038, and ASH-B2046 strains isolated in Thailand throughout 2012 - 2014 were aligned to the viral reference sequences detailed by GenBank. Overall, 10 genes of this Thai G8P[8] strains shared a high degree of nucleotide sequence identity among themselves (98.33 - 100 %). Minimum nucleotide identity (90.52 - 97.99 %) was observed with gene VP6. By applying the nucleotide sequence-based genotyping system, the genotype of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes saw these strains assigned to G8P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2.

To gain insight into their genetic relatedness with the other RVA strains, the nucleotide sequences of 11 genes of these 5 RVAs strains were aligned to the viral reference sequences worldwide from GenBank. These sequences included the genome sequences of the first DS-1-like integenogroup G1P[8] NT004 isolated in Japan in 2012 [14,15,25] and Thai circulating DS-1-like G8P[8] (PCB103, SKT 457, CMH-N165-13)[26,27] and non-G8 strains, i.e., G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133), and Wa-like G1P[8] (PCB118 and SKT98)[16]. Except for VP6, ten genes of these G8P[8] strains shared a high degree of nucleotide sequence identity with the cognate genes of Thai G8P[8] strains, PCB-103 (95.64 - 100 %) and SKT-457 (97.67 - 100 %) isolated in 2013 and 2014, respectively. In addition, 6 genes (VP2, VP3, VP4, NSP1, NSP3, and NSP5) exhibited high nucleotide sequence similarities with DS-1-like G1P[8] NT004 (98.6 - 100 %) and 5 genes (excluding VP4) of the eleven genes shared with authentic genogroup 2, G2P[4] strains isolated in 2013 (> 98 %)[17]. Thus, VP4 is only the gene segment that shared high nucleotide sequence similarities with the cognate gene of circulating Thai human G1P[8], Wa-like, and other G8P[8] strains. Of note, the VP6 gene of only ASH-B1007, ASH-B1025, and ASH-B1032 presented a high degree of identity (> 98 %). Furthermore, ASH-B1038 demonstrated moderately (96.30 - 96.87 %), yet ASH-B2046 showed no identities (88.54 - 89.11%) with the cognate gene sequence of human DS-1-like RVA strains. In addition, nucleotide sequences of NSP4 exhibited relatively low identities (94.32 - 95.57 %), while the other 3 remaining gene segments (VP1, VP7, NSP2) showed no relatedness to those DS-1-like RVA strains (**Table 2**).

Table 2 Percentages of nucleotide identity of the ASH-B1007, ASH-B1025, ASH-B1032, ASH-B1038, ASH-B2046 gene segments with the referenced rotavirus strains.

Reference strains	**Percent identity (%)**										
DS-1-like G2P[4] (BD-20, NP-M51, SKT-138, SSKT-133)	**VP1**	**VP2**	**VP3**	**VP4**	**VP6**	**VP7**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
ASH-B1007	81.18	97.65 - 99.12	97.53 - 98.23	86.41 - 85.58	96.85 - 97.71	73.43 - 72.32	98.38 - 98.71	85.96 - 86.67	96.67 - 99.17	94.32 - 95.06	99.33 - 99.66
ASH-B1025	81.18	97.65 - 99.13	97.17 - 97.88	86.29 - 85.46	95.98 - 96.55	73.43 - 72.32	98.39 - 98.71	86.21 - 86.90	96.94 - 99.44	94.83 - 95.57	98.67 - 99.00
ASH-B1032	81.18	97.36 - 98.83	97.20 - 97.90	86.41 - 85.58	96.83 - 97.69	73.43 - 72.32	98.08 - 98.70	86.64 - 87.36	95.87 - 98.35	94.57 - 95.31	98.68 - 99.01
ASH-B1038	81.18	97.36 - 98.83	96.85 - 97.55	86.29 - 85.70	96.30 - 96.87	73.43 - 72.51	98.41 - 98.73	86.18 - 86.91	96.41 - 98.90	94.83 - 95.57	99.00 - 99.34
ASH-B2046	81.18	97.36 - 98.84	96.85 - 97.55	86.01 - 85.52	88.54 - 89.11	73.80 - 72.69	98.39 - 98.71	86.81 - 87.55	96.19 - 98.64	94.81 - 95.56	98.01 - 98.34
Wa-like G1P[8] (PCB-118, SKT-98)	**VP1**	**VP2**	**VP3**	**VP4**	**VP6**	**VP7**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
ASH-B1007	75.56 - 75.94	79.19	74.43 - 74.81	99.64 - 99.52	75.53 - 75.83	74.54 - 74.35	77.15 - 77.48	81.85	83.43 - 83.71	77.97 - 79.95	90.33 - 91.33
ASH-B1025	75.56 - 75.95	79.19	74.05 - 74.43	99.52 - 99.41	76.03 - 76.34	74.54 - 74.35	76.69 - 77.03	82.21	83.57 - 83.84	78.52 - 80.49	90.69 - 91.72
ASH-B1032	75.56 - 75.96	79.19	74.34 - 74.72	99.64 - 99.52	75.53 - 75.83	74.54 - 74.35	76.90 - 77.23	81.88	82.83 - 83.11	78.22 - 80.20	89.90 - 90.88
ASH-B1038	75.56 - 75.97	79.19	73.96 - 74.34	99.88 - 99.76	75.39 - 75.71	74.72 - 74.54	76.36 - 76.68	81.68	83.43 - 83.70	78.52 - 80.49	90.37 - 91.36
ASH-B2046	75.56 - 75.98	79.19	73.96 - 74.34	99.76 - 99.63	79.29 - 79.59	74.72 - 74.9	76.82 - 77.15	82.22	83.11 - 83.38	78.47 - 80.45	90.34 - 91.38
DS-1-like G8P[8] (PCB-103,SKT-457)	**VP1**	**VP2**	**VP3**	**VP4**	**VP6**	**VP7**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
ASH-B1007	98.96	100	99.65	99.76 - 99.88	88.86 - 98.53	99.64 - 100	99.35	98.97	99.72	99.01 - 99.51	98.99 - 99.66
ASH-B1025	98.96	100	99.65	99.64 - 99.76	89.58 - 97.62	99.64 - 100	99.68	100	100	99.51 - 100	98.33 - 99.00
ASH-B1032	98.96	99.71	99.30	99.76 -99.88	88.76 - 98.52	99.64 - 100	99.04	98.30	98.90	99.26 - 99.75	98.33 - 99.00
ASH-B1038	98.96	99.71	98.95	99.52 - 99.88	88.99 - 96.76	98.57 - 98.93	99.68	98.93	99.72	99.51 - 100	98.66 - 99.33
ASH-B2046	98.96	99.71	98.95	99.63 - 100	88.86 - 98.53	99.64 - 100	99.68	98.31	99.18	99.51 - 100	97.67 - 98.33
DS-1-like G1P[8](NT004)	**VP1**	**VP2**	**VP3**	**VP4**	**VP6**	**VP7**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
ASH-B1007	81.92	100	99.29	99.17	97.34	74.59	99.68	88.13	99.72	94.07	99.66
ASH-B1025	81.92	100	99.29	99.05	97.00	74.59	100	88.17	100	94.58	99.32
ASH-B1032	81.92	99.71	98.95	99.17	97.31	74.59	99.36	87.54	98.90	94.32	99.32
ASH-B1038	81.92	99.71	98.60	99.29	99.11	74.77	100	87.41	99.72	94.58	99.33
ASH-B2046	81.92	99.71	98.60	99.14	89.64	74.95	100	87.94	99.18	94.57	98.64

Phylogenetic analyses of gene segments

Phylogenetic trees were constructed from each of the 11 cognate gene sequences of human and rotavirus isolates from Genbank to gain more insight into the genetic relatedness of these G8P[8] RVA strains. The 10 of 11 genes that exhibited high identities to Thai G8P[8] PCB-103 and SKT-457 also fell into the same cluster in the monophyletic lineage for cognate gene sequences based on their phylogeny. In addition, 5 genes (VP2, VP3, NSP1, NSP3, and NSP5) were arranged into the same cluster in the monophyletic lineage for cognate gene sequences of the first intergenogroup DS-1-like G1P[8] NT004, circulating Thai and Asian G1P[8], G3P[8] strains, and also G2P[4]strains (BD-20, NP-M51, SKT-138 and SSKT-133 (**Figures 1 - 5**, respectively). VP4 fell into the same monophyletic lineage for that of Thai Wa-like G1P[8], Japanese DS-1-like G1P[8] NT004, and other G8P[8] circulating strains (**Figure 6**). VP6, which was the only gene segment displaying a high level of sequence variation among these G8P[8] strains, was arranged into 2 lineages of the phylogenetic tree. In the lineage of VII, VP6 of ASH-B1032, ASH-B1007, and ASH-B1025, strains were subclustered with Thai and Japanese G8P[8] strains. However, ASH-B1038 was grouped with G1P[8] (**Figure 7**). Interestingly, ASH-B2046 was clustered separately in lineage IX with human and animal strains. VP7 and VP1 of these G8P[8] strains were in the same cluster as the Thai human co-circulating G8P[8] and bovine strains in the lineage of V, IV, respectively (**Figures 8** and **9**), respectively); and NSP4 was clustered with bovine and caprine in the lineage of VIII (**Figure 10**). Besides that, NSP2 was arranged with humans and animals, including cows, donkeys, and rabbits in the lineage of III (**Figure 11**).

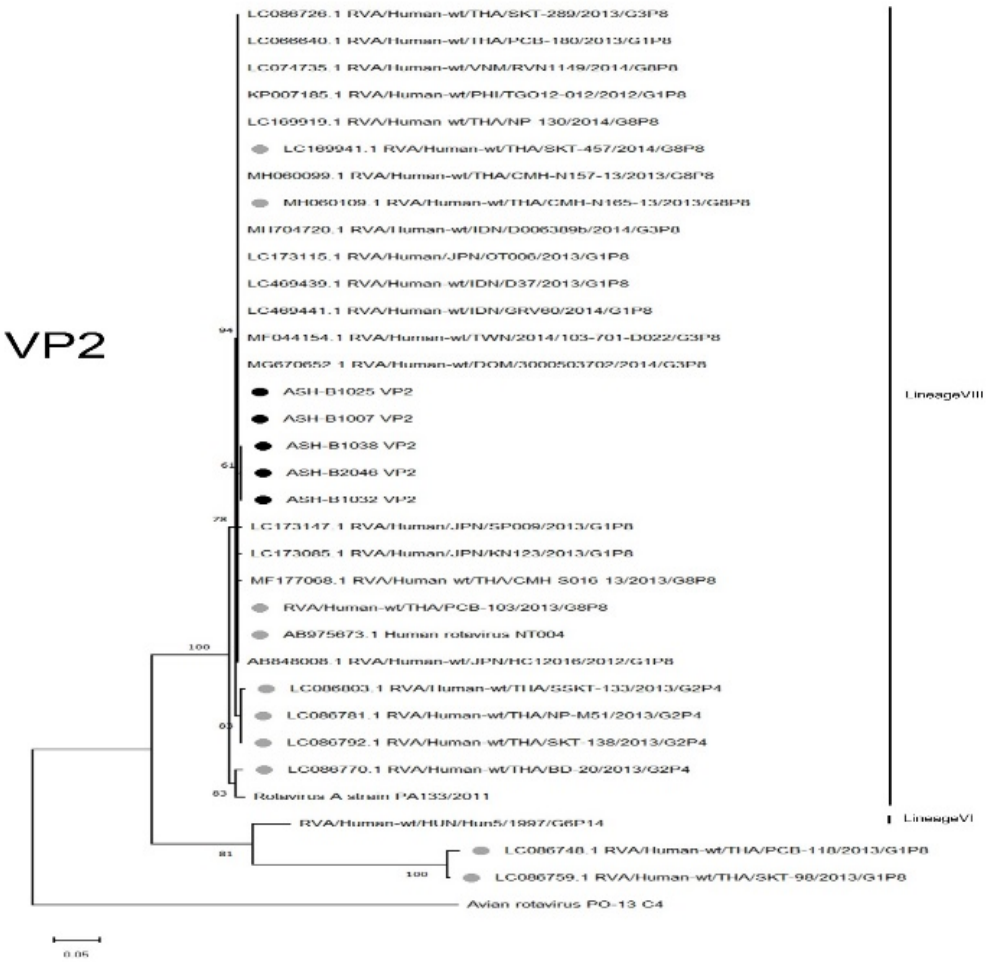

Figure 1 The phylogenetic tree was constructed from the nucleotide sequences of the VP2 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP2 genes of 5 G8P[8] strains. The grey dots show the VP2 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

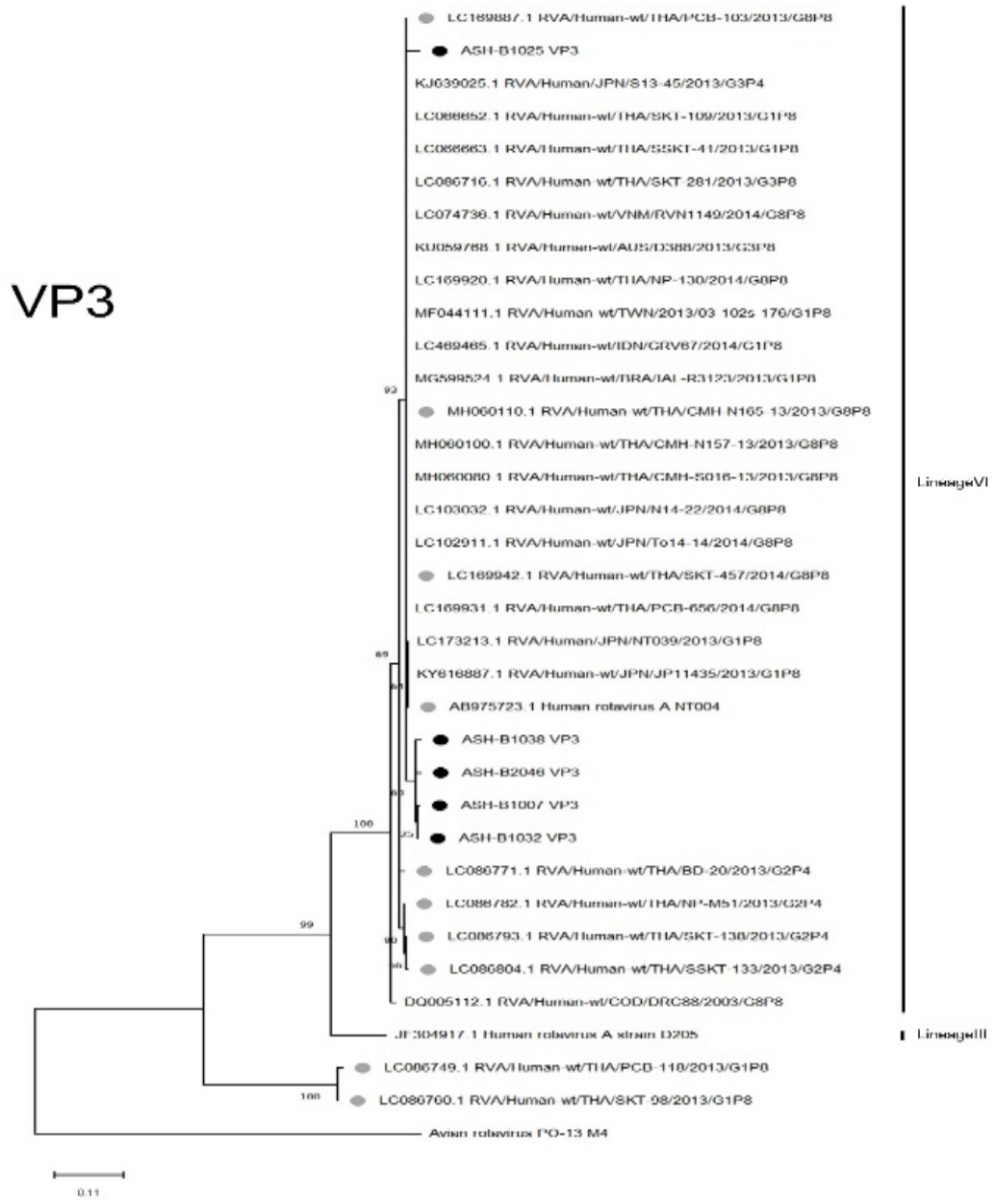

Figure 2 The phylogenetic tree was constructed from the nucleotide sequences of the VP3 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP3 genes of 5 G8P[8] strains. The grey dots show the VP3 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

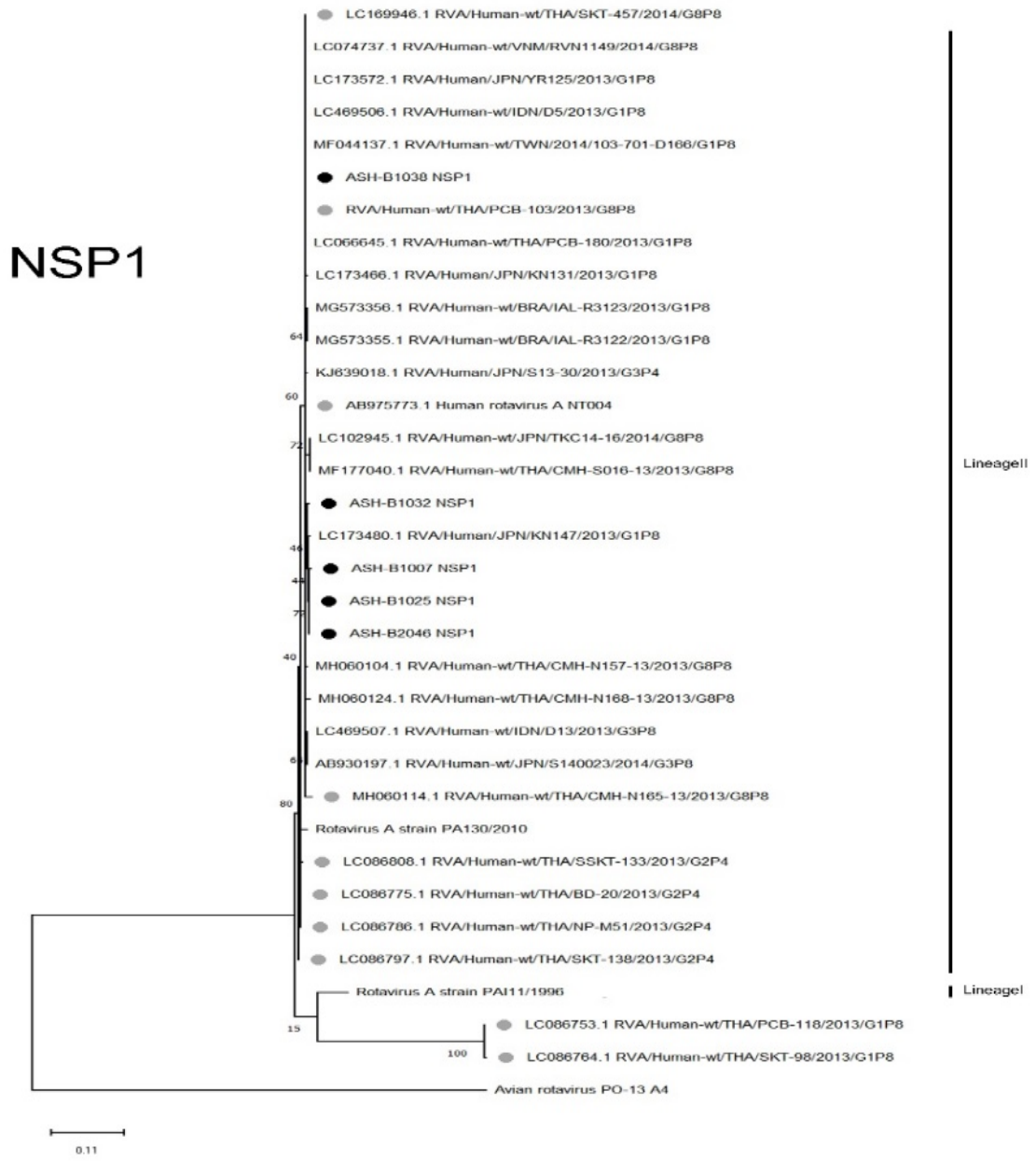

Figure 3 The phylogenetic tree was constructed from the nucleotide sequences of the NSP1 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the NSP1 genes of 5 G8P[8] strains. The grey dots show the NSP1 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

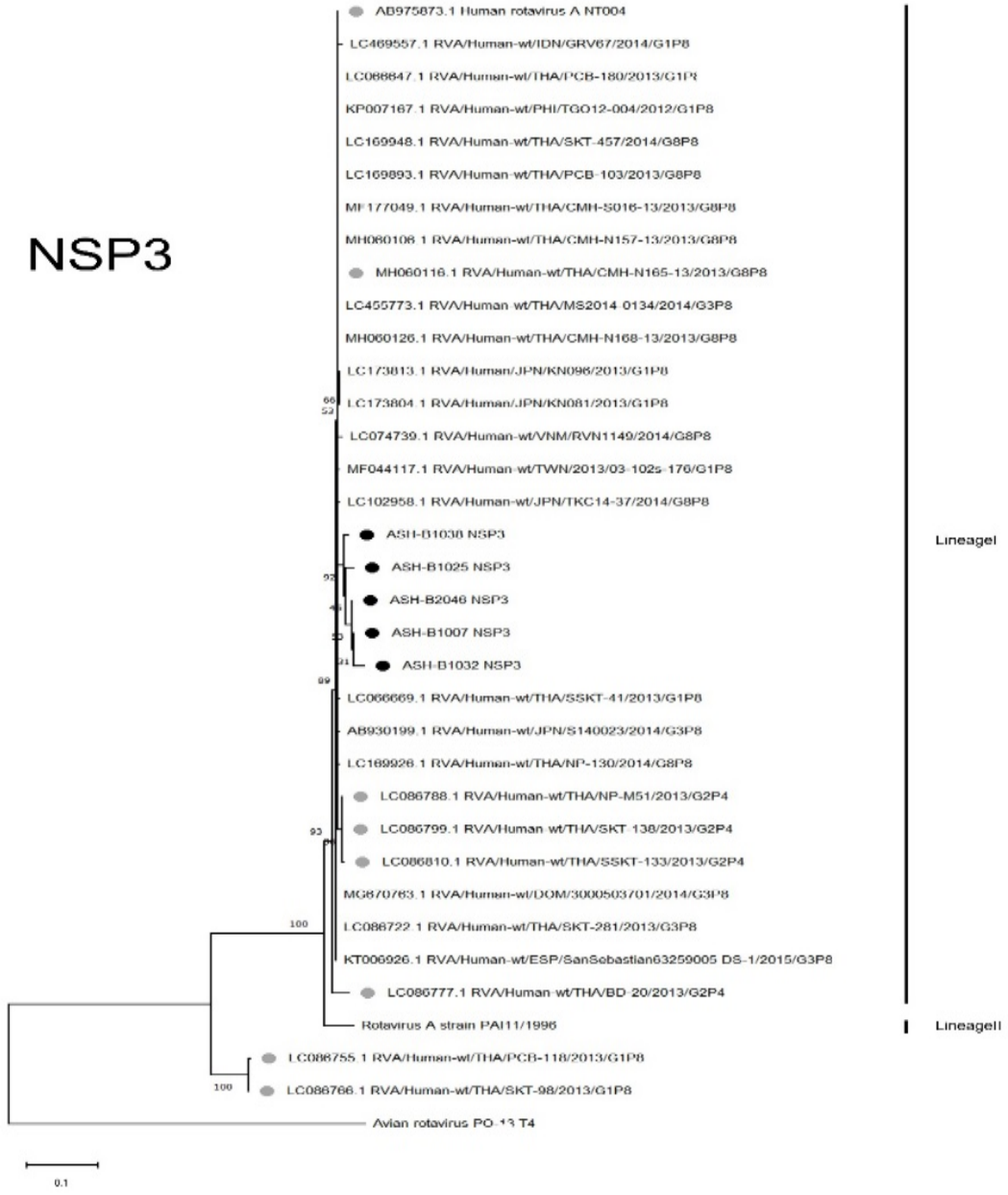

Figure 4 The phylogenetic tree was constructed from the nucleotide sequences of the NSP3 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the NSP3 genes of 5 G8P[8] strains. The grey dots show the NSP3 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

Figure 5 The phylogenetic tree was from the nucleotide sequences of the NSP5 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the NSP5 genes of 5 G8P[8] strains. The grey dots show the NSP5 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

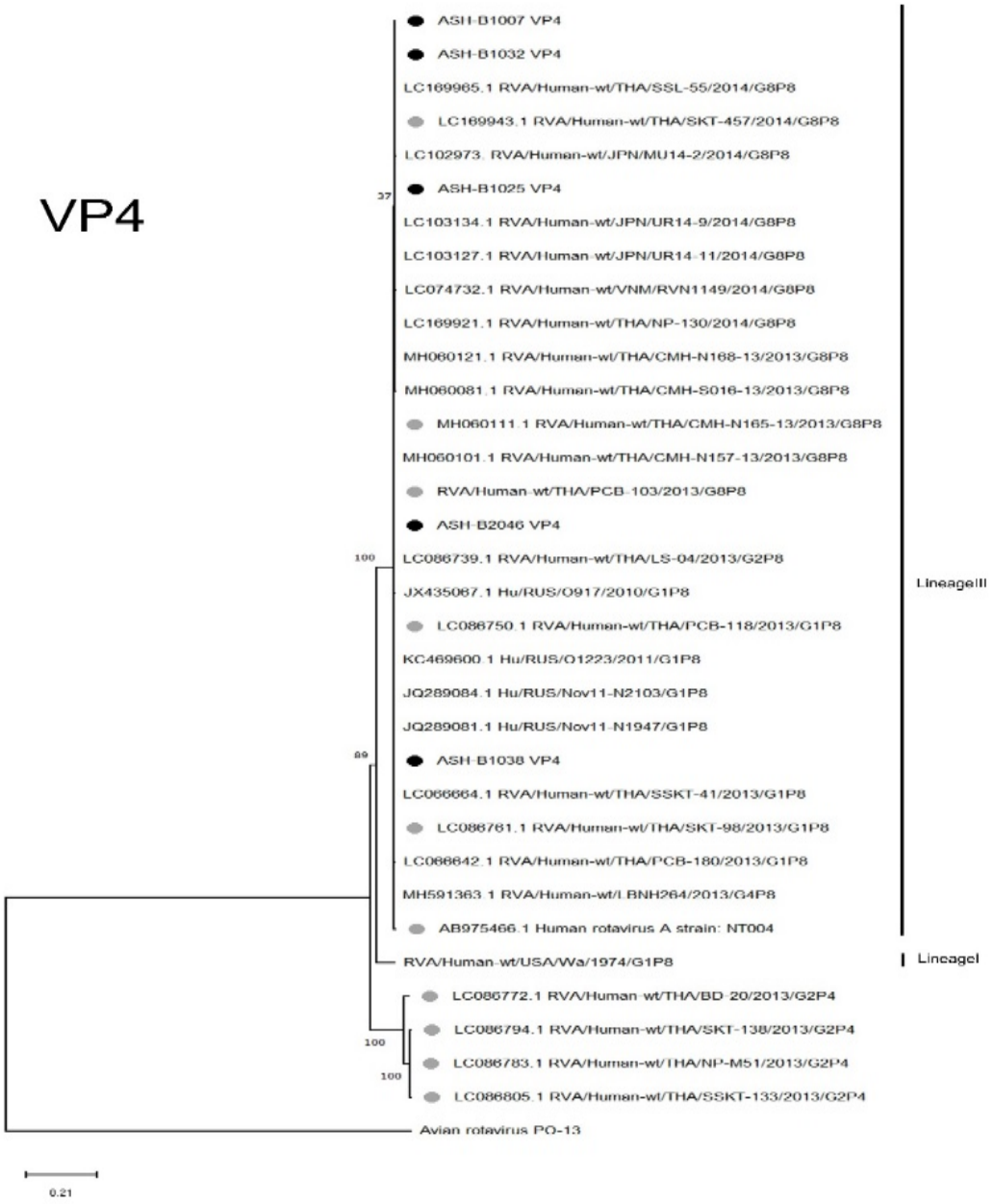

Figure 6 The phylogenetic tree was constructed from the nucleotide sequences of the VP4 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP4 genes of 5 G8P[8] strains. The grey dots show the VP4 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

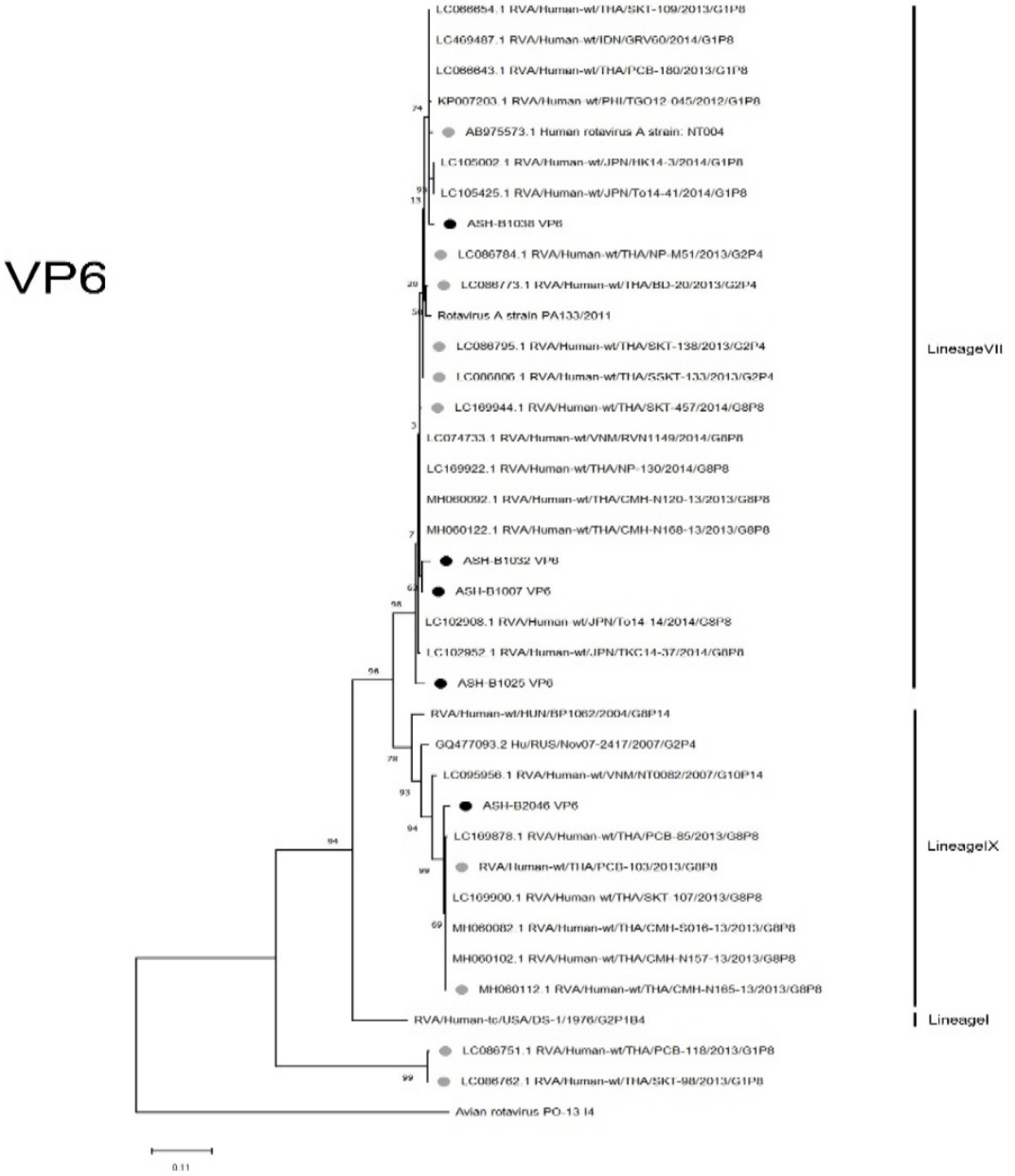

Figure 7 The phylogenetic tree was constructed from the nucleotide sequences of the VP6 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP6 genes of 5 G8P[8] strains. The grey dots show the VP6 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

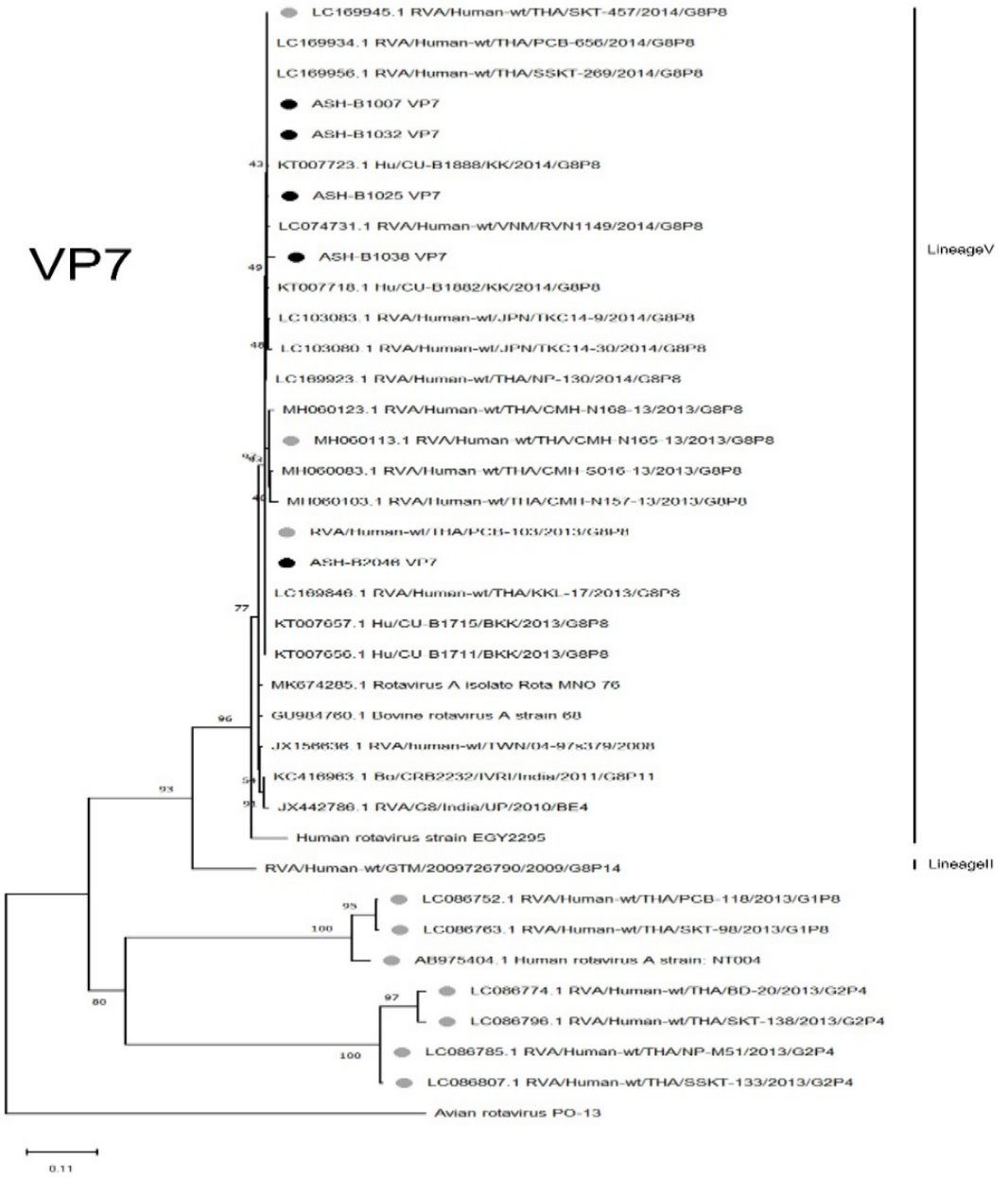

Figure 8 The phylogenetic tree was constructed from the nucleotide sequences of the VP7 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP7 genes of 5 G8P[8] strains. The grey dots show the VP7 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

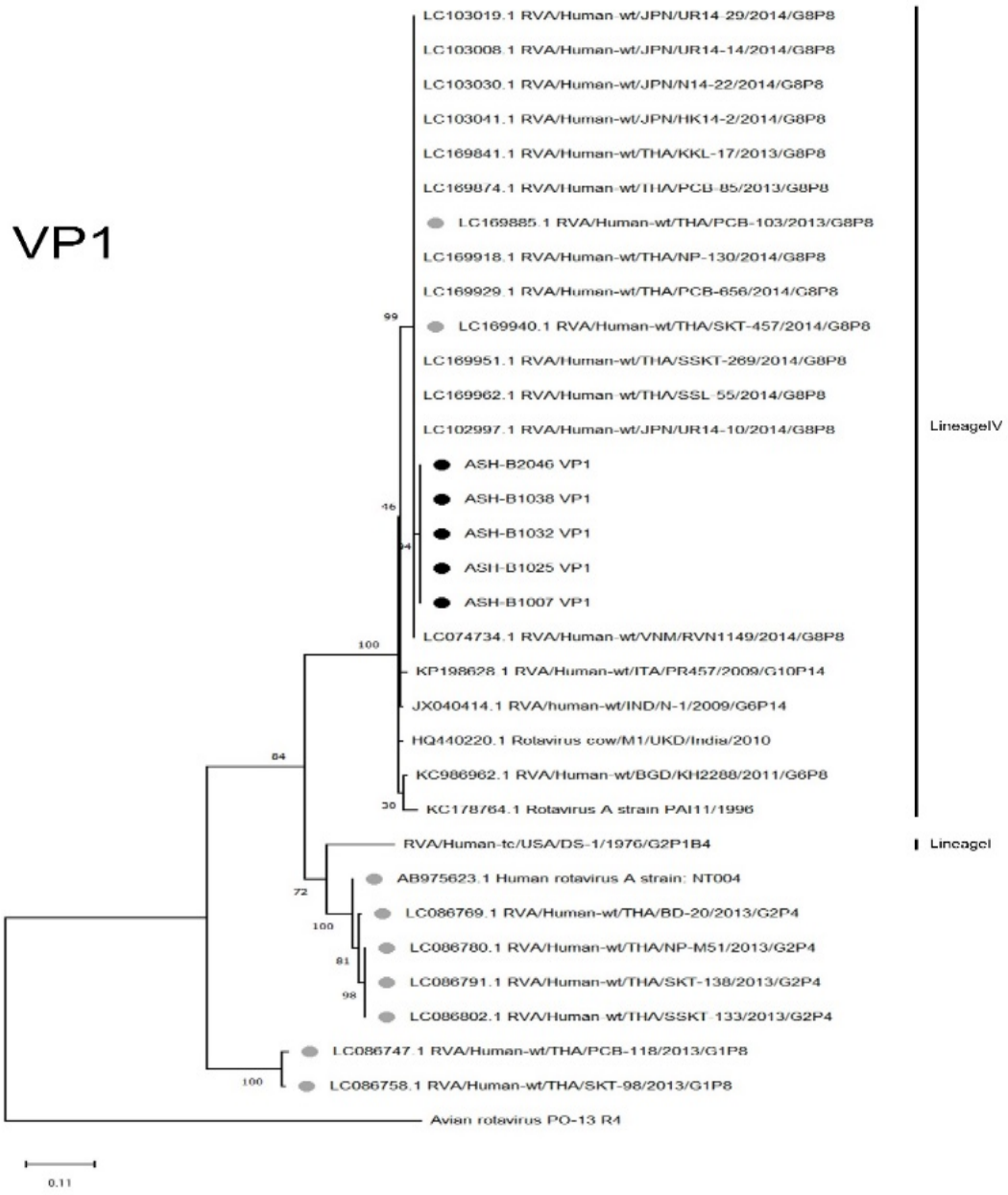

Figure 9 The phylogenetic tree was constructed from the nucleotide sequences of the VP1 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the VP1 genes of 5 G8P[8] strains. The grey dots show the VP1 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

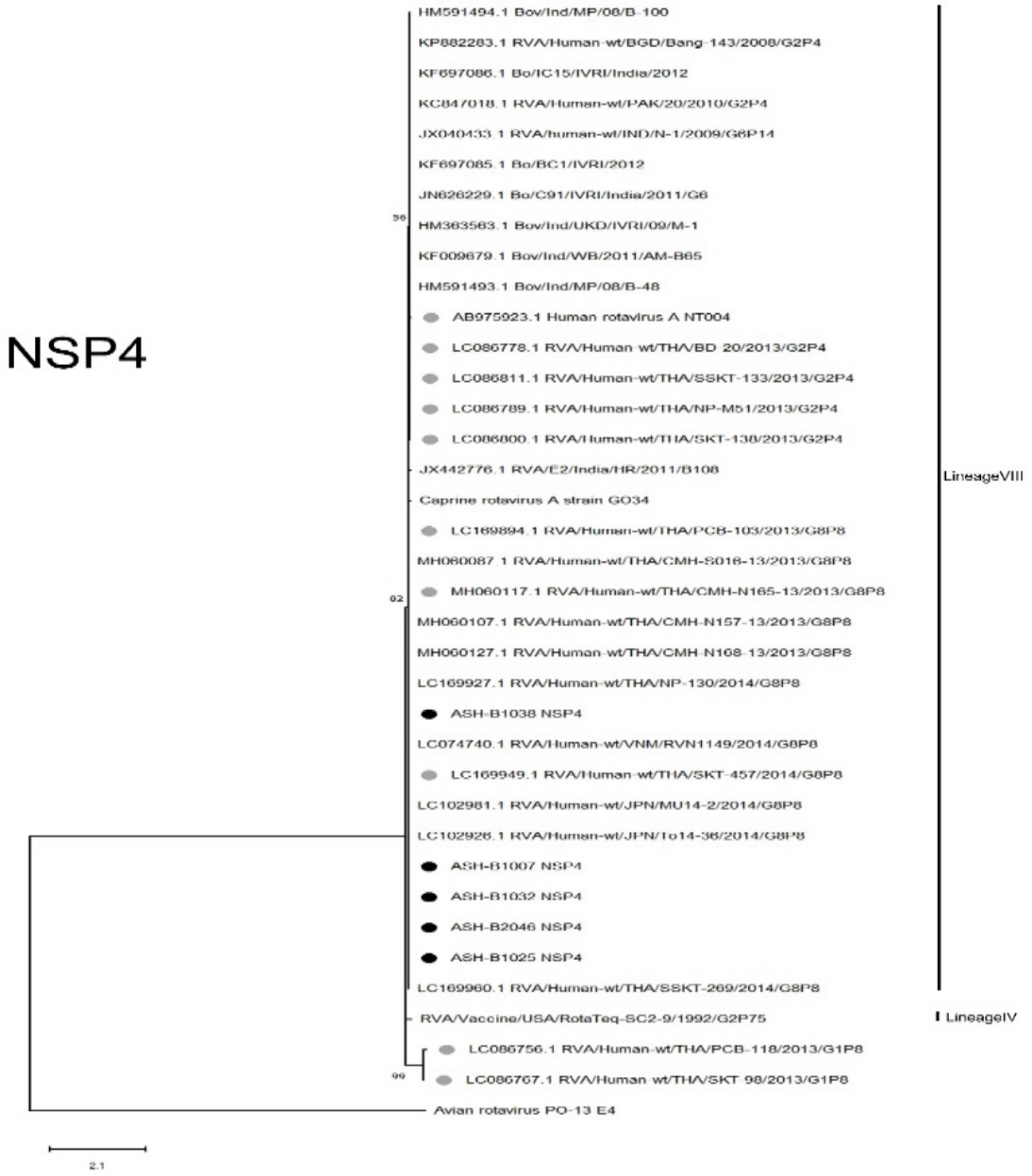

Figure 10 The phylogenetic tree was constructed from the nucleotide sequences of the NSP4 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the NSP4 genes of 5 G8P[8] strains. The grey dots show the NSP4 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

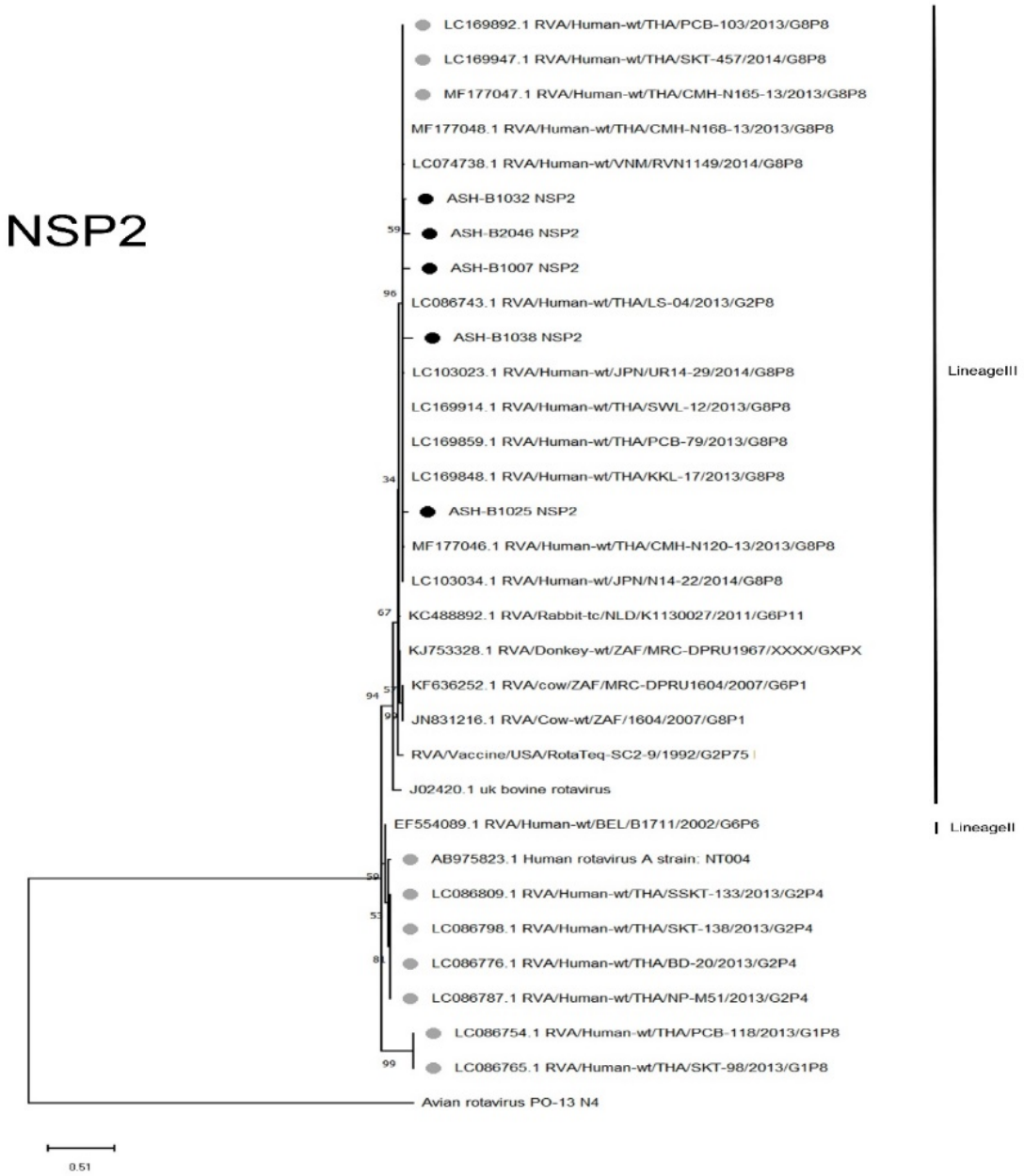

Figure 11 The phylogenetic tree was constructed from the nucleotide sequences of the NSP2 genes of 5 G8P[8] strains analyzed in this study. In the tree, the black dots indicate the NSP2 genes of 5 G8P[8] strains. The grey dots show the NSP2 gene of the Japanese G1P[8] NT004 strain; Thai circulating DS-1 like G8P[8] (PCB-103, SKT-457) strains; Thai circulating DS-1 like G2P[4] (BD-20, NP-M51, SKT-138, and SSKT-133) strains; Thai circulating Wa like G1P[8] (PCB-118 and SKT-98) strains; and the other RVA reference strain.

Results and discussion

Rotaviruses are the leading cause of severe acute dehydrating diarrhea in young children. Despite the global introduction of vaccinations for rotavirus over a decade ago, rotaviruses are continuously evolving to avoid the protective immunity of the host. The genomic diversity of rotavirus genes gradually accumulates from the error-prone nature of RNA polymerases during viral replication. In the total of 11 genes, the VP7 and VP4 genes that code for the outer layer and spikes seem to mutate quickly than the other genes coding proteins obscured in the particle. The nucleotide sequence variabilities of VP7 and VP4 genes are thus used to define at least the 36 G and 51 P genotypes, respectively [3,5,6]. Due to the

segmented nature of the RVA genomes, rotaviruses can exchange (reassort) genes during mixed infection of different circulating strains. An abrupt change of genome diversity of rotaviruses is predicted to create new, possibly more dangerous, virus strains. In the past, most rotavirus genotyping studies have focused mainly on G (VP7) and P (VP4) genotyping, thus limiting understanding of how rotaviruses evolve in nature. Our study revealed the whole picture regarding the diversity, evolution, and origin of viruses by characterizing all segmented genomes.

In this study, stool samples were collected from hospitalized infants and children with acute diarrhea at Bamrajnaradul Institute, Thailand, between 2012 - 2014. With partial genome sequencing, genetic variability was determined in eleven genes of 5 RVAs G8P[8] strains (ASH-B1007, ASH-B1025, ASH-B1032, ASH-B1038, and ASH-B2046). These strains possess the ten genome segments except for VP6, where they are virtually indistinguishable from each other. What's more, they also derived from Thai circulating G8P[8] strains in their nucleotide sequences and phylogenetic lineages, indicating the derivation of G8P[8] strains from a common ancestor. Furthermore, all strains demonstrated the same genotype constellation, G8P[8] -I2-R2-C2-M2-A2-N2-T2-E2-H2, indicating a genomic background of the DS-1 genotype constellation. None of these was shown to be an intergenogroup reassortant.

The DS-1-like backbone was typically associated with G2P[4] combinations [11]. However, non-G2P[4] DS-1-like strains worldwide such as G1P[8][14,15,25], G3P[8], G2P[8][17], G3[6], G1P[4], G12P[6], and G3P[8][18,28,29] and G8 [30] likely originated through the occurrence of reassortment events between DS-1-like G2P[4], and non-G2 strains. The evolution analysis via the phylogenetics tree demonstrates that 5 of the eleven genes (VP2, VP3, NSP1, NSP3, and NSP5) of G8P[8] strains exhibited extremely high nucleotide sequence identities with the cognate genes of G1P[8] NT004. Therefore, our DS-1-like G8P[8] strains were assumed to share common ancestral origins with DS-1-like intergenogroup reassortant strains G1P[8] NT004 and other G8P[8] strains [26].

Interestingly, segment 6 (VP6) nucleotide sequences of these G8P[8] RVA strains showed to have greater sequence diversity when compared to other RVA segments, as seen in the separate lineages. VP6 of 4 G8P[8] strains (ASH-B1032, ASH-B1007, ASH-B1025, and ASH-B1038) belonged to lineage VII, which was closely related to human RVAs, while ASH-B2046 was that of animal (bovine and porcine) strains in lineage IX of I2 genotype. This observation strongly suggested that the VP6 gene of ASH-B2046 might have originated from animal RVA strains. Moreover, the other 4 genes (VP1, VP7, NSP2, and NSP4) shared lower nucleotide level similarities to DS-1-like G1P[8] NT004 and other circulating Thai G3P[8], G8P[8], and G2P[4], yet fell into the same cluster as animal RVA strains on the phylogenetic trees. These 4 genes were likely generated from other intra-reassortment events between original strains and animal strains. Only the VP4 gene segment shared a very close genetic relatedness and phylogenetic clustering with human G1P[8] Wa-like strains. Human G8P[8] strains can possess both the Wa-like and the DS-1-like genetic background [31]. The VP4 genes of these G8P[8] were assumedly transferred from gene V4 from Wa-like to DS-like genetic backbones due to their carrying of the human DS-1 like backbone, which may maintain themselves through human-to-human transmission [32]. Another possibility is that the DS-1 like G8P[8] strains might not have originated from a single ancestor due to their possession of a slightly different VP4 gene.

Intra-genotype reassortant between viruses of the same genogroups is thought to occur more frequently than between different genogroups or inter-genotype reassortants [33,34]. Through intra-genotype reassortment of one or more gene segments, variants of the DS-1-like genotype constellation have been identified [35-39]. In developing countries where people's lifestyles see them living close to animals with inadequate personal hygiene, mixed infections are likely to occur more frequently than in developed countries [9,40]. However, the limitation of this study is that information was obtained from the partial sequencing of all gene segments, thus limiting elucidating the evolutionary behavior of rotaviruses. Therefore, complete genome characterization of RVA strains is required to gain more reliable phylogenetic analysis and deep insight into the complex reassortment events rendering the emergence of RVA strains.

Conclusions

The emergence of G8P[8] rotavirus in Thailand during 2012 - 2014 is evolved from the genetic segments transfer or intra-reassortant event between the co-circulation strains. Evolutionary and genetic analyses carried out in this study provide data useful for the elucidation of evolutionary relationships or dynamics existing in the rotavirus population.

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References

[1] Y Hoshino, RM Chanock and AZ Kapikian. *Rotaviruses*. Lippincott Williams & Wilkins, Philadelphia, 2001, p. 1787-833.

[2] L Liu. Fields virology, 6th edition. *Clin. Infect. Dis*. 2014; **59**, 613.

[3] J Matthijnssens, M Ciarlet, SM McDonald, H Attoui, K Bányai, JR Brister, J Buesa, MD Esona, MK Estes, JR Gentsch, M Iturriza-Gómara, R Johne, CD Kirkwood, V Martella, PPC Mertens, O Nakagomi, V Parreño, M Rahman, FM Ruggeri, LJ Saif, N Santos,… MV Ranst. Uniformity of rotavirus strain nomenclature proposed by the rotavirus classification working group (RCWG). *Arch. Virol*. 2011; **156**, 1397-413.

[4] R Delogu, G Ianiro, A Morea, M Chironna, L Fiore and FM Ruggeri. Molecular characterization of two rare human G8P[14] rotavirus strains, detected in Italy in 2012. *Infect. Genet. Evol*. 2016; **44**, 303-12.

[5] K Li, XD Lin, KY Huang, B Zhang, M Shi, WP Guo, MR Wang, W Wang, JG Xing, MH Li, WS Hong, EC Holmes and YZ Zhang. Identification of novel and diverse rotaviruses in rodents and insectivores, and evidence of cross-species transmission into humans. *Virology* 2016; **494**, 168-77.

[6] Rotavirus Classification Working Group: RCWG, Available from: https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg, accessed March 2019.

[7] V Martella, K Bányai, J Matthijnssens, C Buonavoglia and M Ciarlet. Zoonotic aspects of rotaviruses. *Vet. Microbiol*. 2010; **140**, 246-55.

[8] S Surendran. Rotavirus infection: Molecular changes and pathophysiology. *Exp. Clin. Sci. J.* 2008; **7**, 154.

[9] N Santos and Y Hoshino. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev. Med. Virol*. 2005; **15**, 29-56.

[10] J Matthijnssens, M Ciarlet, E Heiman, I Arijs, T Delbeke, SM McDonald, EA Palombo, M Iturriza-Gómara, P Maes, JT Patton, M Rahman and MV Ranst. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. *J. Virol*. 2008; **82**, 3204-19.

[11] J Matthijnssens, M Ciarlet, M Rahman, H Attoui, K Bányai, MK Estes, JR Gentsch, M Iturriza-Gómara, CD Kirkwood, V Martella, PPC Mertens, O Nakagomi, JT Patton, FM Ruggeri, LJ Saif, N Santos, A Steyer, K Taniguchi, U Desselberger and MV Ranst. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch. Virol*. 2008; **153**, 1621-9.

[12] U Desselberger. Rotaviruses. *Virus Res*. 2014; **190**, 75-96.

[13] SM McDonald, J Matthijnssens, JK McAllen, E Hine, L Overton, S Wang, P Lemey, M Zeller, MV Ranst, DJ Spiro and JT Patton. Evolutionary dynamics of human rotaviruses: Balancing reassortment with preferred genome constellations. *PLoS Pathog*. 2009; **5**, e1000634.

[14] SP Yamamoto, A Kaida, H Kubo and N Iritani. Gastroenteritis outbreaks caused by a DS-1-like G1P[8] rotavirus strain, Japan, 2012 - 2013. *Emerg. Infect. Dis*. 2014; **20**, 1030-3.

[15] Y Fujii, T Nakagomi, N Nishimura, A Noguchi, S Miura, H Ito, YH Doan, T Takahashi, T Ozaki, K Katayama and O Nakagomi. Spread and predominance in Japan of novel G1P[8] double-reassortant rotavirus strains possessing a DS-1-like genotype constellation typical of G2P[4] strains. *Infect. Genet. Evol*. 2014; **28**, 426-33.

[16] S Komoto, R Tacharoenmuang, R Guntapong, T Ide, K Haga, K Katayama, T Kato, Y Ouchi, H Kurahashi, T Tsuji, S Sangkitporn and K Taniguchi. Emergence and characterization of unusual DS-1-Like G1P[8] rotavirus strains in children with Diarrhea in Thailand. *PLos One* 2015; **10**, e0141739.

[17] S Komoto, R Tacharoenmuang, R Guntapong, T Ide, T Tsuji, T Yoshikawa, P Tharmaphornpilas, S Sangkitporn and K Taniguchi. Reassortment of human and animal rotavirus gene segments in emerging DS-1-Like G1P[8] rotavirus strains. *PLos One* 2016; **11**, e0148416.

[18] A Arana, M Montes, KC Jere, M Alkorta, M Iturriza-Gómara and G Cilla. Emergence and spread of G3P[8] rotaviruses possessing an equine-like VP7 and a DS-1-like genetic backbone in the Basque Country (North of Spain), 2015. *Infect. Genet. Evol*. 2016; **44**, 137-44.

[19] D Cowley, CM Donato, S Roczo-Farkas and CD Kirkwood. Emergence of a novel equine-like G3P[8] inter-genogroup reassortant rotavirus strain associated with gastroenteritis in Australian children. *J. Gen. Virol*. 2016; **97**, 403-10.

[20] R Dóró, S Marton, AH Bartókné, G Lengyel, Z Agócs, F Jakab and K Bányai. Equine-like G3 rotavirus in Hungary, 2015 - Is it a novel intergenogroup reassortant pandemic strain? *Acta Microbiol. Immunol. Hung*. 2016; **63**, 243-55.

[21] TN Hoa-Tran, T Nakagomi, HM Vu, LP Do, P Gauchan, CA Agbemabiese, TTT Nguyen, O Nakagomi and NTH Thanh. Abrupt emergence and predominance in Vietnam of rotavirus A strains possessing a bovine-like G8 on a DS-1-like background. *Arch. Virol*. 2016; **161**, 479-82.

[22] U Intamaso, W Poomipak, P Chutoam, P Chotchuang, W Sunkkham and S Srisopha. Genotype distribution and phylogenetic analysis of rotaviruses in Thailand and emergence of uncommon genotypes. *Arch. Clin. Microbiol.* 2017; **8**, 60.

[23] MD Esona, A Geyer, K Bányai, N Page, M Aminu, GE Armah, J Hull, DA Steele, RI Glass and JR Gentsch. Novel human rotavirus genotype G5P[7] from child with diarrhea, Cameroon. *Emerg. Infect. Dis*. 2009; **15**, 83-6.

[24] M Iturriza-Gómara, C Wong, S Blome, U Desselberger and J Gray. Rotavirus subgroup characterisation by restriction endonuclease digestion of a cDNA fragment of the VP6 gene. *J. Virol. Methods*. 2002; **105**, 99-103.

[25] M Kuzuya, R Fujii, M Hamano, K Kida, Y Mizoguchi, T Kanadani, K Nishimura and T Kishimoto. Prevalence and molecular characterization of G1P[8] human rotaviruses possessing DS-1-like VP6, NSP4, and NSP5/6 in Japan. *J. Med. Virol*. 2014; **86**, 1056-64.

[26] R Tacharoenmuang, S Komoto, R Guntapong, T Ide, P Sinchai, S Upachai, T Yoshikawa, P Tharmaphornpilas, S Sangkitporn and K Taniguchi. Full genome characterization of novel DS-1-Like G8P[8] rotavirus strains that have emerged in Thailand: Reassortment of bovine and human rotavirus gene segments in emerging DS-1-Like intergenogroup reassortant strains. *PLos One* 2016; **11**, e0165826.

[27] A Yodmeeklin, P Khamrin, K Kumthip, R Malasao, N Ukarapol, H Ushijima and N Maneekarn. Increasing predominance of G8P[8] species A rotaviruses in children admitted to hospital with acute gastroenteritis in Thailand, 2010 - 2013. *Arch. Virol*. 2018; **163**, 2165-78.

[28] CA Agbemabiese, T Nakagomi, MQ Nguyen, P Gauchan and O Nakagomi. Reassortant DS-1-like G1P[4] Rotavirus A strains generated from co-circulating strains in Vietnam, 2012/2013. *Microbiol. Immunol*. 2017; **61**, 328-36.

[29] J Bwogi, KC Jere, C Karamagi, DK Byarugaba, P Namuwulya, FN Baliraine, U Desselberger and M Iturriza-Gómara. Whole genome analysis of selected human and animal rotaviruses identified in Uganda from 2012 to 2014 reveals complex genome reassortment events between human, bovine, caprine and porcine strains. *PLos One* 2017; **12**, e0178855.

[30] EM Heiman, SM McDonald, M Barro, ZF Taraporewala, T Bar-Magen and JT Patton. Group A human rotavirus genomics: Evidence that gene constellations are influenced by viral protein interactions. *J. Virol*. 2008; **82**, 11106-16.

[31] CA Agbemabiese, T Nakagomi, YH Doan and O Nakagomi. Whole genomic constellation of the first human G8 rotavirus strain detected in Japan. *Infect. Genet. Evol*. 2015; **35**, 184-93.

[32] T Nakagomi, YH Doan, W Dove, B Ngwira, M Iturriza-Gómara, O Nakagomi and NA Cunliffe. G8 rotaviruses with conserved genotype constellations detected in Malawi over 10 years (1997 - 2007) display frequent gene reassortment among strains co-circulating in humans. *J. Gen. Virol*. 2013; **94**, 1273-95.

[33] M Ramachandran, CD Kirkwood, L Unicomb, NA Cunliffe, RL Ward, MK Bhan, HF Clark, RI Glass and JR Gentsch. Molecular characterization of serotype G9 rotavirus strains from a global collection. *Virology* 2000; **278**, 436-44.

[34] M Iturriza-Gómara, B Isherwood, U Desselberger and J Gray. Reassortment *in vivo*: Driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *J. Virol*. 2001; **75**, 3696-705.

[35] A Strydom, ED João, L Motanyane, MM Nyaga, A Christiaan Potgieter, A Cuamba, I Mandomando, M Cassocera, N de Deus and HG O'Neill. Whole genome analyses of DS-1-like rotavirus A strains detected in children with acute diarrhoea in southern Mozambique suggest several reassortment events. *Infect. Genet. Evol*. 2019; **69**, 68-75.

[36] JT Patton. Rotavirus diversity and evolution in the post-vaccine world. *Discov. Med*. 2012; **13**, 85-97.

[37] GM Giammanco, F Bonura, M Zeller, E Heylen, M Van Ranst, V Martella, K Bányai, J Matthijnssens and S De Grazia. Evolution of DS-1-like human G2P[4] rotaviruses assessed by complete genome analyses. *J. Gen. Virol*. 2014; **95**, 91-109.

[38] J Matthijnssens, M Rahman, X Yang, T Delbeke, I Arijs, JP Kabue, JJT Muyembe and MV Ranst. G8 rotavirus strains isolated in the Democratic Republic of Congo belong to the DS-1-like genogroup. *J. Clin. Microbiol*. 2006; **44**, 1801-9.

[39] HJ Dong, Y Qian, T Huang, RN Zhu, LQ Zhao, Y Zhang, RC Li and YP Li. Identification of circulating porcine-human reassortant G4P[6] rotavirus from children with acute diarrhea in China by whole genome analyses. *Infect. Genet. Evol*. 2013; **20**, 155-62.

[40] NM Nielsen, J Eugen-Olsen, P Aaby, K Mølbak, A Rodrigues and TK Fischer. Characterisation of rotavirus strains among hospitalised and non-hospitalised children in Guinea-Bissau, 2002 A high frequency of mixed infections with serotype G8. *J. Clin. Virol*. 2005; **34**, 13-21.