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（学位申請論文）

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Molecular and clinical characteristics of human group A rotavirus detected in two sentinel paediatric clinics in two neighbouring Japanese cities during successive epidemic seasons, Sapporo (2010–2016) and Tomakomai (2014–2016)

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Abstract

Here, we investigated molecular and clinical characteristics of group A rotavirus (RVA) obtained in two sentinel paediatric clinics in Sapporo and Tomakomai in Japan during successive epidemic seasons (Sapporo, 2010–2016; Tomakomai, 2014–2016). In Sapporo, although Wa-like G1P[8] strains were dominant in 2010–2012 (40–95%), DS-1-like G1P[8] strains appeared in 2012 and became the dominant genotype in 2013–2015 (28–82%). G2P[4]I2 and G9P[8]I1 strains increased every 3 years (G2P[4]I2: 2011 and 2014; G9P[8]I1: 2010, 2013 and 2016). In Tomakomai, different trends were observed; G8P[8]I2 strains emerged in 2014 (52.1%) but disappeared thereafter, and a newly emerged equine-like G3P[8]I2 strain was prevalent in 2016 (63.6%). There were no significant differences in clinical characteristics of RVA infections according to genotype.

In phylogenetic analyses, VP7 and VP4 genes of Wa-like G1P[8] strains formed three branches, while those of DS-1-like G1P[8] strains belonged to another branch. DS-1-like G1P[8] strains isolated in this study likely originated from Thailand in 2007. In addition, VP7 gene of the new equine-like G3P[8]I2 strain in Japan shared high nucleotide identity with VP7 genes of equine-like G3P[8]I2 strains detected worldwide (98.9–99.9%).

We observed different trends in predominant RVA GPI genotypes and frequent change in the dominant genotype in two neighbouring Japanese cities (distance about 70 km) during successive epidemic seasons. These findings may be influenced by accumulation of immunological resistance at the city level. Continuous city level surveillance is necessary to elucidate genetic characteristics of RVA.

Introduction

Group A rotaviruses (RVA) are a major cause of severe diarrhea among infants and young children and result in 453,000 deaths among children <5 years of age worldwide [1]. Two vaccines (Rotarix, GlaxoSmithKline Biologicals S.A., Belgium and RotaTeq, Merck & Co., Inc., USA) have been licensed, and RV vaccine effectiveness and RV GP genotype distribution before and after vaccine introduction have been reported in many countries [2-6]. Although they are not included in the national vaccine program in Japan, Rotarix and RotaTeq were licensed in 2011 and 2012, respectively.

The RVA capsid is triple layered, which encloses a genome comprising 11 segments of double-stranded RNA that encodes six structural proteins (VP1–4, VP6, VP7) and six nonstructural proteins (NSP1–5/6) [7]. VP7 and VP4 are outer capsid proteins, which define G and P

types. The five major G and P genotype combinations are G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]; these genotypes account for 90% of all strains [8].

The Rotavirus Classification Working Group devised a classification system based on all 11 RNA segments. In the genotyping system, the genotype constellation is described by the nomenclature Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, which defines the genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, respectively, with x indicating the numbers of corresponding genotypes [9]. Most human RVA strains are classified into two major genogroups constellations, Wa-like (G1/3/9-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) and DS-1-like (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H1). In aditional, a small group of human RVA strains are classified into AU-1-like (G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3) genotype constellation.

In 2012, an unusual human intergenogroup double-gene reassortant DS-1-like G1P[8], which had genotype constellation of G1-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2, were detected [10-12]. Based on full genome analysis, DS-1-like G1P[8] is considered a double-reassortant strain between Wa-like G1P[8] and human DS-1-like backbone strains [11,12]. DS-1like G1P[8] was first detected in Japan in 2012 and has subsequently been detected throughout Japan [10-12] and in Asia [13-15]. Since 2013, a novel equine-like G3P[8] intergenogroup reassortant strain has emerged in several countries [16-21]. We recently reported the first human G8P[8] genotype outbreak in a developed country [22].

However, there are limited reports on annual change in genetic characteristics of RVA in sentinel locations. Here, we investigated molecular characteristics of RVA from several successive epidemic seasons in two sentinel paediatric clinics of neighbouring cities in Hokkaido, Japan. We compared trends of genetic characteristics of epidemic RVA between the two cities. In addition, we examined clinical characteristics according to RVA genotype.

Methods

Sample collection

We collected faecal samples and clinical data from outpatients with acute gastroenteritis in two paediatric clinics, one in Sapporo city (2010–2016) and the other in Tomakomai city (2014–2016), in Hokkaido, Japan. Samples were screened for RV antigen using immunochromatographic test. Written informed consent for collection of stool and acquisition of clinical information was obtained from the patients' parents.

RV detection, RNA extraction, RT-PCR and sequence analyses

Double-stranded viral RNA was

extracted from 10% faecal suspensions by using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Reverse transcription PCR (RT-PCR) was performed using RV-specific primers [23-25]. Two microlitres of extracted double-stranded RNA was added to 1 μL of dimethyl sulfoxide. The mixture was heated at 94°C for 3 min and then placed on ice. Reverse transcription was performed using SuperScript II Reverse Transcriptase (Invitrogen, USA) at 45°C for 45 min, followed by 94°C for 3 min [26]. PCR was performed using GoTaq DNA Polymerase (Promega, USA). The initial denaturation step was performed at 98°C for 10 s, followed by 35 cycles of amplification (10 s at 98°C, 15 s at 55°C and 40 s at 68°C), with a final extension at 68°C for 5 min in a SimpliAmp Thermal Cycler (Applied Biosystems, USA). PCR products were analysed by electrophoresis on a 1% agarose gel stained with ethidium bromide. PCR amplicons were purified with the Wizard SV Gel and PCR Clean-Up system (Promega, USA) and sequenced with the BigDye Terminator v.3.1 Cycle Sequencing Reaction kit (Applied Biosystems,USA) on an ABI PRISM 3130 XL Genetic Analyzer (Applied Biosystems,USA).

Sequence data were analysed using Sequencher v.5.2 (Gene Codes Corporation, USA). RV genotyping of each segment was determined using the online RotaC classification tool [27]. Multiple sequence alignments and phylogenetic analyses with maximum likelihood were performed using MEGA v.7.0.16 software [28]. For phylogenetic analysis, only reads with nucleotide sequences of ≥ 500 bp were analysed. RT-PCR was performed for VP7 and VP4 genes and products were sequenced for G and P genotype determination. Nested PCR was conducted for VP6 genotyping [25, 29].

GenBank/EMBL/DDBJ accession numbers for the sequences of the original fecal samples used in this study have been submitted to the DDBJ nucleotide sequence database under accession numbers LC335973-LC335974, LC342232-LC342263, LC342310-LC342412, LC342692-LC232711, LC342745-LC342786, LC348391-LC348430, LC348559-LC348614, LC348729-LC348762, LC348768-LC348805, LC348873-LC348940, LC350101-LC350210, LC350290-LC350340, LC360240-LC360276.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics v.22 (IBM, Japan). Categorical data were analysed using the χ^2 test or Student's t test, and continuous data were analysed by the Kruskal-Wallis test, as appropriate. All comparisons were two-sided, and a P value of < 0.05 was considered to be statistically significant.

Results

Prevalence of GPI genotypes

RV-positive faecal samples were obtained from 272 children in Sapporo and 132 children in Tomakomai. We successfully determined GPI genotypes for 258 of 272 (94.9%) samples in Sapporo and 126 of 132 (95.5%) samples in Tomakomai. We defined G1P[8]I1 as Wa-like G1P[8] and G1P[8]I2 as DS-1-like G1P[8].

During seven seasons in Sapporo, G1P[8] was the prevalent genotype overall (155/258, 60.1%) (Figure 1). Among these strains, Wa-like G1P[8] was dominant from 2010 to 2012 (40–95%). One DS-1-like G1P[8] strain was detected in 2012, which became the dominant strain from 2013 to 2015 (28–82%) but disappeared in 2016. Following G1P[8], G9P[8]I1 (53/258, 20.5%), G2P[4]I2 (25/258, 9.7%) and G3P[8]I1 (17/258, 6.6%) were predominant. Interestingly, G2P[4]I2 and G9P[8] genotypes were increased every 3 years (G2P[4]I2: 2011 and 2014; G9P[8]I1: 2010, 2013 and 2016).

Tomakomai demonstrated different genotype trends from Sapporo (Figure 1). Overall, G1P[8] was the prevalent genotype (63/126, 50.0%), followed by G8P[8]I2 (24/126, 19.0%), G3P[8]I2 (21/126, 16.7%) and G9P[8]I2 (21/126, 16.7%). G8P[8]I2 genotype emerged and became dominant in 2014 (24/46, 52.1%) but disappeared thereafter [22]. G1P[8] was dominant in 2015 (Wa-like: 19/53, 35.8%; DS-1-like: 31/53, 58.5%), similar to Sapporo. However, in 2016, equine-like G3P[8]I2 emerged and became dominant (21/32, 65.6%).

GPI genotype and clinical data

We obtained clinical data from 154 patients after the 2013 season in Sapporo and from all 132 patients in Tomakomai. Clinical characteristics, such as age, sex, history of RV vaccination, presence of diarrhea, vomiting and fever (> 37.5°C) were examined for patients with RVA infection (Table 1a, b). The numbers of patients who received drip infusion and were hospitalised were also recorded. In Sapporo, there were no significant differences in these clinical characteristics among major RVA genotypes, such as Wa-like G1P[8], DS-1-like G1P[8], G2P[4] and G9P[8] (Table 1a). In Tomakomai, the drip infusion rate was significantly higher in G3P[8] (6/21, 28.6%) RVA infection compared with G8P[8] RVA infection (0/24, 0%) ($P = 0.019$, χ^2 test) (Table 1b). There were no differences in other characteristics. However, the vaccination rate of outpatients with RV gastroenteritis in Sapporo during 2013–2016 (2.0–28.6%) was significantly lower than that of all outpatients (46–74%) ($P = 0.004$, paired t test) (Table 1c).

Sequence and phylogenetic analyses

Sequence analyses were conducted for VP7 and VP4 genes of major prevalent strains Wa-like G1P[8], DS-1-like G1P[8], G9P[8], G3P[8] and equine-like G3P[8]. Phylogenetic analyses of these strains were carried out together with previously reported strains.

VP7 gene sequence analysis showed DS-1-like G1P[8] strains belonged to the same branch (branch a) since first detected in 2012, except for two strains from Sapporo in 2013 (N13-16), which clustered in branch c, and a strain from Tomakomai in 2015 (TKC15-46), which clustered in branch d-1 (Figure 2). Branch a mainly consisted of DS-1-like G1P[8] strains observed throughout Japan and in Asia after 2012 [10-14]. By contrast, VP7 gene sequence analysis demonstrated Wa-like G1P[8] strains mostly belonged to the other three branches (b, c and d). These branches consisted mainly of Wa-like G1P[8] strains detected worldwide. Among them, branch d was located distantly from the other branches and included Wa-like strains observed in Sapporo (N15-2 and N15-4) and Tomakomai in 2015; these strains formed sub-branch d-1 together with Wa-like strains detected in Thailand in 2013 (PCB-118 and SKT-98) [17]. Notably, Wa-like G1P[8] strains from Sapporo in 2014–2015 (N14-33 and N15-6) and those from Tomakomai in 2015 (TKC15-44 and TKC15-49) clustered in branch a.

VP4 gene sequence analysis indicated almost all (93/94) DS-1-like G1P[8] strains belonged to branch a, except for one strain from Sapporo in 2013 (N13-16), which clustered in branch d (Figure 3). Branch a mainly consisted of DS-1-like G1P[8] strains isolated throughout Japan and in Asia after 2012 [10-14]. VP4 gene sequence analysis showed Wa-like G1P[8] strains belonged to three branches (b, c and d) together with Wa-like G1P[8] strains isolated worldwide. Notably, Wa-like G1P[8] strains detected in Sapporo in 2015 (N15-2 and N15-4) and Tomakomai in 2015 (17 strains) belonged to branch a and formed sub-branch a-1, together with both Wa-like and DS-1-like G1P[8] strains in Thailand in 2013 (PCB-118, SKT-98, PCB-180 and SKT-109) [13, 17]. Furthermore, Wa-like G1P[8] strains from Sapporo in 2015 (N15-6) and Tomakomai in 2015 (TKC15-44 and TKC15-49) clustered in branch a, similar to VP7 gene sequence analysis results of these strains.

VP7 gene sequence analysis demonstrated G9P[8] strains from this study and reported strains generally separated into two branches (a and b). Branch a included strains recently detected worldwide (after 2012); however, branch b included strains detected worldwide before 2011 (Figure 4). Our strains from Sapporo (54 strains) and Tomakomai (22 strains) obtained during 2010–2014 belonged to both branches; however, strains from 2015 and 2016 from

Sapporo (26 strains) and Tomakomai (11 strains) clustered in the recent branch a. Although slightly late, recent transition of epidemic G9P[8] strain from conventional to worldwide strains occurred in Sapporo and Tomakomai. Nucleotide identity between these two branches was low at 91.0–93.5%.

VP7 gene sequence analysis indicated G3P[8] strains in this study were clearly separated into two branches (a and b) (Figure 5). G3P[8]I1 strains detected in Sapporo in 2010–2013 (16 strains) clustered in branch a with common human G3P[8] strains. G3P[8]I2 strains detected in Sapporo (1 strain) and Tomakomai (19 strains) in 2016 clustered in branch b together with human equine-like G3 strains isolated in Japan, Thailand, Australia, Spain, Hungary and Brazil after 2013 and shared extremely high nucleotide identity (98.9–99.9%). However, this branch is distinct from the typical animal G3 branch c, including horse strains. Nucleotide identity between common human G3P[8] and equine-like G3P[8] strains was low at 78.4–80.2%.

Discussion

In the present study, we genetically investigated RVA obtained in two neighbouring Japanese cities (distance about 70 km) during several successive epidemic seasons and clarified different trends in RVA GPI genotype distribution and frequent change of dominant genotype (Figure 1). A drastic change from Wa-like G1P[8] to DS-1-like G1P[8] was observed in Sapporo from 2012 through 2013. In addition, G2P[4]I2 and G9P[8]I1 genotypes were detected every 3 years. These phenomena may be influenced by accumulation of immunological resistance at the city level.

Although the two cities Sapporo and Tomakomai were geographically close, their GPI genotype trends were not the same in 2014–2016. The dominant GPI genotype differs according to geographical location [30]. However, to our knowledge, there are no reports of a comparative study at the city level in the same country. From national surveillance in Japan, the trend of G type differs between all Japan and Sapporo. According to the Japanese national surveillance report, G3 was dominant in 2010–2011, G1 was dominant in 2012–2014 and G2 increased from 2014 and became dominant in 2015–2016 [31]. Our observations showed different genotype trends. Thus, to understand the genotype distribution accurately, surveillance at the city level is necessary.

DS-1-like G1P[8] was first detected in Okayama in Japan in 2012 [10]. It subsequently spread to other regions in Japan and has also been reported in other Asian countries [11-15]. In Sapporo, DS-1-like G1P[8] first appeared in 2012 and became the dominant genotype thereafter. DS-1-like G1P[8] appeared just after

vaccine introduction (November 2011) in Japan. Potential reasons for why DS-1-like G1P[8] appeared and became dominant are discussed below from various viewpoints, including the influence of RV vaccines.

DS-1-like G1P[8] strains were detected in many regions in Japan, including this study, in 2012, and showed high VP7 and VP4 nucleotide identity with each other (99.7–100%) [10,11]. In addition, these strains showed high nucleotide identity with Thailand strain CU161-KK/07 isolated in 2007 (VP7: 98.0–99.3%, VP4: 99.2–99.5%) [32]. Many similar G1P[8] strains existed in Thailand between 2007 and 2014 [33, 34]. Therefore, DS-1-like G1P[8] strains detected in this study possibly originated from Thailand. However, I genotype (VP6) of Thailand strains has not been analysed, thus it remains unclear whether the whole virion of DS-1-like G1P[8] strains is derived from Thailand strains or only VP7 and VP4 genes.

Conversely, VP7 and VP4 genes of DS-1-like G1P[8] strains detected in this study shared high nucleotide identity with a Wa-like G1P[8] strain isolated in the United States in 2007 (strain 2007719635; VP7: 98.0–99.2%, VP4: 98.3–99.6%). However, only one strain has been reported, thus it is unlikely that this foreign strain spread to Japan.

Interestingly, both VP7 and VP4 gene sequence analyses showed DS-1-like G1P[8] strains formed an independent branch (branch a) distinct from Wa-like G1P[8] strains since 2012, when DS-1-like G1P[8] first appeared, and no strain isolated before 2007 belonged to this branch (Figures 2 and 3). DS-1-like G1P[8] strains detected in this study belonged to the same branch with DS-1-like G1P[8] strains observed in other regions of Japan and in Asia after 2012 and shared high nucleotide identity (99.0–100%).

Phylogenetic analyses of VP7 and VP4 genes of Wa-like and DS-1-like G1P[8] strains indicated several interesting observations. First, VP7 gene sequence analysis showed Wa-like G1P[8] strains from Sapporo (N15-2 and N15-4) and Tomakomai in 2015 (17 strains) belonged to a distant sub-branch (sub-branch d-1), together with Thailand Wa-like G1P[8] strains in 2013 (SKT-98 and PCB-118) [17] (Figure 2d). By contrast, VP4 gene sequence analysis demonstrated these strains belonged to branch a, which consisted almost exclusively of DS-1-like G1P[8] strains detected in all Japan (Figure 3a). VP7 and VP4 gene sequence analyses of our strains shared high nucleotide identity with these Thailand strains (SKT-98 and PCB-118) in 2013 (VP7: 99.6–99.7%, VP4: 99.6%). Therefore, we considered that these Wa-like G1P[8] strains prevalent in 2015 in Sapporo and Tomakomai could have spread to Japan after a possible reassortant

event in Thailand. Second, VP7 and VP4 gene sequence analyses showed three Wa-like G1P[8] strains in Sapporo and Tomakomai in 2015 (N15-6, TKC15-44 and TKC15-49) clustered in DS-1-like specific branch a with many DS-1-like G1P[8] strains (Figures 2a, 3a). These findings indicate that these three strains were likely created by reassortant events between DS-1-like G1P[8] strain and a Wa-like G1P[8], G3P[8] or G9P[8] strain, which were domestic in the region. Finally, VP7 and VP4 gene sequence analyses demonstrated one DS-1 like G1P[8] strain from Sapporo in 2013 (N13-16) clustered in branches c and d, respectively, which are specific for Wa-like G1P[8] strains. Thus, this strain likely originated from a recent reassortant event between Wa-like G1P[8] strain and DS-1-like G1P[8] or G2P[4] strain (Figure 2c, 3d).

Equine-like G3P[8]I2 strains emerged in 2016 in Sapporo and Tomakomai and were detected as a dominant strain for the first time in Japan. VP7 gene sequence analysis of strains detected in this study indicated our strains differed from common human G3 strains, including strains previously isolated in the same area, and shared high genetic similarity to VP7 gene of equine-like human G3 strains isolated worldwide, including other areas of Japan after 2013 (nucleotide identity: 98.9–99.9%) (Figure 5) [16-21]. Although we could not determine the origin, there is a possibility of domestic spread.

In this study, there was no significant difference in clinical characteristics among major RVA genotypes (Table 1a, b). To our knowledge, only one report has compared the severity of Wa-like G1P[8] and DS-1-like G1P[8] isolates from inpatients, which suggested that disease caused by DS-1-like G1P[8] strains was not more severe than that by Wa-like G1P[8] strains [14]. In the present study, only the drip infusion rate in Tomakomai was significantly higher for patients with equine-like G3P[8] infection compared with other genotypes. The infusion rate of children > 3 years (10/32, 31.3%) was higher than that of children < 3 years (5/92, 5.4%) ($P = 0.0001$, χ^2 test). In addition, children > 3 years more frequently had equine-like G3P[8] infection (10/21, 47.6%) than infection with other genotypes (22/105, 21.0%) ($P = 0.010$, χ^2 test). The reason why equine-like G3P[8] RVA infection required drip infusion in older children remains to be elucidated. Moreover, the observation that the vaccination rate of outpatients with RV gastroenteritis was significantly lower than that of all outpatients might indicate an effect of RV vaccines.

Conclusions

We observed different trends among two neighbouring Japanese cities in RVA GPI genotype distribution, frequent change of dominant genotype and

occasional emergence of new genotypes, some of which were suspected to come from foreign countries such as Thailand.

We also clarified that a few strains were newly created possibly by reassortant events among domestic circulating strains. Continuous city level surveillance and genotyping or sequencing analysis of at least three segments (VP7, VP4 and VP6) are necessary to elucidate genetic characteristics of prevalent RVA and prepare for the emergence of new genotypes.

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Disclosure

The authors declare no conflict of interest.

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Table 1. Clinical characteristics of rotavirus gastroenteritis in paediatric patients from Sapporo (a) and Tomakomai (b)

(a) Wa-like G1P[8] vs. DS-1-like G1P[8] vs. G2P[4]I2 vs. G9P[8] I1 in Sapporo[a]

	Wa-like G1P[8] (n=10)	DS-1-like G1P[8] (n=60)	G2P[4]I2 (n=14)	G9P[8]I1 (n=39)	P value[b]
Male:Female	4:6	31:28	10:4	22:17	0.341
Median age (months)	17.5	27.3	17.1	15.6	0.355
Vaccination [n(%)]	2(20.0%)	7(11.7%)	3(21.4%)	7(17.9%)	0.722
diarrhea [n(%)]	10(100%)	59(98.3%)	14(100%)	39(100%)	1.000
Vomiting [n(%)]	8(80.0%)	56(93.3%)	10(71.4%)	33(84.6%)	0.073
Fever [n(%)]	9(90.0%)	57(95.0%)	13(92.9%)	34(87.2%)	0.553
Three symptoms [n(%)]	7(70.0%)	52(86.7%)	9(64.3%)	28(71.8%)	0.198
Drip infusion [n(%)]	3(30.0%)	30(50.0%)	7(50.0%)	18(46.2%)	0.803
Hospitalisation [n(%)]	1(10.0%)	3(5.0%)	1(7.1%)	8(20.5%)	0.071

[a]Data available after 2013.

[b]Categorical data were analysed by χ^2 or Student's t test; continuous data were analysed by the Kruskal-Wallis test. $P < 0.05$ was considered to be statistically significant.

(b) Wa-like G1P[8] vs. DS-1-like G1P[8] vs. G3P[8] I2 vs. G9P[8] I1 vs. G8P[8]I2 in Tomakomai[a]

	Wa-like G1P[8] (n=23)	DS-1-like G1P[8] (n=37)	Equine-like G3P[8]I2 (n=21)	G9P[8]I1 (n=21)	G8P[8]I2 (n=24)	P value[b]
Male:Female	14:9	21:16	16:5	10:10	12:12	0.441
Median age (months)	27.0	22.7	35.0	26.0	20.3	0.285
Vaccination [n(%)]	4(16.0%)	3(8.3%)	2(9.5%)	0(0%)	1(4.2%)	0.416
diarrhea [n(%)]	21(84.0%)	28(77.8%)	19(90.5%)	18(85.7%)	23(95.8%)	0.361
Vomiting [n(%)]	17(68.0%)	27(75.0%)	18(85.7%)	16(80%)	20(83.3%)	0.634
Fever [n(%)]	21(84.0%)	25(69.4%)	14(66.7%)	13(65%)	12(52.2%)	0.148
Three symptoms [n(%)]	12(48.0%)	16(44.4%)	11(52.3%)	7(33.3%)	10(41.7%)	0.833
Drip infusion [n(%)]	1(4.0%)	6(16.7%)	**6(28.6%)**	2(9.5%)	**0(%)**	**0.019**
Hospitalisation [n(%)]	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	—

[a]Data available after 2013.

[b]Categorical data were analysed by χ^2 or Student's t test; continuous data were analysed by the Kruskal-Wallis test. $P < 0.05$ was considered to be statistically significant.

(c) Comparison of vaccination rates in Sapporo between all outpatients and rotavirus-positive outpatients

	All outpatients	Rotavirus-positive outpatients	P value[a]
2013	46.0% (76/166)	2.2% (1/46)	<0.0001
2014	54.0% (85/156)	20.0% (5/25)	0.0014
2015	70.0% (154/220)	17.6% (6/34)	<0.0001
2016	74.0% (264/355)	30.8% (8/26)	<0.0001
Total	579/897	20/131	<0.0001

[a]Data were analysed by using χ^2 test. $P < 0.05$ was considered to be statistically significant.

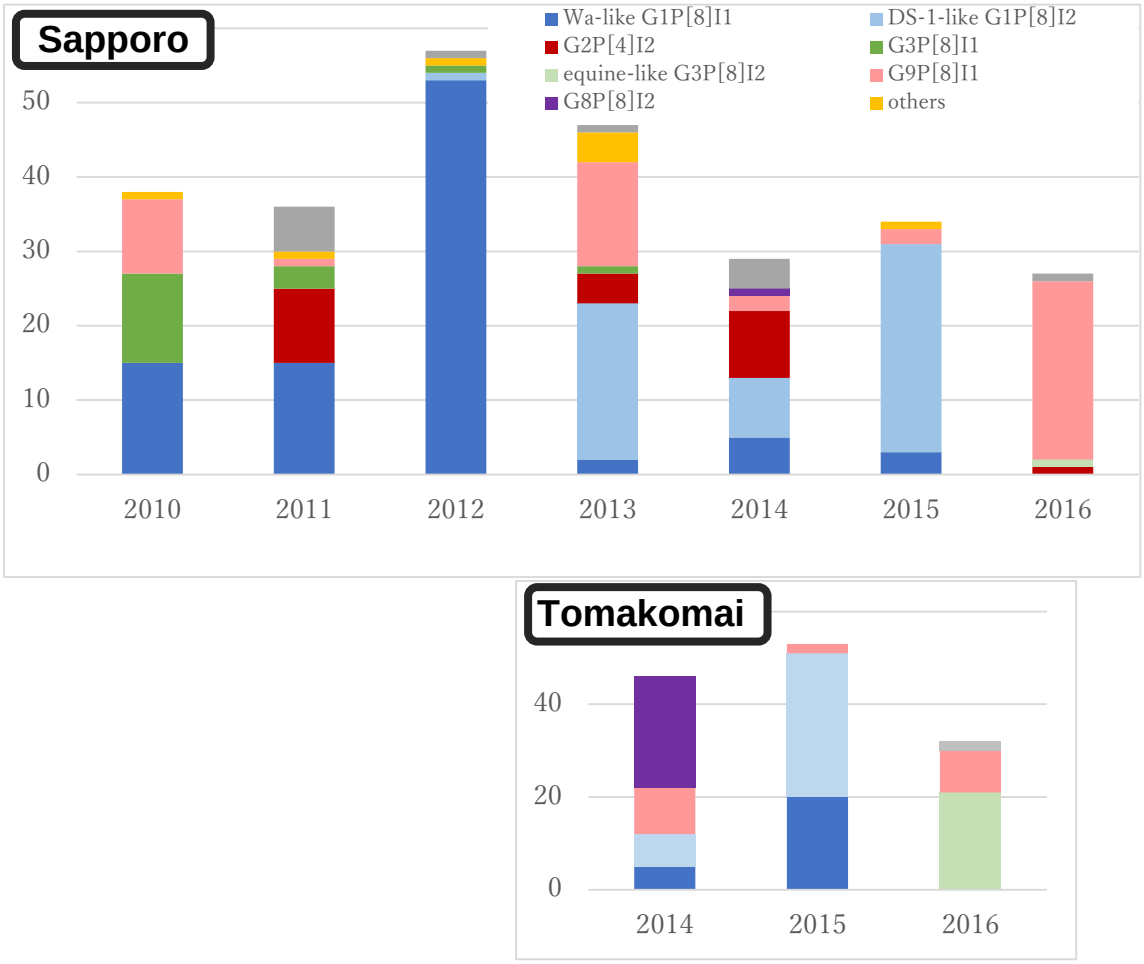

Figure 1. GPI genotype distribution of group A rotavirus strains from Sapporo and Tomakomai, Japan.

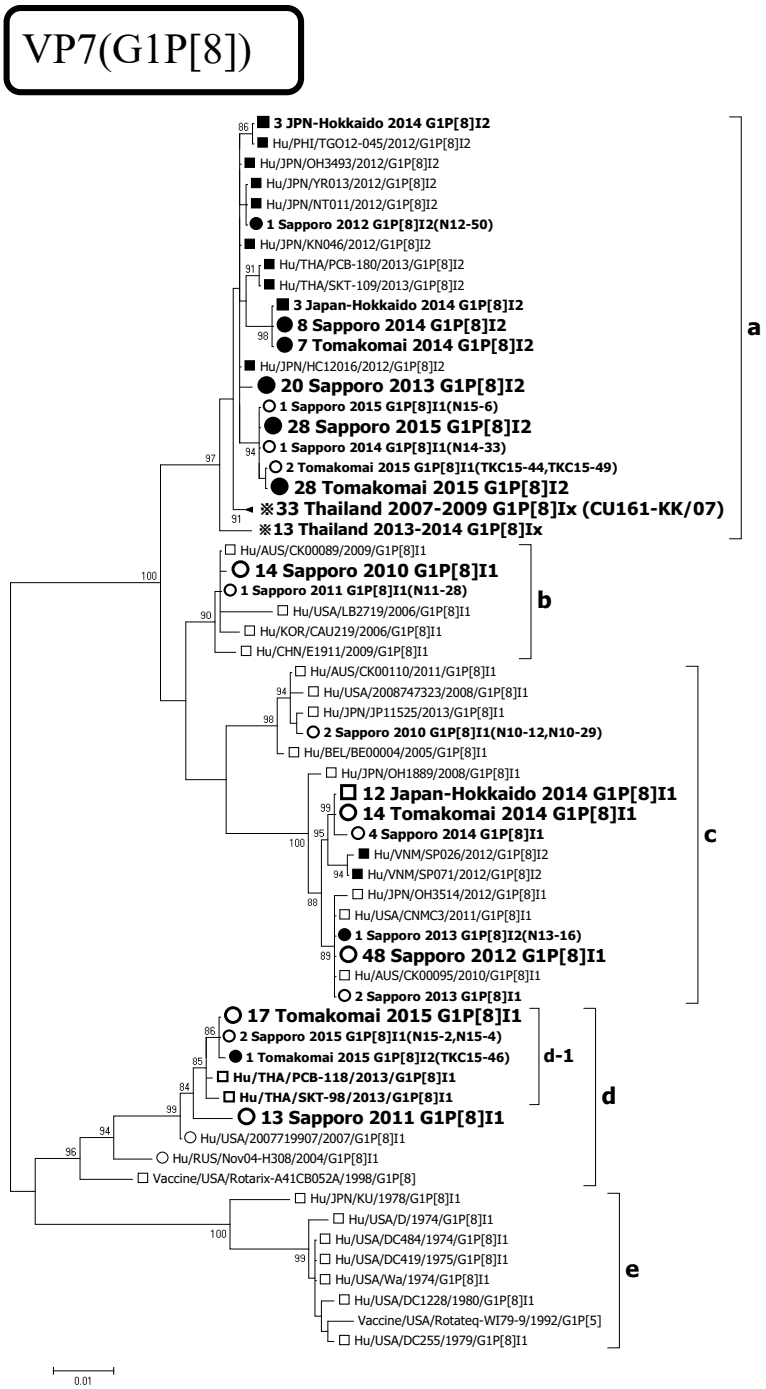

Figure 2. Phylogenetic analysis of VP7 genes in G1P[8] genotype strains.

The tree was constructed using the maximum-likelihood method with Tamura 3-parameter model and 1,000 bootstrap replicates. White and black circles indicate Wa-like and DS-1-like strains detected in this study, respectively. White and black squares indicate previously reported Wa-like and DS-1-like strains, respectively. Star indicates G1P[8]Ix strains prevalent in Thailand in 2007–2014. Bootstrap values are shown at branch nodes (only values >80% are shown). Scale bar indicates genetic distance.

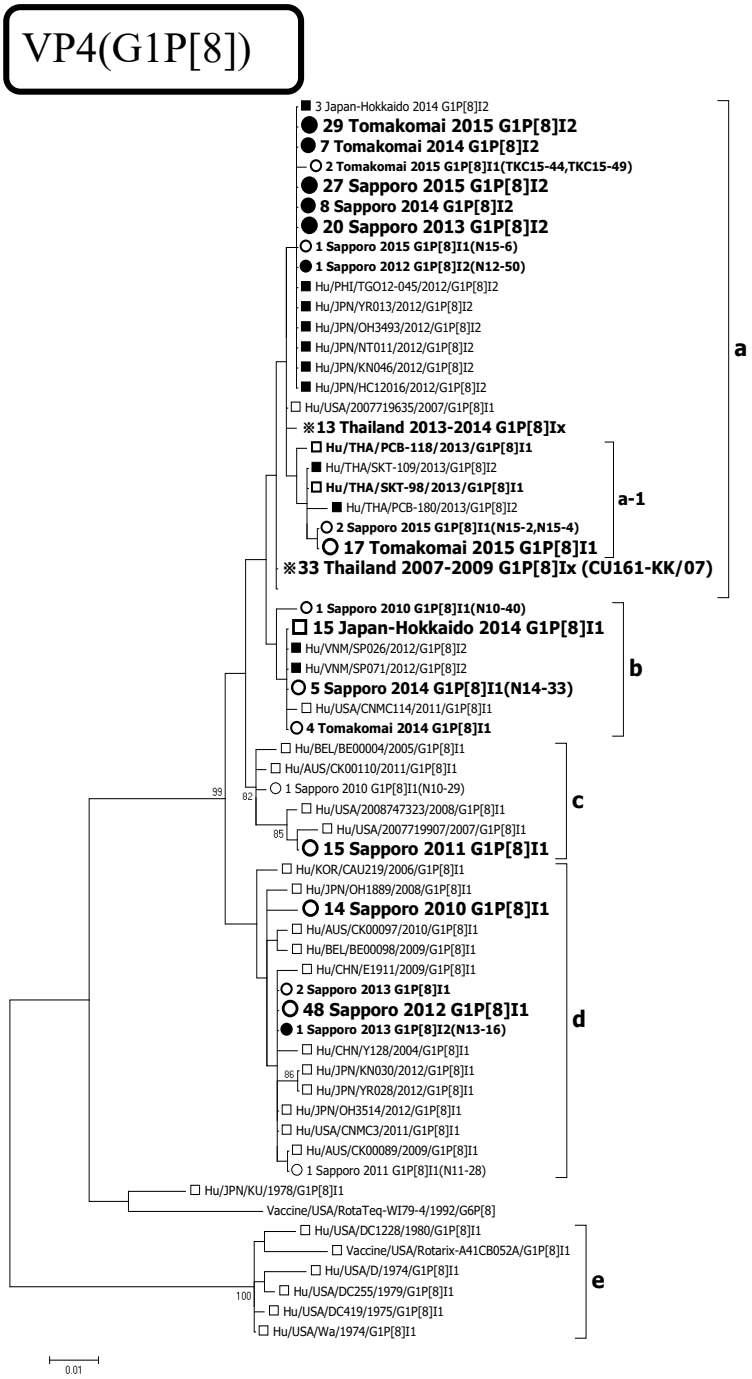

Figure 3. Phylogenetic analysis of VP4 genes in G1P[8] genotype strains.

The tree was constructed using the maximum-likelihood method with Tamura 3-parameter model and 1,000 bootstrap replicates. White and black circles indicate Wa-like and DS-1-like strains detected in this study, respectively. White and black squares indicate previously reported Wa-like and DS-1-like strains, respectively. Star indicates G1P[8]Ix strains prevalent in Thailand in 2007–2014. Bootstrap values are shown at the branch nodes (only values >80% are shown). Scale bar indicates genetic distance.

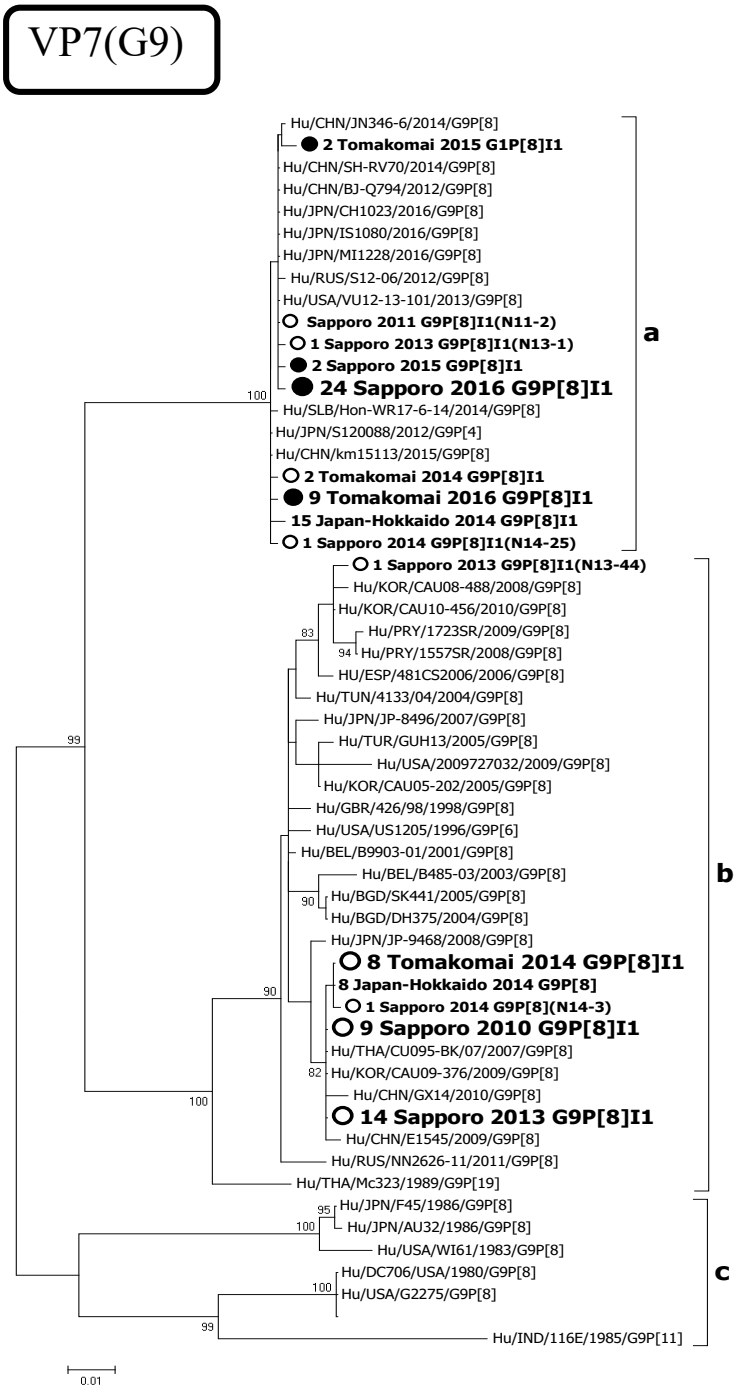

Figure 4. Phylogenetic analysis of VP7 genes in G9 genotype strains.

The tree was constructed using the maximum-likelihood method with Tamura 3-parameter model and 1,000 bootstrap replicates. White and black circles indicate G9P[8]I1 strains detected in 2010–2014 and 2015–2016 in this study, respectively. Bootstrap values are shown at branch nodes (only values >80% are shown). Scale bar indicates genetic distance.

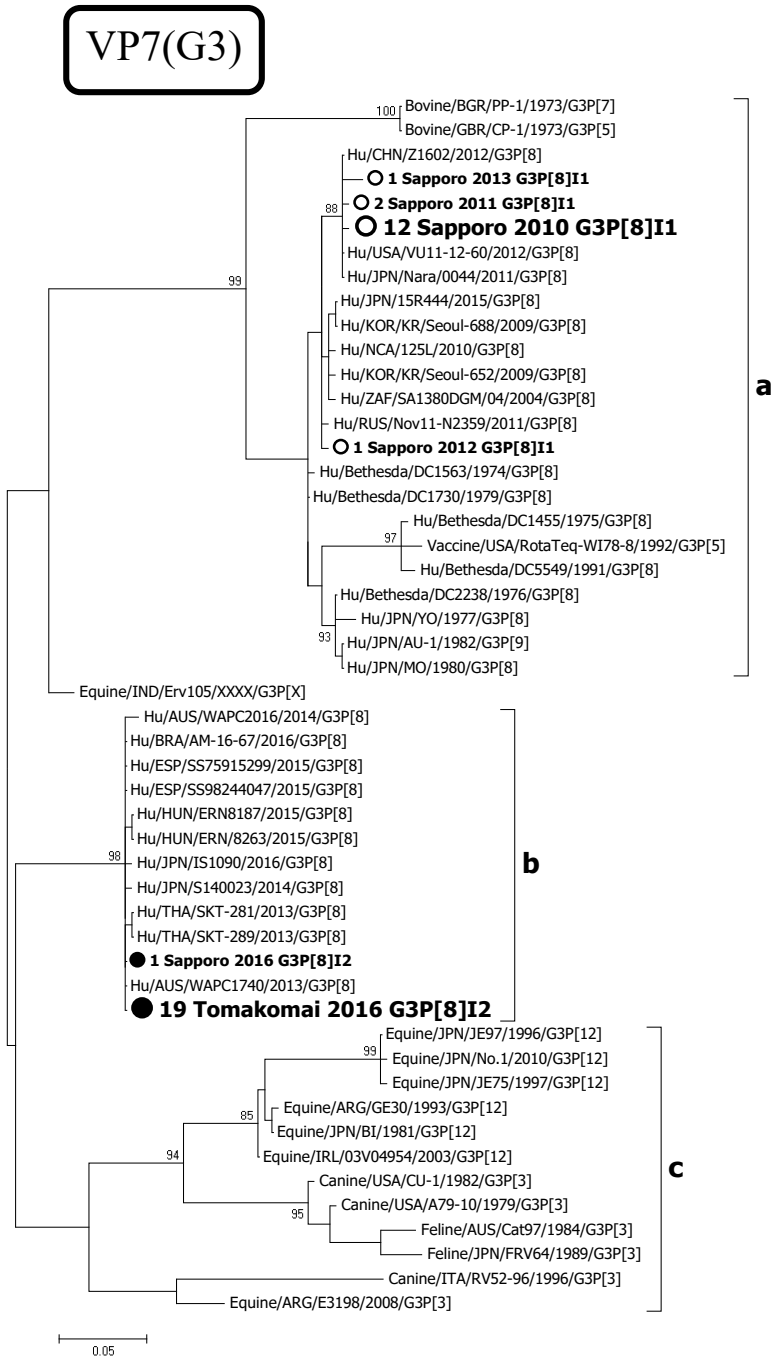

Figure 5. Phylogenetic analysis of VP7 genes in G3 genotype strains.
The tree was constructed using the maximum-likelihood method with Tamura 3-parameter model and 1,000 bootstrap replicates. White and black circles indicate common human G3P[8]I1 and human equine-like G3P[8]I2 strains detected in this study, respectively. Bootstrap values are shown at branch nodes (only values >80% are shown). Scale bar indicates genetic distance.