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Divergence of group a rotavirus with genetic variations before and after introduction of rotavirus vaccines in northern Taiwan

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Abstract

Despite the development of vaccines in 2006, rotavirus is still a major cause of acute gastroenteritis worldwide. This study was performed to analyze the presence of circulating rotaviruses before and after the introduction of rotavirus vaccines to allow phylogenetic comparisons of vaccine strains in northern Taiwan.

Rotavirus genotyping and sequencing of rotavirus VP7 and VP4 PCR products were performed by Reverse Transcriptase Polymerase Chain Reaction and DNA autosequencing. Phylogenies were constructed by the neighbor-joining and maximum-likelihood methods using CLUSTAL W software included in the MEGA software package (version 6.0).

Between April 2004 and December 2012, a total of 101 rotavirus specimens from pediatric patients with acute gastroenteritis hospitalized in Chang Gung Children's Hospital were amplified, and their VP4 and VP7 sequences were determined. These 101 specimens consisted of 55 pre-vaccine strains (G1 [13, 23.6%], G2 [12, 21.8%], G3 [16, 29.1%], and G9 [14, 25.5%]) and 46 post-vaccine strains (G1 [25, 54.3%], G2 [12, 26.1%], G3 [5, 10.9%], and G9 [4, 8.7%]). The most common combination of the G and P types was G2P[4], accounting for 36% cases, followed by G9P[8] (25%), G1P[8] (20%), G3P[4] (15%), G3P[8] (10%), G1P[4] (5%), and G2P[8] (5%). Phylogenetic analysis showed that only the G1 and P[8] genotypes clustered in the same lineages with the rotavirus vaccine strains.

Based on our results, the inclusion of G9, modified G2 and G3 with target lineages, and the combination G2P[4] and G9P[8] in the rotavirus vaccines in Taiwan is warranted as a vaccination strategy.

Abbreviations: AGE = acute gastroenteritis, CGCH = Chang Gung Children's Hospital, ELISA = enzyme-linked immunosorbent assay, RVA = Group A rotavirus.

Keywords: acute gastroenteritis, genetic variations, rotavirus, rotavirus vaccines

1. Introduction

Group A rotavirus is widely recognized as the major etiological agent of serious acute gastroenteritis (AGE) in young children and adults.[1] Group A rotavirus (RVA) infections lead to over 527,000 deaths and 2.4 million hospitalizations globally each year among children <5 years old, resulting in substantial economic burden.[2,3] Rotavirus is classified in the *Rotaviridae* family, and its genome encodes 2 outer capsid proteins, VP7 (glycoprotein; G protein) and VP4 (protease sensitive; P protein), which elicit neutralizing antibodies; the genes are also used to differentiate among rotavirus strains via a binary classification system based on the G and P genotypes.[4] VP4, which forms the head of the VP4 spike, interacts with receptors on host cells and is required for virion attachment and hence rotavirus infection. VP4 is cleaved by proteolysis into 2 fragments designated as VP5 and VP8.[5] So far, 27 G genotypes and 37 P genotypes have been reported in either humans or animals, although 5 common human genotypes (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]) represent 90% of RVA strains globally.[6]

Two live attenuated oral RVA vaccines targeting these common RVA strains were launched in 2006 to prevent disease burden; Rotarix (GlaxoSmithKline Biologicals, [Rixensart], Belgium) is a monovalent vaccine containing strain G1P[8], administered as a 2-dose series, and RotaTeq (Merck & Co., Inc., Whitehouse Station, NJ) is a pentavalent vaccine containing strains G1–G4 and P1A[8], administered as a 3-dose series.[7] In April 2009, the WHO prioritized the introduction of these vaccines in national immunization

Editor: Grant R. Campbell.

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The study was supported by grants from Chang Gung Memorial Hospital (CMRPG34011, CMRPG350391) and MOST 106-2314-B-182A-142 -MY2, Taiwan.

The authors declare that there are no conflicts of interest.

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How to cite this article: Chen YF, Lee CC, Chiu CH, Chang YC, Tsai CN, Chao HC, Kong SS, Chen SY. Divergence of group a rotavirus with genetic variations before and after introduction of rotavirus vaccines in northern Taiwan. Medicine 2020;99:9(e19253).

Received: 21 July 2019 / Received in final form: 30 December 2019 / Accepted: 17 January 2020

http://dx.doi.org/10.1097/MD.0000000000019253

programs worldwide.[8] In the USA and Finland, completed clinical trials showed 98% efficacy of RotaTeq against rotavirus gastroenteritis.[9] RotaTeq has also been introduced in Nicaragua, a developing country in Central America, as well as Asia and Africa, where it was shown to reduce the rate of hospitalizations due to rotavirus gastroenteritis by 44%.[10,11] Rotarix and RotaTeq were demonstrated to have high vaccine efficiency in the United States and Europe.[3,12,13]

In our previous study, we showed that suboptimal use of rotavirus vaccines in the private sector had a slow but modest impact on severe rotavirus AGE since rotavirus vaccine introduced in Taiwan in 2006, but norovirus infection rates continued to increase.[14–16] In this study, we further evaluated the epidemiology and conducted genotyping of circulating RVA before and after the introduction of 2 vaccines in Northern Taiwan between 2004 and 2012.

2. Materials and methods

2.1. Sample and data collection

Pediatric patients with AGE hospitalized at Chang Gung Children's Hospital (CGCH) from April 2004 to December of 2012 were enrolled in this study. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (95-0025B, 96-1518B, 98-3759B, 102-1437B), and either the participants or their guardians provided informed consent for both specimen and clinical data collection. We further enrolled rotavirus-positive AGE patients admitted to the Division of Pediatric Gastroenterology for further rotavirus genotyping and phylogenetic analysis. To understand the relationship between sample collection and climate variation, we calculated monthly average temperatures from the website of the Taiwan Central Weather Bureau (http://www.cwb.gov.tw/V7/climate/monthlyData/mD.htm).

2.2. RNA extraction and sequence analysis

All rotavirus-positive fecal samples were stored at −70 °C before extraction of viral nucleic acids using a commercial kit (QIAamp Viral RNA Mini Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions, as described previously.[17] First-strand cDNA synthesis and PCR were performed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. The PCR primer sets used for detection of rotavirus were described previously.[18,19] The sequences of the RVA VP7 and VP4 PCR products were determined using a DNA autosequencer (ABI 3770; Applied Biosystems, Foster City, CA). We further classified the rotavirus strains into pre-vaccine and post-vaccine periods by the launch of rotavirus vaccine in Taiwan of 2006.

2.3. Phylogenetic analysis

The phylogeny was constructed by the neighbor-joining and maximum-likelihood methods using CLUSTAL W software included in the MEGA software package, version 6.0. (Research Center for Genomics and Bioinformatics, Tokyo Metropolitan University, Hachioji, Tokyo, Japan).[20] The reliability of interior branches in the phylogenetic tree was assessed by the bootstrap method with 1000 resamplings. All reference sequences used in this study were obtained from GenBank and were selected based on previous reports.[21]

3. Results

3.1. Prevalence of rotavirus gastroenteritis

A total of 16,603 children with AGE symptoms visited the Division of Pediatric Gastroenterology, Chang Gung Children's Hospital (CGCH), between 2004 and 2012 (Fig. 1, dashed line). We identified 3273 (19.7%) rotavirus-positive fecal specimens by enzyme-linked immunosorbent assay (ELISA) at the Department of Clinical Pathology, CGCH (Fig. 1, black line). The rate of rotavirus positivity was 18% to 24% from 2004 to 2011, with a winter peak that was observed each year but was no longer apparent in 2012 (7%) (Fig. 1, gray line). The rotavirus disease burden before and after vaccine introduction was pre-vaccine period (753 of 2860, 26.3%) and post-vaccine period (2520 of 13,743, 18.3%) of all AGE. During this 9-year period, increased numbers of AGE and rotavirus gastroenteritis cases were observed when the average temperature was below 17 °C from December to February, until 2012, indicating that rotavirus vaccines applied in the private sector caused a slow but significant reduction in rotavirus gastroenteritis cases 6 years after the vaccine was launched in Taiwan.

We further enrolled 852 patients with AGE admitted to the Division of Pediatric Gastroenterology, CGCH, during this period. These cases included a total of 205 rotavirus-positive cases, accounting for 24% of our total AGE hospitalization cases. We amplified and determined the VP4 and VP7 sequences of 101 rotavirus specimens. These viral strains were divided into pre-vaccine and post-vaccine strains by rotavirus vaccine introduced in 2006.

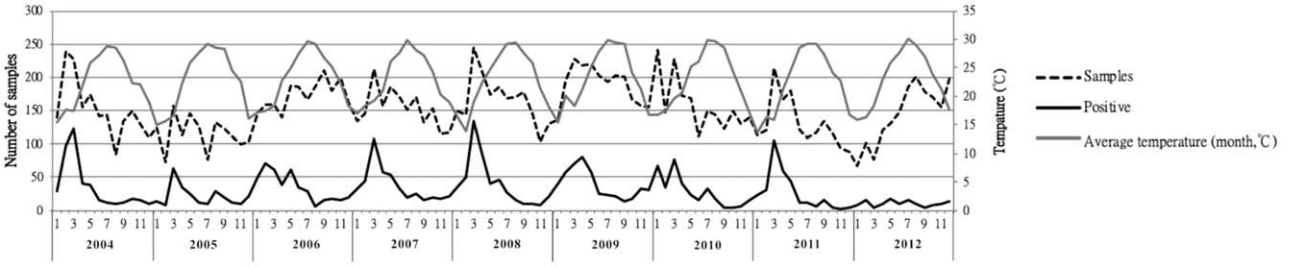


Figure 1. The average temperatures, total AGE cases, and Rotavirus positive cases who visit Chang-Gung Memorial hospital since 2004 to 2012. The average temperature per month (grey line) was downloaded from website of Taiwan central weather bureau (http://www.cwb.gov.tw/V7/climate/monthlyData/mD.htm). The total AGE cases (dash line) and RVA positive cases (black line) were obtained from Department of clinical pathology of Chang-Gung Memorial hospital. AGE = acute gastroenteritis, RVA = Group A rotavirus.

Table 1

Rotavirus genotypes with clusters lineage and representative strains before and after vaccine introduction.

Capsid antigen	Genotype (No.)	Lineage (no.) (pre-vaccine period)	Lineage (no.) (post-vaccine period)	Cluster lineage representative strains
VP7	G1 (38)	LI: 10, LII: 0, LIII: 3	LI: 25, LII: 0, LIII: 0	LI: R833/JPN (EU708571), Mvd9816/Urug (AF480293), CH31/TW (AF183848); RVA/Vaccine/USA/Rotateq-W179-9 (GU565057) LIII: A RMC/G7/USA (AY603150)
	G2 (24)	LI: 6, LII: 5, LIII: 1	LI: 12, LII: 0, LIII: 0	LI: TB Chen/China (AY787646); LII; RVA/Vaccine/USA/Rotateq-SC2-9 (GU565068); LIII: RVA/Human-tc/GBR/ST3/USA (EF672616)
	G3 (21)	LI: 14, LII: 1, LIII: 1	LI: 5, LII: 0, LIII: 0	LI: Human Rotavirus 95-91 Japan (D85266); LII: RVA/Vaccine/USA/Rotateq-W178-8 (GU565079); LIII: Hun4/JPN (AJ487833)
	G9 (18)	LI:14, LII: 0, LIII: 0	LI:4, LII: 0, LIII:0	LI: Human Rotavirus strain BA202/USA (AY695810)
VP4	P4 (13)	LI: 5 (Sub.LA.:4, Sub.LB.:1)	LI: 8 (Sub.LA.:3, Sub.LB.:5)	Sub.LA: Rotavirus strain KO-2/JPN (AF401755), Sub.LB: GRAVP425/Ind. (AB547711)
	P8 (12)	LII: 6	LII: 6	LII: Rotavirus strainCU308-NR/08/Thailand (GQ996827); RVA/Vaccine/USA/Rotarix-A41CB052A (JN849113)

3.2. Distribution of rotavirus genotypes

The 101 rotavirus isolates were clustered based on their lineage (Table 1), comprising 55 pre-vaccine (2004–2005) strains (G1 [13, 23.6%], G2 [12, 21.8%], G3 [16, 29.1%], and G9 [14, 25.5%]), and 46 post-vaccine (2006–2012) strains (G1 [25, 54.3%], G2 [12, 26.1%], G3 [5, 10.9%], and G9 [4, 8.7%]). Rotavirus genotype distribution before and after vaccination. G-typing of rotaviruses revealed the average distributions of G1, G2, G3, and G9 during the pre-vaccine period. During the post-vaccine period, G1 accounted for more than half of all genotypes, followed by G2, with a similar distribution as that during the pre-vaccine period. The most common binary combination of the G and P types was G2P[4], representing 36% of typeable cases, followed by G9P[8] (25%), G1P[8] (20%), G3P[4] (15%), G3P [8] (10%), G1P[4] (5%), and G2P[8] (5%). The pre-vaccine strains included 4 cases of G2P[4], 4 cases of G9P[8], 2 cases of G3P[8], and 1 case of G1P[4], whereas the post-vaccine strains consisted of 5 cases of G2P[4], 4 cases of G1P[8], 3 cases of G3P [4], 1 case of G2P[8], and 1 case of G9P[8].

3.3. Phylogenetic analysis of the human rotavirus VP7 and VP4 genes

Figure 2 showed phylogenetic analysis of VP7 nucleotide sequences. During the pre-vaccine period, the majority of the 13 G1 strains belonged to lineage I (10 in lineage I and 3 in lineage III). In the phylogenetic analysis, clustering indicated that the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-W179-9 [GU565057]) also belonged to lineage I. Most of the 12 G2 strains belonged to lineages I and II (6 in lineage I, 5 in lineage II, and 1 in lineage III), and the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-SC2-9 [GU565068]) clustered in lineage II. The majority of the 16 G3 strains belonged to lineage I (14 in lineage I, 1 in lineage II, and 1 in lineage III), and the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-W178-8 [GU565079]) clustered in lineage II. The 14 G9 strains all belonged to lineage I. The 5 P4 strains clustered into 2 sublineages (4 in sublineage A and 1 in sublineage B), and the 6 P8 strains clustered in lineage II. The rotavirus vaccine strain (RVA/Vaccine/USA/Rotarix-A41CB052A [JN849113]) also clustered in lineage II. During the post-vaccine period, all 25 G1 strains belonged to lineage I, and the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-W179-9 [GU565057]) also belonged to lineage I according to clustering analysis. The 12 G2 strains all belonged to lineage I, and the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-SC2-9 [GU565068]) clustered in lineage II. All 5 of the G3 strains belonged to lineage I, and the rotavirus vaccine strain (RVA/Vaccine/USA/Rotateq-W178-8 [GU565079]) clustered in lineage II. All 4 of the G9 strains belonged to lineage I.

In the Fig. 3, phylogenetic analysis of P-typing showed clustering of the 8 P4 strains in 2 sublineages (3 in sublineage A and 5 in sublineage B) and of the 6 P8 strains in lineage II. The rotavirus vaccine strain (RVA/Vaccine/USA/Rotarix-A41CB052A [JN849113]) also clustered in lineage II.

3.4. Molecular modeling of VP7 and VP4 structures

We analyzed an isolated rotavirus (RVA #764) belonged to G2P [4] genotype from patient received rotavirus vaccination. The amino acid differences in the neutralizing epitopes of the VP7 and VP4 proteins were aligned with Rotarix and RotaTaq strains. The neutralizing epitopes of VP7 has 2 region, 7-1 (divided into 7-1a; 7-1b) and 7-2 (Fig. 4A). The 7-1 epitope of Rotarix includes T87, T91, N94, G96, E97, W98, K99, D100, Q104, K291, Q201, N211, D213, N238, and T242 amino acid. The 7-2 epitope of Rotarix contains D145, Q147, L148, S190, M127, N221, K223, and G264, which were exposed to the surface of VP7 protein. The residues of 7-1a, 7-1b, and 7-2 epitopes of the RVA #764 and the vaccine strains of Rotarix and RotaTeq are compared as revealing via molecular modeling. There were 12 amino acid differences between the RVA #764 and Rotarix or RotaTaq vaccine strain. Mapping of the differences in the VP7 epitopes of the RVA 764 strain and Rotarix vaccine strain onto the VP7 trimer revealed the distinct structure (Fig. 4B).

The VP4 antigenic epitopes of RVA #764 showed a large numbers of differences with Rotarix VP4 epitopes. The VP4 spike protein requires its proteolytic cleavage into VP8 head and VP5 stalk, the VP8 head contains 4 surface-exposed antigenic epitopes region, 8-1 to 8-4 (Fig. 5A), which have been predicted to include the 25 amino acid, there're 13 different residues as compared with Rotarix and RotaTaq vaccine strains, which was concentrated in 8-1 to 8-3 epitopes (Fig. 5A and B). In VP8 region, epitope 8-1 is located near the sialic acid and the positions of proximal sugar residues in an oligosaccharide side chain. It could help the virus binding and entry to cell. In previous study, mAbs

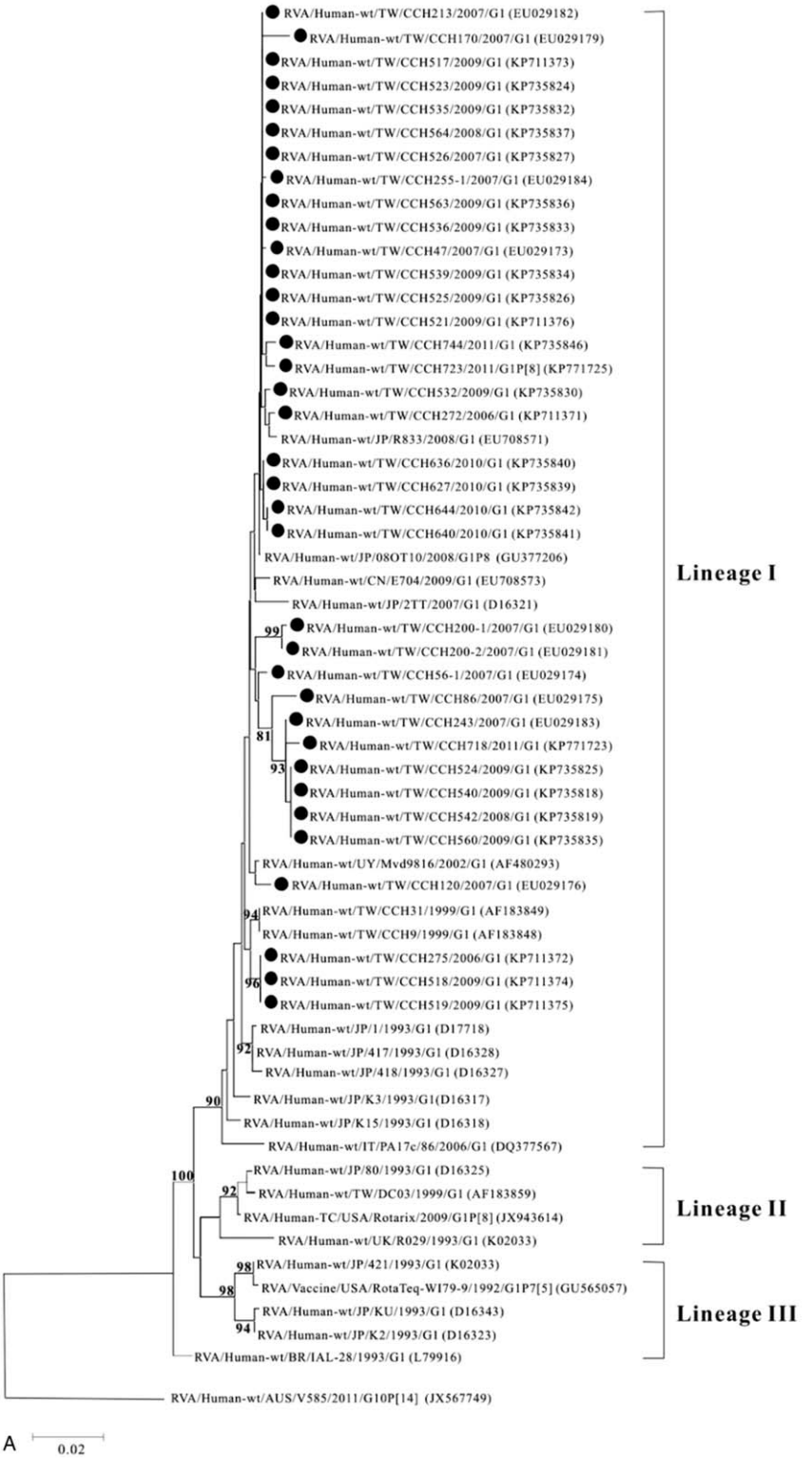


Figure 2. Phylogenetic analysis of VP7 nucleotide sequences of G1 (A), 2AG2 (B), G3 (C), and G9 (D) of rotavirus representative strains circulating in North Taiwan from 2004 to 2012. The phylogenetic analysis was based on the Maximum-likelihood method using the MEGA 6.0 program. The sub-lineages is as indicated. RVA strains isolated from Northern Taiwan were indicated in solid circle. The reference RVA strains were chosen as previously indicated (ref). RVA = Group A rotavirus.

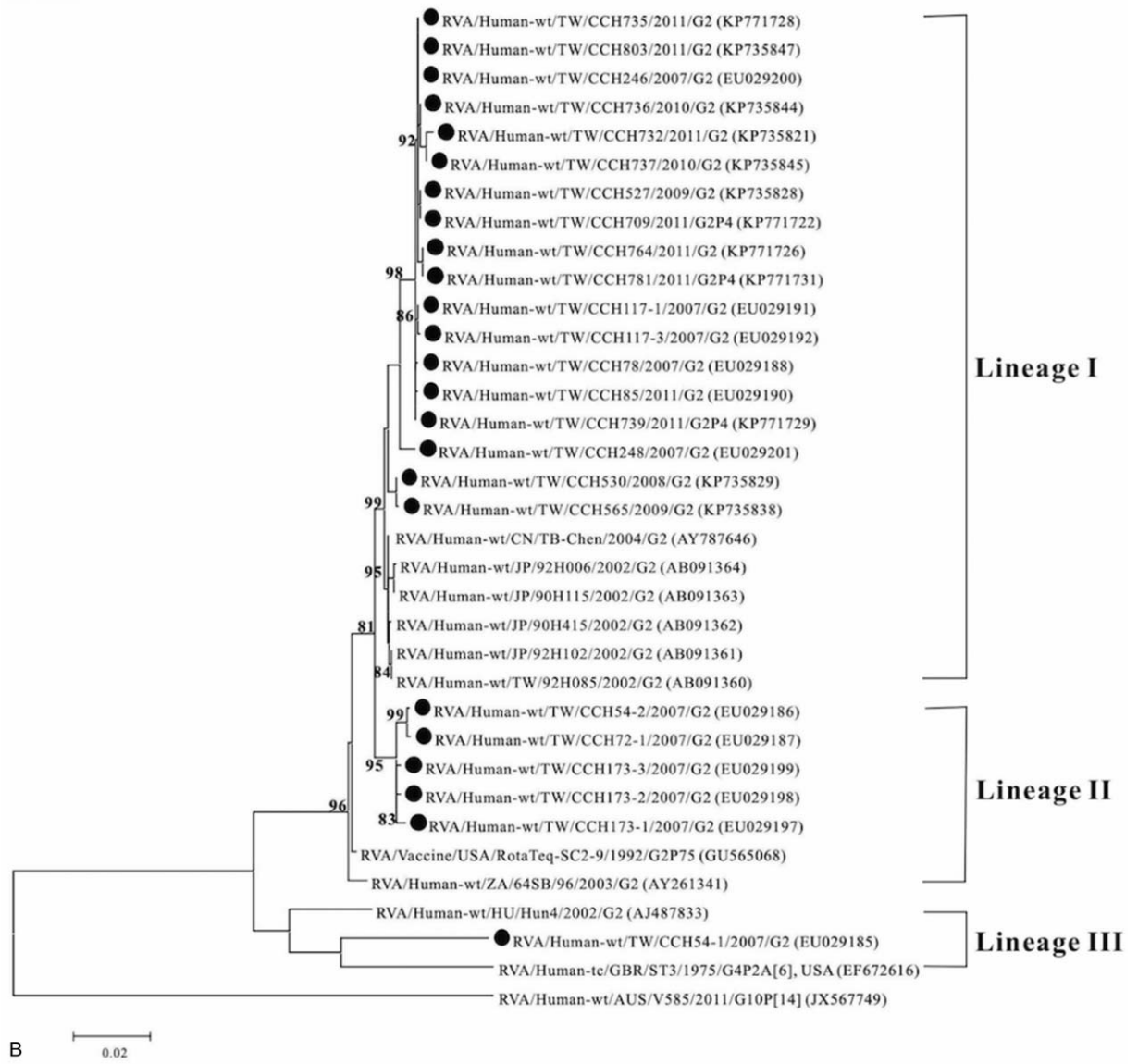


Figure 2. (Continued).

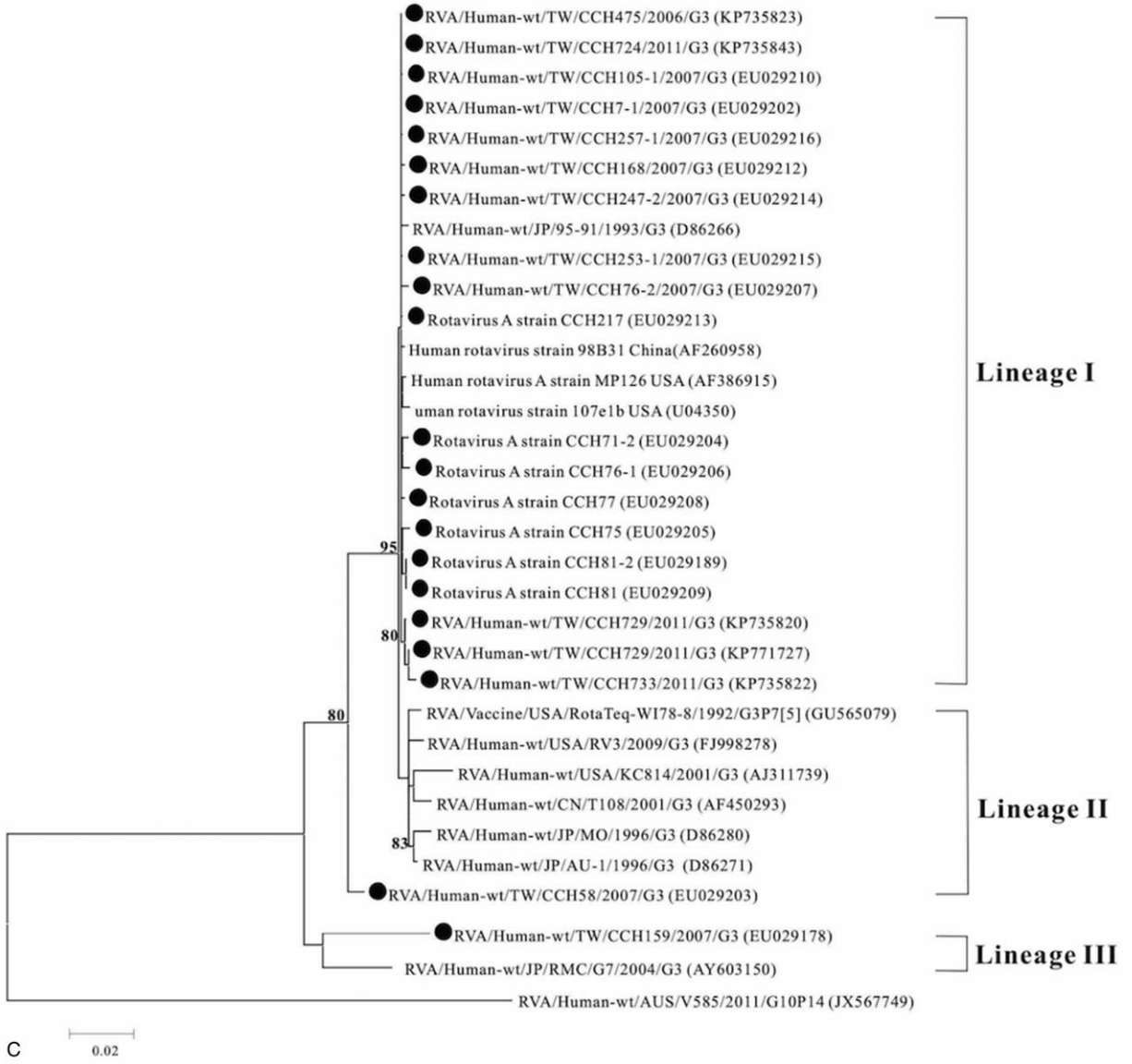
that select escape mutations at residues 100, 148, and 188 block binding to cells. Taken together, the amino acid differences in the neutralizing epitopes of VP4 and VP7 might contribute the structure differences to result in loss of protection of Rotavirus vaccines. Therefore, to continuously monitor local region RVA genotype should be necessary to disclose the protection efficiency of vaccines strains.

4. Discussion

Viral gastroenteritis remains one of the most common infectious diseases worldwide, including in Taiwan. The prevalence of rotavirus gastroenteritis has not decreased significantly despite 5 years of private sector vaccination in Taiwan with the published data encompassing the period from 2004 until 2011 indicate that a major reason for this is the low penetration rate of approximately 20% to 30%.[16] Rotavirus gastroenteritis was found to peak in Northern Taiwan during the winter season, but the annual peak of rotavirus infection has gradually become less prominent.[15,16] According to our continuous surveillance, the winter peak disappeared by 2012.

4.1. Rotavirus genotype distribution before and after vaccination

The average distributions of G1, G2, G3, and G9 during the pre-vaccine period were recognized. Among them, G9 was not included in the compositions of the vaccine strains. During the post-vaccine period, G1 accounted more than half (54.3%) of all genotypes, followed by G2 with similar prevalence to the pre-vaccine period. Meanwhile, the prevalence of G3 was lower than those of G1 and G2, and G9 was still present but at the lowest level of all 4 genotypes (8.7%). According to the genotype distribution, G1 outbreaks indicated that in Taiwan, vaccines



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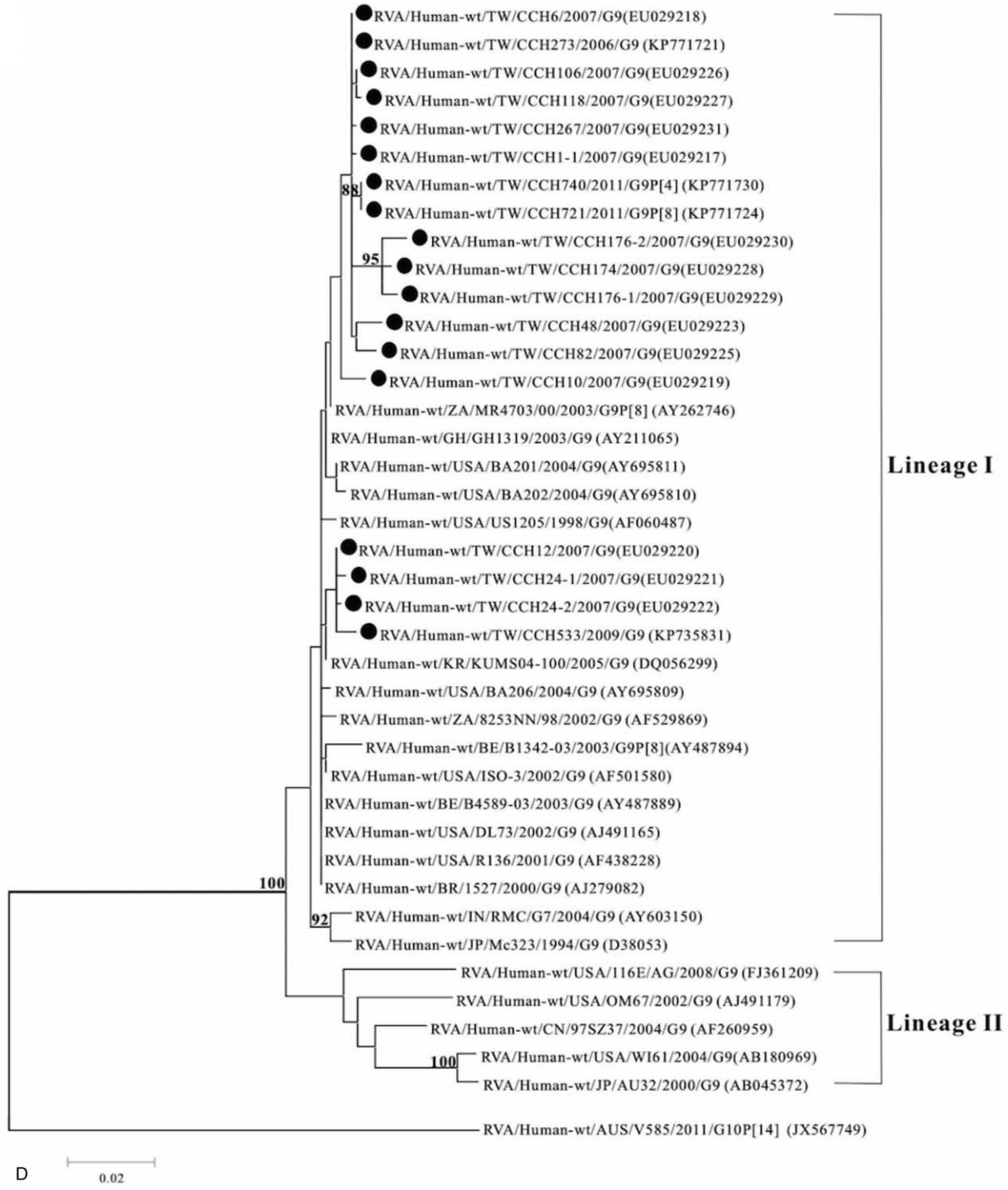
targeting G1 type rotavirus would be vital for future prevention of rotavirus infection, and further investigation is required regarding the inclusion of the G9 genotype in vaccine strains for more complete targeting of rotavirus strains. Based on our results, P-typing of the rotaviruses showed that P[4] and P[8] were near equally distributed; however, the P[4] type, which is not covered by current vaccines, was more common during the post-vaccine period. Further studies on circulating P[4] genotype strains are required for future vaccine coverage.

In regards to G and P combination typing, we found the rotavirus strains were divergent. In the pre-vaccine period, G2P [4] and G9P[8] were the 2 major strains. These 2 major strains were co-circulating in Argentina during 2004 to 2007 and in Ireland in 2011 with emergence, and G9P[8] was circulating in Jiangsu, China between 2010 and 2016.[22–24] G2P[4] and G1P [8] were cocirculating during the post-vaccine period and were isolated in 2006 (early post-vaccine period) and 2011 (late-post-vaccine period). This was similar to the recent epidemics in Venezuela, Japan, Ireland, central Australia, and the United States.[23,25–27] Furthermore, intragenogroup reassortment among cocirculating G1P[8] and G2P[4] strains was ever reported.[28] Other than genetic divergence, the overall predominant combination types of G2P[4] and G9P[8] showed no specificity for either VP7 or VP4 with the vaccine used in Taiwan and may represent a challenge for future vaccination strategies.

4.2. Phylogenetic analysis and correlation of vaccine strain lineage

The G1 vaccine strain (RVA/Vaccine/USA/Rotateq-W179-9 [GU565057]) was found to belong to lineage I, which was the same lineage associated with our isolated strains pre- and post-vaccine. We considered that the high distribution of the G1 type during the post-vaccine period was due to limited vaccine coverage, as only approximately 30% of isolates in Taiwan were



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obtained from unvaccinated infected children. Regarding the G2 and G3 types, the G2 (RVA/Vaccine/USA/Rotateq-SC2-9 [GU565068]) and G3 (RVA/Vaccine/USA/Rotateq-W178-8 [GU565079]) vaccine strains both clustered in lineage II. They belonged partially to the same lineage as our pre-vaccine strains (G2: 5/11 [45.5%]; G3: 1/16 [6.3%]), but none of our post-vaccine strains. Further studies are needed to investigate the G2 and G3 types in the different lineages. Regarding the P types, the rotavirus P[8] vaccine strain (RVA/Vaccine/USA/Rotarix-A41CB052A [JN849113]) clustered in lineage II, and all P[8] isolates belonged to the same lineage, indicating sufficient prevention of P[8] type rotavirus infection. However, P[4] type was found at equivalent or even greater rates than P[8] during the pre- and post-vaccine periods. Further studies evaluating the addition of P[4] to rotavirus vaccines to prevent infections of rotaviruses of different P types are needed.

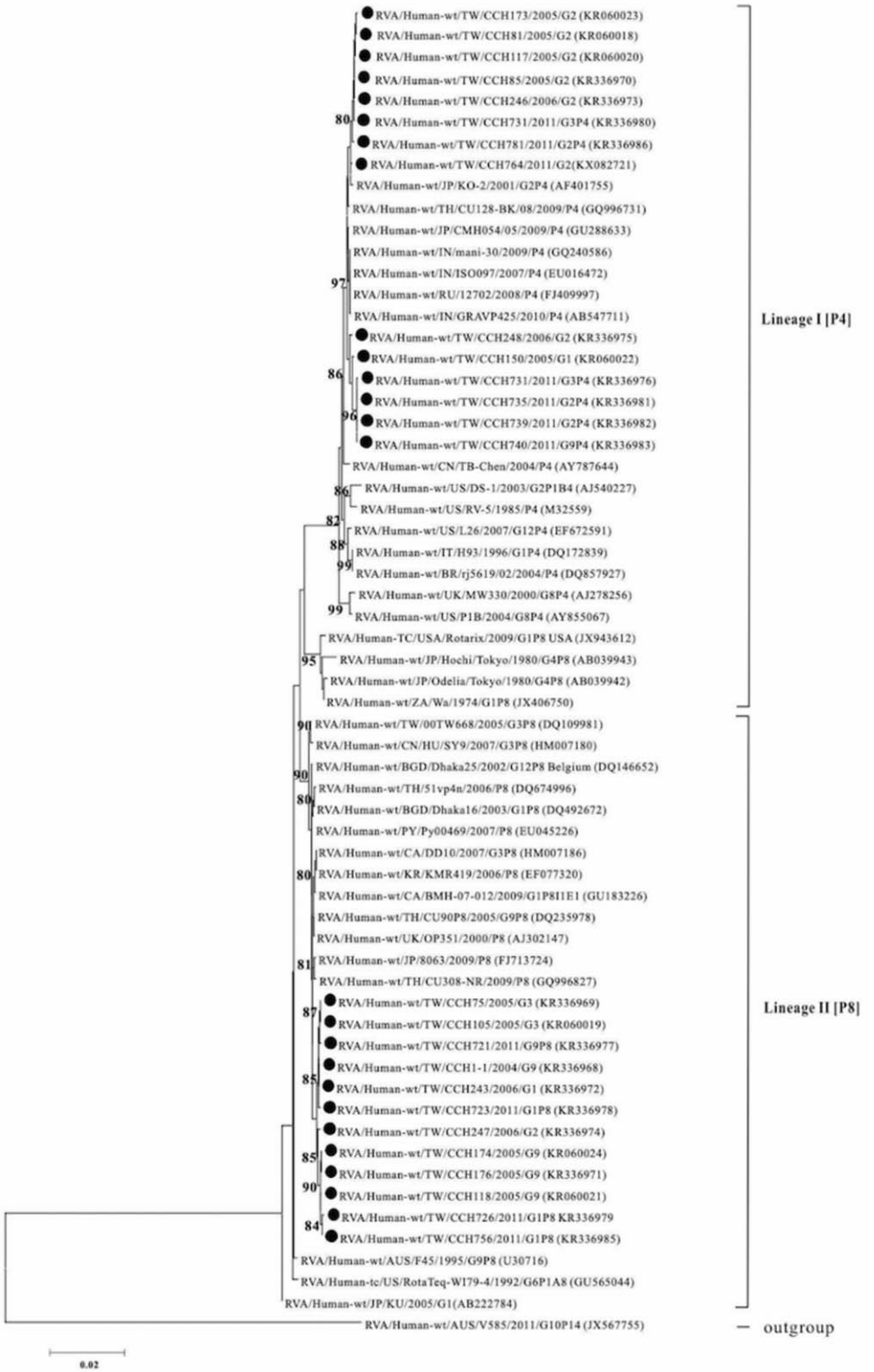

Figure 3. Phylogenetic analysis of VP4 nucleotide sequences of rotavirus representative strains circulating in North Taiwan from 2004 to 2012. The phylogenetic analysis was based on the Maximum-likelihood method using the MEGA 6.0 program. The P[4] and P[8] sub-lineages were as indicated. RVA strains isolated from Northern Taiwan were indicated in solid circle. The reference RVA strains were chosen as previously indicated (ref). RVA = Group A rotavirus.

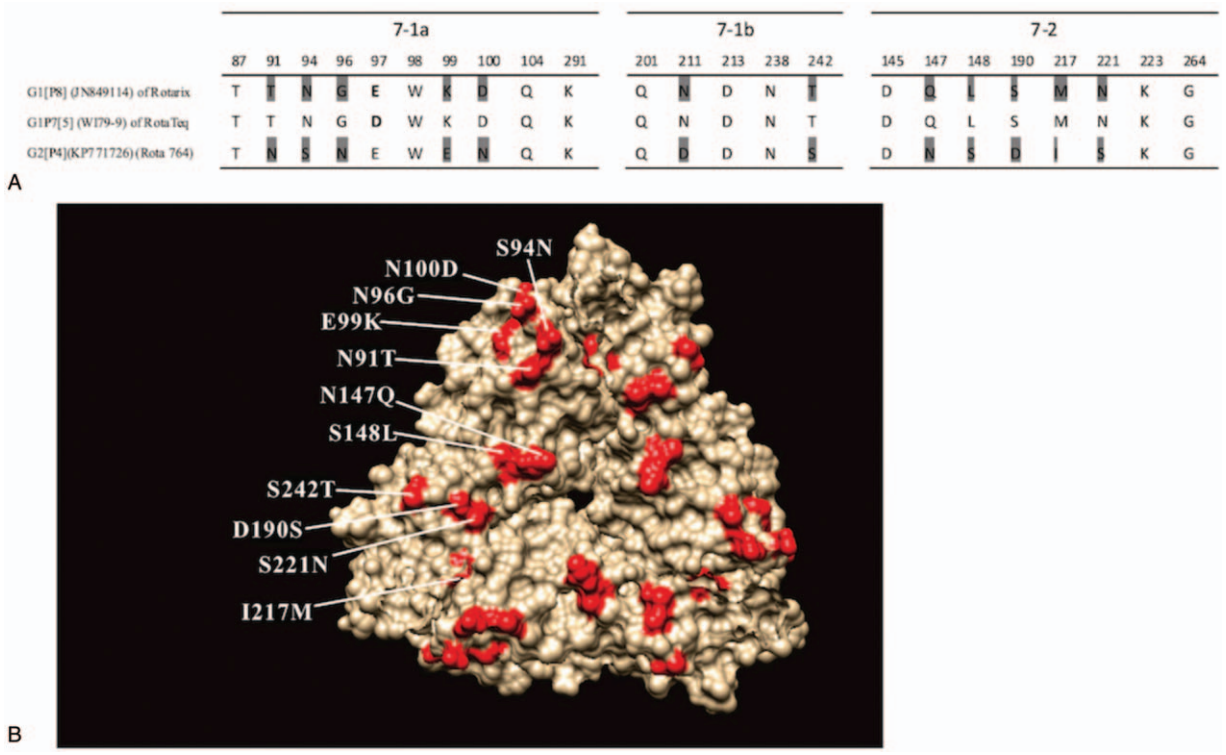

Figure 4. Molecular modeling of VP7 protein in RVA #764 and Rotrix vaccine strain. (A). Protein alignment of neutralizing residues in VP7 protein among the Rotarix, RotaTeq, and RVA 764 strains isolated from Northern Taiwan. There are 3 epitopes (7-1a, 7-1b, and 7-2) within VP7 protein. (B). Surface representation of the VP7 trimer (PDB 3FMG). Surface-exposed residues that differ between RVA 764 and the Rotarix strain. RVA = Group A rotavirus.

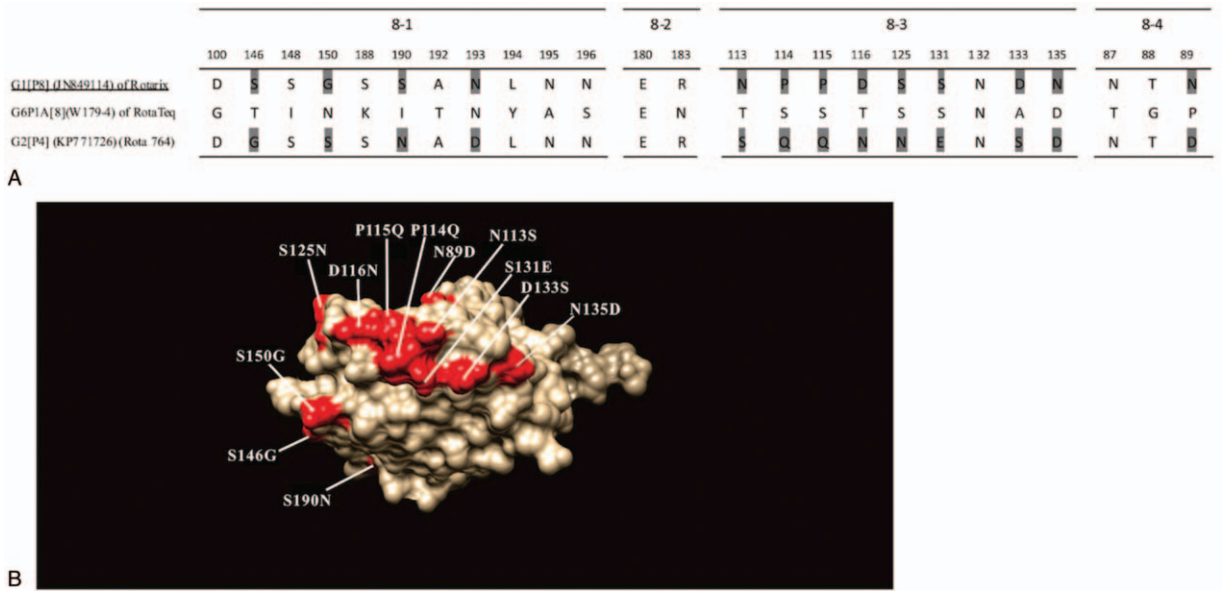

Figure 5. Molecular modeling of VP8 protein in RVA #764 and Rotrix vaccine strain. (A) Protein alignment of neutralizing residues in VP8 protein among the Rotarix, RotaTeq, and RVA 764 strains isolated from Northern Taiwan. There are 4 epitopes (8-1, 8-2, 8-3, and 8-4) within VP8 protein (B). Surface representation of the VP8 trimer (PDB 2AEN). Surface-exposed residues that differ between RVA 764 strain and the Rotarix are shown (B). RVA = Group A rotavirus.

This study provides the first epidemiological evidence of the divergence of rotavirus strains with genetic variations in northern Taiwan during the pre- and post-vaccine periods. Continuous surveillance of antigenic changes in VP7 and VP4 proteins in wild-type rotavirus strains is warranted in Taiwan, where use of the currently approved rotavirus vaccines is suboptimal.

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