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Genetic Evolution of Vaccine-Derived Poliovirus (VDPV) in an Immunodeficient Patient

Abstract

In April 2001, the Center for Disease Control received a report of a patient diagnosed with acute flaccid paralysis at the Chang Gung Linkou Hospital. This patient was immunodeficient. In an 11 month period, one throat swab and eight fecal specimens were collected: on days 5, 17, 18, 52, 54, 179, 224, 261, and 337 after onset of the disease. The difference between this viral genomic sequence and that of the Sabin type 1 vaccine strains was between 1.80 and 2.96%.

The entire genomic sequence did not show genetic recombination. Some neucleotide and amino acids of the Sabin type 1 vaccine strains had mutated to the original strains of Mahoney.

This was the first immunodeficient patient with vaccine-derived poliovirus (iVDPD) recorded in Taiwan. The patient continued to shed the virus. If Polio immunization is discontinued after global eradication of polio is declared, the iVDPD could cause circulating Vaccine Derived Polio Virus (cVDPV) infection. Findings of the present study could serve as a scientific reference for the formulation of immunization strategies after the global eradication of polio.

Introduction

Attenuated oral polio vaccine (OPV) is used in Taiwan. OPV can cause vaccine-associated paralytic poliomyelitis (VAPP)[1,2,3,4,5], particularly in children with B-cell immunodeficiency. Children with normal immunity will generally shed the virus for 3-4 weeks after oral administration of OPV, and when herd immunity is high, the spread of virus will be prevented[6]. The polioviruses isolated from the immunized and their contacts are similar in their nucleotide series to the Sabin OPV strain VP1 region by more than 90%, and are referred to as similar vaccine strains[7]. When similarity is larger than or equal to 99%, they are designated vaccine-derived poliovirus (VDPV). The 1% difference suggests that the vaccine strains have been cloning for at least one year.

In 2001, the Center for Disease Control Acute Flaccid Paralysis (AFP) Monitoring System detected a case with VAPP symptoms. Fecal specimens were collected, and through viral analysis, poliovirus type 1 strain was isolated. By augmentation of the 5' non-coding region with reverse transcriptase polymerase chain reaction (RT-PCR), PCR products were noted in the VP1, VP4, VP3, and VP4 regions as anticipated [18-20]. Gene sequencing was then used to analyze and compare their components.

Materials and Methods

1. Immunodeficient Patient

The patient was born in 1993 and received five doses of oral polio vaccine at age 2, 4, 6, 15 months, and 6 years (Figure 1), without any complications. On April 6, 2001, the child developed fever, cough, and runny nose, and was treated at a clinic. Paralysis of the left hand appeared on April 9, followed by weakness of the right upper arm and both lower limbs. The child became unable to walk. Testing of the presence of Polio antibodies in May showed that the IgG, IgA, and

IgM were 720 mg/dl, <5.88 mg/dl, and 7.78 mg/dl, respectively, and the CD4/CD8 value was 0.3. The child was diagnosed as immunodeficient.

2. Isolation of Virus and Assessment of Types

One throat swab was collected from the patient five days after onset, of illness and fecal specimens were collected on days 17, 18, 52, 54, 179, 224, and 337 after onset. Specimens were processed according to the WHO standard operational procedures for fecal specimens[8], and inoculated onto RD, L20B, and Hep-2 cells for virus isolation. Nine virus strains were obtained. The virus strains were typed by the indirect immunofluorescence method (Chemicon Inc., USA) and polyvalent polio anti-serum neutralization test.

3. Gene Sequencing of Polioviruses

Extraction of RNA

The QIAmp viral RNA kit was used for the purification of RNA. 140 μl of virus fluid was mixed evenly with 560 μl buffer AVL solution and placed at room temperature for 10 minutes, then 560 μl of absolute alcohol was added. The fluid passed through QIAmp spin columns twice, and each column was washed with buffer AW1 and AW2. RNA was finally dissolved in 60 μl of pure water.

Reverse Transcriptase Polychain Reaction (RT-PCR)

The single tube, single step RT-PCR method was used(9). 5 μl of viral RNA was placed in the PCR tube; 20 μM of positive and negative primers, 0.2mM of d-NTP, 2mM of Magnesium Chloride, Tris-HCl, and 10 U of RNA Inhibitor Enzyme was added.. 5 U of polymerase and distilled water were then added to a total volume of 50 μl, to undergo RT reaction at 42℃ for 3 minutes to produce cDNA. The PCR procedure continued at 95℃ for 3 minutes for denaturalization, then 35 secondary reactions proceeded at 94℃ for 30 seconds, 50℃ for 30 seconds, and 70℃ for one minute, and finally at 70℃ for 10

minutes.

Sequencing Analysis

The ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used for nucleic acid products. The ABI model 3730 automatic nucleic acid fluorescent sequence meter was used for bilateral sequencing analysis.

Phylogenetic Analysis

The isolated nine specimens and poliovirus type 1 strain were used with computer soft Molecular Evolutionary Genetics Analysis (MEGA) version 2.1 for drawing VP1, P1 and ORF phylogenetics. The neighbor-joining analysis method was used, and bootstrapped 1,000 times for phlyogenetic analysis.

Results

Genes of the nine isolated poliovirus strains contained 742 neucleotides at the 5' non-transcription zone, primarily for the adjustment of gene cloning and expression. Their internal ribosome entry site (IRES)[10] (Figure 2) was located between 130 and 600 neucleotides. The 5' non-transcription zone was a stem-loop composed of six domains; domains II, IV, and V primarily for transcription. The partial deletion before the 130th and after the 600th neucleotides at the 5' end did not affect the transcription effects. Of the nine poliovirus type 1 strains isolated at different times, the 26th neucleotide in domain I (except D18, the poliovirus type 1 strain isolated on the 18th day after onset) became adenine (A) from guanine (G). The 344th neucleotide in domain IV (except D18) became C from U; the 355th neucleotide became C from U; the 480th neucleotide in the stem-loop V became A from G to increase its neurotoxicity expression. This corresponded to findings of previous studies[13,14] (Table 1, Figure 3). In addition, the 742nd neucleotide in the 5' non-transcription zone, with the exception of D17, the rest had a deletion of eight neucleotides at the

667th position. The D5 deleted eight and 22 neucleotides at the 667th and 695th, respectively (Figure 4). Neucleotides G26A, U355C, and G480A had all mutated to the same as the Mahoney strain.

P1 zone is the structural decoding protein. It comes in four parts; most amino acids of VP1 are exposed on the surface of virus; some of VP2 and VP3 are on the surface; and all of VP4 are within the virus. P2 and P3 are non-structural proteins, and viruses isolated on D5, D17, D18, D52, D54, D179, D224, D261, and D337, differ in their neucleotides and amino acids in the VP1 zone with the Sabin type 1 vaccine strain by 2.76/2.65, 2.43/2.65, 2.43/3.31, 2.76/3.31, 2.76/3.31, 2.98/3.64, 3.20/3.64, 3.20/3.64, and 3.53/3.97, respectively, higher than those of VP2, VP3, and VP4. 2B, not VP1, had the highest variability, the differences were 3.44/3.09, 3.09/2.06, 3.09/3.09, 4.12/3.09, 4.12/3.09, 4.47/4.12, 3.78/3.09,, 4.47/3.09, and 4.81/4.12, respectively; and in the open reading frame (ORF), they were 1.99/1.45, 1.84/1.18, 1.98/1.22, 2.16/1.54, 2.19/1.58, 2.54/1.77, 2.55/1.67, 2.69/1.72, and 3.15/1.81, respectively (Table 2). A large part of the entire gene was silent; they were 72.7%, 72.1%, 75.9%, 73.4%, 73.8%, 75%, 75.7%, 76.4%, and 80.4% respectively. Genes of viruses isolated at different times showed different degrees of evolution, and 3B, 3C, and 3D moved upward by time (Figure 5, Table 2).

In the nine strains, their 95th amino acid (T95I) of 2B, and the 73rd and 250th amino acids (H73Y, E250K) of 3D had become the Mahoney strain; the 165th amino acid of VP2, except D17 one becoming G, the remainder t became wild strain N (D165N); the 225th amino acid of VP3 was the same as the vaccine strain on D17 and D18, the rest became wild strain L; the 99the amino acid of VP1 was, on D17, the same as the vaccine strain, on D5 became M, and the rest became T; amino acid 106 became A on D5, remain unchanged on D17, and the rest became A (Table 3, A, B, and C). In the 3' non-transcription zone, except on D261, the

rest were G7441A.

Phylogenetic analysis was conducted at the VP1, P1, and ORF zones for poliovirus type 1 and nine strains of iVDPD (Figure 5) by the neighbor-joining method, bootstrapped 1,000 times. They revealed four different series.

In the virus RNA genomes, the P1 zone was the antigen structure of the poliovirus. Changes in the amino acid brought about by serial mutation might lead to changes of the antigen protein structure, which could causee antibodies to fail to recognize and unable to produce neutralization reaction[21-23]. There were in the four P1 amino acid zones that determined antigen neutralization (Figure 7, areas indicated with dark lines); the parts of nine isolated strains that are different from the poliovirus type 1 vaccine are indicated in red. In this way, the VP1 amino acids series 96-106 in site 1 (Table 3 B, Figure 7), VP1 amino acids series 221-226 in site 2 (Table 3 B, Figure 7), VP2 amino acids series 164-170 (Table 3 A, Figure 7) in site 2, and VP3 amino acids series 59-67 (Table 3 A, Figure 7) in site 3 could be displayed. They all showed changes in amino acids. To further describe differences in amino acids: they were defined as having experienced non-conservative change if their acidity and alkalinity, ionic charge, and functional base were different; and conservative change if they were similar. Of the total the VP1 amino acid series 96 in site 1 (Ala-Val) was conservative, 98 [Thr (hydroxylic)-Ala (aliphatic)], 99 [Lys (basic)-Met (sulfur)-Thr (hydroxylic)], 100 [Asn (amide)-Ser (hydroxylic)], 104 [Leu (aliphatic)-Gln (amide)], and 106 [Thr (hydroxylic)-Ala (aliphatic)] were non-conservative. The VP1 amino acid series 222 in site 2 [Ala (aliphatic)-Thr (hydroxylic)] was non-conservative; the VP2 amino acid series 165 in site 2 [Asp (amide)-Gly (aliphatic)-Asn (amide)] was non-conservative; the VP3 amino acid series 59 in site 3 [Ala (aliphatic)-Glu (Acidic)] and 60 [Lys (basic)-Gln (amide)-Ser (hydroxylic)] were non-conservative. 61 [Lys-Arg] was conservative. These amino-acid

non-conservative changes induced by nucleic acid evolution in the antigen neutralization zone might be the molecular basis for antibodies that could not produce neutralization to protein structures. The present study used polyclonal poliovirus antibodies; they could be used for typing assessment.

Epidemiological investigation was commenced immediately, and control measures taken upon reporting of the case. The patient was followed up 60 days later. The case was reported in the Epidemiology Bulletin, Vol. 19, No. 11, "The First Vaccine-Derived Poliovirus Case in Taiwan". By 2004 the patient was able to move his left hand; there was muscle atrophy of both lower limbs. He underwent physical rehabilitation at a clinic after discharge from hospital and was injected once every month with IVIG. Due to his immunodeficiency, he was weak. A year later, two tumors of the Aorta were detected; the smaller one was removed at the Chang Gung Hospital. Diarrhea was frequent. The child has to be assisted in tasks of daily living. A schoolteacher makes home visits two days a week to assist him with his schoolwork. Physiotherapy was terminated due to lack of improvement.

Discussion

When individuals with normal immunity are immunized with OPV, they will generally shed poliovirus for three to four weeks. The attenuated virus, when cloned in the intestines, reverts to a pathogenic virus. Studies have found that poliovirus type 3 vaccine is the quickest to revert to a pathogenic virus, followed by type 2 and type 1, and the three subtypes could reorganize together[11, 15-17]. Duration of viral shedding and high herd immunity may restrict the spread of the reverted viruses. Viruses isolated from immunized cases and their contacts are highly associated with the Sabin vaccine. When the neucleotide of the isolate strain VP1 and the Sabin strain are similar by more than 99%, they are called the OPV-like strains[7]. When gene sequences are large and viruses are under

long-term cloning, the VP1 neucleotide series smaller than or equal to 99% of the Sabin vaccine strain and larger than 85%, they are called the vaccine-derived poliovirus (VDPV); and they are considered the wild poliovirus when they are smaller than 85%. VDPV comes in two types, immunodeficient vaccine-derived poliovirus (iVDPV), and circulating vaccine-derived poliovirus (cVDPV).

B cell immunodeficient individuals, individuals receiving OPV or family members in contact with them, may become long-term carriers with cloning of viruses occurring; the carriers may become chronic shedders of iVDPV. In some individuals, the VP1 gene sequences are almost 90% similar to the original OPV strains, thus it is speculated that the viruses have been shed for ten years. In advanced countries with superior medical technology, OPV coverage rate is high, and excellent medical care prolongs the life of the immunodeficient; in developing countries, patients soon die of complications[24,25] of their Immunodeficiency. Thus far, there is no community iVDPV transmission. When IPV is used globally, the risks of being infected with iVDPV are reduced.

An incident of cVDPV polio was noted in the Dominican Republic and in Haiti in 1998-1999 after the administration of one dose of vaccine, leading to a 2000-2001 outbreak by a vaccine-derived type 1 poliovirus strain[14]. In 2001 in the Philippines, a vaccine-derived type 1 poliovirus strain was noted[26]; in 2002 in Madagascar, vaccine-derived type 2 poliovirus was detected[27,28]; and in Egypt in 1980-1991, outbreaks of vaccine-derived type 2 poliovirus occurred[29,30]. The cVDPV isolated from 30 polio patients showed a genetic increase in neurotoxicity, and when analyzed quantitatively with PVR-Tg21, the cVDPV demonstrated neurotoxicity similar to that of wild poliovirus type 2 strain; this strain disappeared however after 1993 with the increase in the coverage rate of OPV. Outbreaks of cVDPV occurred primarily because of lower OPV immunization rate, hence lower herd immunity, and the lack of indigenous wild

poliovirus. Other risk factors are crowded living conditions, high fertility, poor environmental sanitation, and hot weather. Virus strains isolated from outbreaks of cVDPV are often recombinant; three Sabin poliovirus strains recombine at the 5'-UTR and capsid zone[31-33], or at the non-capsid zone[14,28]. Another situation is, co- infection with poliovirus and other species of C enterovirus[14]. In the last ten years of experience in molecular epidemiology, the molecular clock of poliovirus and the global monitoring of poliovirus have made the determination of relationship much faster. This is due primarily to the more powerful nucleic acid sequencing tools. Immunization policies after the global certification of polio eradication must be beneficial and present the least risk to humans. When to use OPV, when to replace OPV with IPV, how to terminate use of OPV, and to develop safe and effective new vaccines to replace OPV, are some of the issues still to be discussed.

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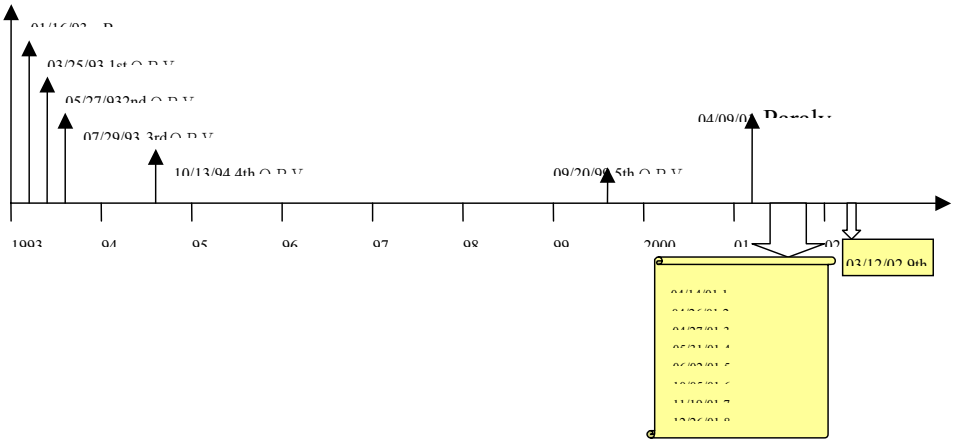


Figure 1. Dates of Five Doses of OPV, Onset, and Specimen Collection of Case

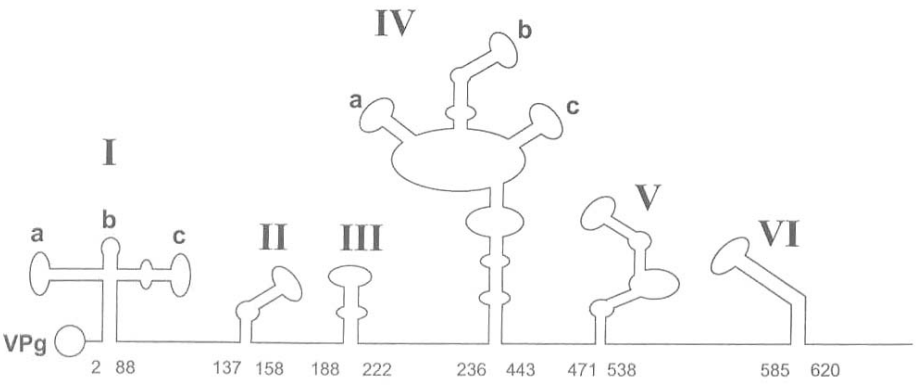
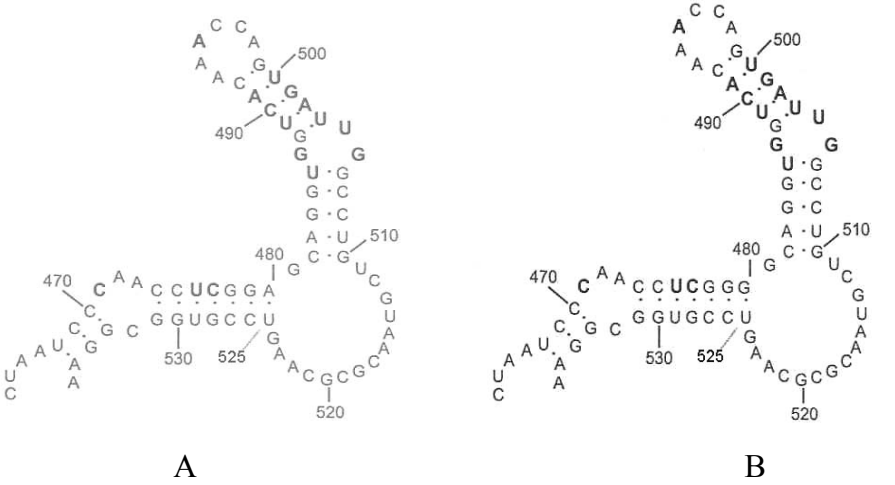


Figure 2. Estimated Two-Level Structures of Poliovirus Genes at the 5' Non-Coding Region

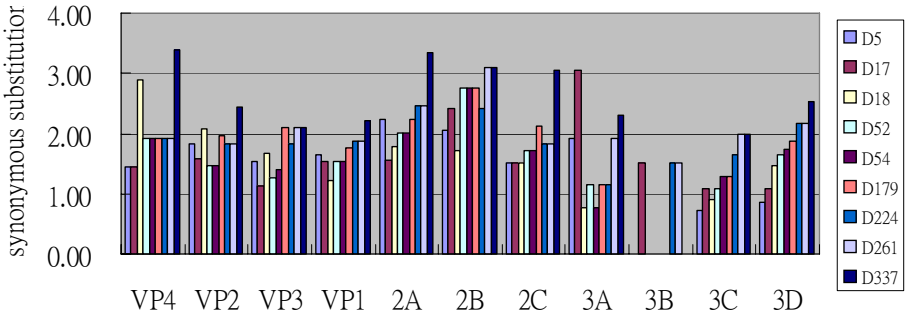


Sabin Type 1 Vaccine Strain (A) and Wild Strain (B) in Domain V

	$^{666\vee}$TGCTG	$^{671\vee}$GATTCGCTCC	$^{681\vee}$ATTGAGTGTG	$^{691\vee}$TTTACTCTAA	$^{701\vee}$GTACAATTTC	$^{711\vee}$AACAGTTA
Sabin-1						
D5	TNNNN	NNNNCGCTCC	ATTGAGTGTG	TTTANNNNNN	NNNNNNNNNN	NNNNNNTA
D17	TGCTG	GATTCGCTCC	ATTGAGTGTG	TTTATTCTAA	GTACAATTTC	AACAGTTA
D18	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTCTAA	GTACAATTTC	AACAGTTA
D52	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA
D54	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA
D179	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA
D224	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA
D261	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA
D337	TNNNN	NNNN CGCTCC	ATTGAGTGTG	TTTATTTTAA	GTACAATTTC	AACAGTTA

Figure 4. Sequences of Nucleic Acid Deficiency in 5' Non-Coding Region

A



B

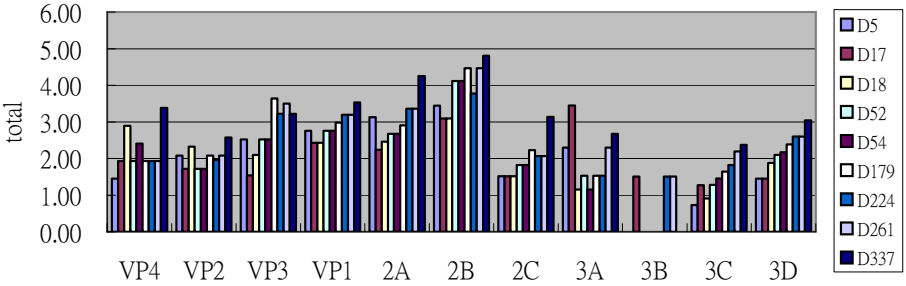
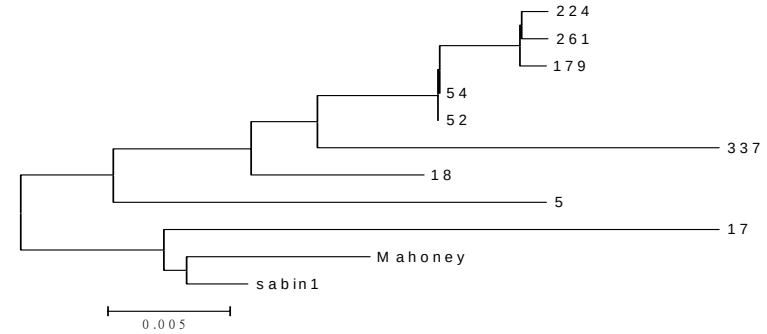


Figure 5. Different Degrees of Evolution in Each Region of Different Vaccine-Derived Polioviruses Type 1 Genes: (A) for all, and (B) for Synonymous Mutation

A.



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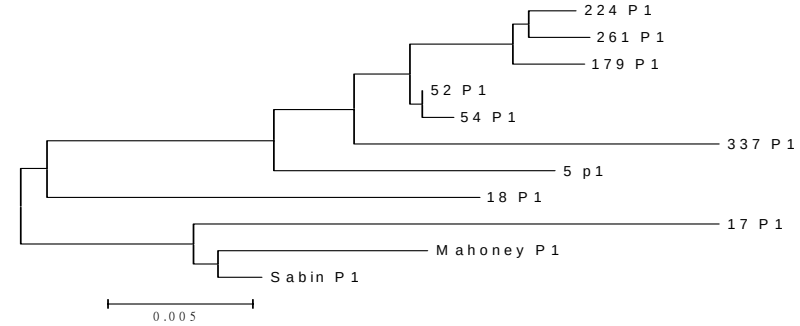


C.

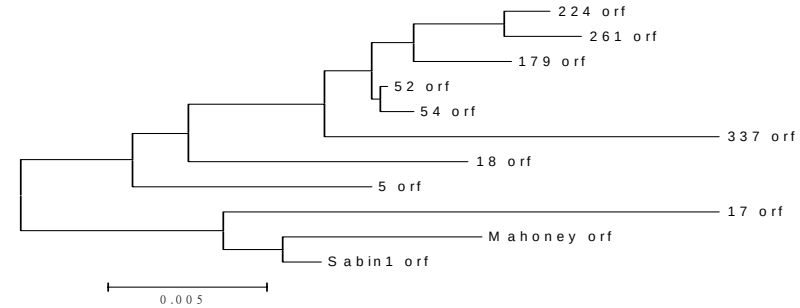
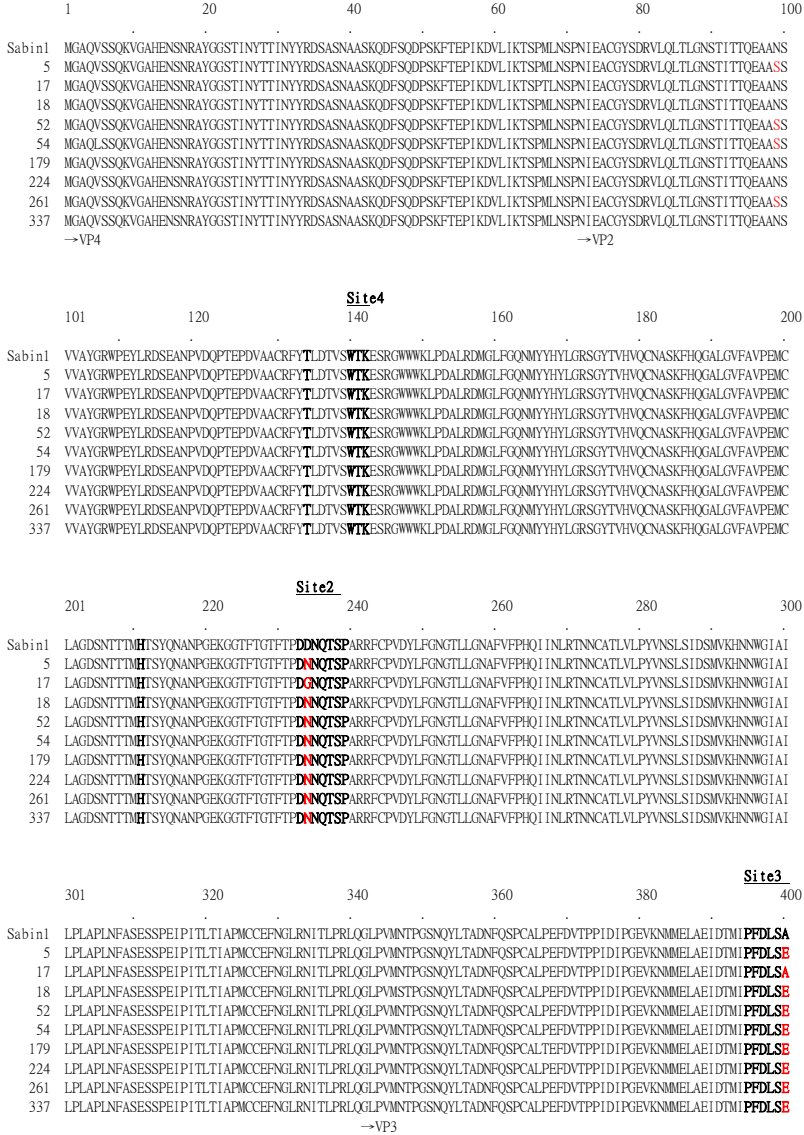


Figure 6. Phylogenetics of (A) VP1, (B) P1, and (C) ORF in Three Regions


1 20 40 60 80 100
Sabin1 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
5 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAASS
17 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPTLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
18 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
52 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAASS
54 MGAQLSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAASS
179 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
224 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
261 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAASS
337 MGAQVSSQKVGAHENSNRAYGGSTINYTTINYYRDSASNAASKQDFSQDPSKFTEPIKDVLIKTSPMLNSPNIEACGYSDRVLQLTLGNSTITTQEAANS
→VP4
→VP2
Site4
101 120 140 160 180 200
Sabin1 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
5 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
17 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
18 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
52 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
54 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
179 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
224 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
261 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
337 VVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESRGWWWKLPDALRDMGLFGQNMYYHYLGRSGYTVHVQCNASKFHQGALGVFAVPEMC
Site2
201 220 240 260 280 300
Sabin1 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDDNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
5 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
17 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDGNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
18 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARKFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
52 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
54 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
179 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
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261 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
337 LAGDSNTTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCPVDYLFGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAI
Site3
301 320 340 360 380 400
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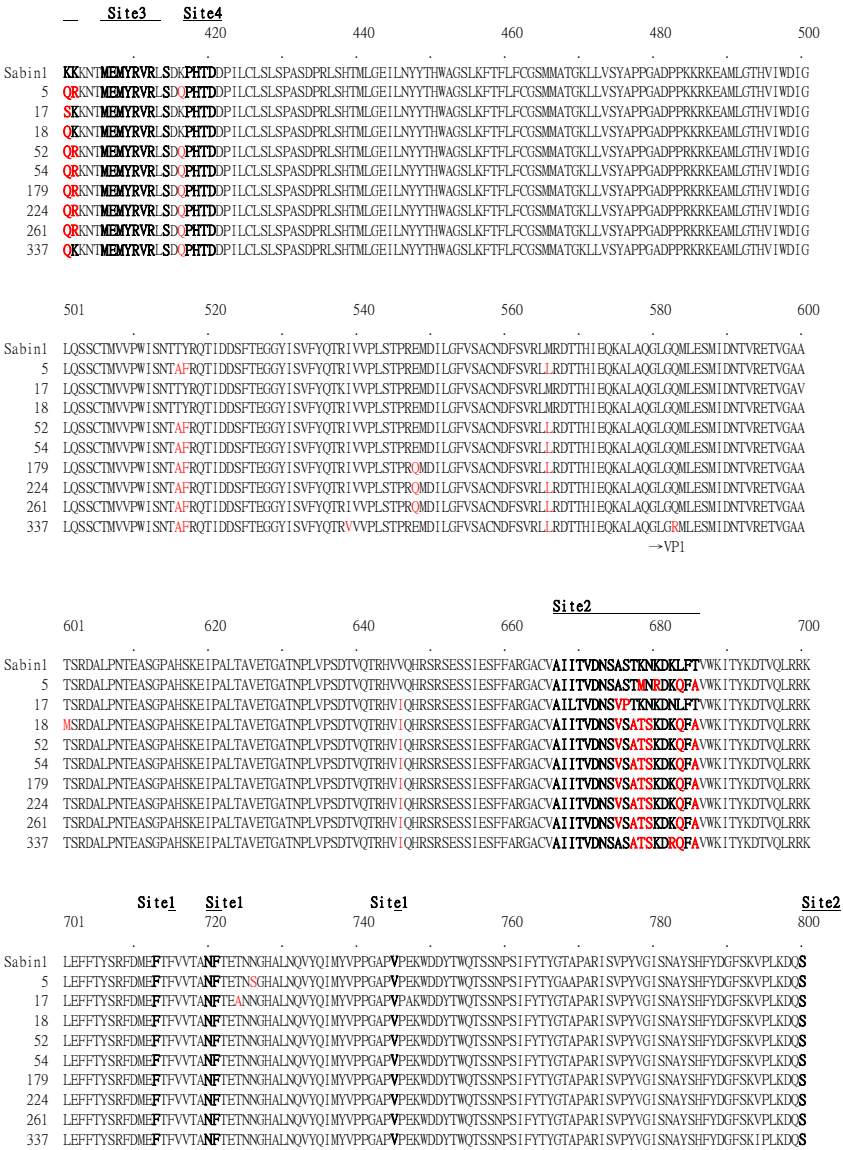
5 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
17 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSA
18 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMSTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
52 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
54 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
179 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALTEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
224 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
261 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
337 LPLAPLNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQGLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLSE
→VP3


Site3
Site4
420
440
460
480
500
Sabin1 KKKNTMEMYRVRLSDKPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPKKRKEAMLGTHVIWDIG
5 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPKKRKEAMLGTHVIWDIG
17 SKKNTMEMYRVRLSDKPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
18 QKKNTMEMYRVRLSDKPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPKKRKEAMLGTHVIWDIG
52 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
54 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
179 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
224 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
261 QRKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
337 QKKNTMEMYRVRLSDQPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGADPPRKRKEAMLGTHVIWDIG
501
520
540
560
580
600
Sabin1 LQSSCTMVVPWISNTTYRQTIDDSFTEGGYISVFYQTRIVVPLSTPREMDILGFVSACNDFSVRLMRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
5 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRIVVPLSTPREMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
17 LQSSCTMVVPWISNTTYRQTIDDSFTEGGYISVFYQTKIVVPLSTPREMDILGFVSACNDFSVRLMRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAV
18 LQSSCTMVVPWISNTTYRQTIDDSFTEGGYISVFYQTRIVVPLSTPREMDILGFVSACNDFSVRLMRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
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54 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRIVVPLSTPREMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
179 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRIVVPLSTPRQMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
224 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRIVVPLSTPRQMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
261 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRIVVPLSTPRQMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGQMLESMIDNTVRETVGAA
337 LQSSCTMVVPWISNTAFRQTIDDSFTEGGYISVFYQTRVVVPLSTPREMDILGFVSACNDFSVRLLRDTTHIEQKALAQGLGRMLESMIDNTVRETVGAA
→VP1
Site2
601
620
640
660
680
700
Sabin1 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVVQHRSRSESSIESFFARGACVAIITVDNSASTKNKDKLFTVWKITYKDTVQLRRK
5 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVVQHRSRSESSIESFFARGACVAIITVDNSASTMNRDKQFAVWKITYKDTVQLRRK
17 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAILTVDNSVPTKNKDNLFTVWKITYKDTVQLRRK
18 MSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
52 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
54 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
179 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
224 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
261 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSVSATSKDKQFAVWKITYKDTVQLRRK
337 TSRDALPNTEASGPAHSKEIPALTAVETGATNPLVPSDTVQTRHVIQHRSRSESSIESFFARGACVAIITVDNSASATSKDRQFAVWKITYKDTVQLRRK
Site1
Site1
Site1
Site2
701
720
740
760
780
800
Sabin1 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
5 LEFFTYSRFDMEFTFVVTANFTETNSGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGAAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
17 LEFFTYSRFDMEFTFVVTANFTEANNGHALNQVYQIMYVPPGAPVPAKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
18 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
52 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
54 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
179 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
224 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKVPLKDQS
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337 LEFFTYSRFDMEFTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEKWDDYTWQTSSNPSIFYTYGTAPARISVPYVGISNAYSHFYDGFSKIPLKDQS

Figure 7. Antigen Neutralization Determination Region in P1 Amino Acid Sequences are Shown in Black Bold; Isolated Strains Different from Polio Vaccine Type 1 are Shown in Red.

Table 1. Differences of Virus Strains Isolated at Different Time from Case from Vaccine Strains and Mahoney at 5' Non-Coding Region

Virus	Position of Nucleic				
	26		355	480	657
Sabin-1	G	T	T	G	C
D5	A	C	C	A	T
D17	A	C	C	A	.
D18	.	.	C	A	T
D52	A	C	C	A	T
D54	A	C	C	A	T
D179	A	C	C	A	T
D224	A	C	C	A	T
D261	A	C	C	A	T
D337	A	C	C	A	T
Mahoney	A	.	C	A	.

Note: Showing amino acid same as that of Sabin-1.

Table 2. Percent of Gene Mutation and Changes of Replaced Amino Acid at Different Regions of Vaccine Strain Type 1 Poliovirus and Vaccine-Derived Strains

Position		D5	D17	D18	D52	D54	D179	D224	D261	D337
5'UTR		5.39	1.61	2.02	2.02	2.29	2.29	2.56	2.43	2.56
P1		2.38/1.93	1.93/1.48	2.34/1.70	2.31/2.27	2.35/2.38	2.80/2.50	2.72/2.38	2.84/2.50	3.14/2.38
	VP4	1.45/0	1.93/1.45	2.89/0	1.93/0	2.41/1.45	1.93/0	1.93/0	1.93/0	3.38/0
	VP2	2.08/0.74	1.72/0.37	2.33/0.74	1.72/0.74	1.72/0.74	2.08/0.37	1.96/0.37	2.08/0.74	2.57/0.37
	VP3	2.52/2.94	1.54/1.26	2.10/1.26	2.52/3.36	2.52/3.36	3.64/4.20	3.22/3.78	3.50/3.78	3.22/3.36
	VP1	2.76/2.65	2.43/2.65	2.43/3.31	2.76/3.31	2.76/3.31	2.98/3.64	3.20/3.64	3.20/3.64	3.53/3.97
P2		2.26/1.22	1.97/0.87	2.03/1.04	2.43/1.22	2.43/1.22	2.78/1.39	2.67/1.57	2.78/1.57	3.7/1.57
	2A	3.13/2.68	2.24/2.01	2.46/2.01	2.68/2.01	2.68/2.01	2.91/2.01	3.36/2.68	3.36/2.68	4.25/2.68
	2B	3.44/3.09	3.09/2.06	3.09/3.09	4.12/3.09	4.12/3.09	4.47/4.12	3.78/3.09	4.47/3.09	4.81/4.12
	2C	1.52/0	1.52/0	1.52/0	1.82/0.30	1.82/0.30	2.23/0.3	2.07/0.61	2.07/0.61	3.14/0.3
P3		1.33/1.06	1.64/1.06	1.51/0.80	1.77/0.93	1.81/0.93	2.04/1.19	2.26/0.93	2.43/0.93	2.74/2.17
	3A	2.3/1.15	3.45/2.3	1.15/1.15/	1.53/1.15	1.15/1.15	1.53/1.15	1.53/1.15	2.30/1.15	2.68/1.15
	3B	0/0	1.51/0	0/0	0/0	0/0	0/0	1.51/0	1.51/0	0/0
	3C	0.73/0	1.27/0.55	0.91/0	1.28/0.55	1.46/0.55	1.64/1.09	1.82/1.55	2.19/1.55	2.37/1.09
	3D	1.45/1.52	1.45/1.08	1.88/1.08	2.10/1.08	2.17/1.08	2.39/1.30	2.60/1.08	2.60/1.08	3.04/1.52
ORF		1.99/1.45	1.84/1.18	1.98/1.22	2.16/1.54	2.19/1.58	2.54/1.77	2.55/1.67	2.69/1.72	3.15/1.81
3'UTR		1.38	1.38	1.38	1.38	1.38	1.38	1.38	0	1.38

Note: Differences between type 1 poliovirus vaccine strain and vaccine-derived strain are shown as

Table 3 A. Comparing Changes in Amino Acid between Vaccine-Derive Strains and Sabin-1 and Mahoney

Virus	VP2	VP3							
	165	59	60	61	75	143	175	176	225
Sabin-1	D	A	K	K	K	K	T	Y	M
D5	N	E	Q	R	Q	•	A	F	L
D17	G	•	S	•	•	R	•	•	•
D18	N	E	Q	•	•	•	•	•	•
D52	N	E	Q	R	Q	R	A	F	L
D54	N	E	Q	R	Q	R	A	F	L
D179	N	E	Q	R	Q	R	A	F	L
D224	N	E	Q	R	Q	R	A	F	L
D261	N	E	Q	R	Q	R	A	F	L
D337	N	E	Q	•	Q	R	A	F	L
Mohoney	N	•	T	•	•	•	•	•	L

Notes: 1. * Amino acid same as that of Sabin-1.

2. The following amino acid changes are considered conservative: Arg=Lys, Ser=Thr; Asp=Glu; Asn=Gln; Ala=Val; Ile=Leu=Met=Phe=Val (R=K; S=T; D=E; N=Q; A=V; I=L=M=F=V)

Table 3 B.

Virus	VP1											
	67	90	96	98	99	100	104	106	222	239	242	298
Sabin-1	V	I	A	T	K	N	L	T	A	I	V	D
D5	•	•	•	•	M	•	Q	A	•	V	•	S
D17	I	L	V	•	•	•	•	•	•	•	•	•
D18	I	•	V	A	T	S	Q	A	•	V	•	S
D52	I	•	V	A	T	S	Q	A	T	V	•	S
D54	I	•	V	A	T	S	Q	A	T	V	•	S
D179	I	•	V	A	T	S	Q	A	T	V	I	G
D224	I	•	V	A	T	S	Q	A	T	V	I	S
D261	I	•	V	A	T	S	Q	A	T	V	I	S
D337	I	•	•	A	T	S	Q	A	T	V	•	N
Mohoney	•	M	•	•	T	•	•	A	•	•	•	•

Notes: 1. * Amino acid same as that of Sabin-1.

2. The following amino acid changes are considered conservative: Arg=Lys; Ser=Thr; Asp=Glu; Asn=Gln; Ala=Val; Ile=Leu=Met=Phe=Val (R=K; S=T; D=E; N=Q; A=V; I=L=M=F=V)

Table 3 C.

Virus	2A				2B			2C	3A	3C	3D					
	36	51	87	134	75	94	95	252	54	182	53	73	123	250	362	441
Sabin-1	N	S	N	T	A	V	T	N	R	S	N	H	M	E	I	I
D5	D	•	D	A	V	A	I	•	K	•	•	Y	L	K	T	L
D17	•	•	D	A	V	•	I	•	K	N	•	Y	•	K	T	•
D18	•	L	D	A	V	A	I	•	K	•	•	Y	L	K	V	L
D52	•	L	D	A	V	A	I	S	K	G	•	Y	L	K	V	L
D54	•	L	D	A	V	A	I	S	K	G	•	Y	L	K	V	L
D179	•	L	D	A	V	A	I	S	K	G	D	Y	L	K	V	L
D224	•	L	D	A	V	A	I	S	K	G	•	Y	L	K	V	L
D261	•	L	D	A	V	A	I	S	K	G	•	Y	L	K	V	L
D337	•	L	D	A	V	A	I	S	K	G	•	Y	L	K	V	L
Mohoney	S	•	•	S	•	•	I	•	•	•	D	Y	•	K	T	•

Notes: 1. *Amino acid same as that of Sabin-1.

2. The following amino acid changes are considered conservative: Arg=Lys; Ser=Thr; Asp=Glu; Asn=Gln; Ala=Val; Ile=Leu=Met=Phe=Val (R=K; S=T; D=E; N=Q; A=V; I=L=M=F=V)