Type 2 Vaccine-Derived Poliovirus from Patients with Acute Flaccid Paralysis in China: Current Immunization Strategy Effectively Prevented Its Sustained Transmission

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In China, 5 patients with acute flaccid paralysis (AFP) associated with type 2 vaccine-derived poliovirus (VDPV) were identified by an AFP surveillance system from 1996 through 2009. A maximum-likelihood tree shows that all 5 Chinese VDPVs were independent. These 5 VDPVs were 100–216 d old according to the number of synonymous substitutions per synonymous site and 176–292 d old according to the number of substitutions per site. This result indicates limited virus replication since the administration of the initiating oral polio vaccine (OPV) dose, which is consistent with the rapid evolution rate of poliovirus genomes. The above-mentioned VDPVs have important implications in the global polio eradication initiative. Localized, limited, and transient circulation may be typical of OPVs; hence, independent VDPVs could be found because of the large population and excellent surveillance system, which permitted early detection and response, but sustained transmission was limited because of high population immunity.

Indigenous wild poliovirus was last isolated in China in 1994 [1], and since then, no indigenous wild polioviruses have been identified by means of careful analysis of polioviruses isolated from patients with acute flaccid paralysis (AFP). In 2000, China was subsequently certificated as a polio-free country by the World Health Organization [2]. The main strategies implemented for polio eradication or for maintaining the polio-free status in China are high oral polio vaccine (OPV) coverage and strong surveillance, including AFP case surveillance and virological surveillance. To elaborate, the strategies are to (1) improve routine immunization coverage, particularly in hard-to-reach populations; (2) continue large-scale supplementary immunization activities (national immunization days [NIDs] and subnational immunization days [sNIDs]) in high-risk areas; (3) conduct sensitive surveillance for cases of AFP; and (4) intensify surveillance and characterization of vaccine-derived polioviruses (VDPVs, which exhibit ≥99% VP1 sequence homology to the ancestral Sabin OPV strains) [1, 3–6].

The genetic instability of polioviruses is mostly due to the nucleotide substitutions that result from a high error frequency during replication [7]; the genetic diversity may also be a result of molecular recombination.
Because of its inherent genetic instability, OPV also undergoes frequent changes throughout its genome during replication in human intestines [9, 10]. The neurovirulence of the polioviruses may increase because of back mutations at key attenuation sites of its genome [11]; thus, in patients, the vaccine variants may reacquire the ability of the ancestral wild-type poliovirus to cause vaccine-associated paralytic poliomyelitis (VAPP, which was defined as occurring in cases of AFP if there was residual paralysis 60 d after the onset of paralysis, and if only vaccine-related poliovirus was isolated from any stool specimen) [12]. The genetic instability of OPV strains due to RNA-dependent RNA polymerase error and recombination coupled with selective pressure also seem to underlie the occurrence of poliomyelitis outbreaks associated with circulating VDPVs (cVDPVs).

There have been several poliomyelitis outbreaks associated with cVDPVs in Egypt [13], Hispaniola (Haiti and the Dominican Republic) [14], the Philippines [15], Madagascar [16, 17], China [18], Indonesia [19], Cambodia [20], Myanmar [21], and Nigeria [21]. Furthermore, ~40 cases of immunodeficiency VDPV (iVDPV) infection have been reported globally [22, 23].

Several observations suggest that the risk of cVDPV emergence may be the highest for type 2 polioviruses [24]. The Sabin 2 strain spreads more readily to unimmunized children than the Sabin 1 and Sabin 3 strains, as shown by frequent detection of type 2 polioviruses among contacts of patients with VAPP [25]. Here, type 2 vaccine-related isolates were studied in more detail to obtain a comprehensive view of the epidemiology, especially of the isolates obtained from the southwestern province of China (which has a very large population, high population density, and tropical or subtropical conditions, which are conducive to the emergence of VDPVs), children who have not received any immunization dose, and areas with gaps in OPV coverage. We found molecular evidence of limited, transient, and localized appearance of VDPVs, but no continuous transmission across polio seasons was found. The results show that the current polio immunization strategy in China is effective in preventing sustained VDPV transmission.

MATERIALS AND METHODS

Test algorithm of stool specimens in AFP surveillance in China. The main objective of the AFP surveillance system is to identify all children with AFP so that their stool specimens can be collected and potential wild polioviruses can be identified. General hospitals, children’s hospitals, infectious disease hospitals, and private clinics are expected to report patients <15 years of age with AFP to the public health departments of the 4-level (county, city, provincial, and national) Center for Disease Control and Prevention (CDC) in China [26].

Stool specimens were collected by county and city CDCs and sent to provincial CDCs for poliovirus isolation and primary identification by a microneutralization test using poliovirus type-specific rabbit polyclonal antiserum (National Institute for Public Health and the Environment, Bilthoven, the Netherlands), which was performed according to standard procedures [27]. If the specimens tested positive for poliovirus, then the viral isolates were sent to the China CDC for intratypic differentiation (ITD), VP1 sequencing, and other laboratory tests to determine whether the poliovirus isolates were wild or of vaccine origin. After identifying patients with AFP, the examination algorithm of stool specimens is performed from the stage when stool specimens are collected to that when laboratory ITD results are reported.

ITD. Two ITD methods, both targeting the VP1 coding region, were used to determine whether the poliovirus isolates were wild or of vaccine origin. One method, polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP), is based on the genetic properties of the polioviruses [28]; this method helped determine whether the poliovirus was typical Sabin-like or atypical non-Sabin-like. The other method, enzyme-linked immunosorbent assay (ELISA) with polyclonal cross-adsorbed rabbit antiserum, detects antigenic differences between wild and Sabin-derived strains [29]; poliovirus isolates were classified into the following 4 groups according to their antigenic properties: Sabin-like, non-Sabin-like, double reactive virus (the virus reacted with both anti–wild poliovirus and anti–Sabin strain antiserum), and nonreactive virus (the virus reacted with other than the expected antiserum). Poliovirus isolates that were found to present ITD results other than Sabin-like were considered to be potential wild polioviruses or antigenically drifted polioviruses with a Sabin origin; hence, the VP1 coding region of these viruses were sequenced.

Nucleotide sequencing. Viral RNA was extracted from poliovirus isolates by use of the QIAamp Mini viral RNA extraction kit (Qiagen). The entire VP1 coding region of the poliovirus isolates was amplified by reverse-transcription polymerase chain reaction with primers that flanked the VP1 coding region, according to the standard method described elsewhere [30]. The primers used for sequencing the whole genome were designed on the basis of the nucleotide sequence of the Sabin 2 strain. Polymerase chain reaction products were purified using the QIAquick Gel extraction kit (Qiagen). The amplicons were then bidirectionally sequenced using an ABI Prism 3100 genetic analyzer (Applied Biosystems). The 5'-end sequences were determined using a 5'-rapid amplification of complementary DNA ends (RACE) core set (Takara Biomedicals), according to the manufacturer’s instructions.

Location of the crossover sites. The sequences of the isolates were aligned with the reference strains by use of Mega software (version 4.0; Sudhir Kumar, Arizona State University, Phoenix, AZ) [31]; the resulting reference strain sequences were
Figure 1. Viral isolation rates of polioviruses (PVs) (triangles) and nonpolio enteroviruses (NPEVs) (circles) and detection rate of acute flaccid paralysis (AFP) cases in children <15 years of age (squares) from 1996 through 2009.

Highly sensitive surveillance reveals that poliovirus type 2 is the predominant serotype. A total of 69,764 cases of AFP were reported by the AFP surveillance system from 1996 through 2009 in mainland China. In 68,974 (98.87%) of these cases, 2 stool specimens were collected at 24-h intervals; hence, a total of 138,738 stool specimens were collected during this period. The detection rate of AFP cases among children <15 years of age, which is generally accepted as a surveillance quality indicator, was 1.4 cases per 100,000 children during this period and stabilized at 1.8–2.0 cases per 100,000 children after 1999 (Figure 1). The isolation rate of polioviruses varied each year and tended to decrease by 0.73% every year from 2002 through 2009. On the other hand, the isolation rate of nonpolio enteroviruses was relatively constant; the average viral isolation rate was 11.19% (Figure 1). All of the data above show that AFP case surveillance and virological surveillance were proved to be highly sensitive. From 1996 through 2009, type 2 poliovirus was the predominant serotype in mainland China, and the number of type 2 polioviruses was larger than the number of type 1 and type 3 polioviruses for most of the years, although the overall incidence of polioviruses decreased from 2002 through 2009 (Figure 2).

Primary characterization of the type 2 VDPVs. Five patients with AFP associated with type 2 VDPVs (strains
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Figure 2. Number of poliovirus isolates (type 1 [P1], type 2 [P2], type 3 [P3], and a poliovirus mixture [P mix]) obtained from patients with acute flaccid paralysis, from 1996 through 2009.

CHN1025 [36], CHN1054, CHN1078, CHN3209, and CHN8316) were identified by the AFP surveillance system from 1996 through 2009 (Table 1). The PCR-RFLP ITD profiles of all 5 isolates were genetically different from the Sabin 2 strain [28], and 3 of them (strains CHN1054, CHN1078, and CHN8316) were also found to have double reactive virus antigenic properties that were identified by the ELISA ITD method [29]. The VP1 coding region of 5 type 2 poliovirus strains differed from that of the polio vaccine strain by 1.00%–1.33% (9–12 substitutions in 903 nucleotides), showing the genomic features of VDPVs (Table 2).

A maximum-likelihood tree of sequence relationships in the complete P1 capsid region for 5 Chinese type 2 VDPVs and other representative international VDPVs was constructed (Figure 3). It clearly shows that all 5 Chinese VDPVs were independent VDPVs, and the epidemiological data show that they did not belong to cVDPVs or iVDPVs—that is, no other patients were found to have AFP associated with these VDPVs and no similar VDPVs could be isolated from their contacts.

In addition, no signs of immunodeficiency (such as abnormal immunoglobulin levels or signs of abnormal T cell and B cell function) appeared in the patients from whom VDPVs were isolated at the time of AFP presentation. Unfortunately, however, their was no information on the immunization status of the other 4 patients with AFP who were detected in the period from 1997 through 1999, when the concept of iVDPV had not yet appeared.

Recombination features of the type 2 VDPVs. Of the 5 type 2 VDPVs, 4 (strains CHN1025, CHN1054, CHN1078, and CHN3209) were found to have recombination in at least 1 crossover site. Strains CHN1078 and CHN3219 were simple; the former had a single crossover site located between nucleotides 5344 and 5352 in the 3A coding region and the latter between nucleotides 5464 and 5492 in the 3C coding region. The 5′ end of the genome contained the Sabin 2 sequence, and the 3′ end contained the Sabin 3 sequence (S2/S3) (Figure 4).

Strain CHN1054 is a multirecombinant poliovirus characterized as S2/S3/S2/S3 trirecombinant on the basis of its ge-

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Table 1. Isolates Obtained from Patients with Acute Flaccid Paralysis Associated with Type 2 Vaccine-Derived Polioviruses in China from 1996 through 2009

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Location</th>
<th>Age, years (sex)</th>
<th>Oral polio vaccination history</th>
<th>Date</th>
<th>Paralysis residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHN1025/1997</td>
<td>Shandong</td>
<td>0.6 (male)</td>
<td>0</td>
<td>3 February 1997</td>
<td>Paralysis</td>
</tr>
<tr>
<td>CHN1054/1997</td>
<td>Guizhou</td>
<td>1.4 (female)</td>
<td>0</td>
<td>21 March 1997</td>
<td>Too young*</td>
</tr>
<tr>
<td>CHN1078/1997</td>
<td>Yunnan</td>
<td>1.2 (male)</td>
<td>1</td>
<td>6 April 1997</td>
<td>Paralysis</td>
</tr>
<tr>
<td>CHN3219/1999</td>
<td>Gansu</td>
<td>1.8 (male)</td>
<td>0</td>
<td>17 July 1999</td>
<td>Recovery</td>
</tr>
<tr>
<td>CHN8316/2004</td>
<td>Guizhou</td>
<td>0.7 (female)</td>
<td>0</td>
<td>15 August 2004</td>
<td>Too young*</td>
</tr>
</tbody>
</table>

* The child was too young to walk during the 60-d follow-up period after the onset of the disease.
Table 2. Genetic and Phenotypic Characterization of Type 2 Vaccine-Derived Polioviruses Isolated from Patients with Acute Flaccid Paralysis in China from 1996 through 2009

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Identificationb</th>
<th>PCR-RFLP</th>
<th>ELISA</th>
<th>No. (%) of NT divergences in VP1c</th>
<th>5'-UTR</th>
<th>VPT</th>
<th>Evolution of the viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabin</td>
<td>P2</td>
<td>Typical</td>
<td>SL</td>
<td>NT (n = 903)</td>
<td>NT</td>
<td>NT</td>
<td>AA</td>
</tr>
<tr>
<td>CHN1025</td>
<td>P2</td>
<td>Atypical</td>
<td>SL</td>
<td>10 (1.11)</td>
<td>G</td>
<td>C</td>
<td>Ile2/S3/S1/S3/S3</td>
</tr>
<tr>
<td>CHN1054</td>
<td>P2</td>
<td>Atypical</td>
<td>DRV</td>
<td>9 (1.00)</td>
<td>G</td>
<td>C</td>
<td>Ile2/S3/S2/S3/S3</td>
</tr>
<tr>
<td>CHN1078</td>
<td>P2</td>
<td>Atypical</td>
<td>DRV</td>
<td>12 (1.33)</td>
<td>G</td>
<td>C</td>
<td>Ile2/S3</td>
</tr>
<tr>
<td>CHN3219</td>
<td>P2</td>
<td>Atypical</td>
<td>DRV</td>
<td>11 (1.22)</td>
<td>G</td>
<td>C</td>
<td>Ile2/S3</td>
</tr>
<tr>
<td>CHN3216</td>
<td>P2</td>
<td>Atypical</td>
<td>DRV</td>
<td>10 (1.11)</td>
<td>G</td>
<td>C</td>
<td>Ile2/S3</td>
</tr>
<tr>
<td>MEF-1</td>
<td>P2</td>
<td>Atypical</td>
<td>NSL</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

NOTE. 5'-UTR, 5' untranslated region; AA, amino acid; DRV, double reactive virus; ELISA, enzyme-linked immunosorbent assay; KS, number of synonymous substitutions per synonymous site; KT, total number of substitutions per site; NSL, non-Sabin-like; NT, nucleotide; P2, type 2 poliovirus; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; SL, Sabin-like.

a Numbered according to the Sabin 2 strain (GenBank accession no. AY184220).
b Determined with a microneutralization test.
c Amount of nucleotide divergence from the relative Sabin strains in the VP1 coding region.

nomic organization. The first and second crossover sites were located in the 2C coding region (between nucleotides 4282 and 4289 and nucleotides 4867 and 4871, respectively), and the third crossover site was in the 3A coding region, as was that in the strain CHN1078 (Figure 4).

Strain CHN1025 is a rare and complicated multirecombinant poliovirus characterized as S2/S3/S1/S3/S1/S3 pentarecombinant on the basis of its genomic organization. The genomic sequence of strain CHN1025 harbors 5 crossover sites [36] (Figure 4).

Reversion of key neurovirulence determination sites by back mutation. Complete genomic sequencing of all 5 Chinese type 2 VDPVs revealed that their genomes were collinear with that of the Sabin 2 strain and that nucleotide substitutions were scattered throughout their genomes. The 2 nucleotide substitutions that had been identified as key determinants of the attenuated phenotype of the Sabin 2 strain had reverted in all Chinese type 2 VDPVs. There was a reversion from adenine to guanine at nucleotide 481 in the 5' untranslated region, and there was a reversion from uracil to cytosine at nucleotide 2909 in the VP1 coding region that led to the substitution of an amino acid in the VP1 coding region at position 143 (isoleucine to threonine). Both substitutions restored the consensus residues for the prototype wild type 2 poliovirus strain, MEF-1/EGY/1942 [37–39] (Table 2).

Antigenic divergence of Chinese type 2 VDPVs. The results of ELISA ITD show that strains CHN1054, CHN1078, CHN3219, and CHN8316 were antigenic variants of the Sabin 2 strain. The amino acid sequences within or near the predicted neutralizing antigenic (NAg) sites [40] were aligned with 5 Chinese type 2 VDPVs (Figure 5). The above antigenic variant strains had at least 1 amino acid substitution at the NAg sites. On the other hand, there was no amino acid substitution at the NAg sites in strain CHN1025; this finding coincided with the Sabin-like result of ELISA ITD. Sabin-specific epitopes were

Figure 3. Maximum-likelihood tree of sequence relationships in the complete P1 capsid region of 5 type 2 vaccine-derived polioviruses (VDPVs) isolated in China from 1996 through 2009 (arrows), 2 type 2 circulating VDPV strains (AF448782 and AF448783) in Egypt, 2 type 2 circulating VDPV strains (AM084223 and AM084225) in Madagascar, 2 type 2 immunodeficiency VDPV strains (EU566941 and EU566950) in Spain, 1 type 2 immunodeficiency VDPV strain (DG890385) in Nigeria, and 1 type 2 immunodeficiency VDPV strain (DG890387) in the United States. The tree was rooted to the sequence of Sabin 2 (AY184220).
Figure 4. Schematic representation of genomes of type 2 vaccine-derived polioviruses (VDPVs) isolated in China. The top part is the genetic organization of the Sabin 2 reference strain (GenBank accession no. AY184220). The single open reading frame, flanked by a 5′ untranslated region (5′-UTR) and a 3′ untranslated region (3′-UTR), is indicated by a rectangle. The bottom part shows the structures of China type 2 VDPVs; sequences of the type 1, type 2, and type 3 polioviruses are indicated (S1, S2, and S3, respectively). Filled triangles indicate the locations of S2/S3 crossover sites. Open triangles indicate the locations of S3/S2 crossover sites. Filled circles indicate the locations of S3/S1 crossover sites. Open circles indicate the locations of S1/S3 crossover sites. The positions of the crossover sites are indicated at the top of each symbol.

present in the strain CHN1025, which suggests that it was antigenically indistinguishable from the Sabin 2 reference strain [36] (Figure 5).

Estimated age of the VDPVs. Molecular clock data can predict the duration of poliovirus replication or transmission, and the pattern of stepwise accumulation of nucleotide substitutions can be used to reconstruct transmission pathways (from evolutionary pathways). The approximate time of the initiating OPV infection was estimated on the basis of differences in the P1 capsid sequence between Chinese type 2 VDPVs and Sabin 2 and the date of specimen collection. The corrected proportion of synonymous substitutions (κ0) was 0.88%–1.89% of synonymous sites in the P1 capsid region, and that of total substitutions (κT) was 0.53%–0.88%. Assuming that the constant nucleotide substitution rate is 3.2% of synonymous substitutions per synonymous site per year, and 1.1% of total substitutions per site per year in the P1 capsid region [35], we estimated that the ages of the 5 Chinese type 2 VDPVs were 100–216 d old according to the κ0 estimate and 176–292 d old according to the κT estimate (Table 2).

DISCUSSION

Polio eradication in China is a public health triumph and demonstrates the soundness of the strategy under challenging conditions: (1) very large population (estimated population in 2009, 1.336 billion; proportion of population aged <15 years, 18.5%; birth rate, 12.09‰; estimated birth cohort, 16.46 million); (2) high population density with unbalanced distribution (mean population density, 134 persons per square kilometer; population density in the eastern coastal area, >1400 persons per square kilometer; population density in the central area, ∼200 persons per square kilometer; population density in the western plateau area, <10 persons per square kilometer); (3) tropical or subtropical conditions in large parts of the country; (4) suboptimal sanitation in some areas, especially rural areas; and (5) hard-to-reach populations in the mountainous area.

The endemicity of indigenous wild polioviruses was confirmed to be restricted to India, Pakistan, Afghanistan, and Nigeria [41]. Of these 4 countries, 3 share a boundary with China; hence, China is at a high risk of wild poliovirus im-
Figure 5. Alignment of amino acid residues of neutralizing antigenic (NAg) sites 1 (VP1, positions 88–106), 2 (VP2, positions 163–172; VP2, positions 268–270; VP1, positions 222–226), 3a (VP3, positions 54–61; VP3, positions 70–74; VP1, positions 287–291), and 3b (VP2, positions 71–73; VP3, positions 75–80) for Sabin 2 (GenBank accession no. AY184220), China type 2 vaccine-derived polioviruses (VDPVs), Egypt type 2 circulating VDPV strains (AF448782 and AF448783), Madagascar type 2 circulating VDPV strains (AM084223 and AM084225), Spain type 2 immunodeficiency VDPV strains (EU566941 and EU566950), a Nigeria type 2 immunodeficiency VDPV strain (DQ890385), a US type 2 immunodeficiency VDPV strain (DQ890387), and the prototype wild type 2 poliovirus strain, MEF-1/EGY/1942 (AY238473).

In fact, patients infected with wild polioviruses imported from Myanmar were found in Yunnan Province of China in 1995 and 1996 [42], and wild polioviruses imported from India were found in Qinghai Province of China in 1999 [43]. But imported wild polioviruses did not spread to other provinces, presumably because of the maintenance of high OPV coverage overall through routine immunization and supplementary immunization activities and mop-ups after imported cases [43]. The reported OPV coverage rate has exceeded 90% since 1988 and has been maintained at >98% since 2001 in whole country. Although the OPV coverage is generally slightly lower in the underdeveloped areas of western China (such as Gansu, Yunnan, and Guizhou provinces) and in areas with many floating populations (such as Shandong Province), the OPV coverage is still >95% in those areas.

In addition to the strategies to improve routine OPV coverage, at least 2 rounds of NIDs with OPV have been conducted every year since 1993. Since regional certification in October 2000, China has conducted 2 rounds of sNIDs instead of NIDs every year, with emphasis on reaching unimmunized children who missed routine immunization services. Because of the reduction of the number of areas with suspected low routine immunization coverage, the amount of OPV used in sNID activities reduced from 111,900,000 doses in 1996 to 51,600,000 doses in 2009, which may be one of the important reasons for the decline in the poliovirus detection rate in the period from 1996 through 2009.

In China, VDPV surveillance has already been implemented by carefully analyzing all polioviruses isolated from patients with AFP and has found preliminary evidence of the above-mentioned independent type 2 VDPVs, 7 independent type 1 VDPVs, and 2 independent type 3 VDPVs during the period from 1996 through 2009. In addition to these independent VDPVs, a limited, highly localized outbreak in China of c-VDPVs (3 case patients and 4 contacts) associated with type 1 virus occurred in Guizhou Province in 2004. The virus circulated when the OPV coverage in a local area was relatively lower, and the circulation ceased after a mass immunization with OPV [18]. The first reported iVDPVs (types 2 and 3) were isolated in Anhui Province in 2005 from a patient with X-linked agammaglobulinemia [26].

All available data indicate that China is polio-free, and sustained transmission of VDPVs has been prevented after the eradication of wild-type polioviruses. High-quality surveillance has permitted very early detection and response, and it has played a key role in stalling the widespread circulation of the emergent cVDPV strains. The present genetic characterization of the above-mentioned type 2 VDPVs reveals data consistent with the short-term survival of these strains.

These type 2 VDPVs were 100–216 d old (according to the
this result indicates that they were not as old as the known cVDPVs in the world [13, 14] because they acquired only a few synonymous substitutions or total substitutions in the VP1 coding region. This result also indicates limited virus replication since administration of the initiating OPV dose, which is consistent with the rapid evolution rate of poliovirus genomes [44, 45] and the normally limited spread of vaccine viruses to individuals in close contact with OPV recipients. This result is also supported by the immunization histories of the 5 patients with AFP; 4 of the patients never received OPV and the fifth had not completed the recommended schedule of 3 doses of OPV. Therefore, it was speculated that a small immunization gap may exist in areas where the AFP cases are located. Localized, limited, and transient circulation may be typical of OPVs in areas with warm climate and high population density; hence, independent VDPVs are detected in China because of its large population and excellent surveillance systems, but the spread of VDPVs was limited because of high population immunity, and therefore independent VDPVs may not be a cause for concern.

Recombination crossover sites can be used as genetic markers for molecular epidemiological studies. For instance, a common crossover site would suggest common ancestral infection, whereas different crossover sites would have 2 possible interpretations. Two independent events or an upstream crossover event occurring at a later time could obscure the epidemiological record of earlier recombination events, and in such cases, evidence for linkage must come from the pattern of base substitutions in nonrecombinant sequences. On the basis of the recombinant pattern in Chinese type 2 VDPVs, 4 VDPVs were considered recombinant but had different crossover sites, and 1 was nonrecombinant. This indicates that they were independent ontogenesis events; this was also confirmed by phylogenetic analysis.

Trivalent attenuated OPVs (strains P1/Sabin, P2/Zhong-II-27, and P3/Zhong-III-2), which are very closely related to the corresponding Sabin strains, were widely used for maintaining the polio-free status in mainland China. The use of OPV in China is likely to continue for a relatively long period until certification of global polio eradication is obtained, and in the meantime, a high OPV coverage rate must be maintained. Indeed, VDPVs will become increasingly important as the prevalence of wild polioviruses declines and OPV becomes the sole source of poliovirus infection. The recent VDPV outbreaks also highlight the importance of maintaining a sensitive poliovirus laboratory surveillance system. Such a system can have major implications in the cessation of immunization with OPV after obtaining certification for the eradication of wild polioviruses. In addition, in the posteradication era in China, it is important to draw a more robust immunization policy for the replacement of OPV with inactive polio vaccine, and inactive polio vaccine intervention may be required for solving problems associated with VAPP and VDPVs and to achieve eradication of all polio cases [46].

The VDPVs found in China have important implications in the global initiative to eradicate polio. Their discovery indicates that a high-quality AFP surveillance system continues to be implemented in China. Circulating VDPVs can be controlled by implementing a good program. High-quality surveillance has permitted very early detection and response and played a key role in stalling widespread circulation of the virus in China. China should maintain its present successful immunization policy until global polio eradication is achieved. Our findings also highlight the need for all polio-free countries to remain vigilant in order to allow early detection of wild polioviruses imported from countries where polio is endemic and to implement rapid control measures. The existing reservoirs for circulation of the wild poliovirus should soon be eliminated to avoid the decline of effective immunization programs.

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References