Epidemiological Survey and Genetic Characterization of Type 3 Vaccine-Derived Poliovirus Isolated from a Patient with Four Doses of Inactivated Polio Vaccine in Henan Province, China

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Abstract

Background: This study aimed to describe the epidemiology and genetic characteristics of the first vaccine-derived poliovirus (VDPV) identified from a patient with acute flaccid paralysis (AFP), with four doses of inactivated polio vaccine immunization in Henan Province, China in 2017.

Methods: The patient was diagnosed with type 3 VDPV. Subsequently, a series of epidemiological approaches was implemented, including a retrospective search of AFP cases, rate of vaccination assessment, study of contacts, and supplementary immunization activities. Fecal samples were collected, viral isolation was performed, and the viral isolates were characterized using full-length genomic sequencing and bioinformatics analysis.

Results: Phylogenetic analysis showed that the viral isolates were different from other reported genetic clusters of type 3 VDPV worldwide. They were identified as a Sabin 3/Sabin 1 recombinant VDPV with a crossover site in the *P2* region. Nucleotide substitutions, including U → C(472) and C → U(2493), have been identified, both of which are frequently observed as reversion mutations in neurovirulent type 3 poliovirus. A unique aspect of this case is that the patient had been vaccinated with four doses of inactive polio vaccine, and the serum neutralizing antibody for Sabin types 1 and 3 were 1:16 and 1:512, respectively. Thus, the patient was speculated to have been infected with type 3 VDPV, and the virus continued to replicate and be excreted for at least 41 d.

Conclusion: The existence of this kind of virus in human population is a serious risk and poses a severe challenge in maintaining a polio-free status in China. To the best of our knowledge, this is the first report of VDPV identified in the Henan province of China. Our results highlight the importance of maintaining a high-level vaccination rate and highly sensitive AFP case surveillance system in intercepting VDPV transmission.

Background

Owing to the success of worldwide vaccination campaigns, the burden of poliomyelitis has radically decreased (1). In October 2000, China and other countries in the Western Pacific Region were polio-free. However, the risk of importation of wild-type poliovirus (WPV) still exists in these countries, such as WPV1 importation to mainland China in 2011(2). Another risk is the emergence of vaccine-derived poliovirus (VDPV) that mutated from live-attenuated oral polio vaccine (OPV) strains (2–4).

The genetic variability of polioviruses is mostly due to nucleotide substitutions and recombination (5–7). Owing to the inherent genetic instability of polioviruses, OPV (a live attenuated poliovirus) also undergoes frequent mutations throughout its genome during replication in the human intestine. OPV has many advantages in polio eradication, including ease of administration, efficient induction of intestinal immunity, triggering of durable humoral immunity, and low cost. However, wider application of OPV is limited by genetic instability, a potential cause of vaccine-associated paralytic poliomyelitis (VAPP), and the emergence of genetically divergent VDPVs (8, 9). VDPVs are defined as OPV-related isolates that

differ from those of the parental strain by 1–15% for serotypes 1 and 3, and 0.5–15% for serotype 2 in the *VP1* coding region. VDPVs have the potential for sustained circulation in areas with low OPV immunization, and many outbreaks of circulating VDPVs (cVDPVs) have been reported worldwide in recent years (10–12). After vaccination with OPV or infection with OPV-related virus in immunodeficient patients, especially patients with B-lymphocyte immunodeficiency, immunodeficient VDPV (iVDPV) is produced due to the long-term existence of vaccine in the body, continuous replication of the virus, and viral excretion from the body (13, 14). If neither cVDPV nor iVDPV is evident, such VDPV is classified as ambiguous VDPV (aVDPV) (15).

In this study, we reported an acute flaccid paralysis (AFP) case with four doses of inactive polio vaccine in Runan County, Zhumadian City, Henan Province, in February 2017. Although the patient was diagnosed with viral myositis by a provincial Polio Expert Committee after a 60-day follow-up physical examination, this case was diagnosed as VDPV patient due to the isolated type 3 VDPV, in accordance with the latest recommendations from the World Health Organization (WHO). This report described the results of epidemiological and laboratory investigations and the actions taken to prevent the circulation of VDPV.

Materials And Methods

Case investigation algorithm

Cases of paralytic polio were identified by an AFP case surveillance system in China. Staff at the county-level CDC routinely investigate AFP cases reported by healthcare centers and hospitals, collect stool specimens, and investigate residual paralysis 60 days after the onset of paralysis. Clinical and epidemiological information of AFP cases was extracted from medical records, and cases were investigated using standard case-investigation forms.

A series of fecal samples were collected from a patient on February 23, February 24, April 5, April 23 May 3, May 10, and May 19, 2017. After VDPV was identified, stool samples were collected successively from 19 relatives and 20 close contacts of the patient on April 21, 2017.

The neutralizing antibodies for immunoglobulin (IgG) of serotypes 1 and 3 were determined using a microneutralization assay with authentic Sabin strains, in accordance with the WHO guidelines in the Henan provincial CDC. A serum sample was considered positive if the neutralizing antibody level was determined at a dilution of ≥1:4.

Detection of viruses in surrounding environment

Three sewage samples (1 liter each) were obtained via grab sampling from local environmental water sources, including household wastewater and nearby river water. The samples were immediately transferred to the laboratory under refrigerated conditions. The 1-liter sample was concentrated through improved negative-charge filter membrane absorption, and then the virus was eluted in 10 ml of 3% beef

extract solution (pH 9.6) after sonication and centrifugation (16). Thereafter, 200 µl of each concentrated eluent was used to inoculate a standard monolayer of cells for virus isolation.

Isolation and characterization of poliovirus isolates

Stool specimens were forwarded to the Henan Provincial Polio Laboratory, where viral isolates were obtained from L20B and RD cell cultures. L20B cell-positive isolates underwent intratypic differentiation (ITD) and VDPV screening using real-time polymerase chain reaction (rRT-PCR) (Poliovirus rRT-PCR ITD/VDPV-V5.0 kits, Centre for Disease Control and Prevention, USA) to determine whether the isolates were wild or of vaccine origin (17, 18).

Total RNA was extracted from 140 ml of the infected cell culture using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's recommended procedure. The entire *VP1* region of the poliovirus isolates was amplified using RT-PCR with primers that flanked the *VP1*-coding region, and the amplicons were bidirectionally sequenced using the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR products were sequenced in both directions to avoid possible ambiguous nucleotides.

Full-length genome sequencing of the VDPV strains

The full-length genome of the Henan strains (CHN21006-1, CHN21006-2, and CHN21006-3) was amplified using two long-distance PCR reactions using the TaqPlus Precision PCR system (Stratagene, USA). The primer pairs Y7/7500A and 0010S48/Q8 were used to amplify the 5.5 kb and 3.5 kb fragments, respectively (19). The combined sequences of the two fragments yielded the full-length genome sequence. The PCR products were purified for sequencing using the QIAquick Gel Extraction Kit (Qiagen, Germany), and the amplicons were sequenced on the ABI 3130 Genetic Analyzer (Applied Biosystems) as described above. The 5' -end of the genome was amplified using the 5'-Full RACE Kit (Takara, Shiga, Japan) according to the manufacturer's instructions. The 3' end of the genome was amplified using an oligo-dT primer (7500A), as previously reported in another study (20).

Phylogenetic and recombination analysis

Full-length genomic sequences of the Henan VDPV strains were aligned with other type 3 VDPV strains using the ClustalW algorithm implemented in MEGA11 (21). Maximum likelihood trees were constructed using the GTR+I+G model suggested by jModelTest2 (22) and performed in MEGA11 with 1000 bootstrap replicates. Maximum likelihood trees were also constructed using RAxML (v8.2.10) to verify the best tree topology (23).

Nucleotide sequence accession number

The full-length genome sequences of Henan type 3 VDPV strains CHN21006-1, CHN21006-2, and CHN21006-3 described in this study were deposited in the GenBank database under the accession numbers ON221307- ON221309.

Results

VDPV case investigation

The two-year-old boy was born on August 19, 2014. He received four scheduled doses of IPV (at 4, 5, 6, and 18 months) and completed routine polio immunization. His last vaccination date was March 14, 2016. On February 14, 2017, the patient experienced weakness and claudication of the right lower extremity. On February 16, his condition deteriorated with fever (37.5°C–38.5°C), diarrhea, and difficulty in walking. The patient was sent to a hospital in Zhumadian City and diagnosed with AFP. Nutritional myocardial and comprehensive rehabilitation treatments were administered after the diagnosis of viral myositis upon admission. The patient gradually recovered with regained strength in the right lower extremity and no residual paresis. On March 12, the fully-recovered patient was discharged from the hospital with a discharge diagnosis of viral myositis.

Viruses were isolated from the first two stool samples collected during hospitalization (February 23 and 24, 2017) and were identified as poliovirus serotype 3 Sabin-like using rRT-PCR; however, sequencing of the *VP1* region of the virus revealed nine nucleotide changes consistent with VDPV3 classification. After the patient was discharged from the hospital, type 3 VDPV was detected again in the third stool sample collected during follow-up (April 5, 2017), with 10/900 (1.11%) nucleotide substitutions in the *VP1* region compared with the type 3 Sabin strain. These three viral strains (CHN21006-1, CHN21006-2, and CHN21006-3) isolated at three time points shared eight nucleotide substitution sites. In the stool samples collected from the fourth to the seventh time periods (April 23, May 3, May 10, and May 19, 2017), the viral isolation results were negative, and no virus was detected. Therefore, it is considered that the excretion of poliovirus has been terminated.

Humoral immunity function was measured using blood samples from the patient collected on April 21, 2017. The results showed that the serum neutralizing antibody for Sabin type 1 was 1:16 and Sabin 3 was 1:512 (1: 4 is considered positive), while antibodies against hepatitis B, hepatitis C, and HIV were all negative. Based on all the results, the patient was diagnosed with VDPV classified as VDPV.

Investigation for AFP in close contacts and the environment

To investigate the scope of the virus epidemic, after identification of the VDPV case, investigations among 19 relatives and 20 close contacts who had contact with the patient were performed by testing stool samples for poliovirus. The vaccination certificates of the individuals who had contact with the patient were examined. Furthermore, sewage samples from household wastewater and nearby river water were also collected. All samples were negative for polioviruses.

Retrospective analysis of AFP cases and assessment of vaccination coverage

AFP cases were actively searched and crosschecked using the records of all county- and prefecture-level hospitals in Zhumadian city, Henan province. Medical records for both inpatients and outpatients were also reviewed in 23 county-level hospitals in Zhumadian. No additional AFP cases were found during

active case searches in hospitals, and no additional AFP cases were observed during house-to-house searches in the villages and neighboring areas.

Through a vaccination information management system for children, the inoculation clinic A and adjacent inoculation clinic B were investigated. The vaccination rate of the third dose of polio vaccine for children under 1 year of age was 80.51%, while the rate of the fourth dose was only 68.93% in vaccination clinic A. The vaccination rates of the second and third doses of polio vaccine were 80.24% and 74.47% in vaccination clinic B respectively. The rate of complete polio vaccination in children under 5 years of age was above 97% in vaccination clinic B.

Phylogenetic analysis

To elucidate the divergence and evolution of type 3 Henan aVDPV, a maximum-likelihood tree of sequence relationships in the entire *P1* capsid region for Henan type 3 VDPVs and a set of divergent type 3 VDPVs was constructed (Figure 1). Representative VDPVs include Russian cVDPVs (GenBank accession numbers: MT645947–MT645951), Iranian iVDPVs (GenBank accession numbers: EU684056–EU684057), Belarusian aVDPV (GenBank accession numbers: FJ460226–FJ460227), and Indian aVDPV (GenBank accession number: KR259358).

The phylogenetic tree, which was based on the *P1*-coding region, revealed that all three Henan type 3 VDPVs could be grouped into a single cluster with divergence pathways different from those of Sabin 3, and they were distinct from the genetic clusters of other representative type 3 VDPVs (Figure 1).

Reversions of key neurovirulence determination sites

Based on the full-length genome sequencing analysis, CHN21006-1, CHN21006-2, and CHN21006-3 contained 41, 43, and 60 nucleotide substitutions, respectively, compared to Sabin3/Sabin1 strain. Most of the nucleotide substitutions in the coding region of the genome were synonymous, resulting in 9, 9, and 12 amino acid substitutions, respectively (Supplementary Table 1).

Notably, the nucleotide U at position 472 in the 5' noncoding region was mutated to C. Moreover, the nucleotide C at position 2493 in the *VP1* coding region was mutated to U, causing an amino acid substitution in VP1–6: Thr to Ile. Both mutations are neurovirulent reversion mutations usually observed in VDPV-associated paralytic poliomyelitis cases (24). However, the third nucleotide substitution identified as a key determinant of the attenuated phenotype of Sabin 3 (a U-to-C reversion at nucleotide 2034 in the VP3 coding region, which caused an amino acid substitution in VP3–91: Phe to Ser) did not revert.

Recombination features of Henan type 3 VDPVs

A similarity plot and bootscanning analysis of the complete genomes of the three Henan type 3 VDPVs indicated that they were Sabin 3/Sabin 1 recombinants (Figure 2). The upstream sequences (i.e., 5'-UTR, P1/capsid, and 5' part of the P2/noncapsid sequences) up to nucleotide position 4899 were derived from

the Sabin 3 strain (28, 30, and 38 nucleotide substitutions, respectively), and sequences downstream of nucleotide position 4899 (i.e., 3' part of the P2/noncapsid sequences, P3/noncapsid sequences, and 3'-UTR) were derived from the Sabin 1 strain (13, 13, and 22 nucleotide substitutions, respectively) (Figure 2).

Antigenic divergence of Henan type 3 VDPVs

The amino acid sequences within or near the predicted neutralizing antigenic (NAg) sites were aligned with those of the Henan type 3 VDPV strains, Sabin 3 strain (GenBank accession No. AY184221), and type 3 wild poliovirus prototype strain P3/Leon/37 (GenBank accession No. K01392) (Figure 3). The Henan type 3 VDPVs had two shared amino acid replacement in the NAg sites, one is in NAg2 (VP2–164: Asn to Asp, and), and the other is in NAg3b (VP3–77: Asp to Asn). Strain CHN21006-3 also had another amino acid replacement in NAg3b (VP2–172: Glu to Lys) (Figure 3).

Supplementary immunization activities (SIAs)

To prevent possible circulating VDPV, SIAs were conducted throughout the county. House-to-house SIAs were conducted in urban regions, covering 54,916 children under 7 years of age. The staff at all levels were trained to ensure the high quality of SIAs, including planning the SIA, overseeing the obligation and responsibility of staff, providing technical guidelines, and monitoring adverse events of OPV. It is worth mentioning that the bivalent OPV (bOPV, containing Sabin virus types 1 and 3) was used in SIAs, instead of the trivalent OPV (tOPV).

Discussion

This is the first report of a VDPV case detected by AFP case surveillance in Henan Province. After the case was identified as type 3 VDPV by the National Polio Laboratory, the emergency response of grade IV was immediately launched in Henan province. A timely emergency response was conducted, including an epidemiological investigation, and SIAs were performed in time to prevent the possible circulation of VDPV in the population and outside environment. Based on active searches of AFP cases, these preventive approaches proved to be effective and successful, and no poliovirus was detected in the close contacts and surrounding environment of the patient. In addition, through a comprehensive risk assessment, the risk of VDPV transmission was small, and cVDPV did not occur during the epidemic events.

In 2000, the WHO Western Pacific region, which includes China, declared that China had entered the stage of maintaining the polio-free status. In the process of eradicating polio and maintaining polio-free status, the occurrence of VDPV cases in some countries and regions in recent years is a newly emerging problem. For many years, massive OPV immunization campaigns worldwide have substantially reduced the number of cases of poliomyelitis caused by WPV. Nevertheless, challenges originating from low polio vaccine coverage could lead to the emergence of VDPVs or the reintroduction of WPV from disease-endemic countries, which might threaten the success of poliomyelitis eradication programs (2, 25). Based

on the characteristics of OPV, VDPV will inevitably appear as long as OPV are used. Meanwhile, poliomyelitis outbreaks caused by pathogenic VDPVs are primarily caused by low polio vaccine coverage. Low coverage enables interhuman circulation of polioviruses from the OPV strains and the genetic drift of the viruses, with the potential danger of subsequent reversion to neurovirulent phenotypes. If VDPV exists or causes a single case, it will not constitute a major threat to public health. However, once cVDPVs that cause disease outbreaks are present, they become a major public health emergency.

Reports of type 3 VDPV are relatively rare compared to those of types 1 and 2 VDPVs. In addition, only 11 records of type 3 VDPV sequences are available in GenBank as of April 2022. Both Chinese and global data show that type 2 VDPV account for the vast majority of all VDPVs. However, since the global switch from tOPV to bOPV in April 2016, the prevalence of types 1 and 3 VDPV may have changed (26).

A unique aspect of this case is that the patient had been vaccinated with four doses of IPV, and the serum neutralizing antibody in the serum for Sabin types 1 and 3 were 1:16 and 1:512, respectively. Since the titer of anti-type 3 poliovirus antibody was significantly higher than that of anti-type 1 poliovirus antibody, it is speculated that the patient was infected with type 3 VDPV, and the virus continued to replicate and be excreted for at least 41 days (from the date of the first stool sample collection to the date of the third stool sample collection). The presence of this kind of virus in the human population is a serious risk and undoubtedly poses a severe challenge in maintaining a polio-free status in China.

How the virus infected this patient remains unclear. All stool samples collected from patients with AFP, meningitis, or HFMD in 2016–2017 were tested for virus isolation; however, no signs of poliovirus circulation were found before or after the isolation of CHN21006. Environmental surveillance was strengthened by collecting more wastewater samples from different areas of Zhumadian City; however, this did not lead to the isolation of similar viruses for the second time.

The emergence of the Henan VDPV has important implications for present and future polio immunization policies. This case report clearly showed that Henan type 3 VDPVs were aVDPVs, and the epidemiological data reported that they did not belong to cVDPVs or iVDPVs, that is, no other patients were found to have AFP associated with these VDPVs. Furthermore, 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 patients from whom VDPVs were isolated at the time of AFP presentation. Fortunately, no evidence of transmission of this VDPV among the general population was observed. Nevertheless, finding the source of the virus is difficult and remains unclear.

As the world approaches the goal of polio eradication, the WHO is developing corresponding strategies to minimize the risk of paralysis caused by poliovirus, including WPV and VDPVs. Stopping the use of type II OPV is an important part of the strategies in the later stages of polio eradication, thereby switching from tOPV to bOPV. Since May 1, 2016, China has begun to implement the sequential immunization program of one dose of IPV plus three doses of bivalent OPV (containing type 1 and 3 OPV). Since January 1, 2020, China changed its immunization strategy again and began the sequential immunization procedure of two doses of IPV plus two doses of bivalent OPV (containing type I and type III OPV). This program

has a good protective effect, prevents the transmission of imported poliovirus, and reduces the incidence of VAPP and VDPV cases. As the last case of WPV 3 was observed in 2012, the WHO declares type 3 poliovirus eradicated after a 31-year campaign (27). Under these circumstances, consideration should be given to hastening the global withdrawal of Sabin serotype 3 from OPV.

In conclusion, we described the genetic characteristics of Sabin 3/Sabin 1 recombinant VDPV, which seems to have the characteristics of an iVDPV. Although the observed antigenic changes will help the virus escape vaccine-induced immunity in human populations, evidence of virus transmission was not observed. Moreover, maintaining a high vaccination rate and sensitive AFP case surveillance system is always the most effective measure to detect and intercept the importation of wild poliovirus or the transmission of VDPV.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Review Committee of the Henan Center for Disease Control and Prevention (CDC) Institutional Review Board. Written informed consent regarding anonymizing the publication of VDPV infection and the use of clinical samples was obtained from the parents of the children involved in this study. All experimental protocols were approved by the Henan CDC, and the study were performed in accordance with the approved guidelines.

Consent for publication

Not applicable

Availability of data and materials

The full-length genome sequences of Henan type 3 VDPV strains CHN21006-1, CHN21006-2, and CHN21006-3 described in this study were deposited in the GenBank database under the accession numbers ON221307- ON221309. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Yong Zhang、Yanyang Zhang and Mingyu Zhang conceived and designed the plan and experiments. Yiran Bai, Changshuang Wang, Mingxia Lu, Yating Ma and Yan Wang conducted epidemiological investigations and collected stool samples. Janhui Yang, Hui Zhu, Lu Zhang, Jin Xu performed the experiments. Zhanpei Xiao, Xiaoxiao Zhang, Xiaolei Li, Dongyan Wang, Shuangli Zhu, Dongmei Yan and Wenbo Xu analyzed the data. Mingyu Zhang and Yong Zhang wrote the main manuscript and prepared all the figures. All authors read and approved the final manuscript.

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Figures

Figure 1

Maximum-likelihood tree showing the phylogenetic relationship between Henan aVDPV isolates and type 3 VDPV strains. The evolutionary history based on the P1-coding region (2635 nucleotides) was inferred

using the Maximum Likelihood method and Kimura 2-parameter model conducted in MEGA 11. The accession numbers of the other type 3 VDPV strains are as follows: Russian cVDPVs (GenBank accession numbers: MT645947–MT645951), Iranian iVDPVs (GenBank accession numbers: EU684056–EU684057), Belarusian aVDPV (GenBank accession numbers: FJ460226–FJ460227), and Indian aVDPV (GenBank accession number: KR259358). The tree was rooted in Sabin 3 sequence (AY184221).

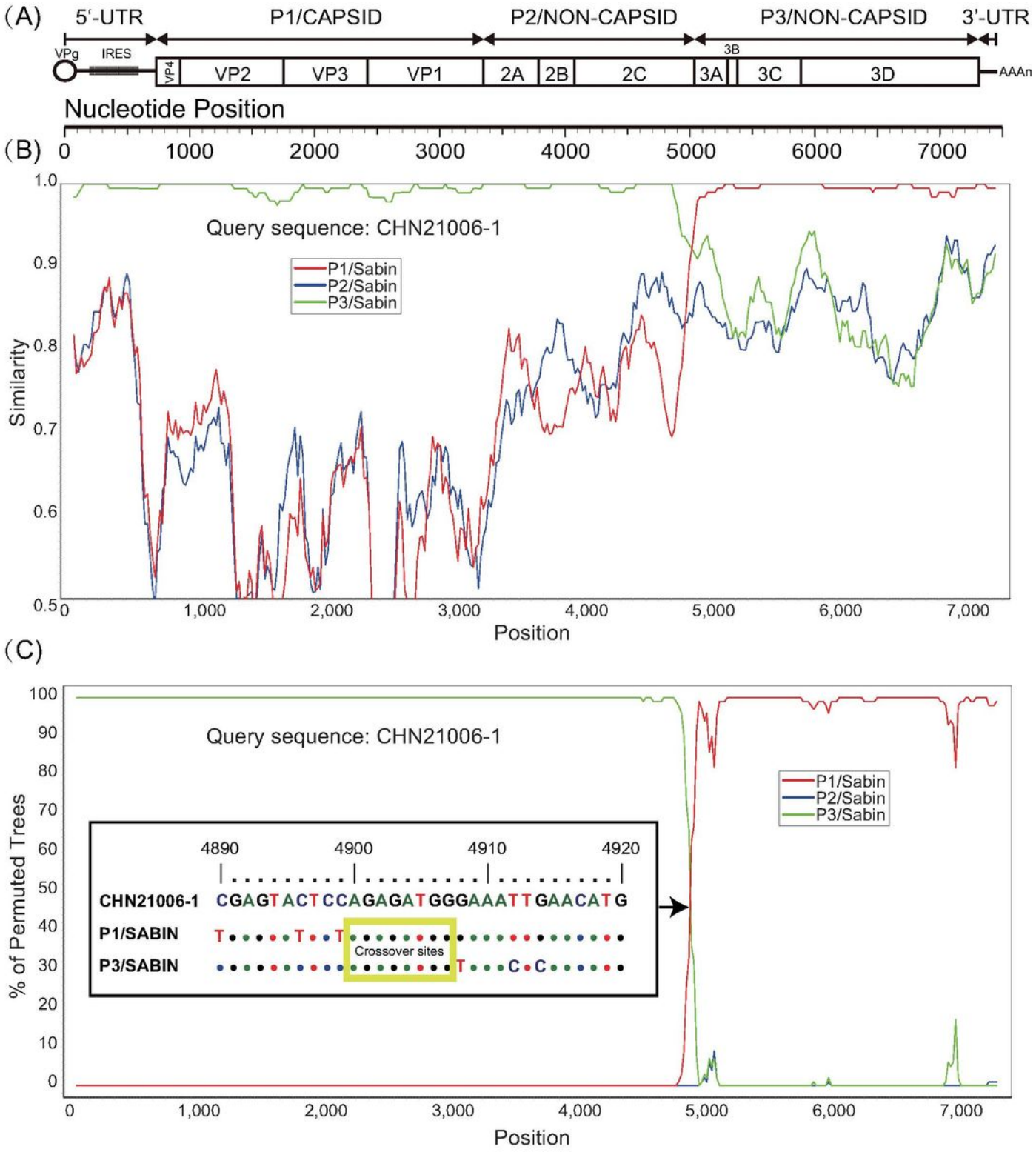

Figure 2

Similarity plot and bootscanning analysis of complete genomes of Henan type 3 VDPVs. For the plot and bootscanning analysis, the names of viruses of the query sequence are indicated in the upper left corner. The yellow solid line rectangle indicates possible crossover site.

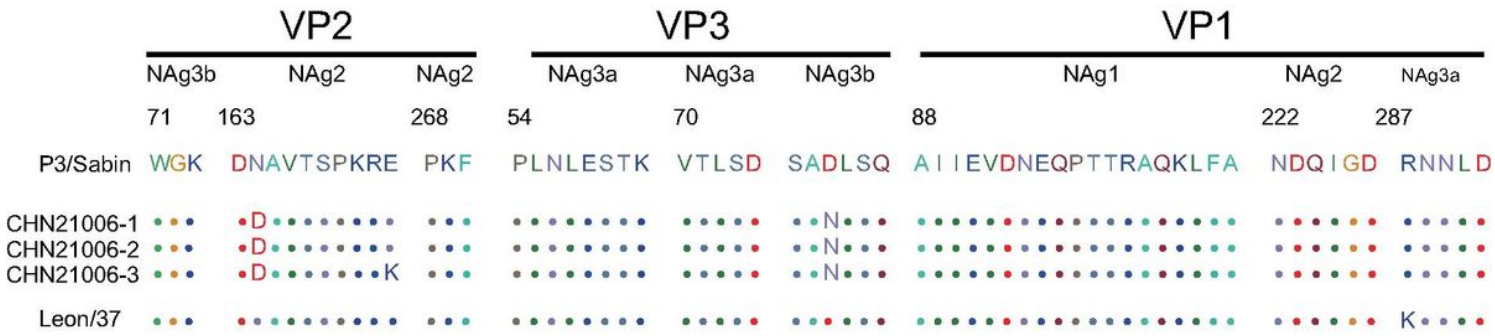

Figure 3

Alignment of amino acid residues of neutralizing antigenic (NAg) sites.

NAg1 (VP1: 88–106), NAg2 (VP2: 163–172; VP2: 268–270; VP1: 222–227), NAg3a (VP3: 54–61; VP3: 70–74; VP1: 287–291), and NAg3b (VP2: 71–73; VP3: 75–80) were identified in Sabin 3 (GenBank accession no. AY184221), Henan type 3 VDPVs, and the type 3 wild poliovirus prototype strain P3/Leon/37 (GenBank accession no. K01392).

Supplementary Files

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- Supplementarytable.docx