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Evaluation of the genetic stability of Sabin strains and the consistency of inactivated poliomyelitis vaccine made from Sabin strains using direct deep-sequencing

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ABSTRACT

Inactivated poliomyelitis vaccine made from Sabin strains (sIPV) has been encouraged to introduce in the "Global Polio Eradication & Endgame Strategic Plan" and increasingly used worldwide. Attenuated Sabin strains used in manufacture of oral poliovirus vaccine (OPV) and sIPV may regain full or partial neurovirulence during growth in vaccine recipients and the vaccine manufacturing processes. Ensuring the molecular consistency of sIPV batches and that no mutation accumulates beyond the level present in past batches are important for quality control of vaccine manufacture process. Direct deep-sequencing allows the construction of a library of virus RNA and the detection of genetic mutations throughout the viral genome. In the present study, direct deep-sequencing was conducted to detect molecular mutations in virus passages, multiple sIPV monovalent lots, and virus monovalent lots from different polio type III strains. The results indicated that direct deep-sequencing can be used to identify and quantify small amounts of mutant viruses in vaccine preparations, trace the source of a specific virus seed, and monitor the batch-to-batch consistency of vaccines, suggesting that this technique could be suitable for the quality control and consistency monitoring of sIPV production.

1. Introduction

Polio is expected to be the second infectious disease eradicated after smallpox due to the use of vaccines. Large efforts have been undertaken by the World Health Organization (WHO) Global Polio Eradication Initiative to obtain new vaccines that are safe and available at low cost [1]. The use of alternative strains to produce inactivated poliomyelitis vaccines, such as the attenuated Sabin strain (sIPV), is encouraged by the WHO [2]. sIPV was licensed in 2015 and is widely used in China, becoming one of the best tools in the "Global Polio Eradication & Endgame Strategic Plan" with increasing usage [3].

Attenuated Sabin strains have been used in the manufacture of oral poliovirus vaccine (OPV) and sIPV. It may regain full or partial neurovirulence during growth in vaccine recipients and vaccine manufacturing processes [4–6]. Because of this genetic instability, every batch of OPV must be tested for neurovirulence by the Monkey Neurovirulence Test (MNVT) [7] or through the use of transgenic mice [8,9]. A highly sensitive molecular method of mutant analysis by polymerase chain reaction (PCR) and restriction enzyme cleavage (MAPREC) is currently recommended by the WHO for screening during OPV production before this vaccine can be released for use in humans [7]. Despite the high sensitivity and accuracy of MAPREC, this method can only monitor mutations

Abbreviations: WHO, World Health Organization; sIPV, inactivated poliomyelitis vaccine made from Sabin strains; OPV, oral poliovirus vaccine; MNVT, Monkey Neurovirulence Test; PCR, polymerase chain reaction; MAPREC, mutant analysis by polymerase chain reaction and restriction enzyme cleavage; MPS, Massively Parallel Sequencing; NGS, Next Generation Sequencing.

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at a few genomic loci, thereby missing mutations at other sites that could adversely affect vaccine quality [10].

Massively Parallel Sequencing (MPS), also known as Deep Sequencing or Next Generation Sequencing (NGS), is a large-scale high-throughput gene analysis technology based on DNA sequencing that has been developed in recent years [11]. Identifying the sequence of nucleotides by capturing newly synthesized terminal labels, MPS requires no electrophoresis and is simple to conduct. MPS has been used to sequence and detect various viral genes [12–15]. In 2010, Neverov et al. [10] detected and quantified mutations in the whole viral genome of OPV through MPS and confirmed that the results of MPS were consistent with those of the MAPREC test.

Ensuring the molecular consistency of sIPV batches and that no mutation accumulates beyond the level present in past batches could be useful for quality control of the vaccine manufacture progress. In the present study, direct deep-sequencing, a MPS using RNA sequencing directly was conducted to detect the molecular mutation of viral passages, multiple sIPV monovalent lots, and virus monovalent lots from different polio type III strains. We demonstrated that direct deep-sequencing was able to identify and quantify small amounts of mutant viruses in vaccine preparations. Moreover, this technique was also able to identify specific virus seeds during the vaccine manufacture process, suggesting that direct deep-sequencing will be suitable for quality control and consistency monitoring of sIPV production.

2. Materials and methods

2.1. Preparation of serial virus passages from Sabin strains

The sub-master virus seeds of type I Sabin SO + 2, type II Sabin SO + 2, and type III Pfizer RSO2 were obtained from the WHO and cultured on Vero cells to prepare working seeds (type I Sabin SO + 3, type II Sabin SO + 3, type III Pfizer RSO3). Both the sub master and working seeds passed the MNVT.

The sIPV monovalent lots (type I and type II Sabin SO + 4, type III Pfizer RSO4) were prepared as previously reported [16]. Briefly, Vero cells were scale-up in culture on microcarriers in 7, 75 and 550 L bioreactors. Three types of working virus seeds were inoculated into 550 L bioreactors after the cells reached a certain concentration. The viral suspension was incubated at 33–34 °C for 2–3 days and subsequently harvested.

In the present study, Vero cells were cultured in culture flasks and then inoculated with the vaccine lot virus (SO + 4, RSO4) at a multiplicity of infection (MOI) of 0.01 and cultured at 33 ± 0.5 °C to obtain fifth passage polioviruses (type I and type II Sabin SO + 5, type III Pfizer RSO5) and sixth passage polioviruses (type I and type II Sabin SO + 6, type III Pfizer RSO6).

2.2. Preparation of the virus monovalent bulk using the Zhong-3 strain

The Zhong-3 strain is a plaque purification strain that belongs to poliovirus type III, which was established by Institute of Medical Biology, Chinese Academy of Medical Sciences in 1961 and approved for use in OPV production in China since 1971 [7]. Vero cells were inoculated with Type III Zhong-3 working seed lots (2 passages from primary seed) at a MOI of 0.01, and the virus bulk was harvested.

2.3. RNA extraction and sequencing

Viral RNA was extracted from each sample using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). For each sIPV virus sample, the extracted RNA was purified using a RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany) and then quantified by using a Qubit RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA). The purified viral RNA was used to prepare an mRNA-Seq library with a KAPA Stranded mRNA-Seq Kit (KAPA Biosystems, Boston, Massachusetts, United States) according to the manufacturer's instructions. Briefly, the following seven major steps were performed: (1) 5 ng of purified viral RNA, KAPA Fragment, Prime and Elute Buffer (2×) were mixed and incubated at 94 °C for 6 min, immediately followed by incubation on ice to fragment RNA. (2) KAPA 1st Strand Synthesis Buffer was added to synthesize first-strand cDNA by incubating at 25 °C for 10 min, 42 °C for 15 min, and 70 °C for 15 min. (3) KAPA 2nd Strand Marking Buffer and KAPA 2nd Strand Synthesis Enzyme Mix were added to synthesize second-strand cDNA at 16 °C for 1 h. 4) After purification using Agencourt AMPure XP Beads (Beckman Coulter, California, USA), KAPA A-Tailing Buffer (10×) and A-Tailing Enzyme were added for terminal repair and the addition of A-Tailing. The reaction was incubated at 30 °C for 30 min and 60 °C for 30 min. (5) Adaptor, KAPA Ligation Buffer (5×) and KAPA DNA Ligase were subsequently added to start adapter ligation with incubation at 20 °C for 15 min. (6) Ligated cDNA between 200 and 300 bp in length was obtained after two-step AMPure XP Bead purification involving the addition of KAPA HiFi HotStart Ready Mix (2×) and KAPA Library Amplification Primer Mix (10×) to induce PCR amplification under the following conditions: pre-denaturation at 98 °C for 45 s, denaturation at 98 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. (7) AMPure XP Beads were added at a ratio of 1:1 to purify the material, and a library ready for sequencing was obtained. Finally, the established library was sequenced on a HiSeq X Ten (Illumina, San Diego, CA) with a read length of 150 bp.

2.4. Statistics analysis

Paired-end reads from each sample were first pre-processed using sickle software v1.200 with argument-t Sanger-q30-l50 parameters (https://github.com/najoshi/sickle). The filtered data were then aligned to the poliovirus genome by Bowtie2 v2.1.0 with default parameters [17]. Samtools v0.1.16 was used to further filter aligned reads on the basis of the mapping quality with the -q20 parameter [18]. SNP callings of each sample were conducted by VarScan 2.3.7 and in-house PerlScripts [19]. Finally, SNP statistics were performed via in-house R scripts.

A total of 3 batches of type I, 6 batches of type II, and 5 batches of type III monovalent vaccine lots of sIPV were sequenced, and the mean value, standard deviation (SD), and 2- and 3-fold SD of percent mutants at critical virulence sites were calculated using Excel to analyse the genetic stability of the batches.

3. Results

3.1. Sequencing of working seeds and reliability analysis

To assess the reliability of the direct deep-sequencing method, RNA from three types of working seeds (type I Sabin SO + 3, type II Sabin SO + 3, and type III Pfizer RSO3) was extracted, and each sample was sequenced three times. The average length was 150 bp, number of sequences (pair-end) was approximately 31×10^6, total nucleotides read was approximately 9266×10^6, average coverage per nucleotide was approximately 16×10^4, site mutation rate was 0.04%, and percentage of insertions and deletions was approximately 0.003%. The detailed sequence results for the 9 samples are shown in Table 1.

The repeatability of the direct deep-sequencing method was evaluated by comparing the sequencing results for 3 types of work-

Table 1
Direct deep-sequencing results of sIPV working seeds.

sample	Average length	Number of sequences (pair-end), 10^3	Total nucleotides read, 10^6	Average coverage per nucleotide	% mutations (substitutions)	% insertions and deletions
I SO + 3-1	150	35,749	10,725	101,056	0.0443	0.0059
I SO + 3-2	150	37,021	11,106	118,183	0.0449	0.0055
I SO + 3-3	150	35,294	10,588	111,612	0.0451	0.0058
II SO + 3-1	150	29,462	8839	104,667	0.0388	0.0027
II SO + 3-2	150	28,071	8421	87,145	0.0396	0.0031
II SO + 3-3	150	32,816	9845	105,959	0.0399	0.0028
III RSO3-1	150	27,648	8295	258,139	0.0385	0.0015
III RSO3-2	150	24,818	7446	275,801	0.0372	0.0015
III RSO3-3	150	27,099	8130	275,711	0.0367	0.0015
Average	150	30,886	9266	159,808	0.0405	0.0033

ing seed lots 3 times. The comparison of the mutation rates of 3 type I Sabin strain samples is shown in Fig. 2. The R^2 values for the type I Sabin strain, type II Sabin strain and type III Pfizer strain were 0.97–0.98, 0.99, and 0.98–0.99 (Table 2), respectively, indicating that the direct deep-sequencing method has high repeatability (Fig. 1).

Table 2
R^2 of deep direct deep-sequencing in sIPV working seeds.

	1 vs 2	1 vs 3	2 vs 3
I SO + 3	0.9765	0.9804	0.9765
II SO + 3	0.9932	0.9922	0.9941
III RSO3	0.9882	0.9922	0.9891

1 vs 2, the first mutation rate versus the second mutation rate.
1 vs 3, the first mutation rate versus the third mutation rate.
2 vs 3, the second mutation rate versus the third mutation rate.

3.2. Virus genetic stability after passaging

Three types of serial passages of viruses, including sub-master seeds, working seeds, monovalent lots, and passage 5 and 6 poliovirus were sequenced through direct deep-sequencing, and then, the mutation rates for each viral gene passage were compared.

3.2.1. Mutations in type I Sabin strain after passaging

The two major attenuation determinants of neurovirulence in the 5′NCR, $G_{480}A$ and $U_{525}C$ increased after passaging (Fig. 2). The

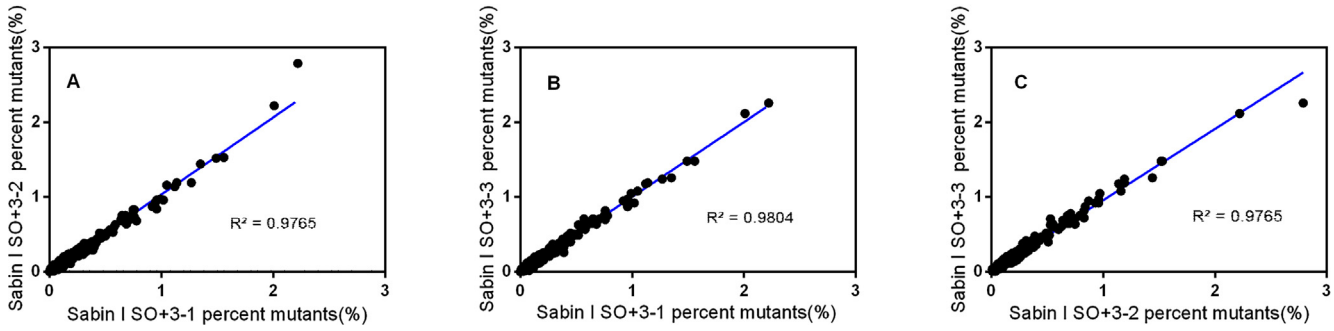

Fig. 1. Repeatability analysis of direct deep-sequencing using Sabin I SO + 3. Sabin I SO + 3-1 compared to SO + 3-2 (A); Sabin I SO + 3-1 compared to SO + 3-3 (B); Sabin I SO + 3-2 compared to SO + 3-3 (C).

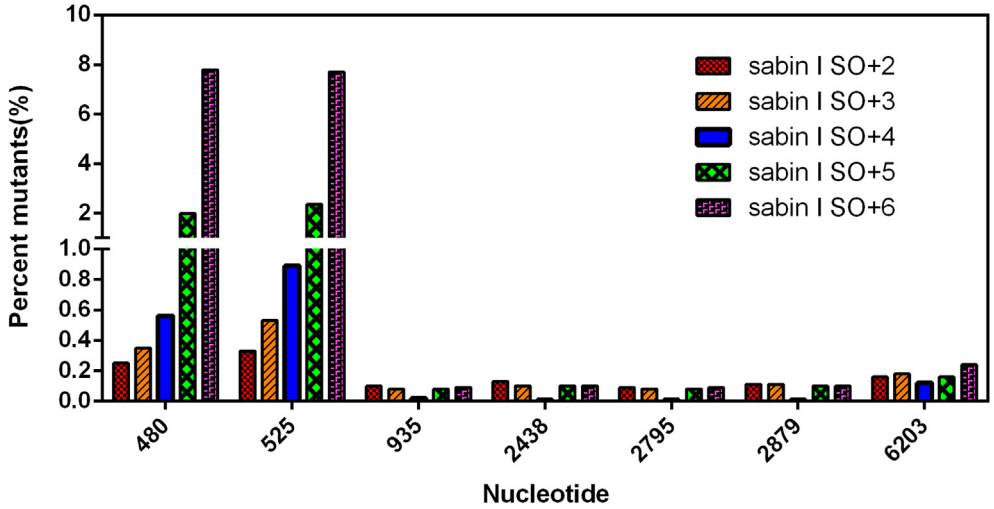

Fig. 2. Patterns of mutations at neurovirulence determinants of the type I Sabin strain of passage SO + 2 to SO + 6.

mutation content at 480-A for SO + 2 was 0.24% and increased to 1.98% and 7.79% for SO + 5 and SO + 6, respectively; at position 525-C, the mutation content for SO + 2 was 0.33% and increased to 2.35% and 7.70% for SO + 5 and SO + 6, respectively. Mutations in the coding region, including $U_{935}G$ in VP4, $A_{2438}C$ in VP3, $A_{2795}C$ and $U_{2879}G$ in VP1, and $C_{6203}A$ in 3D, showed no significant difference between different passages, with approximately 0.1% mutations at all nucleotide positions (Fig. 2).

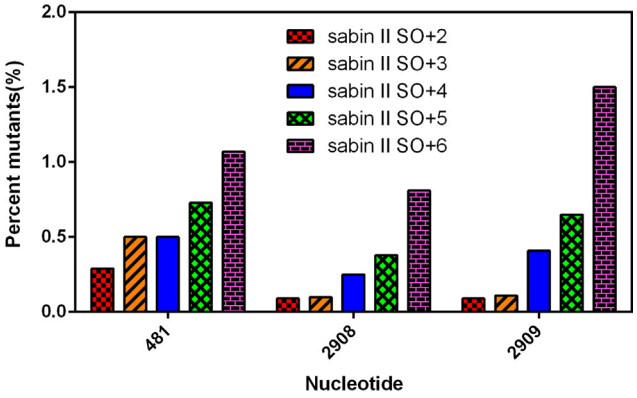

Fig. 3. Mutation contents at neurovirulence determinants of the type II Sabin strain of passage SO + 2 to SO + 6.

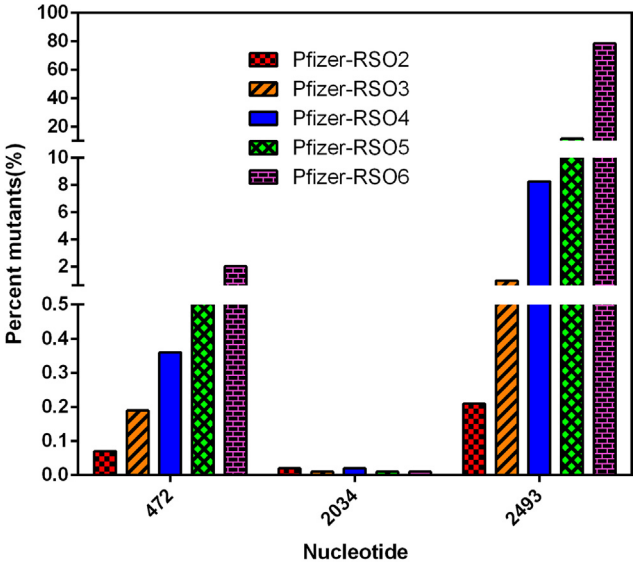

Fig. 4. Mutation contents of neurovirulence determinants of the type III Pfizer strain of passage SO + 2 to SO + 6.

3.2.2. Mutations in type II Sabin strain after passaging

The mutant $A_{481}G$, a major attenuation determinant of neurovirulence in the 5′NCR of type II Sabin, gradually increased with the number of passages (Fig. 3). The mutation content at 481-G increased from 0.29% for SO + 2 to 1.07% for SO + 6. At $A_{2908}G$, the mutation content increased from 0.09% (SO + 2) to 0.81% (SO + 6), and at $U_{2909}C$ in VP1, the mutation content increased from 0.09% (SO + 2) to 1.50% (SO + 6) (Fig. 3).

3.2.3. Mutations in type III Pfizer strain after passaging

The critical important 472-C mutation in the 5′NCR, which determines the neurovirulence of type III virus, mutated from 0.07% at RSO2 to 2.02% at RSO6. In the coding region, the neurovirulence determinants $U_{2034}C$ in VP3 showed almost no difference between different passages, which was approximately 0.01%; however, the mutation frequency at $C_{2493}U$ in VP1 increased from 0.21% (RSO2) to 78.62% (RSO6) (Fig. 4).

3.3. Genetic consistency of multiple sIPV monovalent batches

Direct deep-sequencing was used to analyse the genetic consistency of sIPV. A total of 3 batches of type I, 6 batches of type II, and 5 batches of the type III monovalent bulk of sIPV were sequenced. The nucleotide mutation frequency showed no significant difference between batches of each type, particularly for the critical mutants $G_{480}A$ and $U_{525}C$ of type I, $A_{481}G$ of type II, and $U_{472}C$ of type III, suggesting that the batch difference was relatively small. The average content of the 480A and 525C mutations in 3 batches was 1.45 ± 0.04%, while the content of 481G mutations in 6 batches was 0.74 ± 0.08% and the content of 472C mutations in 5 batches was 0.43 ± 0.07% (Fig. 5).

3.4. Comparison of the patterns of mutations between Pfizer and Zhong-3

To distinguish the patterns of mutations that were characteristic for specific virus seeds, the monovalent bulks produced from Pfizer and Zhong-3 were sequenced and their genomic mutations were compared.

In the 5′NCR, the monovalent bulk prepared using Pfizer or Zhong-3 showed no significant difference in genomic mutations, and the mutation frequencies at $U_{472}C$ were all lower than 0.5% (Fig. 6A). In the coding region, the mutation frequency at $U_{2034}C$ showed almost no significant difference, remaining at a relatively low level of 0.02%. However, at the unstable genomic position 2493, the frequency was 13.46% for Pfizer and as high as 99.95% for Zhong-3 (Fig. 6B). A significant difference in the mutation

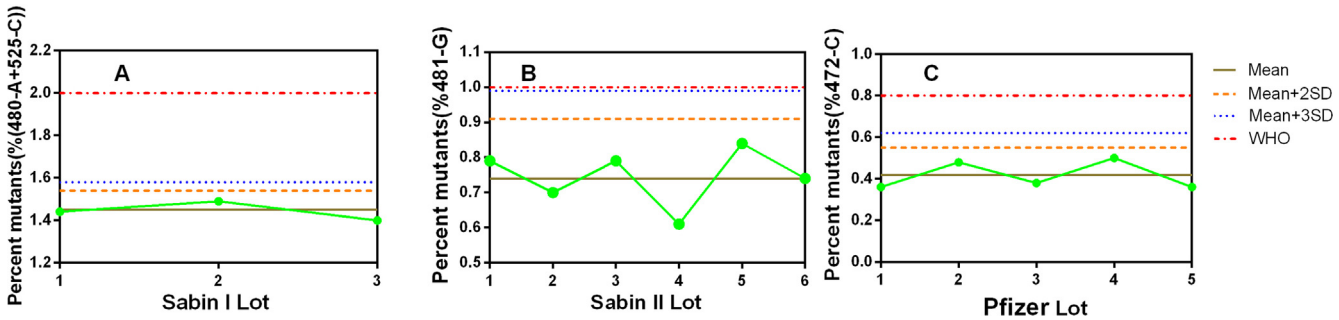

Fig. 5. Mutant trend analysis of multiple sIPV monovalent batches. Sum of the mutants of 480-A and 525-C for type I Sabin strain in three batches of the monovalent bulk (A); Mutants of $A_{481}G$ for type II Sabin strain in six batches of the monovalent bulk (B); Mutants of $U_{472}C$ for Pfizer strain in five batches of the monovalent bulk (C).

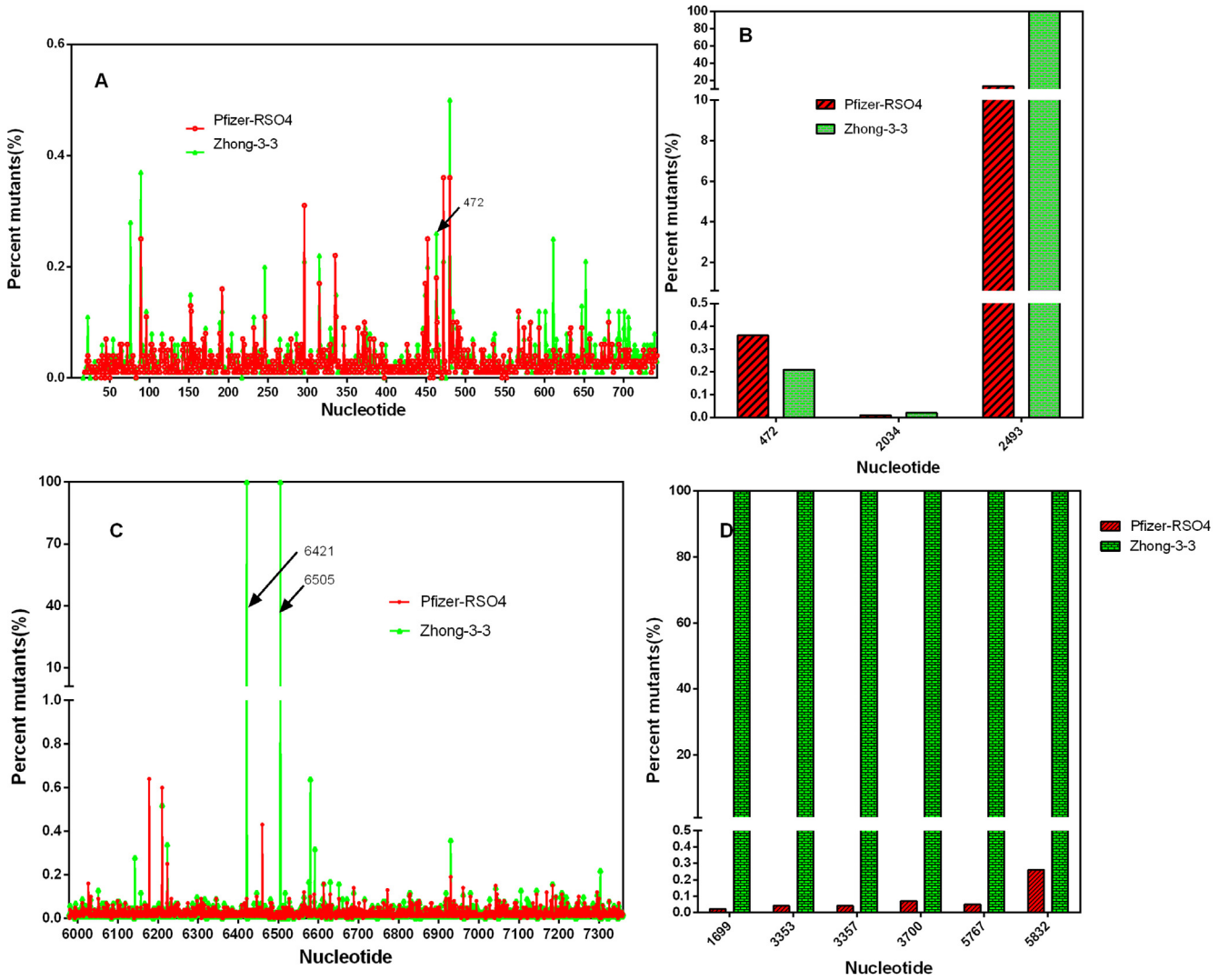

Fig. 6. Mutation contents in virus harvests prepared from different virus seed strains. Frequency of mutations of different virus seeds at important determinant sites (A); Frequency of mutations in the 5′NCR (B); Frequency of mutations in the 3D region (C); Mutation sites of different virus harvests that showed significant differences (D).

frequency was also observed at $C_{6421}U$ and $U_{6505}C$, and the mutation frequencies at $C_{6421}U$ and $U_{6505}C$ of Pfizer were 0.09% and 0.03%, respectively, while those of Zhong-3 were both 99.95% (Fig. 6C).

At other genomic loci, a significant difference in the mutation content at $G_{1699}A$ in VP2, $U_{3353}C$ and $A_{3357}G$ in VP1, $C_{3700}U$ in 2A, $U_{5767}C$ and $U_{5832}C$ in 3C was observed. These positions contained 0.5% mutations for Pfizer, but reached 99.91% mutations for Zhong-3 (Fig. 6D).

4. Discussion

Direct deep-sequencing, which was initially used to examine mammalian transcriptomes, revolutionized the study of transcriptomics and has been extended to sequencing the viral RNA genome [20–22]. Due to the low-fidelity viral polymerase used for genomic replication, RNA viral genomes, although small, undergo a high level of evolution and diversity, resulting in multiple slightly different viral variant quasispecies [23]. The information for viral quasispecies relies on obtaining single nucleotide variations of viral genomes. Genome walking was traditionally used to produce a virus sequence [24,25], and recently, Massively Parallel Sequencing (MPS) has been widely used for virus sequencing [26–28]. A recent study verified the viral genetic consistency and stability of OPV through DNA sequencing, indicating that MPS could be a useful tool for monitoring the genetic consistency of live viral vaccines [10,29,30].

In the present study, we used direct deep-sequencing, a MPS method using RNA directly to monitor genomic variations of three types of serial passages of viruses from sIPV production. Unlike traditional DNA-sequencing methods, direct deep-sequencing directly constructs virus RNA libraries, which can reduce mutations derived from PCR amplification prior to cDNA library construction. Sarcey E et al. [30] detected OPV III type virus using a DNA-sequencing method and found that the 472C mutation rate in the plasmid was 0.02% without using PCR, but reached approximately 0.10% after using PCR. In studies of deep sequencing of OPV using DNA-sequencing, Neverov et al. [10] found that with an average base read of 56,659 times, the base insertion and deletion rates were 0.01%, while in the present study using direct deep-sequencing, the base read increased to 159,808 times on average and the insertion and deletion rates were only approximately 0.005%. The only disadvantage in the present study was that a higher sequencing coverage of virus RNA was required due to host cell-derived RNAs, which increased sequencing costs. However, with increasing sequencing throughput, additional costs could be ignored.

During virus culture for sIPV production, the accumulation of genomic mutations may result in neurovirulence reversion [5,6]. To examine the effect of virus passage on genetic stability, 5 passages of viruses were tested. Mutations of neurovirulence determinants in all of the tested viruses increased with the number of passages, and the mutation rate of critically important positions in virus seed and vaccine lots were consistent with the WHO recommended specifications (the sum of the mutation rate of 480-A and 525-C for type I was lower than 2.0%, and the mutation rates of 481-G for type II and 472-C for type III were lower than 1.5% and 1.0%, respectively) [31]. However, the sum of the mutation rate of 480-A and 525-C for type I was 4.33% for SO + 5 and 15.49% for SO + 6, while that of 472-C for type III was 2.02% for RSO6; these values were beyond the WHO recommended specifications. Therefore, strict control of virus passaging is important to ensure the attenuated character of Sabin strain poliovirus.

From the perspective of biosafety, sIPV, a vaccine that is inactivated and produced by the Sabin strain, maintains the attenuation characteristic of vaccine viruses. Additionally, controlling the molecular consistency of monovalent bulk production is critical for quality control during sIPV manufacture. According to the WHO Expert Committee on Biological Standardization of IPV, the mutation contents should be tested from batch-to-batch [31]. We used direct deep-sequencing to perform deep sequencing of monovalent bulks from different production batches of sIPV. The results indicated that the mutation rates at neurovirulence determinants in both the 5′NCR and coding regions were maintained at relatively stable levels. The two-fold and three-fold standard deviations of the average major attenuated determinants of neurovirulence mutation rates of the three virus types in every batch met the WHO recommended qualified range, indicating that the viruses have good genetic consistency among batches of sIPV and maintain stable virulence. In the future, direct deep-sequencing methods should be used to sequence a certain number of batches of vaccines annually, and the trend of mutation rates of major attenuated determinants of neurovirulence should also be plotted. Furthermore, more data on the batch-to-batch consistency are needed to establish and improve the alert limit.

Direct deep-sequencing can also be used to identify seed viruses. To date, the Sabin origin seed plaque-purified rederived Sabin original seed (RSO, or Pfizer) and Zhong-3 seed have been widely used in vaccine production. As a marker of a seed virus, the mutant $C_{2493}U$ has shown a significant difference between Sabin and Pfizer strains: The Sabin-derived seed contains 30%-90% of this mutant, while the Pfizer seed only contains 1–4% of 2493-U [10,32]. In the present study, the contents of the mutant $C_{2493}U$ in Pfizer and Zhong-3 were 13.46% and 99.95%, respectively, indicating that Zhong-3 is significantly different from Pfizer but more similar to the Sabin strain. Using NGS through a DNA-sequencing method, Neverov et al. [10] showed different mutant rates for $A_{5467}G$, $U_{5473}C$, and $U_{5476}C$ in the 3C region in vaccines using different virus seeds produced by different manufacturers. In the present study, we did not identify the above three mutations, but other mutations, including $G_{1699}A$ in VP2, $U_{3353}C$ and $A_{3357}G$ in VP1, $C_{3700}U$ in 2A, $U_{5767}C$ and $U_{5832}C$ in 3C, and $C_{6421}U$ and $U_{6505}A$ in 3D, were different between Pfizer and Zhong-3. The mutation rates at over 8 sites in the Pfizer strain ranged from 0.01 to 0.50%, whereas those in the Zhong-3 strain were as high as 99.85–99.97%. The present results suggested that these 8 genomic sites may possess strain specificity and could be used in future identity tests.

Direct deep-sequencing is able to quickly and sensitively detect genetic mutations of poliovirus and can be used in the genetic stability test of sIPV virus seeds and serial passages as well as for the monitoring of the batch-to-batch consistency of vaccines. Since the WHO recommends performing viral mutation assays of purified bulks with a high virus concentration and low interference from host cells, we will focus on exploring mutation differences between the viral harvest and purified bulk of sIPV to establish a novel and routine RNA-Seq method to improve quality control during sIPV manufacture.

Author contributions

Conceived and designed the experiments: Li Shi and Mingbo Sun. Virus Preparation: Wei Cai, Xiaolei Yang, Lujie Yang, and Yan Deng. Sample detection: Yan Deng, Jinyan Li, Yin Li, and Yan Ma. Data Analysis: Yan Deng, Yufeng Yao, Li Shi, and Mingbo Sun. Manuscript writing: Li Shi and Mingbo Sun.

Conflicts of interest

We confirm that all of the listed authors have actively participated in the present study, and have read and approved the submitted manuscript. The authors do not have any potential conflicts of interest to declare.

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References

[1] Organization WH. Polio eradication & endgame strategic plan 2013–2018. Global Polio Eradication Initiative, working draft 2013;23:1–99.
[2] Organization WH. WHO global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. GAPIII; 2015.
[3] WHO Representative Office in China. New poliovirus vaccine licensed in China. [updated 2015-01-15; cited 2018-01-08] http://www.wpro.who.int/china/mediacentre/releases/2015/20150115/en/.
[4] Benyesh-Melnick M, Melnick JL. The use of in vitro markers and monkey neurovirulence tests to follow genetic changes in attenuated poliovirus multiplying in the human alimentary tract. PAHO Scientific Publication: Pan American Health Organization; 1959. p. 179–98.
[5] Cann A, Stanway G, Hughes P, Minor P, Evans D, Gt Schild, et al. Reversion to neurovirulence of the live-attenuated Sabin type 3 oral pollovirus vaccine. Nucl Acids Res 1984;12:7787–92.
[6] Evans D, Dunn G, Minor P, Schild G, Cann A, Stanway G, et al. Increased neurovirulence associated with a single nucleotide change in a noncoding region of the Sabin type 3 poliovaccine genome. Nature 1985;314:548.
[7] Organization WH. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated). WHO Expert Committee on Biological Standardization Sixty-third report 2014;TRS 980:49–140.
[8] Dragunsky E, Taffs R, Chernokhvostova Y, Nomura T, Hioki K, Gardner D, et al. A poliovirus-susceptible transgenic mouse model as a possible replacement for the Monkey Neurovirulence Test of oral poliovirus vaccine. Biologicals 1996;24:77–86.
[9] Levenbook I, Dragunsky E, Pervikov Y. Development of a transgenic mouse neurovirulence test for oral poliovirus vaccine: international collaborative study 1993–1999. Vaccine 2000;19:163–6.
[10] Neverov A, Chumakov K. Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines. Proc Natl Acad Sci 2010;107:20063–8.
[11] Schuster SC. Next-generation sequencing transforms today's biology. Nat Methods 2007;5:16.
[12] Margeridon-Thermet S, Shulman NS, Ahmed A, Shahriar R, Liu T, Wang C, et al. Ultra-deep pyrosequencing of hepatitis B virus quasispecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)–treated patients and NRTI-naive patients. J Infect Dis 2009;199:1275–85.
[13] Rozera G, Abbate I, Bruselles A, Vlassi C, D'Offizi G, Narciso P, et al. Massively parallel pyrosequencing highlights minority variants in the HIV-1 env quasispecies deriving from lymphomonocyte sub-populations. Retrovirology 2009;6:15.

[14] Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, et al. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS Pathog 2010;6:e1000896.
[15] Ninomiya M, Ueno Y, Funayama R, Nagashima T, Nishida Y, Kondo Y, et al. Use of illumina deep sequencing technology to differentiate hepatitis C virus variants. J Clin Microbiol 2012;50:857–66.
[16] Liao G, Li R, Li C, Sun M, Li Y, Chu J, et al. Safety and immunogenicity of inactivated poliovirus vaccine made from Sabin strains: a phase II, randomized, positive-controlled trial. J Infect Dis 2012;205:237–43.
[17] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 2012;9:357–9.
[18] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics 2009;25:2078–9.
[19] Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER, et al. VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics 2009;25:2283–5.
[20] Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008;5:621–8.
[21] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 2009;10:57–63.
[22] Wilson JAC, Prow NA, Schroder WA, Ellis JJ, Cumming HE, Gearing LJ, et al. RNA-Seq analysis of chikungunya virus infection and identification of granzyme A as a major promoter of arthritic inflammation. PLoS Pathog 2017;13:e1006155.
[23] Domingo E, Martínez-Salas E, Sobrino F, Jc DLT, Portela A, Ortín J, et al. The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: biological relevance–a review. Gene 1985;40:1–8.
[24] Conzelmann KK, Cox JH, Schneider LG, Thiel HJ. Molecular cloning and complete nucleotide sequence of the attenuated rabies virus SAD B19. Virology 1990;175:485–99.
[25] Tordo N, Poch O, Ermine A, Keith G, Rougeon F. Walking along the rabies genome: is the large G-L intergenic region a remnant gene? Proc Natl Acad Sci U S A 1986;83:3914–8.
[26] Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, et al. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. J Virol 2009;83:4642–51.
[27] Eisen JA. Environmental shotgun sequencing: its potential and challenges for studying the hidden world of microbes. PLoS Biol 2007;5:e82.
[28] Marston DA, McElhinney LM, Ellis RJ, Horton DL, Wise EL, Leech SL, et al. Next generation sequencing of viral RNA genomes. BMC Genomics 2013;14:444.
[29] Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, et al. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. J Virol 2010;84:6033–40.
[30] Sarcey E, Serres A, Tindy F, Chareyre A, Ng S, Nicolas M, et al. Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing. J Virol Methods 2017;246:75–80.
[31] Organization WH. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated). WHO Expert Committee on Biological Standardization Sixty-fifth report. 2015;TRS 993:89–172.
[32] Chumakov KM, Norwood LP, Parker ML, Dragunsky EM, Ran Y, Levenbook IS. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. J Virol 1992;66:966–70.