· Article ·

Comparison of Ion Torrent, Illumina, and Nanopore platforms for sequencing SARS-CoV-2 JN.1 lineage variants from Dalian, China

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Abstract

Objectives Dominant strains of SARS-CoV-2 have been replaced by SARS-CoV-2 JN.1 lineage variants, which have spread widely in a short period of time. However, an accurate detection method and the reason for rapid spread have not been clarified.

Methods Ion Torrent, Illumina, and Nanopore platforms were used to sequence the whole genome of SARS-CoV-2 from samples collected from positive cases in the Dalian Center for Disease Control from December 2023 to January 2024.

Results The Ion Torrent and Illumina platforms failed to detect some of the variation sites, whereas the optimized Ion Torrent and Nanopore platforms detected all the sites. The Ion Torrent platform had higher sensitivity than the other two platforms and was more suitable for short-read length sequencing; however, the amplicon primers had to be optimized. The Illumina platform was also suitable for short-read length but prone to miss some variation sites. The Nanopore platform was more suitable for long-read length sequencing and had high compatibility for more variation sites. By comparing JN.1 lineage variants with BA.2.86, multiple specific variation sites were detected for the first time in the non-S protein region.

Conclusions Timely optimized primers and supplement variation sites provide a more effective means for monitoring and controlling the prevalence of JN.1 lineage variants in advance. The discovery of specific variation sites may indicate the reasons for immune escape and rapid transmission.

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Introduction

In late 2023, the SARS-CoV-2 JN.1 lineage replaced XBB.1.5 as the dominant SARS-CoV-2 strain worldwide. JN.1 variants spread rapidly in the Americas, Europe, and the Western Pacific, with prevalence as high as 65.6% in the Western Pacific [1-2]. According to literature reported that the JN.1 lineage spike (S) protein had up to 30 mutations, which was more serious than the immune escape of BA.2.86 and more likely to spread. There was only one more mutation (L455S) in the JN.1 S protein than in the BA.2.86 S protein, which enhanced the ability of the virus to bind to the ACE2 receptor, the gateway for SARS-CoV-2 to enter cells, increasing its transmission and immune escape capacity [3-5]. As a result, JN.1 is more infectious than any other branch of the Omicron variants, which also challenged the immune system Therefore, it is essential to constantly monitor and prepare to deal with the threat of emerging JN.1 lineage variants.

Since the first SARS-CoV-2 genome sequence was obtained [6-8], various next-generation sequencing technologies and protocols for SARS-CoV-2 have been developed and optimized to improve the technical performance, such as sensitivity, genome coverage, detection time, and cost [9-12]. However, studies comparing the various methods are relatively limited and usually involve schemes that compare available single type sequencing techniques or cross-platform studies of amplicon sequencing schemes [13-18]. For JN.1 variantion sites with strong transmission, no studies comparing the genome sequencing technologies have been reported, especially short- and long-read sequencing platforms and metagenomics cross-platform studies.

A preliminary study of overseas-imported cases was conducted in Dalian, Liaoning Province, China in 2022. Whole-genome targeted amplification and Ion Torrent sequencing were performed on samples of locally associated cases, and the genomic characteristics, nucleotide and amino acid mutation sites, and genotype of SARS-CoV-2 were analyzed [19]. An evolutionary tree of the BF.21 clade was constructed, and the epidemic strain of SARS-CoV-2 was traced based on the epidemiological data of the cases. In another study, nanopore sequencing and high-throughput sequencing methods were used to detect the genetic characteristics of the first local outbreak of novel coronavirus caused by Omicron variant strains in Dalian, and to determine the transmission chain [19]. Therefore, there is rich experience of using different methods to sequence novel SARS-CoV-2 variants.

In this study, we performed a cross-platform study of three different sequencing methods, Ion Torrent, Illumina, and Nanopore sequencing. Multiple sequencing regimens were compared using clinical samples by considering a wide range of throughput, accuracy, and run time. The performances were evaluated by comparing genome coverage, read depth, amplicon distribution, mutation call, and target read segment ratio.

Materials and Methods

Ethical statement

The viral samples were obtained from outpatient, emergency, and inpatient cases of SARS-CoV-2 infection in Dalian hospitals without epidemiological investigation.

The samples were redundant samples and retained for assay to detect new variants and unexplained clusters. The experimental design and scheme of this study fully considered the principles of safety and fairness, and the qualification, ability and experience of researchers met the experimental requirements. According to the National Health and Family Planning Commission's Ethical Review Measures for Biomedical Research Involving Humans, the identity information of both the specimen provider and the subject are anonymized, and the project research does not involve personal privacy or commercial interests, and the principle of voluntary and informed consent of the subject is not applicable. The study design was approved by the Ethics Committee of the Dalian Center for Disease Control and Prevention.

Specimen collection

A total of 59 oral/nasopharyngeal swab samples were collected from 13 December 2023 to 31 January 2024. Overseas input sample numbers were h CoV-19/China/LNDL A-20xx-xx-xx-xx, and Dalian local samples numbers were h CoV-19/China/LNDL M-20xx-xx-xx-xx. Details of all the specimens are provided in Supplementary material Table 1.

Nucleic acid extraction and amplification

We used the same nucleic acid extraction method for all sequencing protocols to rule out potential differences due to different nucleic acid extraction methods. The specimens were stored in the Biosafety Level p2+ laboratory of the Dalian Center for Disease Control (CDC). Nucleic acid was extracted from 200 μL input material using a Shuoshi Kit (SDKF60101, Jiangsu) according to the manufacturer's manual. Amplification was performed using a Mingde (Wuhan) fluorescence quantitative PCR nucleic acid detection kit.

Library construction and Ion Torrent sequencing

Construction of SARS-CoV-2 library and whole genomes sequencing.

The extracted RNA was used as the template, and cDNA was synthesized by reverse transcription. The Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay-GS Chef-Ready and Ion AmpliSeq™ Kit for Chef DL8 were used to construct libraries in the Ion Chef System (Thermo Fisher Scientific). The Ion 510™ & Ion 520™ & Ion 530™ Kit-Chef and Ion 530™ Chip Kit were used for template preparation and sequencing. A high pass measurement sequence plate was prepared in Ion Chef System, and Ion GeneStudio S5 Plus (Thermo Fisher Scientific) was used for targeted next-generation sequencing.

Whole genome sequence splicing and variation analysis.

The Ion GeneStudio S5 Plus built-in software was used to analyze the sequence data. Base-caller was used for data quality control and low quality sequences were removed. Then, the remaining high-quality sequences were passed through the torrent mapping alignment program (TMAP) software. The obtained sequence was compared with the whole genome sequence of the SARS-CoV-2 reference Wuhan-Hu-1 isolate (GenBank: NC_045512). Sequence quality control was performed using SARS-CoV-2 coverage. The analysis plug-in was used to measure sequence depth, specific alignment rate, and homogeneity index. Variation analysis of the quality control sequence was performed with SARS-CoV-2 variant Caller insert, and the obtained results were annotated with SARS-CoV-2 annotate SnpEff insert. Finally, the generated Consensus plug-in group was installed with a common sequence as a Fasta file. Integrative Genomics Viewer (IGV)

v12.0 software was used to analyze the initial Bam file, verify the untrustworthy mutation sites, and finally confirm the whole genome sequence of SARS-CoV-2.

Library construction and nanopore sequencing (GridION platform)

Construction of SARS-CoV-2 library and whole genomes sequencing.

SARS-CoV-2 library construction and whole genome sequencing were performed with specific amplification using an ultra-sensitive novel variation whole genome capture kit (BaYi) SARS-CoV-2 total basis group. The fast strip code test box (SQK-RBK004, ONT, UK) and R9.4.1 chip were used for the genomic DNA. Nanopore sequencing was performed on a Nanopore GridION sequencing platform.

Whole genome sequence splicing and variation analysis.

Quality control included low-quality results filtration, long reads filtration, label separation, and base identification. The high-quality reads were obtained as a Fastq file. NanoPlot and NanoFilt were used to filter, trim, and plot the long-read sequences to obtain the filtered Fastq files. Minimap2 was used to map and align the reads in the Fastq files to the SARS-CoV-2 reference genome (NC_045512) in the National Center for Biotechnology Information (NCBI) GenBank database. The results were obtained as a Bam file. Bam files were joined using SAMtools, and BCFtools was used to calculate coverage and sequence depth. Medaka was used to create consensus sequences and variant calls. The consensus sequence was obtained as a Fasta file and identified mutations were annotated with SnpEff.

Library construction and Illumina sequencing

Library construction, cDNA reverse transcription, and whole genome sequencing were performed using a SARS-CoV-2 whole genome capture kit (MicroFuture) according to the manufacturer's instructions. Product purification was done with AMPure XP beads, and the library was prepared using a Nextera XT DNA Library Preparation Kit (Illumina) and sequenced on a MiniSeq platform (Illumina) with the pair-end sequencing option.

Data analysis was performed using bcl2fastq v2.20 software (Illumina) according to the manufacturer's protocol and combined with the MicroFuture server. Duplicate reads were removed and quality control and read trimmings were performed using Trimmomatic v0.36. The reads were mapped to the human reference genome GRCh38 using Bowtie2 v2.1.0, and reads that mapped to the genome were counted and removed. Mapped reads were indexed in a genome sorted bam file using SAMtools v1.7. Variant calling was done using BCFtools v.1.7.

Virus typing and phylogenetic trees analysis

The Nextclade website (https://clades.nextstrain.org/) was used to analyze the SARS-CoV-2 data. MAFFT v7.475 software was used for multiple sequence alignment, and MEGA 10.1.8 software was used to construct a phylogenetic tree based on the adjacent method.

Results

Epidemic trend of SARS-CoV-2 variation

A search of the GISAID database (data were from 04 February 2024) showed that the top five global SARS-CoV-2 variants changed from JN.1, HV.1, JN.1.4, JN.1.1, and HK.3 in December 2023 to JN.1, HV.1, JN.1.4, JN.1.1, and BA.2.86.1 in January 2024. In China, the top five SARS-CoV-2 variants are HK.3.2,

HK.3, JN.1, EG.5.1.1, and JN.1.1. In Liaoning Province, the top five SARS-CoV-2 variants changed from HK.3.2, HK.3, EG.5.1.1, EG.5.1.8, and JN.1.1 in December 2023 to HK.3.2, HK.3, EG.5.1.1, JN.1, and JN.1.1 in January 2024 (Figure 1A). A total of 59 oral/nasopharyngeal swab samples were sequenced between 13 December 2023 and 31 January 2024, including samples from overseas outpatient, and Dalian local outpatient, emergency, and inpatient cases. The results showed that JN.1 lineage variants gradually replaced EG.5 lineage variants to become the dominant strain (Figure 1B). We analyzed the number of confirmed cases in Dalian and found that the proportion of JN.1 variants reached the inflection point of the growth of local cases on approximately 10 January 2024, whereas the inflection point of the growth of JN.1 variants was on 25 December 2023 after the first detection on 20 December 2023. The second rapid growth of local cases occurred on 22 January 2024 (Figure 1C). By searching the GISAID database, we further analyzed the effective series of COVID-19 cases in the world, China, and Liaoning Province, and found consistency among trends (Figure 1D).

Performance comparison of multiple sequencing platforms for SARS-CoV-2

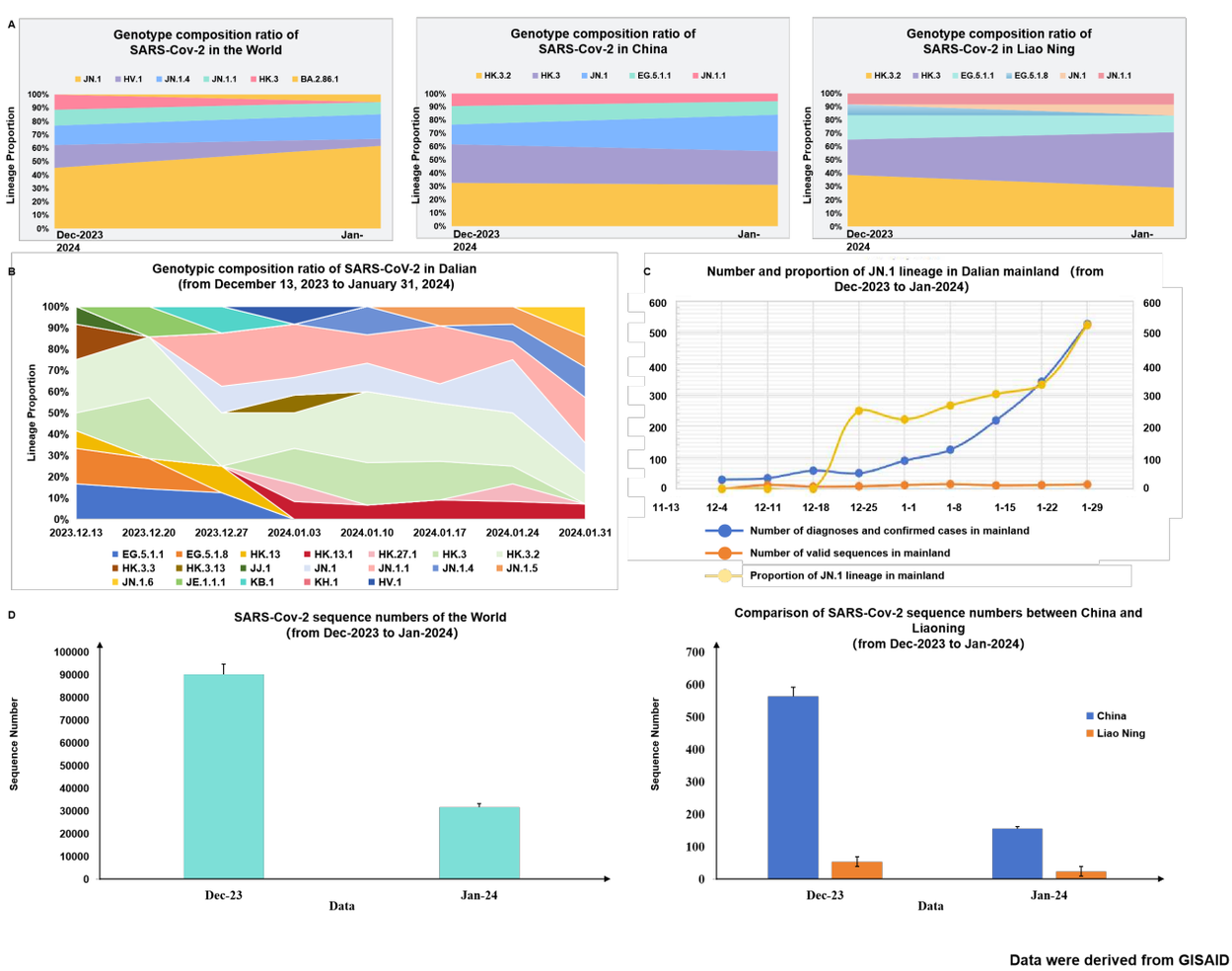


Figure 1. Epidemic trends of SARS-CoV-2 genotypes from December 2023 to January 2024.

A. Genotype composition ratio of the World, China, and Liaoning Province (data were derived from GISAID 2024-02-04). **B.** Genotype composition ratio of Dalian (data were derived from Dalian Center for Disease Control). **C.** Number and proportion of JN.1 lineage variants in Dalian mainland samples. **D.** SARS-CoV-2 sequence numbers of the World, China, and Liaoning Province.

whole genome sequencing

We used multiple sequencing platforms, namely Ion Torrent, and optimized amplification primers Ion Torrent V8, Illumina, and Nanopore platforms. A flow chart of the multiple sequencing platforms is shown in Figure 2.

We compared the performance of the three sequencing platforms in detecting variation sites of SARS-CoV-2 variants. We first analyzed the range difference of cycle threshold (Ct) values in the samples with valid sequences (coverage rate >96% according to national CDC requirements). The results showed that the Ct values of the ORF1ab polyprotein and N genes on the Ion Torrent platform were 16.91–35.44 and 15.37–34.49, respectively. On the Illumina and Nanopore platforms, the Ct values of the ORF1ab gene were 18.35–29 and 18.13–29.03, and those of the N gene were 16.96–29.82 and 17.43–27.93, respectively (Figure 3A). There was almost no difference in the coverage rates among the three platforms at 1×, 4×, and 10×, but at 30×, the coverage rate of the Nanopore platform dropped, and at 100×, the Nanopore platform coverage rate showed a big difference compared with that of the other two sequencing platforms (Figure 3B).

The Ion Torrent platform had the best coverage rate for Ct values >30. For 30 < Ct < 35, the short-augmented library can be considered for use on the Ion Torrent platform; the sequence coverage rate was 98.5%–99.5% and was not affected by the Ct value, and met the requirements of high-throughput sequencing (Figure 3C). The Illumina platform had the largest total number of reads, whereas the Nanopore platform had the lowest total number of reads (14497–43584 nt). The mean read length for the Ion Torrent platform was 182–218 nt, and 1183–1520 nt for the Nanopore platform (Table 1).

Oral/nasopharyngeal swab samples were taken from patients. RNA was extracted and amplified to prepare reverse transcription templates. Capture kits were used for reverse transcription and library preparation. The Ion Torrent platform has a specialized Ion AmpliSeqTM SARS-CoV-2 Research Panel, not an ultra-sensitive capture kit. The Illumina platform was combined with a MicroFuture capture kit. The Nanopore platform was combined a with BaiYi capture kit. The three platforms were used to sequence the genomes of the SARS-CoV-2 variants.

Molecular tracing of SARS-CoV-2 JN.1

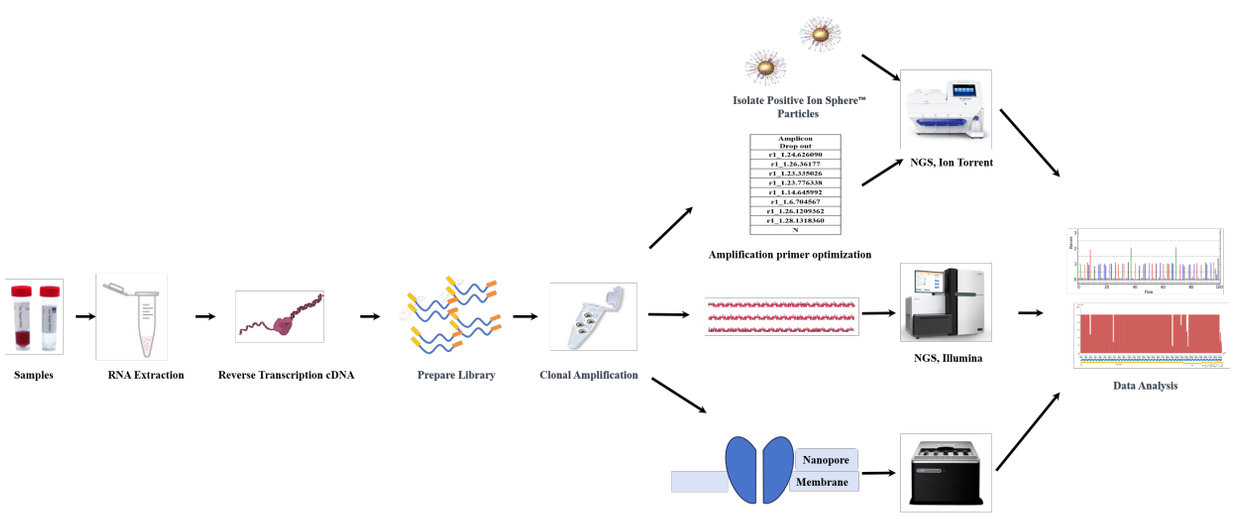


Figure 2. Flow chart of the multiple sequencing platforms.

Table 1. Performance comparison of multiple sequencing platforms.

Sequencing Platform	Sample Type	Total Reads	Read Mean Length	Mean Depth	1 X Coverage Rate (%)	10 X Coverage Rate (%)	30 X Coverage Rate (%)	100 X Coverage Rate (%)	Ct Value (ORFlab)	Ct Value (N)
Ion Torrent Sequencing Platform	Oral/ Nasopharyngeal Swab	470374-3862985	182-218	2752-26669	99.54-99.99	98.43-99.93	97.42-99.81	96.79-99.61	16.91-35.44	15.37-34.49
Optimized Ion Torrent V8 Sequencing Platform		470374-3862985	182-218	2752-26669	99.54-99.99	98.43-99.93	97.42-99.81	96.79-99.61	16.91-35.44	15.37-34.49
Illumina Platform		2442888-3476212	NA	3162.254-4141.38	99.6-99.94	98.76-99.92	96.58-99.88	89.64-98.67	18.35-29	16.96-29.82
Nanopore Platform		14497-43584	1183.3-1520.2	338.922-1201.8668	99.93-100	99.87-99.92	84.77-99.92	48.25-99.91	18.13-29.03	17.43-27.93

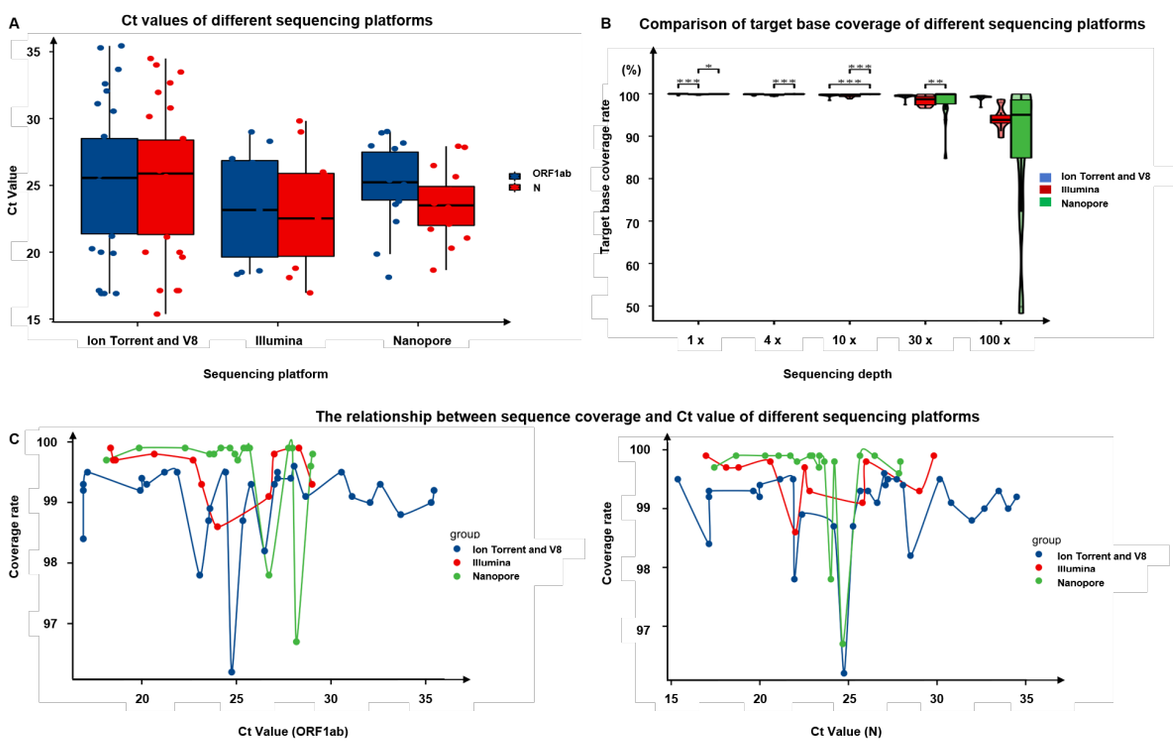


Figure 3. Performance comparison of multiple sequencing platforms.

A. Cycle threshold (*Ct*) values of the ORF1ab and N genes. **B**. Coverage rate of different sequencing depths. **C**. Relationship between sequence coverage rates and *Ct* values.

lineage variants

As of January 2024, five SARS-CoV-2 variants were listed as Variants of Concern by the World Health Organization (WHO), namely B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and Omicron (B.1.1.529). Molecular tracking based on viral genomes has an important role in investigating the chain of transmission of SARS-CoV-2. To explore the origin of our JN.1 cases, we compared the nucleotide sequences (e.g. single nucleotide differences) of the newly detected JN.1 variants with the GISAID data, including 30 strains from the Dalian cases, and constructed a phylogeny tree based on the alignment (Figure 4). These results showed that the JN.1 variants clustered in different branches within the same clade. 98% of the variant



Figure 4. Phylogenetic tree of the JN.1 lineage variants and variants from GISAID.

strains were from other branches, and there were also some unmarked Omicron strains.

Analysis of SARS-CoV-2 JN.1 lineage variation sites with multiple sequencing platforms

We evaluated the accuracy of the three sequencing platforms in detecting variation sites among the SARS-CoV-2 JN.1 variants compared with the SARS-CoV-2 reference genome (NC_045512). The Ion Torrent platform failed to detect variations in two regions: 22686–22917 nt and 23055–23075 nt. After optimizing the amplification primer, the detection rate was fully covered. The Illumina platform combined with MicroFuture capture kit primer 1 failed to detect completely or partially sites 7842, 13339, and 21618–21711. However, when primer 1+2 was used, the detection rates of these sites improved to some degree, but it still cannot cover all detection sequences, and there were still some variation sites cannot be detected. The Nanopore platform combined with BaiYi capture kit had a detection rate of 100%. Overall, 126 nucleotide variation sites were detected among the JN.1 variants, including 1 MPLF, 9 Del, and 116 synonymous/non-synonymous mutations. Sixteen of the sites were not detected by the Ion Torrent platform and six of the sites were not detected by the Illumina platform Supplementarymaterial.Table-2.

When the non-S protein regions of JN.1.5, BA-2, and BA.2.86 were compared, 10 more site-point mutations were identified in the JN.1.5 variants. Namely,

A211D, V1056L, N2526S, A2710T, V3593F, T4175I in ORF1a; D3H, T30A, A104V in the M region, which contributes to the shape of the virus envelope and promotes the completion of the virus assembly, and Q229K in the N region, which participates in viral genome assembly and acts as a viral RNAi inhibitor to antagonize the host immune defense system. Compared with BA.2, both BA.2.86 and JN.1 had one less variation site (L3201F in ORF1a), and compared with BA.2 and BA.2.86, JN.1 had three more site-point mutations (K1973R in ORF1a NSP3; R3821K in ORF1a NSP6; F19L in ORF7b). The JN.1.1-specific site F499L in NSP2 is involved in disrupting the host cell environment, and the JN.1.4-specific site T170I in NSP1 inhibits the IFN signal and blocks host innate immune response. Other variant-specific sites were the JN.1.5-specific site V1271T in ORF1b NSP13 and the JN.1.6-specific site G2387D in ORF1b NSP16.

When the S protein regions of JN.1.5 and BA.2.86 were compared with that of BA-2, 1 new insertion (16 MPLF), 5 deletions (H69, V70, Y144, N211, V483), and 24 point mutations (R21T, S50L, V127F, F157S, R158G, L212I, L216F, H245N, A264D, I332V, K356T, R403K, V445H, G446S, N450D, L452W, N460K, N481K, F486P, E554K, A570V, P621S, S939F, P1143L) were detected from BA.2.86 to JN.1.6. Compared with BA.2, BA.2.86 to JN.1.6 had four less site mutations (G339D, E484A, Q493R, P681H). Interestingly, BA.2.86 and JN.1 variants had different amino acid variations, G339H, E484K, and P681R mutation, which the same site showed different amino acid variations. Compared with BA.2 and BA.2.86, JN.1 variants had only one more site mutation (L455S). The new variation sites were located mainly in the N-terminal and receptor binding domain regions, resulting in changes in receptor binding, which led to immune escape and enhanced transmission (Figure 5).

Discussion

We conduced whole genome sequencing of 59 SARS-CoV-2 positive samples from the Dalian CDC, China from December 2023 to January 2024 using the Ion Torrent, Illumina, and Nanopore platforms. This is the first time the performances of different sequencing platforms have been comprehensively analyzed for detection of SARS-CoV-2 variation, and different sequencing platforms showed advantages and disadvantages. All the variation sites of the JN.1 variants (JN.1–JN.1.6) are described. Our results provide a theoretical basis for the accurate detection of SARS-CoV-2 variants and the rapid spread of the JN.1 lineage, and offer sufficient guidance for precise prevention and control strategies, such as the design of vaccine development targets.

With the numbers of new SARS-CoV-2 variation sites continuously increasing, the conditions of sequencing platforms need to be continuously optimized for accurate and rapid detection. We found that the Ion Torrent platform has the advantages of shorter amplicon primers, higher coverage rate, and higher compatibility than the other two sequencing platforms at high Ct values, but has the disadvantage of primers needing to be continuously optimized to more accurately identify variation sites. The Illumina platform also does short read sequencing and needs to be optimized, but Ion Torrent needs to be combined with its own library kit and analysis platform, whereas Illumina is more flexible and open, and can be combined with other manufacturers' capture kits, although the capture kit needs to be continuously optimized.

The Nanopore platform does not need frequent optimization because it uses long amplicon primers, and it had a 100% detection rate unlike those of the other sequencing platforms. There was no major difference in base coverage rate among the three

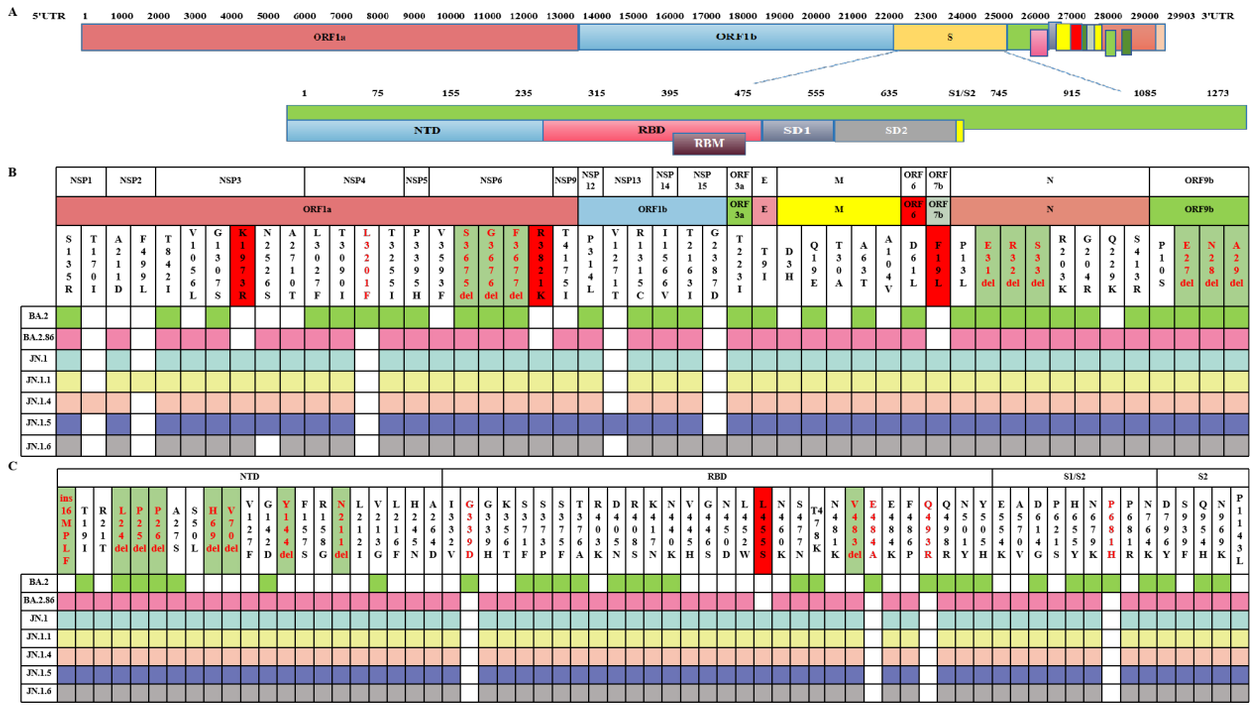


Figure 5. Amino acid variation sites among the BA.2, BA.2.86, and JN.1 variants.

A. Diagram of the full-length SARS-CoV-2 genome sequence. **B**. Amino acid variation sites in non-S protein regions among BA.2, BA.2.86, and JN.1 variants. **C**. Amino acid variation sites in the S protein region among BA.2 BA.2.86, and JN.1 variants.

sequencing platforms at low sequencing depth. The detection depth recommended by China CDC for SARS-CoV-2 genome detection is 10×, so all three sequencing platforms met the requirements. Appropriate sequencing platforms and timely optimization are crucial to improve the coverage rate, reduce the error rate, and shorten detection time for SARS-CoV-2 variations.

SARS-CoV-2 variations are constantly emerging. The JN.1 lineage is the second-generation subbranch of the Omicron BA.2.86 variation with strong transmission. The variation was first detected in Luxembourg on 25 August 2023. Because of the rapid growth of the JN.1 lineage globally, the WHO upgraded the BA.2.86 variation from Variant Under Monitoring to Variant of Interest on 21 November 2023[1-2]. The JN.1 lineage has shown significant antigenic drift and enhanced ACE2-binding affinity advantages compared with those of BA.2.86, but immune escape is relatively moderate compared with the EG.5 lineage [3-5]. The emergence of new JN.1 sub-variants optimizes the probability of this mutation, with a significantly higher L455S mutation rate in the JN.1 lineage compared with the rate in the BA.2.86 lineage (detected in 96% of strains). This dominant mutation enabled the JN.1 variants to effectively evade Class 1 neutralizing antibodies and counteracted the sensitivity of BA.2.86, thus enabling JN.1 to outperform its precursor BA.2.86 and popular variants XBB.1.5 and XBB.1, among others. This has led to a significant increase in the prevalence of JN.1 in Europe, especially France, and in North America [21-22].

Analysis of the variation showed that it was not only in the S protein, but also in non-S proteins, including Nsp2, Nsp3, Nsp6, M, ORF7b, and N regions. Specific mutations of Nsp3, Nsp6, N may enhance the suppression of the immune system and promote viral infection. The specific mutations of Nsp2 and M may promote

the process of virus assembly and replication, whereas the specific mutations in ORF7b may make the virus more virulent to cells. Although the mutation of the S protein may cause some difficulty for the virus to enter the cell, once inside the cell, it may cause strong replication efficiency and damage the immune system, possibly producing acute symptoms. This scenario suggests that the virus may be highly virulent to some people, and therefore needs to be observed so that appropriate preventive measures can be implemented [23-25].

Among these mutations, P681R appears to enhance S protein function; for example, viral cell membrane fusion may be improved. K356T, N450D, L452W, V445H, E484K, and V483del are associated with enhanced antibodies. Two variation sites on the S protein, N501Y and P681H, were seen previously on B.1.1.7 (Alpha) and were associated with increased transmission of the Alpha variant. E484K appeared in the B.1.351 (Beta) variant and was associated with immune escape. Q493 is a receptor binding motif residue that is critical for interacting with host cells, and Q493R/K is the only mutation to date that causes resistance to the monoclonal antibody bamlanivimab [22, 26].

There are concerns about the potential of JN.1 being able to better evade existing immunity from vaccines and previous infections compared with other recent variants [22]. The CDC believes that the continued growth of the JN.1 lineage indicates that it is either more contagious or more able to evade the human immune system. Professor Samuel Hyman has suggested that the S protein mutation means that after infection with JN.1, patients take longer to recover, or develop more severe disease. Professor Razai MS has noted that the symptoms of JN.1 infection "may be similar to those of other mutated viruses" [28-29]. This means that the symptoms of infection in JN.1 may include fever, runny nose, headache, loss of smell, and diarrhea. Doctors warn that although most infected people recover on their own, if symptoms worsen, patients should seek medical attention immediately. Chinese experts expected that JN.1 infections would peak around the Spring Festival in 2024. The emergence of new JN.1 sub-variants that are more infections and pathogenic cannot be ruled out. Therefore, the evolution of SARS-CoV-2 variation strain should be continuously and comprehensively monitored to prepared for future threats.

Our findings highlight the importance of selecting an appropriate sequencing platform to comprehensively identify SARS-CoV-2 variation sites. The multi-platform comparison strategy developed here can be extended to detect variants of other pathogens such as influenza and monkey pox. The detection of the lineage-specific variation sites of SARS-CoV-2 JN.1 provides new ideas for the selection of targets for vaccine development and epidemic prevention and control. However, outbreak prevention still depends on a variety of strategies, including vaccination, good hand and respiratory hygiene, surveillance, avoiding poorly ventilated enclosed areas, adequate indoor ventilation, isolating SARS-CoV-2 patients, avoiding close contact with patients with respiratory symptoms, and wearing masks [27].

Conclusion

The variation of SARS-CoV-2 strains presents a challenge for global public health and human well-being. The more recent JN.1 lineage has emerged as the predominant variant and has spread rapidly across multiple countries. Our results clearly indicate the applicability of multiple sequencing platforms in detecting specific site variations of JN.1. The specific variation sites in the whole genome may enable JN.1 variants to evade antibody effects obtained by vaccination or infection, thereby enhancing its immune escape ability and promoting widespread transmission. Research on

the selection and optimization of detection methods for ongoing SARS-CoV-2 variation is continuously being expanded, as well as the continuous evaluation and development of new strategies for antibody therapy and vaccine development targeting specific mutation sites.

Supplementary Information

The online version contains supplementary material available at http://www.infectioncontrol.org.cn/col.jsp?id=112.

Supplementary Material Table 1

Information of the SARS-CoV-2 patients involved in the study.

Supplementary Material Table 2

Variations in the JN.1 nucleotied sequences detected by the on Torrent, Optimized on Torrent, Illumina.and Nanopore platforms.

Acknowledgements

We thank all the participants who agreed to contribute to this study. Special thanks to Thermo Fisher Scientific for optimizing amplicon primer design. Thank you to Ge Teng from the Dalian International Travel Health Care Center (Dalian Customs Port Clinic) for providing some sequencing data from the Nanopore platform. We also thank the authors and originating and submitting laboratories for making the genetic sequences and metadata available through GISAID.

Author Contributions

Xingying Lang and Nan Xia conceived the idea for the study. Xingying Lang conducted bioinformatics analysis, operated the multiple sequencing platforms, data collation and summary, statistical analysis, and mapping. Yue Wang performed Illumina sequencing. Huan Wang and Xue Teng performed Ion Torrent sequencing. Mingchun Luan performed Nanopore sequencing. Zhijian Bo and Xuehong Meng conducted bioinformatics analysis. Shumin Niu pre-treated the specimens. Xiaoman Cui oversaw sequencing sample scheduling and overall arrangement. Nan Xia wrote the manuscript, drew figures, and data analysis. Xingying Lang and Nan Xia revised the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The original contributions presented in the study are included in the article or Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Dalian CDC (Dalian, China). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Use of Experimental Human Statement

According to the National Health and Family Planning

Commission's Ethical Review Measures for Biomedical Research Involving Humans, the strain used does not involve the identity information of the specimen provider, and the subject cannot be found. The project research does not involve personal privacy and commercial interests, and the principle of voluntary and informed consent of the subject is not applicable.

Consent for Publication

All authors agreed with the results and conclusions. All authors approved the final version of the manuscript to be published.

Conflicts of Interests

The authors have declared that no conflict of interest exist.

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