Study on the Complete Transmission Chain and Clinical Characteristics of the SARS-CoV-2 Delta Variant

MGI's ATOPlex Technology and DNBSEQ-G400 Sequencing Platform Enable Deciphering of the Genome of Delta Strain

Based on the MGI ATOPlex technology and DNBSEQ-G400 sequencing platform, researchers from the Guangzhou Eighth People's Hospital affiliated to Guangzhou Medical University and the First Affiliated Hospital of Guangzhou Medical University performed virus identification, genotyping and tracing of 159 locally transmitted aggregated cases of SARS-CoV-2 delta VOC in Guangzhou using multiplex PCR amplicon library preparation technology.

Related results have been published in 2021 in the journal *EClinicalMedicine*, entitled "Transmission, viral kinetics and clinical characteristics of the emergent SARS-CoV-2 Delta VOC in Guangzhou, China".

Recommended applications: Pathogenic microorganisms -SARS-CoV-2

Recommended model: DNBSEQ-G400RS

- **SARS-CoV-2 targeted sequencing specialist**

  MGI's SARS-CoV-2 amplicon-based library preparation technology, DNBSEQ sequencing platform and the supporting analysis software facilitate the identification, genotyping and tracing of COVID-19.

- **Compatible with automated sample preparation system**

  MGI's SARS-CoV-2 targeted sequencing solution is compatible with MGI's self-developed automated equipment, thus endowing efficient sample extraction and library preparation.

Background

Since the outbreak of novel coronavirus pneumonia (COVID-19), several prevalent SARS-CoV-2 variants worldwide have imposed a considerable economic burden on society[1]. The SARS-CoV-2 delta lineage (B.1.617.2) was identified by the World Health Organization as one of the four lineages that needed to be considered for variation of concern (VOC), and it has been the main strain emerging in many countries for a period[2] and continues to evolve and mutate[3]. Domestic and international health system data show strong transmission of Delta VOC[4]. However, the transmission chain, viral kinetics, and clinical characteristics of delta variant strains are not yet fully understood. Therefore, it is extremely important to monitor the epidemiological trends of SARS-CoV-2, to track virus variations, and to provide early warning of novel viruses. High-throughput sequencing technology can perfectly solve the related problems and provide data support for the recommendations of COVID-19 vaccine strains and the use of antiviral drugs worldwide.

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Research description

From May 21, 2021 to June 18, 2021, a total of 159 cases of delta virus infected COVID-19 patients were diagnosed in the Guangzhou Eighth People's Hospital affiliated to Guangzhou Medical University. The hospital, together with the First Affiliated Hospital of Guangzhou Medical University and other institutes, used MGI's ATOPlex technology and DNBSEQ-G400 sequencer to achieve the identification, genotyping and tracing of the delta virus for this COVID-19 outbreak[5].

Materials and Methods

Sample collection and RNA preparation

A total of 419 swab samples with Ct values <40 in RT-PCR test were collected for this study. Among them, 159 confirmed cases of delta virus were from Delta-positive patients diagnosed from May 21, 2021 to June 18, 2021 at the Guangzhou Eighth People's Hospital affiliated to Guangzhou Medical University; the rest 260 cases were wild-type SARS-CoV-2 infections from the diagnosed cases in the hospital from January to February 2020. Samples were subjected to RNA extraction within two hours of collection using a nucleic acid extraction kit.

Library preparation and sequencing

Sequencing libraries were prepared using either amplicon or hybridization capture-based methods. Amplicon sequencing libraries were constructed using the ATOPlex RNA Multiplex PCR-based Library Preparation Set, while hybridization capture libraries were prepared according to the previous protocol.

All samples were sequenced on the DNBSEQ-G400 genetic sequencer. The SARS-CoV-2 genome was assembled using the nCoV Finder pipeline (https://github.com/BGI-IORI/nCoV_Meta). The amplicon-based sequencing data and the hybrid capture-based sequencing data were analyzed using the UWIC SARS-CoV-2Multi-PCRv1.0(https://github.com/MGI-tech-bioinformat- ics/SARS-CoV-2_Multi-PCR_v1.0) and the nCoV Variant detection pipeline (https://github.com/BGI-IORI/nCoV_Variants) to evaluate SARS- CoV-2 mutations, respectively. In this study, only mutation loci with sequencing depth greater than 100× were reported to ensure the reliability of mutation detection in positive retest samples.

Phylogenetic tree construction

Representative SARS-CoV-2 genomic sequence was downloaded from the GISAID database (http://gisaid.org) as reference sequence. The whole genome sequence of SARS-CoV-2 was compared with the above reference sequence and manually compared using BioEdit software (Version 7.0.5). Based on the Kimura two-parameter model, a neighbor - joining (NJ) phylogenetic tree was constructed using the MEGA 6.0.6 program. Bootstrap support values are calculated from 1000 pseudo replication trees.

Sample collection	Library preparation and sequencing	Bioinformatics analysis	Results analysis
Total 419 patient samples:159 diagnosed cases with delta virus, 260 cases with wild-type SARS-CoV-2 infection.	 ATOPlex RNA Multiplex PCR-based Library Preparation Set Hybridization Capture Program  DNBSEQ-G400RS Genetic Sequencer	nCoV Finder pipeline, SARS-CoV-2 Multi-PCR v1.0, nCoV Variant detection pipeline, BioEdit software, MEGA 6.0.6	Phylogenetic tree construction

Results

Delta virus transmission is mainly through close contact

This virus transmission network showed a clear chain of transmission in 157 infected patients (Figure 1). The transmission of each generation is indicated by a diamond or circle of a different color. The first-generation patient (black solid diamond, G1) is located in the middle, and G1 is phylogenetically associated with 1 imported case (red dashed diamond, G0), with colored arrows indicating the different transmission pathways. Transmission methods include dinners, family, community (chatting, chance encounters, elevator rides) and other ways (work, socializing). Serious condition (dashed line) and critical condition (solid line) are marked with a square. The asterisk indicates that the patient is located in another city. The main routes of virus transmission are direct and indirect close contact, such as diet, household contact and community contact. Transmission was most frequent through diet (30.8%), followed by household contact (29.6%) and community transmission (18.2%). The critical patients were identified in every generation. Phylogenetically identical virus strains could be detected in each generation compared to the virus strains of the second generation.

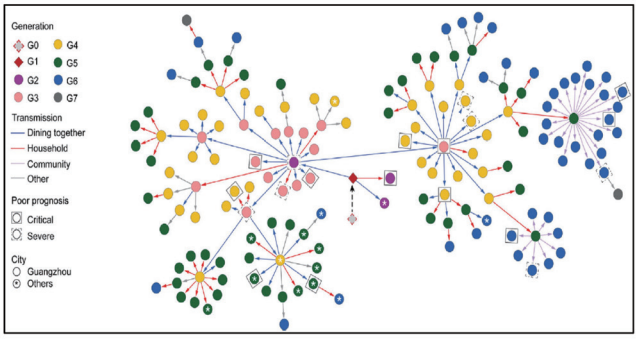

Figure 1. Epidemiological transmission network of SARS-CoV-2 delta variant in Guangzhou.

Shorter incubation period and faster transmission of delta

Figure 2 shows the absolute counts (a) and proportions (b) of patients in three age groups: under eighteen years, eighteen to fifty-nine years, and greater than or equal to sixty years. Box/violin plots showed the difference in latency between the wild strain (dark blue) and the delta variant (earthy yellow) in all patients (c), non-serious (d), serious (e) and critically ill (f) patients, $p<0.001$ calculated by Wilcoxon test.

According to Figure 2, the latency period of the delta variant was significantly shorter than that of the wild strain (4.0 days vs. 6.0 days, $p<0.001$); in the non-serious group, the latency period of the delta variant was also significantly shorter than that of the wild strain (4.0 days vs. 7.0 days, $p<0.001$). In addition, the transmission rate of the delta mutant strain was rapid with transmission of four generations in just ten days, of which the shortest intergenerational transmission was less than one day.

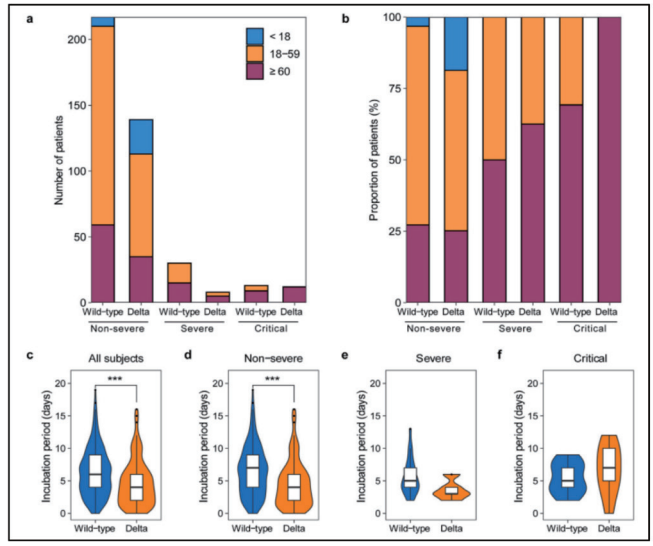

Figure 2. Incubation period of SARS-CoV-2 wild strain and delta variant infected patients.

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Higher delta viral load

As shown in the box/violin plots of Figure 3 showing the Ct values of the highest viral load during hospitalization, the overall (a), non-severe (b), severe (c) and critical (d) patients with the delta variant were significantly shifted upwards compared to patients infected with the wild strain. In the box line diagram, the box boundary closest to zero is the 25% quantile, the black line inside the box indicates the median, and the box boundary farthest from zero is indicated as the 75% quantile. To show the dynamics of Ct values in patients infected with wild-type or delta VOC, arithmetic means (dots) and standard errors (colored ranges) were first calculated for each day, and then the moving means were calculated for three consecutive days for all subjects (e) and patients with different disease severity (f, h). The viral load was expressed by the Ct value of ORF1a/b gene.

As shown in Figure 3, the delta variant had a higher viral load compared to the wild strain (median Ct value 20.6 vs. 34.0; p<0.001), and this trend was consistent among non-serious, serious and critically ill patients. The time for nucleic acid conversion to negative (Ct value >40) was also longer in those infected patients with the delta mutant strain.

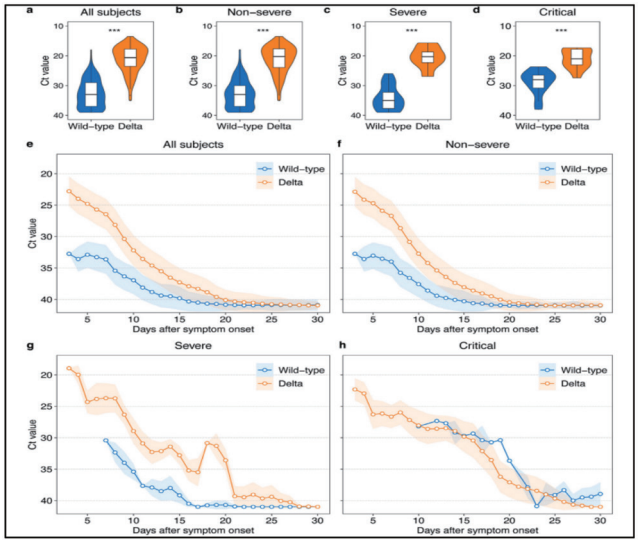

Figure 3. Peak viral load and dynamic changes of SARS-CoV-2 wild strain and delta variant infected patients.

Higher risk of serious illness in patients over 60 years of age

Figure 4 shows Kaplan-Meier survival plots from symptom onset to critical status time by viral lineage (delta variant versus wild strain) for patients aged at and younger than 60 years. Mono-factor analysis showed that age, gender, comorbidities (including chronic respiratory disease, hypertension, and diabetes) and symptoms within 3 days of admission (including fever and dyspnea) were associated with worsening of the disease (p<0.05). Multivariate Cox regression analysis revealed that delta variant infection (HR2.98 [95% CI 1.29-6.86]), age >60 years (HR11.13 [95% CI 3.78-32.82]), male (HR3.49 [95% CI 1.45-8.41]), dyspnea (HR2.60 [95% CI 1.14-5.93]) and fever within 3 days of admission (HR4.77 [95% CI 1.10-20.64]) were independent risk factors associated with worsening of disease.

All patients infected with the delta variant in critically ill patients were ≥60 years of age, while in the wild strain, this percentage was only 69%. Among them, the risk of progressing to critical illness was 2.98 times higher for infection with the delta variant than for infection with the wild type. It can be assumed that elderly patients infected with the delta variant are more likely to progress to critical illness.

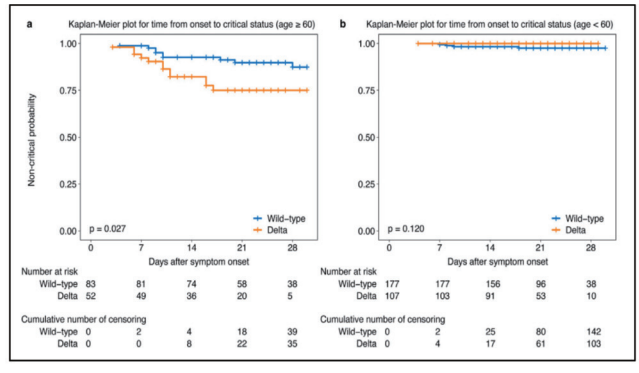

Figure 4. Peak viral load and dynamics of Kaplan-Meier survival plots for prognostic factors.

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Conclusion

In response to the epidemic, the Guangzhou Eighth People's Hospital affiliated to Guangzhou Medical University and the First Affiliated Hospital of Guangzhou Medical University have successfully achieved the sequencing, comparison, genotyping and tracing of the whole genome of SARS-CoV-2 by using the combinational products based on ATOPlex technology and DNBSEQ-G400 genetic sequencer developed by MGI, which provides a reliable theoretical basis and advanced experience for further combating the virus and preventing and controlling the epidemic nationwide and globally.

The high-throughput sequencing technology of DNBSEQ-G400 genetic sequencer developed by MGI can facilitate rapid monitoring of the epidemic trend of COVID-19 and tracking of virus mutations. Meanwhile, the automated sample preparation system provided by MGI can satisfy the needs of rapid, automatic and safe prevention and control while significantly reducing the time of virus nucleic acid extraction and library preparation.

Gene Sequencer DNBSEQ-G400RS

References

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G400RS	900-000170-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
	metargetCOVID	970-000228-00
Library Prep	ATOPlex RNA Multiplex PCR-based Library Preparation Set V3.1 (16 RXN)	940-000132-00
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE100)	1000016943

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