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Variation and evolution of SARS and coronavirus genomes

2 Materials and Methods

2. Materials

2.1.1 Cases and samples

The 10 SARS-CoV samples were obtained from Beijing Youan Hospital and Zhejiang Center for Disease Control and Prevention, respectively. All

The patients all met the WwWHO definition of a suspected SARS case (http:/www.who.inycsr/sars/guidelines

/em), the information is as follows:

Patient 1BJ162, BJ202), male, 63 years old, was admitted to Beijing on April 29, 2003, on the seventh day of illness.

Youan Hospital. After clinical diagnosis, combined with serological and molecular biological methods of testing, the diagnosis of SARS patient was confirmed. In order to

To exclude the increase in genomic mutation loci due to artificial culture, day 24 (BJ162) and day 31 (BJ202) classes were collected respectively.

The specimens were directly extracted for genomic analysis.

Patient 2 (BJ182, BJ182-1, BJ182-5 and BJ182-9) was admitted to Beijing Youji Medical Center on April 30, 2003.

In the hospital, the cadaveric stool specimens were collected on the 28th day of disease onset and cultured for direct RNA extraction for genomic analysis (BJ182).

At the same time, we did the passaged culture. The whole genome was sequenced and analyzed in 10 successive generations of 1, 5 and 9 generations.

Patient 3 (ZJ01-1, ZJ01-5 and ZJ01-10), female, 52 years old, arrived in Wuchang on April 12, 2003 to meet with Beijing

He returned to Hangzhou on April 14.

The patient was diagnosed with SARS on April 19. It was the first case in Hangzhou and died on June 1. The throat swab was collected

ZJ01 was isolated and cultured as a virus for 10 generations, and the genome was also sequenced for 1, 5 and 10 generations.

Analysis (ZJ01-1, ZJ01-5 and ZJ01-10).

Patient 4ZJ02), female, 49 years old, was homozygous for ZJ01. She was discharged from the hospital on June 17. Her pharynx was collected

Swabs were isolated and sequenced as viruses (ZJ02) (see Table 1).

2.2 Methodology

2.2.1 PCR primer design

Based on the initially available sequence of the working framework map of the SARS-CoV BJO01 genome and the WHO April 11 publication

The sketches of SARS-CoV Tor2 (AY274119) and BJ0O1 (AY278488) genome sequences were used as templates to design PCR special

Seventy-five pairs of primers were used to cover the entire genome, and each pair of primers amplified a fragment of about 450 bp. The RTPCR products were directly

Put for forward and reverse sequencing.

Variation and evolution of the SARS coronavirus genome

Table 1 Origin of the 10 SARS coronavirus strains in this study.

Tabie 1 The Sources of ten SARS-CoVs strins used in this study-

\_ Isolated strain number Specimen , , , , Specimen type . , , , , , , , Source of sample Flash bed results

BJj62 and the original generation of patients | rehabilitation

BJ202 Raising Original Generation Benefit 1 Rehabilitation

BJI182 fitted Original generation suffering from 2 deaths

BJ182- Ginger first generation suffering from 2 NA

BJ182-5 and 5th generation suffering from 2 NA

BJ182-9 feces Ninth generation Wheat 2 NA

ZJ01 1 swabbing first generation suffering 3 death

ZJ01 5 swab fifth generation toe 3 death

ZJ01 10 Swabbing Tenth generation Affected3 Death

ZJ02 Swabbed cells Affected 4 Rehabilitation

Wide: Fecal matter, swabbing a throat swab, NA with no results.

2.2.2 PCR product cloning

The PCR products were purified (using millipore membrane 9-well 384-well purification plates), and the pGEM-T Easy

The vectot carriers were ligated overnight at 4C and the receptor cells were E.coli cell line DH5au. The procedure was performed according to the kit requirements.

Le

But.

223 DNA sequencing

The system is 10 kb, including 2 BigDye reagents, 0.5 nmolL of sequencing primers, 200ng of plasmid template DNA.

or PCR product template 3-$ ng/100bp, sequencing reaction buffer (5x) 2 by, add water to 10hl, thermal cycling conditions are

Denaturation at 967C for 2min, denaturation at 96TC for 10sec, annealing at 50C for 10sec, extension at 60 for 4min, 35 cycles. Reaction

The product was purified by 70% ethacrynic precipitation, dried and dissolved in 3hi of formamide-EDTA loading buffer.

AABI-3730 sequencer or MegaBACE 1000 capillary sequencer sequencing.

.2.4 Genome sequence splicing

Genome sequencing was performed using the Phred/Phrap/Consed DNA Sequence Splicing Package developed at the University of Washington, USA.

Sequencing reactions (reads) were assembled for splicing. Gaps were filled by direct sequencing with PCR products, and low quality regions were re

The sequence was sold. Using GZ02 as the reference sequence, for the SNPs locus, the region near the locus was cloned and resequenced to confirm

All 10 SARS-Cov genomes were sequenced with 6X or more coverage and individual nucleotide quality values of 20 or more

The final complete genome sequence was obtained (see Table 2?).

Variation and evolution of the genome of SARS status quo virus

Table 2 Sequencing data of the genome of 10 SARS-CoV strains in this study.

Table 2 SequencingE Coverage of our 10 SARS-CoV strains.

Virus strain Number of sequencing reactions Effective total length (nD) Cumulative coverage (X) Genome size (ng)

Bl 1 24027 84 29606

BJ202 673 221880 up.49 29608

bj182 772 233848 7.89 29650

bjl182-1 621 222114 7.54 29464

bj182-5 624 227949 7.73 29472

bj182-9 604 222614 7.55 29492

zj01-1 494 180551 6.08 29674

zj01-5 593 219345 7.41 29604

zj01-10 559 208101 7.03 29604

ZJ]02 548 196586 6.64 29605

2.2.5 Sequence annotation and comparative analysis

Open reading frame (open reading frame, orchang by ORF Finder (http://wwwncbi.nlmnih.gow/

gorfygorfhtmD and Glimmer (http:WWWtigrorg/software/glimmer) OK, run BLAST locally

Compare nr database (non-meta residual database) for nucleic acid and and protein sequence alignment analysis. Local runs of CiustalW

1.83 (ftp:/ ftp-igbmc.u-strasbe.frjpub/ClustalW) for multiple sequence alignment and for different research purposes

A Perl program was written to find SNP loci using GZ02 (AY390556) as the reference sequence.

2.2.6 Evolution and variation analysis

Global alignment of 125 SARS-CoYV whole genome sequences was performed using Clustal W 1.83. Based on the concatenation

As a result, the Neiborjoining Method was used to determine the results of the study using MEGA3 (http:/www.

megasofware.neVmega.html) and Phylip 3.63 http:/evolution.genetics.washington.edu/

phylip.htmJ), and the bootstrap method was used to verify the kinship trees (replicate=1000) and cross-referenced to obtain the final

to a consistent gui. Ka and Ks values were calculated between the sequences using MEGA3.

2.2.7 SARS-CoV comparative genomic analysis data sources

The whole genome sequences of 115 published SARS coronavirus strains were downloaded from the Genebank database (Table 3, Hungary).

Together with the 10 strains sequenced in this study, the whole genome sequences of 125 SARS viruses were used for comparative genomic differentiation.

Analysis.

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