SARS-Associated Coronavirus Quasispecies in Individual Patients

TO THE EDITOR: Quasispecies are known in RNA viruses such as hepatitis C virus and human immunodeficiency virus.[1] Owing to poor fidelity of RNA polymerases, RNA-virus populations typically contain genetic variants that form a heterogeneous virus pool. The severe acute respiratory syndrome (SARS)–associated coronavirus, as a newly identified RNA virus,[2] however, has been reported with relatively limited variations,[3,4] and no published data have recorded the existence of quasispecies.

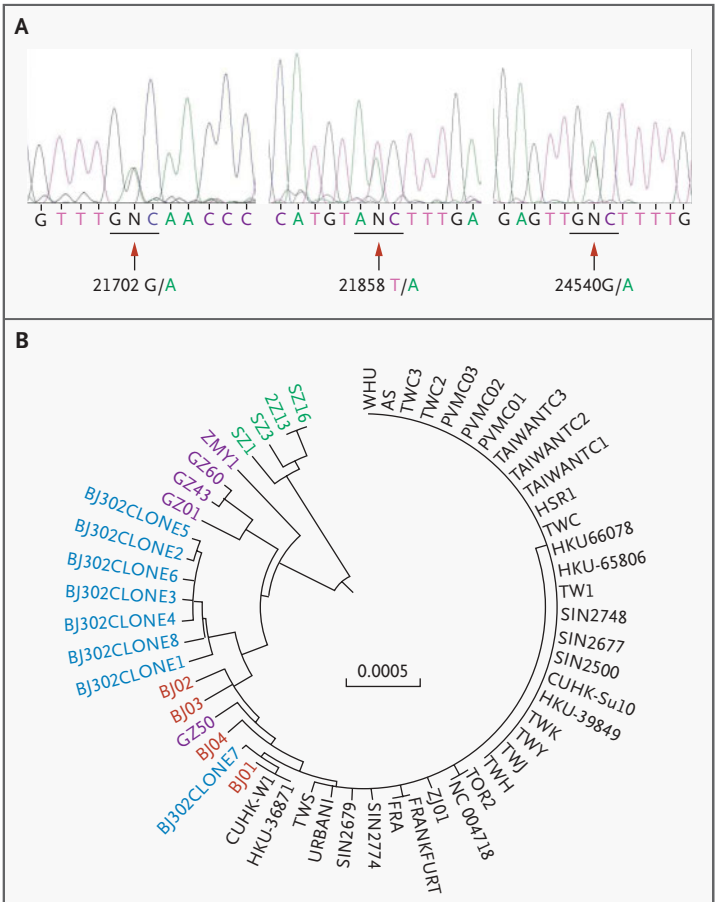

Figure 1. Sequence Variations in the S Gene of the Severe Acute Respiratory Syndrome (SARS)–Associated Coronavirus from Patients with SARS in China.

Panel A shows typical heterozygous sequences in three variant sites of the S gene, amplified from a single subject. The heterozygous sequence profile, derived from direct analyses of RT-PCR products and confirmed by TA-cloning and sequencing, showed the coexistence of variant and reference sequences at three sites (with the BJ01 strain as the reference strain). Panel B shows the phylogenetic relationship of strains of the SARS-associated coronavirus, on the basis of the S-gene variations. The analysis was performed with the use of the MEGA-2 program and was based on 56 entire S-gene sequences from updated data in GenBank (as of Nov. 30, 2003), including 8 representative sequences obtained from our study (shown in blue), 4 from other patients in the Beijing area (red), 5 from the Guangdong area (purple), 4 from wild animals (green), and the remaining 35 from other regions of the world (black).

During the SARS outbreak from March to June in 2003, 132 patients with SARS were treated in our unit, including those with the first cluster of cases in the Beijing, China, area.[5] We sequenced 28 full-length spike (S) glycoprotein genes of the SARS-associated coronavirus from 19 individual hospitalized patients. Viral RNA was directly extracted from clinical samples, including plasma, throat swabs, sputum, and stool. The full-length S gene was amplified as six overlapping fragments by means of a nested reverse-transcriptase polymerase chain reaction (RT-PCR). Both a TA-cloning assay and direct screening of PCR products were performed. The sequencing results were verified in three independent experiments with the use of different RT-PCR products and were confirmed by the use of platinum Pfx DNA polymerase, if necessary.

A total of 107 sequence variations with 9 recurrent variant sites were identified in analyzed sequences compared with the S gene of the BJ01 strain (GenBank accession number AY278488), including 7 nonsynonymous variants (21494 C→T, 21702 A→G, 21858 A→T, 22908 A→G, 23198 T→C, 24018 A→T, 24540 A→G [numbered on the basis of the full-length genomic sequence]). With the exception of one site (position 21702), the variant sites were first documented in humans, so far as we know.

We speculate that the higher frequency of variations in the S gene than in previous reports might be due to a broader sample collection over a longer period of time. In particular, the coexistence of sequences with and those without substitutions (with BJ01 as the reference strain) was observed in 7 of the 19 subjects. In one subject, the variant and reference sequences for three variant sites coexisted (Fig. 1A). In addition, the sequences of the S gene from different samples collected at different times from the same patient showed similar, but not identical, variation profiles (data not shown).

Phylogenetic analysis based on the S gene of the SARS-associated coronavirus showed that the new-

ly identified variant sequences are closest to the isolates from the Beijing and Guangdong areas in China (Fig. 1B). Taken together, our observations suggest that the SARS-associated coronavirus may consist of complex and dynamic distributions of mutants in vivo, rather than a single, defined genomic sequence — this is a characteristic typical of RNA-virus quasispecies.

Dongping Xu, M.D.
Zheng Zhang, Ph.D.
Fu-Sheng Wang, M.D., Ph.D.

Beijing 302 Hospital
Beijing 100039, China
fswang@public.bta.net.cn

1. Domingo E. Quasispecies and the development of new antiviral strategies. Prog Drug Res 2003;60:133-58.
2. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003;348:1967-76.
3. Ruan YJ, Wei CL, Ee AL, et al. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 2003;361:1779-85. [Erratum, Lancet 2003;361:1832.]
4. Tsui SKW, Chim SSC, Lo YMD. Coronavirus genomic-sequence variations and the epidemiology of the severe acute respiratory syndrome. N Engl J Med 2003;349:187-8.
5. Zhou XZ, Zhao M, Wang FS, et al. Epidemiologic features, clinical diagnosis and therapy of first cluster of patients with severe acute respiratory syndrome in Beijing area. Zhonghua Yi Xue Za Zhi 2003; 83:1018-22. (In Chinese.)