Comprehensive evolution and molecular characteristics of a large number of

SARS-CoV-2 genomes revealed its epidemic trend and possible origins

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Abstract

Objectives:

To reveal epidemic trend and possible origins of SARS-CoV-2 by exploring its evolution and molecular characteristics based on a large number of genomes since it has infected millions of people and spread quickly all over the world.

Methods:

Various evolution analysis methods were employed.

Results:

22 The estimated Ka/Ks ratio of SARS-CoV-2 is 1.008 or 1.094 based on 622 or 3624 SARS-CoV-2
23 genomes, and the time to the most recent common ancestor (tMRCA) was inferred in late
24 September 2019. Further 9 key specific sites of highly linkage and four major haplotypes H1, H2,
25 H3 and H4 were found. The Ka/Ks, detected population size and development trends of each
26 major haplotype showed H3 and H4 subgroups were going through a purify evolution and almost
27 disappeared after detection, indicating H3 and H4 might have existed for a long time, while H1
28 and H2 subgroups were going through a near neutral or neutral evolution and globally increased
29 with time. Notably the frequency of H1 was generally high in Europe and correlated to death rate
30 ($r>0.37$).

31 *Conclusions:*

32 In this study, the evolution and molecular characteristics of more than 16000 genomic sequences
33 provided a new perspective for revealing epidemiology of SARS-CoV-2.

34

35 **KEYWORDS:** SARS-CoV-2; evolution; classification; haplotype

36

37 **Introduction**

38 The global outbreak of SARS-CoV-2 is currently and increasingly recognized as a serious, public
39 health concern worldwide. Coronaviruses exist widely around the world, and to date, a total of seven
40 types of coronaviruses that can infect humans have been found, of which four coronaviruses including
41 hCoV-229E, hCoV-NL63, hCoV-OC43 and hCoV-HKU1 cause a cold, while the other three including
42 SARS-CoV, MERS-CoV and SARS-CoV-2 usually cause mild to severe respiratory diseases. The total

43 number of SARS-CoV and MERS-CoV infections are only 8,069 and 2,494 with reproduction number
44 (R_0) fluctuates 2.5-3.9 and 0.3-0.8, respectively. However, SARS-CoV-2 has infected 2471136 people
45 in 212 countries up to April 22th(WHO, 2020), with the basic R_0 ranging from 1.4 to 6.49(Liu, et al.,
46 2020). Among these three typical coronavirus, MERS-CoV has the highest death rate of 34.40%,
47 SARS-CoV has the modest death rate of 9.59%, SARS-CoV-2 is about 6.99% and 7.69% death rate of
48 the global and Wuhan, but is quite high death rate in some countries of Europe, such as Belgium, Italy,
49 United Kingdom, Netherlands, Spain and France, which even reaches 14.95%, 13.39%, 13.48%,
50 11.61%, 10.42% and 13.60% respectively according to the data of April 22th, 2020. Except for the
51 shortage of medical supplies, aging and other factors, it is not clear whether there is virus mutation
52 effect in these countries with such a significantly increased death rate.

53 It has been reported that SARS-CoV-2 belongs to beta-coronavirus and is mainly transmitted by the
54 respiratory tract, which belongs to the same subgenus (SarbecoVirus) as SARS-CoV(Lu, et al., 2020).
55 Through analyzing the genome and structure of SARS-CoV-2, its receptor-binding domain (RBD) was
56 found to bind with angiotensin-converting enzyme 2 (ACE2), which is also one of the receptors for
57 binding SARS-CoV(Wrapp, et al., 2020). Some early genomic studies have shown that SARS-CoV-2 is
58 similar to certain bat viruses (RaTG13, with the whole genome homology of 96.2%(Zhou, et al., 2020))
59 and Malayan pangolins coronaviruses (GD/P1L and GDP2S, with the whole genome homology of
60 92.4%(Lam, et al., 2020)). They have speculated several possible origins of SARS-CoV-2 based on its
61 spike protein characteristics (cleavage sites or the RBD)(Lam, et al., 2020, Zhang and Holmes, 2020,
62 Zhou, et al., 2020), in particular, SARS-CoV-2 exhibits 97.4% amino acid similarity to the Guangdong
63 pangolin coronaviruses in RBD, even though it is most closely related to bat coronavirus RaTG13 at

64 the whole genome level. However, it is not enough to present genome-wide evolution by a single gene
65 or local evolution of RBD, whether bats or pangolins play an important role in the zoonotic origin of
66 SARS-CoV-2 remains uncertain(Andersen, et al., 2020). Furthermore, since there is little molecular
67 characteristics analysis of SARS-CoV-2 based on a large number of genomes, it is difficult to
68 determine whether the virus has significant variation that affects its phenotype. Thereby, it is necessary
69 to further reveal the phylogenetic evolution and molecular characteristics of the whole genome of
70 SARS-CoV-2 in order to develop a comprehensive understanding of the virus and provide a basis for
71 the prevention and treatment of SARS-CoV-2.

72

73 **Results**

74 **Genome sequences**

75 We got a total of 1053 genomic sequences up to March 22th, 2020. According to the filter criteria, 37
76 sequences with ambiguous time, 314 with low quality and 78 with similarity of 100% were removed. A
77 total of 624 sequences were obtained to perform multiple sequences alignment. Two highly divergent
78 sequences (EPI_ISL_414690, EPI_ISL_415710) according to the firstly constructed phylogenetic tree
79 were also filtered out (Table S1). The remaining 622 sequences were used to reconstruct a phylogenetic
80 tree. In addition, total of 3624 and 16373 genomic sequences were redownloaded up to April 6th, 2020
81 and May 10th, 2020 respectively for further exploring the evolution and molecular characteristics of
82 SARS-CoV-2.

83 **Estimate of evolution rate and the time to the most recent common ancestor for SARS-CoV,**

84 **MERS-CoV, and SARS-CoV-2**

85 In this study, date for SARS-CoV-2 ranged from 2019/12/26 to 2020/03/18 was collected. The average
86 Ka/Ks of all the coding sequences was closer to 1 (1.008), which indicating the genome was going
87 through a neutral evolution. We also reevaluated the Ka/Ks of SARS-CoV and MERS-CoV through the
88 whole period, and found the ratio were smaller than SARS-CoV-2 (Table 1). To estimate more credible
89 Ka/Ks for SARS-CoV-2, we recalculated it using redownloaded 3624 genome sequences ranged from
90 2019/12/26 to 2020/04/06, and the average Ka/Ks of it was 1.094 (Table 1), which was almost same
91 with the above result.

92 We assessed the temporal signal using TempEst v1.5.3(Rambaut, et al., 2016). All three datasets
93 exhibit a positive correlation between root-to-tip divergence and sample collecting time (Figure S1),
94 hence they are suitable for molecular clock analysis in BEAST(Bouckaert, et al., 2019, Rambaut, et al.,
95 2016). The substitution rate of SARS-CoV-2 genome was estimated to be 1.601×10^{-3} (95% CI: $1.418 \times$
96 10^{-3} - 1.796×10^{-3} , Table 2, Figure S2A) substitution/site/year, which is as in the same order of
97 magnitude as SARS-CoV and MERS-CoV. The tMRCA was inferred on the late September, 2019 (95%
98 CI: 2019/08/28- 2019/10/26, Table 2, Figure S2B), about 2 months before the early cases of
99 SARS-CoV-2(Huang, et al., 2020).

100 **Phylogenetic tree and clusters of SARS-CoV-2**

101 The no-root phylogenetic trees constructed by the maximum likelihood method with PhyML
102 3.1(Guindon, et al., 2010) and MEGA(Kumar, et al., 2018) were showed in Figure 1, Figure S3A and
103 3B. According to the shape of phylogenetic trees, we divided 622 sequences into three clusters: Cluster

104 1 including 76 sequences mainly from North America, Cluster 2 including 367 sequences from all
105 regions of the world, and Cluster 3 including 179 sequences mainly from Europe (Table S2).

106 **The specific sites of each Cluster**

107 The *Fst* and population frequency of a total of 9 sites (NC_045512.2 as reference genome) were
108 detected (Table 3, Table S3). Thereinto, three (C17747T, A17858G and C18060T) are the specific sites
109 of Cluster 1, and four (C241T, C3037T, C14408T and A23403G) are the specific sites of Cluster 3.
110 Notably, C241T was located in the 5'-UTR region and the others were located in coding regions (6 in
111 *ofr1ab* gene, 1 in *S* gene and 1 in *ORF8* gene). Five of them were missense variant, including C14408T,
112 C17747T and A17858G in *ofr1ab* gene, A23403G in *S* gene, and T28144C in *ORF8* gene. The PCA
113 results showed that these 9 specific sites could clearly separate the three Clusters, while all SNV
114 dataset could not clearly separate Cluster 1 and Cluster 2 (Figure S4), which further suggested that
115 these 9 specific sites are the key sites for separating the three Clusters.

116 **Linkage of specific sites**

117 We found the 9 specific sites are highly linkage based on 622 genome sequences (Figure 2A), then we
118 carried out a further linkage analysis using the 3624 genome sequences (Figure 2B). As a result, for the
119 3624 genome sequences, 3 specific sites in the Cluster 1 were almost complete linkage, and haplotype
120 CAC and TGT accounted for 98.65% of all the 3 site haplotypes. The same phenomenon was also
121 found in 4 specific sites of Cluster 3, and haplotype CCCA and TTTG accounted for 97.68% of all the
122 4 site haplotypes. Intriguingly, the 9 specific sites were still highly linkage, and four haplotypes,
123 including TTCTCACGT (H1), CCCCCACAT (H2), CCTCTGTAC (H3) and CCTCCACAC (H4),
124 accounted for 95.89% of all the 9 site haplotypes. Thereinto, H1 and H3 had completely different bases

125 at the 9 specific sites. The frequencies of each site and major haplotype for each country were showed
126 in Figure 3 and Table S4. The data showed that the haplotype TTTG of the 4 specific sites in Cluster 3
127 had existed globally at present, and still exhibited high frequencies in most European countries but
128 quite low in Asian countries. While the haplotype TGT of the 3 specific sites in Cluster 1 existed
129 almost only in North America and Australia. For the 9 specific sites, most countries had only two or
130 three major haplotypes, except America and Australia had all of four major haplotypes and with
131 relative higher frequencies.

132 **Characteristics and epidemic trends of major haplotype subgroups**

133 All haplotypes of 9 specific sites for 3624 or 16373 genomes and the numbers of them were shown in
134 Table S5. Four major haplotypes H1, H2, H3 and H4, three minor haplotypes H5, H7 and H8 close to
135 H1 and one minor haplotype H6 close to H3 were found in both datasets. The numbers of these
136 haplotypes for 16373 genomes with clear collection data detected in each country in chronological
137 order were shown in Figure 4A. From these results, H2 and H4 haplotype subgroups had existed for a
138 long time (2019-12-24 to 2020-04-28), the detected population size of H2 subgroup was far greater
139 than that of H4 subgroup, and the H3 haplotype subgroup almost disappeared after detection
140 (2020-02-18 to 2020-04-28), while H1 haplotype subgroup was globally increasing with time
141 (2020-02-18 to 2020-05-05), which indicated H1 subgroup had adapted to the human hosts, and was
142 under an adaptive growth period worldwide. However, due to the nonrandom sampling on early phase
143 (only patients having a recent travel to Wuhan were detected), some earlier cases of H3 may be lost, the
144 high proportion of H3 subgroup during February 18 to March 10 could confirm this point.

145 H3 and H4 subgroups had the lowest Ka/Ks ratio in 3624 or 16373 genomes among the 4 major

subgroups (Table 4), suggesting that H3 and H4 subgroups might be going through a purifying evolution and have existed for a long time, while H2 and H1 subgroups might be going through a near neutral or neutral evolution according to their Ka/Ks ratios (Table 4), which were consistent with the above phenomenon that only H1 and H2 subgroups were expanding with time around the world. From the whole genome mutations in each major haplotype subgroup (Figure 4B, Table S6), we found that except the 9 specific sites, there was no common mutations with frequencies more than 0.05 in these haplotype subgroups but between H2 and H4 subgroups.

153 **Phylogenetic network of haplotype subgroups**

Phylogenetic networks were inferred with 697 mutations called from 3624 genomes dataset. For these datasets, the network structures of TCS and MSN were similar. The major haplotype subgroups H4 and H2 were in the middle of the network, while H1 and H3 were in the end nodes of the network (Figure 5). According to the phylogenetic networks, we proposed four hypothesis: (1) the ancestral haplotypes evolved in four directions to obtain H1, H2, H3 and H4 respectively or evolved in two or more directions to obtain two or more major haplotypes and then involved into the other major haplotype(s); (2) H2 or H4 evolved in two directions respectively and finally generated H1 and H3; (3) H1 evolved in one direction to generate H2 and H4, and then evolved into H3; (4) H3 evolved in one direction to generate H4 and H2, and then evolved into H1. Although we cannot exclude the first hypothesis based on the present data, but if there are evolutionary relationships among the four major haplotypes, according to the Ka/Ks, detected population size and development trends in chronological order of each major haplotype subgroup, we speculate that the most likely evolution hypothesis is that H3 and H4 are the earliest haplotypes, which is gradually eliminated with selection, while H2 is the transitional

167 haplotype in the evolution process, and H1 may be the haplotype to be finally fixed.

168 **Correlation analysis of specific sites with death rate and infectivity**

169 To explore the relationship between death rate and the 9 specific sites, we used Pearson method to
 170 calculate the correlation coefficient between death rate and frequency of each specific site or major
 171 haplotype in 17 countries with 3624 genomes at early stage. As a result, all r values of 241T, 3037T,
 172 14408T, 23403G and haplotype TTTG and H1 were more than 0.4 (Figure 6, Table S4). We also
 173 evaluated the correlation coefficient with 16373 genomes in 30 countries, the r values of haplotype
 174 TTTG and H1 were still more than 0.37 (Table S4), which might be increased when the death rates of
 175 some countries were further stabilized with time. These finding integrating their high frequencies in
 176 most European countries indicated that the 4 sites and haplotype TTTG and H1 might be related to the
 177 pathogenicity of SARS-CoV-2.

178 To explore the relationship between infectivity and the 9 specific sites, we used population size of
 179 major haplotypes to deduce the possible specific sites related infectivity. We assumed that these major
 180 haplotype in each country were subject to similar virus transmission and control patterns, while the
 181 population sizes of H1 and H2 subgroups were far greater than those of H3 and H4 subgroups (Figure
 182 4A, Table S4), then the common different specific sites of H1 and H2 subgroups with H3 and H4
 183 subgroups, C8782T and T28144C, might be related to the infectivity of SARS-CoV-2, and the virus
 184 with C8782 and T28144 might be more infectious than those virus with 8782T and 28144C.

185

186 **Discussion**

187 SARS-CoV-2 poses a great threat to the production, living and even survival of human beings(Guo, et

188 al., 2020). With the further outbreak of SARS-CoV-2 in the world, further understanding on the
189 evolution and molecular characteristics of SARS-CoV-2 based on a large number of genome sequences
190 will help us cope better with the challenges brought by SARS-CoV-2.

191 Exploring evolution rate, tMRCA and phylogenetic tree of SARS-CoV-2 would help us better
192 understand the virus(Yuen, et al., 2020). The average Ka/Ks for all the coding sequences of 622 and
193 3624 SARS-CoV-2 genomes was 1.008 and 1.094, which was higher than those of SARS-CoV and
194 MERS-CoV, indicating that the SARS-CoV-2 was going through a neutral evolution. In general,
195 SARS-CoV would show purifying selection pressures and the Ka/Ks would go down at the middle or
196 end of the epidemic(He, et al., 2004). Therefore, it seemed to be reasonable considering that
197 SARS-CoV-2 was under an exponential growth period around the world. Interestingly, we also found
198 that the subgroups of different haplotypes (H1, H2, H3 and H4) seemed to experience different
199 evolutionary patterns according to their Ka/Ks. The H3 haplotype subgroup disappeared soon after
200 detection (2020-02-18 to 2020-04-28, Figure 4A), while H1 haplotype subgroup was globally
201 increasing with time, these evolution and changes should be considered in developing therapeutic drugs
202 and vaccines. The tMRCA of SARS-CoV-2 was inferred on the late September, 2019 (95% CI:
203 2019/08/28- 2019/10/26), about 2 months before the early cases of SARS-CoV-2(Huang, et al., 2020).
204 We also estimated tMRCA of SARS-CoV and MERS-CoV with the same methods, both were about 3
205 months later than the corresponding tMRCAs estimated by previous studied(Cotten, et al., 2014, Zhao,
206 et al., 2004). A recent study used TreeDater method to estimate tMRCA for more than 7000
207 SARS-CoV-2 genomes and indicated the tMRCA of SARS-CoV-2 was around 6 October 2019 to 11
208 December 2019, which were about one and a half months later than ours and in broad agreement with

209 six previous studies all performed on no more than 120 early SARS-CoV-2 genomes with BEAST
210 method(van Dorp, et al., 2020). While we used the most common method BEAST to estimate tMRCA
211 of SARS-CoV-2 based on 622 genomes and combined with the comparison of tMRCA of SARS-CoV
212 and MERS-CoV, indicating that the estimated tMRCA in the present study are more reliable.

213 A recent study clustered 160 SARS-CoV-2 whole-genome sequences into A, B and C groups by a
214 phylogenetic network analysis by taking bat RaTG13 as root (Forster, et al., 2020). The cluster result
215 was similar to our study: both the samples in Cluster A and our Cluster 1 were mainly from the United
216 States; the samples in Cluster C and our Cluster 3 were mainly from European countries, while Cluster
217 B and our Cluster 2 were mainly from China and the other regions. It was interesting that the markers
218 C8782T and T28144C, which were also discovered by Yu et al(Yu, et al., 2020) , in Cluster B were
219 also found in our study, but the other markers in Cluster A (T29095C) and Cluster C (G26144T) were
220 not significantly in our study. That may be caused by different sample sizes and different constructing
221 methods of phylogenetic tree. Based on the base substitution model, the ML method avoids the
222 possible "long-branch attraction" problem in the maximum parsimony method and is faster than the
223 Bayesian method(Holder and Lewis, 2003), hence it could be used as a reliable method for
224 phylogenetic analysis. Some studies used the genome of bat SARS-like-CoV(Zhang, et al., 2020),
225 RaTG13(Zhang, et al., 2020) or MT019529 (<https://bigd.big.ac.cn/ncov/tree>) as the root of
226 phylogenetic tree. Unfortunately, there was no obvious evidence showing that SARS-CoV-2 was from
227 the bat coronavirus even the identity between SARS-CoV-2 and RaTG13 was up to 96.2%(Zhou, et al.,
228 2020). In our study, the tMRCA of SARS-CoV-2 was inferred on the late September 2019, which
229 indicated there might exist an earlier SARS-CoV-2 strain we didn't find. Then in the case of unclear

230 source of SARS-CoV-2 and high homology of its genomes (> 99.9% homology mostly), it may be
231 inappropriate to identify the evolutionary characteristics inside the genomes by taking bat
232 SARS-like-CoV, RaTG13 or MT019529 as root. Therefore, the maximum likelihood method was used
233 in current study to construct a no-root tree to obtain the reliable clusters with different characteristics.
234 Based on the no-root tree, we identified 9 specific sites of highly linkage that play a decisive role in the
235 classification of clusters successfully. Among the four major haplotypes, H1 and H3 were in Cluster 3
236 and Cluster 1, respectively, while there were two haplotypes H2 and H4 in Cluster 2 (Figure 1, Figure
237 S3B).

238 Among the 9 specific sites, 8 of them were located in coding regions (6 in *orf1ab* gene, 1 in *S* gene
239 and 1 in *ORF8* gene), and 5 of them were missense variant, including C14408T, C17747T and
240 A17858G in *orf1ab* gene, A23403G in *S* gene, and C28144T in *ORF8* gene. *Orf1ab* is composed of
241 two partially overlapping open reading frames (orf), *orf1a* and *1b*. It is proteolytic cleaved into 16
242 non-structural proteins (nsp), including nsp1 (suppress antiviral host response), nsp9 (RNA/DNA
243 binding activity), nsp12 (RNA-dependent RNA polymerase), nsp13 (helicase) and others(Chan, et al.,
244 2020), indicating the vital role of it in transcription, replication, innate immune response and
245 virulence(Graham, et al., 2008). C14408T with high frequencies of T in European countries were
246 located at nsp12 region, indicating that this missense variant might influence the role of RNA
247 polymerase. Spike glycoprotein, the largest structural protein on the surface of coronaviruses,
248 comprises of S1 and S2 subunits which mediating binding the receptor on the host cell surface and
249 fusing membranes, respectively(Li, 2016). It has been reported that S protein of SARS-CoV-2 can bind
250 angiotensin-converting enzyme 2 (ACE2) with higher affinity than that of SARS(Wrapp, et al., 2020,

251 Zhou, et al., 2020). Whether the missense variant of A23403G in *S* gene, which with high frequencies
252 of G in European countries, will change the affinity between S protein and ACE2 remains to be further
253 investigated.

254 It seems to take a long time to finally fix mutations according to the mutation frequency of each
255 subgroup. For example, H2 and H4 subgroups, which have been detected for more than four months
256 from December 24, 2019 to May 5, 2020 (Figure 4A), have more mutations with higher frequencies,
257 but the highest mutation frequency is only 0.486 at the position of 11083 (Figure 4B, Table S6). From
258 these phenomena, it can be inferred that it takes a long time for the specific sites of each major
259 subgroup to be fixed, but it may be faster if the early population is small. In addition, there is also the
260 possibility that an ancestor strain evolved in four directions by obtaining the specific mutations directly
261 and produced the four current major haplotypes, so the evolution time for obtaining four major
262 haplotypes may be shorter, which seems to be consistent with the phenomenon that four major
263 haplotypes can be detected in two months (Figure 4A). However, if there exist evolution relationship
264 among the major haplotypes of SARS-CoV-2, it is difficult to complete the evolution among the four
265 major haplotypes within two months (2019-12-24 to 2020-02-18, Figure 4A) at the current evolution
266 rate of each major haplotype population (Table 4). Therefore, we speculate that the transformation
267 among the four major haplotypes may have been completed for a long time, which have not been
268 detected. What's interesting is that only United States and Australia among 29 countries had all of four
269 major haplotypes and with relative higher frequency (Figure 4A, Table S4), which indicated that the
270 two countries are the most likely places where the virus appeared earlier based on all the present data.

271 Previous studies showed that the death rate of SARS-CoV-2 is affected by many factors, such as

272 medical supplies, aging, life style and other factors(Liu, et al., 2020, Malavolta, et al., 2020). However,
273 our study found that the 4 specific sites (C241T, C3037T, C14408T and A23403G) in the Cluster 3
274 were almost complete linkage, and the frequency of haplotype TTTG was generally high in European
275 countries and correlated to death rate ($r>0.37$) both based on 3624 or 16373 SARS-CoV-2 genomes,
276 which provides a new perspective to the reasons of relatively high death rate in Europe, and these
277 specific sites should be considered in designing new vaccine and drug development of SARS-CoV-2.
278 Two possible specific sites C8782T and T28144C related to the infectivity of SARS-CoV-2 were also
279 deduced in the present study, which would provide some basis for SARS-CoV-2 epidemiology.

280

281 **Conclusion and prospective**

282 The Ka/Ks ratio of SARS-CoV-2 and tMRCA of SARS-CoV-2 (95% CI: 2019/08/28- 2019/10/26)
283 indicated that SARS-CoV-2 might have completed the selection pressure of cross-host evolution in the
284 early stage and be going through a neutral evolution at present. The 9 specific sites with highly linkage
285 were found to play a decisive role in the classification of clusters. Thereinto, 3 of them are the specific
286 sites of Cluster 1 mainly from North America and Australia, 4 of them are the specific sites of Cluster 3
287 mainly from Europe in the early stage, both of these 3 and 4 specific sites are almost complete linkage.
288 The frequencies of haplotype TTTG for the 4 specific sites and H1 for 9 specific sites were generally
289 high in European countries and correlated to death rate ($r>0.37$) based on 3624 or 16373 SARS-CoV-2
290 genomes, which suggested these haplotypes might relate to pathogenicity of SARS-CoV-2 and
291 provided a new perspective to the reasons of relatively high death rates in Europe. The relationship
292 between the haplotype TTTG or H1 and the pathogenicity of SARS-CoV-2 needs to be further verified

293 by clinical samples or virus virulence test experiment, and the 9 specific sites with highly linkage may
294 provide a starting point for the traceability research of SARS-CoV-2. Given that the different evolution
295 patterns of different haplotypes subgroups, we should consider these evolution and changes in the
296 development of therapeutic drugs and vaccines.

297

298 **Materials and methods**

299 **Genome sequences**

300 The complete genome sequences of SARS-CoV-2 were downloaded from China National Center for
301 Bioinformation (https://bigd.big.ac.cn/ncov/release_genome/) and GISAID (<https://www.gisaid.org/>)
302 up to March 22th, 2020. The sequences were filtered out according to the following criteria: (1)
303 sequences with ambiguous time; (2) sequences with low quality which contained the counts of
304 unknown bases > 15 and degenerate bases > 50 (https://bigd.big.ac.cn/ncov/release_genome/); (3)
305 sequences with similarity of 100% were removed to unique one. Finally, 624 high quality genomes
306 with precise collection time were selected and aligned using MAFFT v7 with automatic parameters.
307 Besides, the genome sequences of 7 SARS-CoV and 475 MERS-CoV were also downloaded from
308 NCBI (<https://www.ncbi.nlm.nih.gov/>), and the MERS-CoV dataset including samples collected from
309 both human and camel. In addition, for further exploring evolution and molecular characteristics of
310 SARS-CoV-2 based on the larger amount of genomic data, we redownloaded validation datasets of the
311 genome sequences from GISAID up to April 6th, 2020 and May 10th, 2020 respectively.

312 **Estimate of evolution rate and the time to the most recent common ancestor for SARS-CoV,**

313 **MERS-CoV, and SARS-CoV-2**

314 The average Ka, Ks and Ka/Ks for all coding sequences were calculated using KaKs_Calculator

315 v1.2(Zhang, et al., 2006), and the substitution rate and tMRCA were estimated using BEAST

316 v2.6.2(Bouckaert, et al., 2019). The temporal signal with root-to-tip divergence was visualized in

317 TempEst v1.5.3(Rambaut, et al., 2016) using a ML whole genome tree with bootstrap value as input.

318 For SARS-CoV and SARS-CoV-2, we selected a strict molecular clock and Coalescent Exponential

319 Population Model. For MERS-CoV, we selected a relaxed molecular clock and Birth Death Skyline

320 Serial Cond Root Model. We used the tip dates and chose the HKY as the site substitution model in all

321 these analyses. Markov Chain Monte Carlo (MCMC) chain length was set to 10,000,000 steps

322 sampling after every 1000 steps. The output was examined in Tracer v1.6

323 (<http://tree.bio.ed.ac.uk/software/tracer/>).

324 **Variants calling of SARS-CoV-2 genome sequences**

325 Each genome sequence was aligned to the reference genome (NC_045512.2) using bowtie2 with

326 default parameters(Langmead and Salzberg, 2012), and variants were called by samtools (sort; mpileup

327 -gf) and bcftools (call -vm). The merge VCF files were created by bgzip and bcftools (merge

328 --missing-to-ref)(Li, 2011, Li, et al., 2009).

329 **Phylogenetic tree construction and virus isolates clustering for SARS-CoV-2**

330 After alignment of 624 SARS-CoV-2 genomes with high quality and manually deleted 2 highly

331 divergent genomes (EPI_ISL_415710, EPI_ISL_414690) according to the firstly constructed

332 phylogenetic tree, the aligned dataset of 622 sequences was phylogenetically analyzed. The SMS

method was used to select GTR+G as the base substitution model(Lefort, et al., 2017), and the PhyML 3.1(Guindon, et al., 2010) and MEGA(Kumar, et al., 2018) were used to construct the no-root phylogenetic tree by the maximum likelihood method with the bootstrap value of 100. The online tool iTOL(Letunic and Bork, 2019) was used to visualized the phylogenetic tree. The clusters were defined by the shape of phylogenetic tree.

Detection of specific sites from each Cluster

Information (ID, countries/regions and collection time) and variants (NC_045512.2 as reference genome) of each genome from each Cluster were extracted. The allele frequency and nucleotide divergency (pi) for each site in the virus population of each Cluster were measured by vcftools(Danecek, et al., 2011). The *Fst* were also calculated by vcftools(Danecek, et al., 2011) to assess the diversity between the Clusters. Sites with high level of *Fst* together with different major allele in each Cluster were filtered as the specific sites. PCA were analyzed by the GCTA v1.93.1beta(Yang, et al., 2011) with the specific sites and all SNV dataset respectively.

Linkage analysis of specific sites and characteristics of major haplotype subgroups

The linkage disequilibrium of the specific sites were analyzed by haploview(Barrett, et al., 2005), and the statistics of the haplotype of the specific sites for each Cluster or country were used in-house perl script.

Phylogenetic network of haplotype subgroups

The phylogenetic networks were inferred by PopART package v1.7.2(Leigh, et al., 2015) using TCS and minimum spanning network (MSN) methods respectively.

353 **Frequencies of specific sites or haplotypes and correlation with death rate**

354 The frequencies of specific sites for each country were calculated. The death rate was estimated with
 355 Total Deaths /Confirmed Cases based on the data from Johns Hopkins resources on May 12th, 2020
 356 (<https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6>).The
 357 correlation coefficient between death rate and frequencies of specific site or haplotype in different
 358 countries was calculated using Pearson method.

359

360 **Authors' contributions**

361 HD conceived the study. YM, YB, DJ, JL and XC carried out the data analysis and wrote the
 362 manuscript. MH, SL, and ZC collected data and revised the manuscript. XW attended the discussions.
 363 HD and YM supervised the whole work and revised the manuscript.

364 **Conflict of Interest**

365 The authors declare no conflict of interest.

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370 **Ethical Approval**

371 Not required.

372

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453

454 **Figure Legends**

455 **Figure 1 Phylogenetic tree and clusters of 622 SARS-CoV-2 genomes**

456 The 622 sequences were clustered into three clusters: Cluster 1 were mainly from North America,
457 Cluster 2 were from regions all over the world, and Cluster 3 were mainly from Europe.

458 **Figure 2 Linkage disequilibrium plot of haplotypes of the 9 specific sites**

459 **A.** The plot for 622 genome sequences; **B.** The plot for 3624 genome sequences.

460 **Figure 3 The frequencies of both the 9 specific sites and haplotypes**

461 The frequencies of the 9 specific sites (**A**) and haplotypes (**B**) in each country for 3624 genomes.

462 **Figure 4 The characteristics of haplotype subgroups**

463 **A.** The numbers of haplotypes of the 9 specific sites for 16373 genomes with clear collection data
464 detected in each country in chronological order; **B.** The whole genome mutations in each major
465 haplotype subgroup.

466 **Figure 5 Phylogenetic network of haplotype subgroups for 3624 genomes**

467 The network was inferred by POPART using TCS method. Each colored vertex represents a haplotype,
468 with different colors indicating the different sampling areas. Hatch marks along edge indicated the
469 number of mutations. Small black circles within the network indicated unsampled haplotypes. H1-H5
470 subgroups were point out according haplotypes of the 9 specific sites, and other small subgroups were
471 not specially pointed out.

472 **Figure 6 The correlation between death rate and frequencies of both the 9 specific sites and**

473 **haplotypes**

474

475 **Tables**

476 **Table 1 Statistics of Ka, Ks and Ka/Ks ratios for all coding regions of the SARS-CoV,**

477 **MERS-CoV and SARS-CoV-2 genome sequences**

478 **Table 2 Substitution rate and tMRCA estimated by BEAST v2.6.2**

479 **Table 3 The information of the 9 specific sites in each Cluster**

480 **Table 4 Statistics of Ka, Ks and Ka/Ks ratios for all coding regions of each 4 major haplotype**

481 **subgroup with 3624 and 16373 genomes respectively**

482

483 **Supplementary material**

484 **Figure S1 Regression analyses of the root-to-tip divergence with the maximum-likelihood**

485 **phylogenetic tree of SARS-CoV (A), MERS-CoV (B) and SARS-CoV-2 (C)**

486 The root-to-tip genetic distances were inferred in TempEst v1.5.3. Three data sets exhibit a positive

487 correlation between root-to-tip divergence and sample collecting time, and they appear to be suitable

488 for molecular clock analysis.

489 **Figure S2 Substitution rate and tMRCA estimate of SARS-CoV-2**

490 **A.** Estimated substitution rate of SARS-CoV-2 using BEAST v2.6.2 under a strict molecular clock. The

491 mean rate was 1.601×10^{-3} (substitutions per site per year). **B.** The estimated tMRCA of SARS-CoV-2

492 using BEAST v2.6.2 under a strict molecular clock. The mean tMRCA was 2019.74 (date:

493 2019-09-27).

494 **Figure S3 Phylogenetic tree of 622 SARS-CoV-2 genomes**

495 A. Phylogenetic tree constructed by PhyML with bootstrap value of 100; B. Phylogenetic tree
496 constructed by MEGA with bootstrap value of 100.

497 **Figure S4 The PCAs analysis result based on the 9 specific sites (A) and all SNVs (B)**

498 **Table S1 The information of the first downloaded complete genome sequences**

499 **Table S2 The sequence IDs in each Cluster**

500 **Table S3 The details information of the SNVs in all Clusters**

501 **Table S4 The information of death rate, frequencies of the 9 specific sites and major haplotypes**
502 **in each country for 3624 and 16373 genomes respectively**

503 **Table S5 All haplotypes of the 9 specific sites and numbers of them for 3624 and 16373 genomes**
504 **respectively**

505 **Table S6 Whole genome mutations and frequencies in each major haplotype subgroup of 3624**
506 **and 16373 genomes respectively**

Tree scale: 0.0001

Category	Value
Category 1 (Blue)	.423
Category 2 (Red)	.288
Category 3 (Blue)	.148
Category 4 (Red)	.122

[illegible]

The figure displays four horizontal stacked bar charts, each representing a different value: .454, .324, .123, and .063. The x-axis for each chart is labeled with numbers 1 through 9. The bars are composed of red and blue segments, indicating the proportion of each category. The total length of the bars decreases as the value decreases.

Value	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6	Category 7	Category 8	Category 9
.454	0.15	0.05	0.05	0.10	0.15	0.10	0.10	0.10	0.05
.324	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.123	0.10	0.05	0.05	0.10	0.10	0.10	0.10	0.10	0.05
.063	0.05	0.05	0.05	0.10	0.10	0.10	0.10	0.10	0.05

A

B

A

B

Estimated death rate

	Ka (mean) 10⁻³	s.e. (Ka) 10⁻³	Ks (mean) 10⁻³	s.e. (Ks) 10⁻³	Ka/Ks (mean)	s.e. (Ka/Ks)	Hypothesis	P-value* 622 genomes	P-value* 3624 genomes
SARS-CoV	0.985	0.018	1.310	0.049	0.760	0.038	Ka/Ks (SARS-CoV < SARS-CoV-2)	0.139	0.084
MERS-CoV	1.319	0.040	4.887	0.096	0.260	0.003	Ka/Ks (MERS-CoV < SARS-CoV-2)	0.000	0.000
622 genomes	0.231	0.004	0.265	0.005	1.008	0.020			
SARS-CoV-2 3624 genomes	0.287	0.002	0.298	0.002	1.094	0.010			

Note: *One-sided Mann-Whitney U-test for the means of two independent samples.

	substitution rate (10^{-3})	95% CI (10^{-3})	tMRCA	95% CI	references
SARS-CoV	1.050	0.489, 1.654	2002/6/28	2002/1/19, 2002/11/3	this study
	-	0.800, 2.380	spring of 2002	-	¹⁷
MERS-CoV	1.516	1.392, 1.632	2012/6/7	2012/6/4, 2012/6/9	this study
	1.120	0.876, 1.370	2012/3	2011/12, 2012/6	¹⁸
SARS-CoV-2	1.601	1.418, 1.796	2019/9/27	2019/8/28, 2019/10/26	this study

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Pos	Ref	Alt	Cluster 1			Cluster 2			Cluster 3			Fst	Gene region	Mutation type	Protein changed	codon changed	Impact
			pi	Major allele	frequency	pi	Major allele	frequency	pi	Major allele	frequency						
241	C	T	0.0000	C	1.0000	0.0109	C	0.9945	0.0000	T	1.0000	0.9912	5'UTR	upstream	NA	NA	MODIFIER
3037	C	T	0.0000	C	1.0000	0.0109	C	0.9945	0.0000	T	1.0000	0.9912	gene-orf1ab	synonymous	924F	2772ttC>ttT	LOW
8782	C	T	0.0000	T	1.0000	0.3863	C	0.7390	0.0000	C	1.0000	0.5821	gene-orf1ab	synonymous	2839S	8517agC>agT	LOW
14408	C	T	0.0000	C	1.0000	0.0055	C	0.9973	0.0000	T	1.0000	0.9956	gene-orf1ab	missense	4715P>L	14144cCt>cTt	MODIFIER
17747	C	T	0.0735	T	0.9620	0.0000	C	1.0000	0.0000	C	1.0000	0.9752	gene-orf1ab	missense	5828P>L	17483cCt>cTt	MODIFIER
17858	A	G	0.0497	G	0.9747	0.0000	A	1.0000	0.0000	A	1.0000	0.9836	gene-orf1ab	missense	5865Y>C	17594tAt>tGt	MODIFIER
18060	C	T	0.0000	T	1.0000	0.0164	C	0.9918	0.0000	C	1.0000	0.9761	gene-orf1ab	synonymous	5932L	17796ctC>ctT	LOW
23403	A	G	0.0000	A	1.0000	0.0164	A	0.9918	0.0000	G	1.0000	0.9869	gene-S	missense	614D>G	1841gAt>gGt	MODIFIER
28144	T	C	0.0000	C	1.0000	0.3915	T	0.7335	0.0000	T	1.0000	0.5785	gene-ORF8	missense	84L>S	251tTa>tCa	MODIFIER

		Ka (mean) 10⁻³	s.e. (Ka)10⁻³	Ks (mean) 10⁻³	s.e. (Ks)10⁻³	Ka/Ks (mean)	s.e. (Ka/Ks)	Hypothesis	P-value*
3624 genomes	H1	0.510	0.057	0.498	0.056	1.099	0.010	Ka/Ks (H3 < H1)	0.000
	H2	0.347	0.042	0.334	0.044	1.268	0.023	Ka/Ks (H3 < H2)	0.000
	H3	0.298	0.007	0.397	0.009	0.795	0.010	-	-
	H4	0.334	0.023	0.414	0.019	0.796	0.019	-	-
16373 genomes	H1	0.416	0.011	0.384	0.011	1.178	0.005	Ka/Ks (H3 < H1)	0.000
	H2	0.332	0.014	0.316	0.014	1.224	0.012	Ka/Ks (H3 < H2)	0.000
	H3	0.305	0.004	0.401	0.005	0.809	0.007	-	-
	H4	0.354	0.010	0.477	0.009	0.749	0.010	-	-

Note: *One-sided Mann-Whitney U-test for the means of two independent samples.