

# 1 SARS-CoV-2 genetic variations associated 2 with COVID-19 severity

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15

## 16 Abstract

17 Herein, we performed a genome-wide association study on SARS-CoV-2 genomes to identify genetic  
18 variations that might be associated with the COVID-19 severity. 152 full-length genomes of SARS-  
19 CoV-2 that were generated from original clinical samples and whose patient status could be  
20 determined conclusively as either “asymptomatic” or “symptomatic” were retrieved from the GISAID  
21 database. We found that nucleotide variations at the genomic position 11,083, locating in the coding  
22 region of non-structural protein 6, were associated with the COVID-19 severity. While the 11083G  
23 variant (i.e. having G at the position 11,083) was more commonly found in symptomatic patients,  
24 the 11083T variant appeared to associate more often with asymptomatic infections. We also  
25 identified three microRNAs that differentially target the two variants, namely miR-485-3p, miR-539-  
26 3p, and miR-3149. This may in part contribute to the differential association of the two SARS-CoV-2  
27 variants with the disease severity.

28

## 29 Keywords

30 SARS-CoV-2, GWAS, nsp6, miRNA

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

## 31 Introduction

32 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus  
33 disease 2019 (COVID-19), was first reported in Wuhun, Hubei, China in late December 2019 [1,2].  
34 SARS-CoV-2 is a positive-sense single-stranded RNA virus in the family *Coronaviridae* [3]. It is the  
35 seventh known coronavirus capable of infecting human, after HCoV-229E, HCoV-OC43, HCoV NL63,  
36 HKU1, MERS-CoV, and the original SARS-CoV. The former four typically cause non-lethal mild upper  
37 respiratory diseases, while the latter two and SARS-CoV-2 can cause severe lethal respiratory  
38 illnesses [4,5].

39  
40 On 30 January 2020, approximately one month after the first reported outbreak of SARS-CoV-2 in  
41 China, the virus was found to spread to 19 countries, and the World Health Organization (WHO)  
42 declared the outbreak to be a Public Health Emergency of International Concern [6]. After the virus  
43 was found to spread to 114 countries, the WHO recognised COVID-19 as a pandemic on 11 March  
44 2020, the first one to be caused by a coronavirus [7]. As of now (27 May 2020), the virus had spread  
45 to 213 countries and territories around the world, infecting more than 5,700,000 people [8], and this  
46 rapid surge of patients had quickly overwhelmed hospitals in many countries. Although the case-  
47 fatality ratio of SARS-CoV-2 (~3–6% [8,9]) is lower than those of SARS-CoV (11%) [10,11] and MERS-  
48 CoV (34–37%) [12], due to the great number of infected cases, the number of deaths caused by  
49 SARS-CoV-2 is much greater than those by SARS-CoV and MERS-CoV. To date, at least 350,000  
50 deaths had been reported to be associated with SARS-CoV-2 infection [8].

51  
52 The incubation period for COVID-19 is ~4–5 days, with most cases (97.5%) develop symptoms within  
53 11–12 days of infection [13]. However, studies have reported that 5–80% of infected cases might be  
54 asymptomatic [14]. Several asymptomatic (and presymptomatic) transmissions have been reported  
55 [15], suggesting roles of asymptomatic infections in the transmission and spread of the disease.  
56 Indeed, it has been proposed that “asymptomatic carriers is a challenge to containment” [16] and  
57 that “asymptomatic transmission of SARS-CoV-2 is the Achilles’ heel of Covid-19 pandemic control”  
58 [17]. To effectively combat with the spread of the disease, this is therefore important to understand  
59 the COVID-19 pathogenesis, and its underlying factors.

60  
61 Several host factors that are positively correlated with the COVID-19 severity have been identified,  
62 including patient age [9,18], low level of CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts, and the high levels of IL-6 and  
63 IL-8 [19]. On the other hand, viral factors associated with the COVID-19 severity are still yet to be  
64 determined. There are currently more than 30,000 genomes of SARS-CoV-2 sampled from around

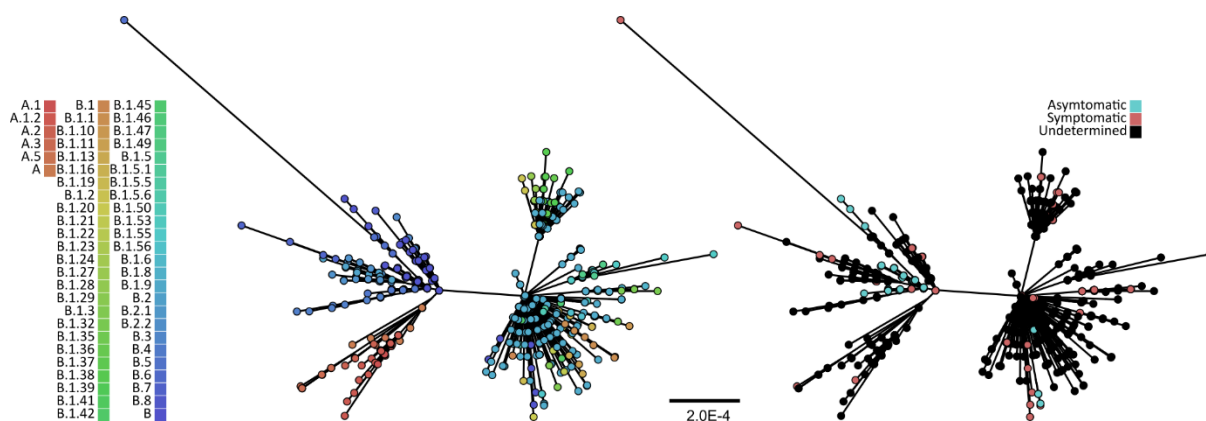
65 the globe made publicly available on the database of the Global Initiative on Sharing All Influenza  
66 Data (GISAID) initiative [20], and many of these sequences had patient data. This enabled us to  
67 examine viral genetic variations that might be correlated with the severity of COVID-19 on the global  
68 scale. In this study, we performed a genome-wide association study (GWAS) on 152 SARS-CoV-2  
69 genomes to identify potential viral genetic variations that might be associated with the COVID-19  
70 severity.

## 71 Results and discussion

### 72 SARS-CoV-2 genome sequences with patient status

73 SARS-CoV-2 genomes with patient status were retrieved from GISAID [20]. At the time of this study,  
74 152 genome sequences were full-length (>29,000 nucleotides (nt)), generated from original clinical  
75 samples, and had unambiguous patient status, which could be confidently determined either as  
76 “asymptomatic” or “symptomatic” (see **Materials and Methods**). To allow for accurate identification  
77 of viral genetic factors associated with COVID-19 severity, only these sequences were analysed.  
78 Together with 500 randomly sampled SARS-CoV-2 genomes from GISAID, all which were full-length  
79 and had a high sequencing coverage (but did not have clear patient information), we reconstructed a  
80 maximum likelihood (ML) phylogeny to examine how these 152 SARS-CoV-2 isolates are related to  
81 one another and to other sequences. We found that they covered a wide diversity of SARS-CoV-2,  
82 distributing across the entire tree (**Figure 1**). According to the classification scheme and method  
83 described in [21], these 152 genomes comprised 16 distinct lineages of SARS-CoV-2 (**Table 1**). The  
84 distribution of asymptomatic and symptomatic SARS-CoV-2 were different across lineages however  
85 ( $\chi^2$  test: score = 50.67, degree of freedom = 28, p-value = 0.005). While symptomatic SARS-CoV-2  
86 were found across almost all 16 lineages, majority of asymptomatic SARS-CoV-2 in this dataset were  
87 predominantly found to be those of lineage B and B.5. Mirroring this observation, asymptomatic  
88 viruses in this dataset were mostly isolated from patients exposed to the virus in Japan (60/72 =  
89 83.33%) and India (6/72 = 8.33%), while symptomatic cases were found around the globe (**Table 2**).

90



91

92 **Figure 1. Maximum likelihood phylogeny of 625 SARS-CoV-2 full-length genomes.** The tree was reconstructed using IQ-  
93 TREE [22] and the GRT+I nucleotide substitution model, the best-fit model as determined under the Bayesian information  
94 criterion by ModelFinder [23]. The bootstrap clade support values were computed based on 1,000 pseudoreplicate  
95 datasets, and only branches with >70% bootstrap support are shown. The scales bar is in the units of substitutions per site.  
96 The tips were coloured either by their lineages (**left**) as identified by pangolin ([github.com/hCoV-2019/pangolin](https://github.com/hCoV-2019/pangolin)), or by  
97 COVID-19 severity (**right**).

98 **Table 1: Lineages of the 152 SARS-CoV-2 genomes with patient status information according to the**  
 99 **classification scheme and method described in [21].**

Severity \ Lineage	A	A.1	A.2	A.3	B	B.1	B.1.22	B.1.3	B.1.36	B.1.5	B.1.6	B.2	B.3	B.5	B.6	B.7	Total
<b>Asymptomatic</b>	1	1	1	0	50	4	0	0	0	0	1	0	0	14	0	0	72
<b>Symptomatic</b>	0	4	0	1	17	37	1	2	5	2	0	2	1	2	3	3	80
<b>Total</b>	1	5	1	1	67	41	1	2	5	2	1	2	1	16	3	3	152

100

101 **Table 2: Country of exposure of the 152 SARS-CoV-2 genomes with patient status information.**

Severity \ Country	Austria	Brazil	China	Colombia	Egypt	Gambia	Hong Kong	India	Indonesia	Israel	Italy	Japan	Malaysia	Mexico	Sri Lanka	UK	USA	Total
<b>Asymptomatic</b>		1	1					6			1	60				1	2	72
<b>Symptomatic</b>	16	1	1	6	10	1	3	4	1	3		17	1	1	1		14	80
<b>Total</b>	16	2	2	6	10	1	3	10	1	3	1	77	1	1	1	1	16	152

102

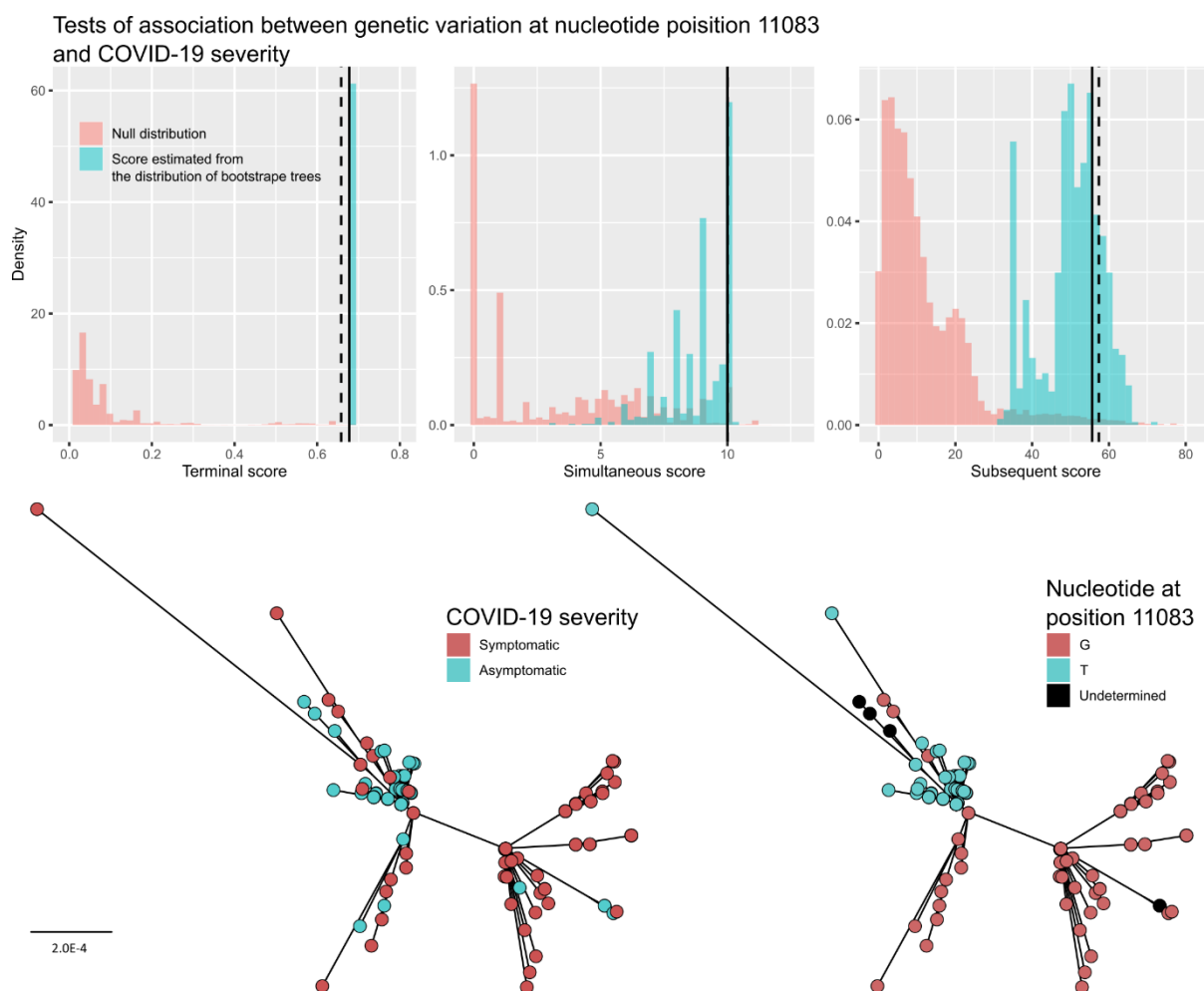
103 **Genetic variations at nucleotide position 11,083 is associated with COVID-19 severity**

104 To identify potential viral genetic variations associated with the COVID-19 severity, we performed a  
 105 GWAS by using TreeWAS [24] – a phylogenetic-based approach for GWAS of microbial genomes,  
 106 which can take into account the observed virus population structure. The estimated ML tree (**Figure**  
 107 **1**) was used for the population structure correction. Our analysis identified genetic variations at the  
 108 nucleotide position 11,083 (with respect to the reference SARS-CoV-2 genome sequence, accession  
 109 number: NC\_045512) to be significantly associated with the disease severity (**Figure 2**). By applying  
 110 this analysis to all bootstrap trees, we found that this genomic position was identified 751/1,000 =  
 111 75.1% of the times, suggesting that our result was robust to the population structure uncertainty.

112

113 Two genetic variations were observed at the nucleotide position 11,083, namely thymine (11083T,  
 114 75/152 = 49.34%) and guanine (11083G, 72/152 = 47.37%). 5 sequences (5/152 = 3.29%) had  
 115 undetermined nucleotides at this site. We found that asymptomatic SARS-CoV-2 tended to have  
 116 11083T ( $N(11083T)/N(11083G) = 60/7$ ), while viruses causing symptomatic cases tended to have  
 117 11083G ( $N(11083T)/N(11083G) = 15/65$ ) (**Figure 2**). The relative risk ratio of developing symptoms  
 118 given 11083G to 11083T is  $(65/72)/(15/75) = 4.51$  times (95% confident interval = 2.85–7.14), and  
 119 the odd ratio was estimated to be 37.14 by the Wald method (95% confident interval = 14.17–  
 120 97.33). A study of SARS-CoV-2 from a Shanghai cohort has indeed identified the 11083T variant to be  
 121 more prevalent in asymptomatic cases (9 in 91 cases = 9.89%) compared to symptomatic cases (1 in  
 122 21 cases = 4.76%), but the association was not significant [19]. This was likely due to their relatively  
 123 small data set ( $N = 112$ ), and different groupings of the disease outcome (where mild symptomatic

124 and asymptomatic cases were grouped together as one and was compared against severe and  
125 critical cases), which could potentially mask the effect we observed. Another study identified this  
126 nucleotide position as being under a positive selection pressure [25], consistent with our finding. The  
127 closest relative of SAR-CoV-2 currently known is the bat coronavirus RaTG13 (GenBank accession  
128 number: MN996532) [26], and it has a G at this position, suggesting that G is the ancestral state. We  
129 hence designated this mutation 11083G>T. This mutation locates in the coding region of non-  
130 structural protein 6 (nsp6, nt 10973–11842), coded by ORF1ab (nt 266–21,555). 11083G>T is a non-  
131 synonymous mutation, conferring an amino acid change from leucine (L) to phenylalanine (F) in the  
132 nsp6 protein (L37F).  
133



134

135 **Figure 2. Genetic variations at nucleotide position 11,083 is associated with COVID-19 severity.** (Top) three tests of  
136 genome-wide association analysis were performed, namely “Terminal test” (left), “Simultaneous test”, and “Subsequent  
137 test” to identify potential genetic variations associated with COVID-19 severity by using TreeWAS [24]. Across all three  
138 analyses, we identified genetic variations at the nucleotide position 11,083 (with respect to the reference SARS-CoV-2  
139 genome, NC\_045512) to be associated with COVID-19 severity. The null distributions of the scores are shown in red. The  
140 scores estimated based on the consensus tree (Figure 1) are indicated by black vertical lines. The significant thresholds  
141 corrected for multiple testing are indicated by dotted black vertical lines. The distributions of the scores estimated from  
142 the 1,000 bootstrap trees are shown in blue. Trees were pruned to contain only those with patient status before the  
143 analysis as recommended. (Bottom) the maximum likelihood tree of SARS-CoV-2 pruned to contain only those with patient  
144 status (152 sequences). The scales bar is in the units of substitutions per site. The tips were coloured by COVID-19 severity  
145 (left) or by the nucleotide variant at the position 11,083 (right).

146

### 147 **11083G and 11083T variants may interact with human miRNAs differently**

148 Interaction between host microRNAs (miRNAs) and viral transcripts has been implicated in  
149 pathogenesis of viral diseases [27]. In this study, we examined whether the two virus variants have  
150 different modes of binding to human miRNAs by querying their nucleotide sequences against the  
151 miRNA database miRDB [28]. Although a coronavirus genome is a positive-sense RNA, negative-  
152 sense genomic RNAs are nonetheless generated during their replication, presenting a possibility that  
153 it might also interact with human miRNAs. We therefore examined both positive- and negative-  
154 strands of SARS-CoV-2 genome sequences.

155

156 On the positive strand, two miRNAs were predicted to uniquely target the 11083G with the same  
157 (highest) scores, namely miR-485-3p (5'-GUCAUACACGGCUCUCCUCUCU-3') and miR-539-3p (5'-  
158 AUCAUACAAGGACAAUUUCUUU-3'). These two miRNAs belong to the same miRNA cluster (chr14:  
159 101,047,321–101,047,398, and chr14: 101,055,419–101,055,491, respectively), and share identical  
160 seed sequences (5'-UCAUACA-3'), spanning the nt 11,083 (11,082–11,088) (**Figure 3**). By using the  
161 negative-sense sequence as a query, miR-3149 (5'-UUUGUAUGGAUAUGUGUGUGUAU-3') was  
162 predicted to bind to the 11083G variant, and not the 11083T variant (nt c11087–11080). Our  
163 analyses did not identify miRNAs that specifically target the 11083T variant but not the 11083G  
164 variant. This suggested that the two variants might interact with these three miRNAs differently.

165

### 166 **Potential biological significance of 11083G>T mutation in COVID-19 severity**

167 A study demonstrated that miR-485 can down regulate antiviral immunity by interacting with  
168 *retinoic acid-inducible gene 1 (RIG-I)* mRNA [29], the protein product of which can sense viral RNAs  
169 inside the cell, and activates the cell antiviral immune response. It is possible that the two variants of  
170 SARS-CoV-2 may interact with miR-485-3p differently, leading to differential host antiviral immune  
171 response, and subsequently differential COVID-19 severity. Moreover, one of the most common  
172 symptoms of severe COVID-19 is the cytokine release syndrome [30,31]. RIG-I signalling pathway is  
173 known to induce production of TNF $\alpha$  [32], which is a pro-inflammatory cytokine involved in the  
174 cytokine release syndrome [30]. It is thus also possible that the RNA molecules produced by the  
175 11083G variant might sequester miR-485-3p, and ultimately lead to over-production of TNF $\alpha$   
176 through an unregulated upregulation of the RIG-I pathway, resulting in a severe COVID-19 disease.  
177 This result warrants further experimental investigations how the SARS-CoV-2 11083G variant  
178 interacts with miR-485-3p.

179



Positive strand viral RNA

miR-485-3p

```

3' -UCUCUCCUCUCGGCACAUACUG-5'
      |           | | | | |
5' -AAUGGUCUUUGUUCUUUUUUUUGUAUGAAAAUGCCUUUUUACCUUUUGC-3'
    
```

miR-539-3p

```

3' -UUUCUUUAACAGGAACAUACUA-5'
      | | | | | | | | |
5' -AAUGGUCUUUGUUCUUUUUUUUGUAUGAAAAUGCCUUUUUACCUUUUGC-3'
    
```

Negative strand viral RNA

miR-3149

```

3' -UAUGUGUGUGUAUAGGUAUGUUU-5'
      | | | | | | | | |
5' -GCAAAAGGUAAAAAGGCAUUUCAUACAAAAAAAGAACAAGACCAUU-3'
    
```

180

181 **Figure 3. miR-485-3p, and miR-539-3p, and miR-3149 miRNAs specially target the 11083G variant of SARS-CoV-2, but not**  
 182 **the 11083T variant.** Sequences of the two SARS-CoV-2 variants as defined by the genetic variations at the nucleotide  
 183 position 11,083 (11083G and 11083T variants) were searched against the miRNA database miRDB [28]. We found that miR-  
 184 485-3p, and miR-539-3p were predicted to specially target the 11083G variant and not the 11083T variant on the positive  
 185 strand. miR-3149 was predicted to uniquely target the 11083G variant on the negative strand. The viral genetic variations  
 186 are written in bold and underlined. The seed sequences of miRNAs are shown in bold.  
 187

188 Regarding miR-539-3p, a recent study showed that it suppresses expression of the pro-angiogenic  
 189 factor Jagged1 [33] – a ligand for the Notch signalling pathway which controls cell proliferation and  
 190 differentiation of various cell lineages, including blood vessel formation and sprouting [34,35].  
 191 Similar to the case of miR-485-3p, the viral RNAs produced by the 11083G variant might sequester  
 192 miR-539-3p, leading to up-regulation of Jagged1 and subsequently angiogenesis, as observed in  
 193 several symptomatic COVID-19 patients [36]. In addition to cell proliferation, miR-539 is also known  
 194 to upregulate autophagy [37], the process by which cells degrade and recycle cellular components  
 195 [38]. Incidentally, nsp6, the viral protein which the 11083G>T mutation directly affects, has also  
 196 been reported to interfere with the host autophagy, restricting autophagosome expansion [39,40].  
 197 This restriction of autophagosome size likely compromises the cell ability to deliver viral components  
 198 to lysosomes for degradation, and hence favouring virus infection [39]. Together, our results  
 199 suggested potential roles of autophagy in pathogenesis of SARS-CoV-2, and should be further  
 200 investigated.

201

202 In comparison to miR-485-3p and miR-539-3p, the functions of miR-3149 are much less known and  
 203 well-understood. Nevertheless, it has been reported that patients with acute coronary syndrome  
 204 had high levels of miR-3149 in their plasma [41]. Whether miR-3149 plays a role in COVID-19  
 205 pathogenesis is yet to be determined. Similarly, it is still unclear how the 11083T variant interacts  
 206 with the host and causes an asymptomatic infection. Functional investigation of this mutation is  
 207 warranted.



208

## 209 Conclusion and final remarks

210 An unprecedented number of SARS-CoV-2 genomes have been generated at a rapid rate and made  
211 publicly available in near real-time like never before. To date, there are more than 30,000 sequences  
212 of SARS-CoV-2 genomes made publicly available on the GISAID database [20], and many of these  
213 sequences have patient information available. This allowed us to investigate viral genetic factors  
214 that might be associated with the COVID-19 severity.

215

216 In this study, we performed a GWAS on 152 SARS-CoV-2 genomes, and identified nucleotide  
217 variations at the genomic position 11,083 to be associated with the disease severity. Most of  
218 symptomatic cases were found to be infected with the 11083G variant, while the 11083T variant  
219 appeared to be associated more often with asymptomatic infections (relative risk ratio = 4.51 (2.85–  
220 7.14); odd ratio = 37.14 (14.17–97.33)). The two nucleotide variants, 11083G and 11083T, are non-  
221 synonymous, corresponding to L and F at the amino acid position 37 in the nsp6 protein,  
222 respectively. Our results have potential applications for the development of better, and more  
223 informative test kits, potentially allowing for asymptomatic cases to be distinguished from  
224 symptomatic cases. Continual surveillance of COVID-19 should monitor this genomic region as well  
225 as its surrounding neighbourhood as they might affect the pathogenesis of COVID-19.

226

227 Bioinformatic analyses suggested that the two variants might interact with the human host miRNAs  
228 differently. In particular, the 11083G variant was identified as a potential target of miR-485-3p, miR-  
229 539-3p, and miR-3149, while the 11083T variant was not. These differences might contribute to the  
230 observed differential association between the two variants and the disease severity. Our results  
231 warrant further experimental confirmations to validate biological significance of these genetic  
232 variations and their consequences.

## 233 Methods

### 234 SARS-CoV-2 genome sequences with patient status

235 Genome sequences of SARS-CoV-2 with patient status were downloaded from the GISAID database  
236 [20] on 18/05/2020 with their metadata. To allow for accurate determination of genetic factors  
237 associated with COVID-19 severity, we only analysed sequences whose patient status could be  
238 unambiguously determined as either “asymptomatic” or “symptomatic”. We designated a virus to  
239 cause an asymptomatic infection if its patient status was either “Asymptomatic/Released” or  
240 “asymptomatic”. A virus was determined to cause a symptomatic infection if the patient status was  
241 either “Hospitalized in ICU”, “Hospitalized/Deceased”, “ICU; Serious”, “Intensive Care Unit”,  
242 “pneumonia (chest X-ray)”, “Severe/ICU”, or “Symptomatic”. Sequences that were not generated  
243 from original clinical samples were excluded. Those with ambiguous nucleotides greater than 5% of  
244 the total sequence length, and whose total lengths were less than 29,500 nucleotides were also  
245 excluded from downstream analyses. Although EPI\_ISL\_417919 was found to fit the inclusion  
246 criteria, manual inspection revealed that it contained many unique nucleotide variants surrounding  
247 its multiple undetermined regions of “N”s, likely due to sequencing and / or assembly errors, and  
248 had about 4.28% of undetermined nucleotides. It was thus also excluded from our dataset. In total,  
249 our dataset comprised 152 sequences. A table of acknowledgements for the sequences used in this  
250 study can be found in **Table S1**.

251

### 252 Lineage assignment

253 The lineage of all genomes were determined based on the methodology described in [21] by using  
254 pangolin ([github.com/hCoV-2019/pangolin](https://github.com/hCoV-2019/pangolin)) with the reference lineage version 07/05/2020 under  
255 the default setting. All prediction passed the quality control.

256

### 257 Phylogenetic analysis

258 500 randomly sampled SARS-CoV-2 genomes were downloaded from GISAID on 21/05/2020 (**Table**  
259 **S1**), all of which were full-length and had a high sequencing coverage. Together with the 152  
260 genomes with patient status, a manually-curated multiple sequence alignment of 652 SARS-CoV-2  
261 genomes was constructed. Potential recombination within the alignment was checked by using RDP,  
262 GENECONV, Chimera, MaxChi, and 3Seq, all implemented in Recombination Detection Program 4  
263 [42]. Sites with more than 50% ambiguous nucleotides were excluded from recombination analysis.  
264 Sites with the most common nucleotide present in more than 99% of the sequences were also

265 excluded. None of the program found evidence for recombination events within the data, suggesting  
266 that our dataset was recombinant-free.

267

268 A maximum likelihood phylogeny was estimated from the prepared full-length alignment by using  
269 IQ-TREE [22]. The best-fit nucleotide substitution models was determined to be GTR+I+F under the  
270 Bayesian information criterion by using ModelFinder [23] and was used for the tree reconstruction.  
271 The bootstrap clade support was computed by using 1,000 pseudoreplicate datasets.

272

### 273 Identification of genetic variations associated with COVID-19 severity

274 TreeWAS [24] was used to identify potential genetic variations associated with COVID-19 severity,  
275 defined as two discrete traits: "asymptomatic" and "symptomatic". The estimated ML tree was used  
276 for the population structure correction. The tree was rooted by assuming that lineage A and B of  
277 SARS-CoV-2 are monophyletic, and was pruned to contain only those with patient status before the  
278 analysis as recommended. Three tests of association were performed, including the "terminal",  
279 "simultaneous", and "subsequent" tests. Sites with more than 50% ambiguous nucleotides were  
280 removed. Sites with the most common nucleotide present in more than 95% of the sequences were  
281 also removed. Only 15 variant loci remained after the filtering. Ancestral states of both genetic and  
282 phenotypic data were inferred under a maximum likelihood framework as implemented in the  
283 package. The number of sites simulated for estimating the null distribution was  $1,000 \times 15 = 15,000$   
284 sites. The overall threshold of significance was set to 5%, and corrected to be  $5\%/15/3 = 0.11\%$  in  
285 each test under the Bonferonni multiple-testing correction criteria as recommended. An association  
286 was considered significant if it was detected by at least one of the three tests aforementioned. We  
287 also apply this analysis to all of the 1,000 trees in the bootstrap tree distribution obtained from the  
288 phylogenetic analysis described above to examine the robustness of the result. Our analyses robustly  
289 identified nucleotide variations at the genomic position 11,083 (with respect to the reference SARS-  
290 CoV-2 genome, NC\_045512) to be significantly associated with the disease severity.

291

### 292 microRNA analyses

293 To examine potential biological significance of the identified genetic variations, we searched the two  
294 sequence variants against the microRNA database miRDB [28] under the default settings, available at  
295 <http://mirdb.org/>.

## 296 Data Availability

297 All sequence data used in this study were retrieved from GISAID. The table of acknowledgement of  
298 the sequences used can be found in **Table S1**.

299

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302

## 303 Author contributions

304 P.A., P.W., A.T. conceived the study. P.A., Y.T, P.W. performed data curation, analysis, and  
305 interpretation of the results. P.A., P.W. drafted the manuscript. All revised and approved of the final  
306 manuscript.

307

## 308 Competing Interests

309 The authors declare no conflict of interest.

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