Genetic variations from successive whole genome sequencing during COVID-19 treatment in five individuals

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Abstract

We report multiple single nucleotide polymorphism taken at different time interval during treatment of COVID-19. Gene sequencing showed mutation within ORF1b at position P314L. Mutation at this point has been shown to impose structural remodelling that increases the affinity for remdesivir binding and may also affect binding affinity for favipiravir.

Keywords: COVID-19, genetic variations, next generation sequencing, SARS-CoV-2
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In 2020, first two outbreaks in Thailand were effectively controlled with isolation and contact restriction. Previous experience in our COVID-19 facility, patients were responding to favipiravir, a purine nucleotide analogue with a small fraction progressing to severe pneumonia. On the third and current outbreaks, several factors including new delta variant, lack of active surveillance and inadequate vaccination, number of infected were out of control. The situation is difficult with more healthcare workers becoming infected and people dying in their home and on the street.

COVID-19 is caused by enveloped positive sense single-stranded RNA virus with multiple variants emerging and circulating around the world [1]. The viral genome consists of spike (S) protein responsible for host cell viral entry, along with envelope (E) and membrane (M) forming viral envelope. The genome nucleocapsid (N) protein holds viral RNA genome in place and non-structural open reading frames (ORFs) govern RNA transcription via RNA polymerase [2]. ORF1ab occupies majority of ORF region and is the target for binding and inhibition of antivirals such as favipiravir and remdesivir. Mutation within the drug binding site may increase or reduce binding affinity of antiviral and its effect on inhibiting viral replication theoretically could be altered [3].

On this outbreak, we observed many more patients progressing to severe pneumonia with successive nasopharyngeal and throat (NP and T) swabs showing reduced cycle threshold (CT) from RT-PCR reflecting higher viral load after five days of Favipiravir. Patient who progresses while on favipiravir will be re-swab and if CT remains low, they will be switched to remdesivir, a ribonucleotide analogue and increase in dexamethasone. As a result, many patients were showing improvement in pneumonia with higher CT value. This approach is necessary due to limited supply of remdesivir in Thailand.

To explain this differing response to antiviral, nasopharyngeal and throat swab samples from patients with worsening COVID-19 pneumonia, defined as significant increase in oxygen requirement from nasal prong to high flow nasal cannula (HFNC) despite receiving favipiravir were examined. Initial and consecutive swab of five patients with CT showing reduction despite at least 5 days of favipiravir undergo SARS-CoV-2 whole genome sequence library construction using QIAseq SARS-CoV-2 Primer Panel for next-generation sequencing (Table 1). In this evaluation, we found single nucleotide polymorphisms (SNPs) in the same individual taken at different time interval during active treatment of COVID-19 with favipiravir. Two consecutive samples were taken from four patients with first sample for diagnosis and second sample taken on clinical deterioration. Three consecutive samples were taken from one patient, additional sample was taken during clinical improvement.

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TABLE 1. Variant with specific genetic markers associated with increased transmissibility, morbidity and mortality, and ability to evade natural immunity. Spike (S), nucleotide (N), open reading frames (ORFs)

Patient ID	Patient 1		Patient 2			Patient 3		Patient 4		Patient 5	
Specimen ID	**SI2124803-NT**	**SI2127342-NT**	**SI2129241-NT**	**SI2130606-NT**	**SI2131209-NT**	**SI2129950-NST**	**SI2130745-NT**	**SI2129253-NT**	**SI2130446-NT**	**SI2126070-NST**	**SI2127539-NT**
Collection date	**20/05/2021**	**04/06/2021**	**15/06/2021**	**22/06/2021**	**27/06/2021**	**19/06/2021**	**23/06/2021**	**15/06/2021**	**22/06/2021**	**27/05/2021**	**06/06/2021**
Amionacid substitutions	20I (Alpha, V1) N:D3L N:G204R N:S255A ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF1a:A2249V ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:N501Y S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H	20I (Alpha, V1) N:D3L N:R203K N:G204R N:S235F ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF1a:A2249V ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H	20I (Alpha, V1) N:D3L N:R203K N:G204R N:S235F ORF1a:A1708D ORF1a:I2230T ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:L5F S:A570D S:D614G S:P681H S:T716I S:S982A S:K1073Q S:D1118H	20I (Alpha, V1) N:D3L N:R203K N:G204R N:S235F ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:L5F S:N501Y S:A570D S:D614G S:P681H S:T716I S:S982A S:K1073Q S:D1118H	20I (Alpha, V1) N:D3L N:G204R ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:L5F S:A570D S:D614G S:P681H S:T716I S:S982A S:K1073Q S:D1118H	21A (Delta) M:I82T N:D63G N:L139F N:R203M N:D377Y N:R385K ORF1a:P309L ORF1a:P1640L ORF1a:A3209V ORF1a:V3718A ORF1b:P314L ORF1b:G662S ORF1b:P1000L ORF1b:H2285Y ORF3a:S26L ORF7a:V82A ORF7a:L116F ORF7a:T120I ORF9b:T60A S:T19R S:R158G S:L452R S:T478K S:D614G S:P681R S:D950N	21A (Delta) M:I82T N:D63G N:R203M N:D377Y N:R385K ORF1a:P309L ORF1a:A3209V ORF1a:V3718A ORF1b:P314L ORF1b:G662S ORF1b:P1000L ORF1b:H2285Y ORF3a:S26L ORF7a:V82A ORF7a:T120I ORF9b:T60A S:T19R S:R158G S:L452R S:T478K S:D614G S:P681R S:D950N	20I (Alpha, V1) N:D3L N:R203K N:G204R N:Q229H N:S235F ORF1a:T1001I ORF1a:Q1129R ORF1a:A1708D ORF1a:I2230T ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:N501Y S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H	20I (Alpha, V1) N:D3L N:R203K N:G204R N:Q229H N:S235F ORF1a:T1001I ORF1a:Q1129R ORF1a:A1708D ORF1a:I2230T ORF1b:P314L ORF3a:G254 ORF8:R52I ORF8:Y73C S:N501Y S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H	20I (Alpha, V1) N:D3Q N:R14C N:G204R ORF1a:A565V ORF1a:S944L ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF1b:P314L ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:Y73C S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H	20I (Alpha, V1) N:D3L N:R14C N:R203K N:G204R ORF1a:A565V ORF1a:S944L ORF1a:T1001I ORF1a:A1708D ORF1a:I2230T ORF3a:G254 ORF8:Q27 ORF8:R52I ORF8:K68 ORF8:Y73C S:A570D S:D614G S:P681H S:T716I S:S982A S:D1118H
Aminoacid deletions	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF8:D119- ORF8:F120- S:E156- S:F157-	ORF8:D119- ORF8:F120- S:E156- S:F157-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-	ORF1a:S3675- ORF1a:G3676- ORF1a:F3677- S:H69- S:V70- S:Y144-

In the first patient, we found substitution at position N:S255A and S:N501Y on the first swab and revert back to its original sequence on the second swab and additional substitution at N:R203K and N:S235F. Second patient, we found substitution at position N:R203K and N:S235F on the first swab, additional substitution at ORF1a:T10011 and S:N501Y on the second swab. We found reversion of all substitution except at ORF1a:T10011 on the third swab perform during recovery. Third patient, we found substitution at N:L139F, ORF1a:P1640L and ORF7a:L116F on the first swab and all substitution revert to original on the second swab. Fourth patient, we found substitution at ORF1b:P314L on the second swab. Last patient, we found substitutions at N:D3Q and ORF1b:P314L on the first swab and reversion of both substitutions with addition substitution at N:R203K on the second swab.

Mutations in COVID-19 during treatment were observed. Significance of mutations is not known, but widespread use of antiviral may have driven selective pressure. Analysis is also showing mutation within ORF1b at position P314L (P323L) in the initial swab sample in four out of five individuals. Mutation at this point has been shown to impose structural remodelling that increases the affinity for remdesivir binding [4,5]. Further mutation will occur in every replicative cycle due to its RNA structure and the pandemic will provide a platform for rapid turnover and accelerated mutations. Constant global systematic surveillance of significant mutation is urgently needed to study the viral behaviors and assists in epidemiological data gathering.

Transparency declaration

The authors report no relevant disclosures or conflict of interest.

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