# Whole Genome Sequence Analyses of SARS-CoV-2

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# Whole genome sequence analyses of Indonesian isolates SARS-CoV-2 variants and their clinical manifestations

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**ABSTRACT** The SARS-CoV-2 virus has been the cause of the global pandemic since the end of 2019. Since then, the virus has mutated to create different types of variants with numerous effects on those infected. This has complicated human intervention for prevention. Indonesia was heavily affected by the pandemic, specifically from May to August 2021, and as a country has recorded many distinct isolates. Thus, characterization of the virus strains from Indonesia is important. GISAID, NCBI BLAST, and MAFFT version 7 were used. There were 9,488 isolates in Indonesia as of November 2021, with the majority including the Delta variant. While most of the isolates have mutations common to those from other countries, there are some atypical ones, such as mutation V1264L in the Delta variant that was suspected to play a role in worsening the pandemic. The Delta variant had the most mutations in the spike protein when compared to the Alpha and Beta variants, giving it important roles in infectivity and vigorous entry into cells, with some general clinical manifestations like fever and sore throat; however, the severity of the Delta variant is attributable to its rapid growth. This is why, from May to November 2021 in Indonesia, cases of the Delta variant rocketed, unlike the other variants.

**KEYWORDS** Mutation; SARS-CoV-2; Sequence alignment; Whole genome sequence; Wild type; Variants

## 1. Introduction

Whole-genome sequencing (WGS) can give more understanding of the biology and epidemiology of the SARS-CoV-2 virus, it is a very significant method of analysis. The infamous COVID-19 virus has a +ssRNA genome that is linear and it is from a family called coronaviruses (CoVs) which was named after its spikes on the surfaces that look like crowns. Coronaviruses can spread respiratory diseases in mammals and birds, and were discovered in humans in the mid-1960s. Seven common human coronaviruses (HCoVs) are divided again into either alpha or beta coronaviruses. Though in the focus of this COVID-19 pandemic, the three main greatly pathogenic HCoVs manifested in 2002, 2012, and 2019, include respectively the SARS-CoV, MERS-CoV, and the SARS-CoV-2 viruses (Yin 2020).

From the various lineages evident by the summer of 2020, it was revealed that the SARS-CoV-2 has gone and is going through genetic mutations (Rantam et al. 2021). Thus, characterization of the virus strains from different places is needed, Indonesia included. Based on the GISAID SARS-CoV-2 genome database, the lineage that is predominant in Indonesia is the B.1 lineage which varies from the Wuhan isolate (Rantam et al. 2021), more specifically the B.1.466.2 variants (Cahyani et al. 2022).

Suitable actions can be taken when the virus is understood deeply. Following its different variants and pinpointing them can help us in preventing the pandemic from worsening in Indonesia. With the whole genome sequence of Indonesian isolates analyzed, including its wild type, differences and similarities can be examined. Between each isolate, there will be mutations in the sequences that form various variants and types of the virus, which can affect people differently. The whole genome sequence will contain the nucleotide sequences for the structural proteins of the virus as well. These structural proteins include the spike, envelope, membrane, and nucleocapsid proteins labelled S, E, M, and N, respectively.

Specific to Indonesia, the determination of isolates and the study of their genomics, mutations, and types can bring about a much clearer distinction or observation of similarities. Any changes in the virus's genome that translate to the virus proteins, for example, can cause different clinical manifestations. The distinction between the Indonesian isolates, or the similarities observed, can help people know and plan better for prevention in the country, such as in the process of vaccine design or development.

This research aims to give an overview of the gen-

eral characteristics, including the number of SARS-CoV-2 variants found throughout the specified period, the distinct mutations, and the symptoms of individual variants. Providing general information such as the aim of this research has been very few in Indonesia, and by filling in the gaps in knowledge regarding this area, it will open doors for further research and analysis on the field.

## 2. Materials and Methods

Figure 1 shows the diagram of the research's workflow, the methods, steps, and the pipeline used. Whole genome sequences (WGS) of SARS-CoV-2 viruses of Indonesia isolates were obtained from the GISAID (https://www.gisaid.org/) and NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The randomly chosen isolates were selected and analyzed. The Wuhan SARS-CoV-2 genome NC_045512.2 from the NCBI database and the Indonesian genome EPI_ISL_435282 from the GISAID database were used as references. Genome sequences of each variant found in Indonesia were obtained from the GISAID database. From the GISAID database, FASTA files were acquired. These genome sequences were compared to each other, as well as the Wuhan and Indonesian genome references, with Nucleotide BLAST and MAFFT version 7. The bioinformatics tools, GISAID database, NCBI BLAST, and MAFFT version 7, were used to compare the genome sequences for two and multiple alignments, respectively.

## 3. Results and Discussion

### 3.1. SARS-CoV-2 variants found in Indonesia

As of November 2021, around 9,488 SARS-CoV-2 viruses were obtained from the GISAID database with the number of variants as shown in Table 1. From the collected isolates, there were three variants identified, namely: the Delta, Alpha, and Beta variants. The other variants, such as the Lambda, Omicron and Mu variants of the virus had not been found in Indonesia when the data was retrieved.

Figure 2 shows that the highest amount of Delta variant of the SARS-CoV-2 virus collected in Indonesia was in the period from May 1 until August 1, 2021. The second highest was also of the Delta variant from August 1 until November 1, 2021. It was shown that the Delta variant is the variant with the highest number of infections when compared to the others, and Table 1 supports this as well with the Delta variant capturing 58.1% out of all viruses collected and submitted into the GISAID database. Thus, this finding was found consistent with other studies that state that the Delta variant was the most severe variant during the pandemic period because of many factors, such as its specific mutations in certain genes. The factor that caused its great severity was the mutations of the Delta variant that affect binding, making it much easier to attach to human cells. The spike proteins from the virus have region binding domains (RBDs) that attach to the angiotensin-converting enzyme 2 (ACE2) receptor which can be found in a person's lung cells. Aside from that, the Delta variant also has mutations that help the stability

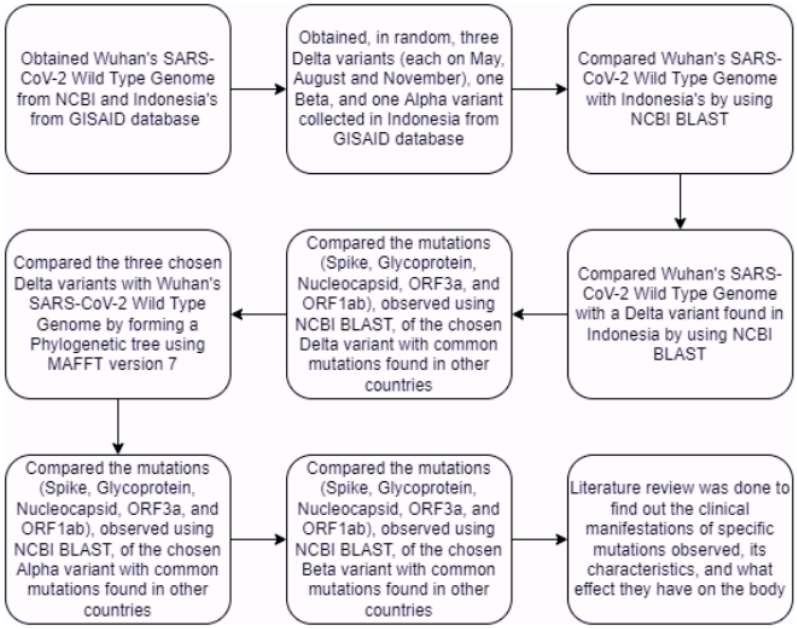


**FIGURE 1** Methodology diagram.

**TABLE 1** Percentage of SARS-CoV-2 variants from Indonesian isolates deposited in the GISAID database as of November 2021.

| Database | Variants | Number of Viruses Collected | Percentage (%) |
|---|---|---|---|
| GISAID | Delta | 5,512 | 58.1 |
| | Alpha | 78 | 0.822 |
| | Beta | 22 | 0.232 |
| | Gamma | 0 | 0 |
| | Lambda | 0 | 0 |
| | Mu | 0 | 0 |
| | Wild Type | 3,876 | 40.9 |

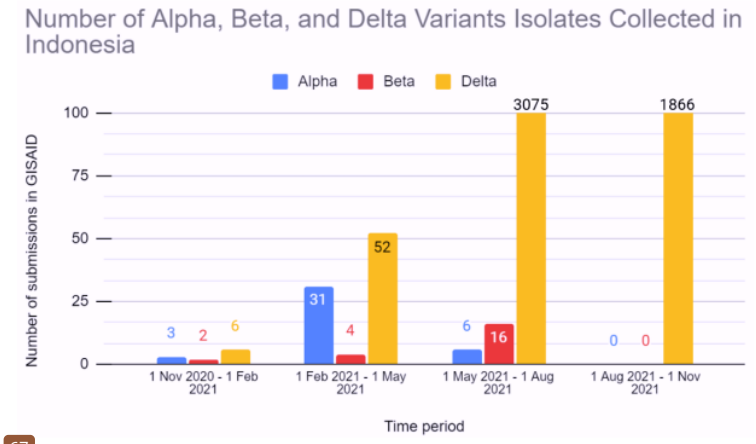


**FIGURE 2** Number of Alpha, Beta, and Delta variants in Indonesia collected and submitted into the GISAID database with the horizontal axis representing the period the virus sample was collected.

of the virus with increased mRNA packaging, making it more stable and resistant to antibodies, increasing infectivity by lessening antibody function, improving the lives of the virus by escalating the taking in of glucose, and many more. These mutations and their effects will be covered in detail.

### 3.2. *Comparison of Wuhan isolate (reference) with Indonesian wild-type*

Using NCBI BLAST, the first Wuhan SARS-CoV-2 Wild-Type Isolate (NC_045512.2) from NCBI was compared with an Indonesian Wild-Type Isolate (ESP_ISL_435282) from GISAID and it showed a 99% query cover having slight differences on the N gene. The only differences include, from the Indonesian isolate nucleotide range of 29866 to 29920, the presence of two substitutions of nucleotides. The first was from adenine (A) to guanine (G) and the second was from guanine (G) to cytosine (C). Due to these, there were two substitutions of amino acids, the first from lysine (K) to arginine (R) and the second from aspartic acid (D) to histidine (H). At the beginning and last of the Wuhan isolate nucleotide range of 29292 to 29346, aspartic acid was found, which was not present in the Indonesian isolate. The GISAID database indicated that the isolate ESP_ISL_435282 had an amino acid substitution on the non-structural protein 2 (NSP 2) from isoleucine (I) to valine (V), NSP2 I281V, and non-structural protein 12 (NSP12) from alanine (A) to valine (V) NSP12 A399V. There had not been much research on NSP2 I128V mutation, however, NSP2 is claimed to be the key place of viral pathogenicity which could explain why the SARS-CoV-2 virus is more contagious than other SARS (Angeletti et al. 2020). By comparison with other research, NSP2 had been researched to be involved in many biological processes, such as endosome transport and translation, and had been claimed to take part in binding with human mRNA and disturbing splicing, translation, and protein trafficking (Zheng et al. 2021).

### 3.3. *Comparison and analysis of Wuhan isolate (reference) with Indonesian Delta variant*

The Wuhan isolate was then blasted with a Delta variant sample obtained from the GISAID database from Indonesia with high non-coding sequences. Pairwise similarities are shown in the line graph in Figure 2 and the empty spaces indicate the differences in genome or nucleotide sequences. The query cover was 96%; Thus, it suggested that the Wuhan isolate had more differences when compared to the Indonesian Delta variant sample, rather than the Indonesian isolate genome that was chosen. These dif-

ferences include substitutions and deletions of nucleotides, wherein some did not cause any amino acid changes, but some did. There are patterns of substitutions as well that can be observed in Supplementary Table 1. The most frequent substitutions in the ORF1ab gene include substitutions from nucleotide C to G, with seven occurrences out of twelve, and amino acid proline (P) substitutions which occurred five times out of twelve mutations.

In addition, the Indonesian virus isolate EPI_ISL_2537488.2 had an amino acid substitution from S26L on the ORF3a protein due to a C to T nucleotide substitution.

Supplementary Table 1 shows that there was a well-known mutation on the S gene that coded for the spike protein of the Delta variant, which was the D614G amino acid substitution. D614G substitution increases the infectivity and stability of virions, which resulted in a more vigorous entry of the virus into lung epithelial cells and causes viral replication enhancement (Plante et al. 2021b). Other important substitutions in the spike protein from EPI_ISL_2537488.2 include L452R, P681R, T478K (Cherian et al. 2021). P681R mutation causes an increase in infectivity because of the slight increase in proteolytic processing (Tada et al. 2021). L452R mutation could also possibly reduce the binding ability of REGN10933 and P2B-2F6 antibodies to the variant strains (Tada et al. 2021), which causes an increase in infectivity and a decrease in the neutralizing activity of RBD-specific mAbs

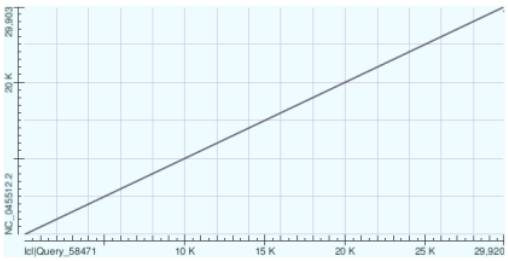


**FIGURE 3** The nucleotide similarity range of Wuhan SARS-CoV-2 Wild-Type Isolate (NC_045512.2 from NCBI) vs an Indonesian Wild-Type Isolate (ESP_ISL_435282 from GISAID) obtained from NCBI BLAST.

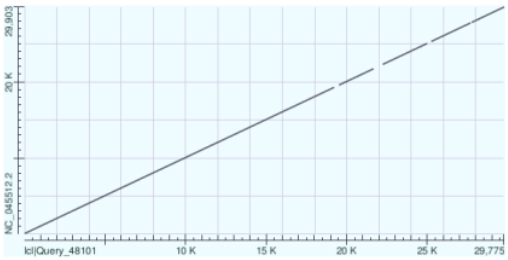


**FIGURE 4** The nucleotide similarity range of Wuhan SARS-CoV-2 Wild-Type Isolate (NC_045512.2 from NCBI) vs an Indonesian Delta Variant Sample (EPI_ISL_2537488.2 from GISAID) obtained from NCBI BLAST.

(Motozono et al. 2021). L452R and T478K mutations affect ACE2 binding, showing increased stabilization of the RBD-ACE2 complex (Cherian et al. 2021). P681R mutation, on the furin cleavage site, was also proven to aid transmissibility due to a higher rate of membrane fusion and internalization. This was because of an increase in the basicity of the poly-basic stretch and the higher rate of S1-S2 cleavage (Cherian et al. 2021). Aside from those common mutations found in the S gene, other mutations found from the EPI_ISL_2537488.2 include T19R, D950N, and V1264L.

It can be observed from the mutations of the ORF1ab gene in Supplementary Table 1 that there were many cytosine (C) to thymine (T) nucleotide substitutions and the next leading one was A to G. These high frequencies of mutation happening on the ORF1ab gene were explained because its codes for non-structural proteins (NSP1 to NSP16) had been studied to have the most missense mutation (Khailany et al. 2020), as can be observed in Supplementary Table 1, which explained why the ORF1ab gene had the most substitution mutations. The prominent C to T and A to G mutations can result in mutations of amino acids that affect the replication of the viruses' RNA (Yin 2020). Bakhshandeh et al. (2021) stated that C to T and A to G nucleotide substitutions in some positions of the *ORF1ab* gene are common in isolated genomes of Europe which causes severe infection with higher intensity than in other regions (Bakhshandeh et al. 2021). Mutations due to nucleotide and amino acid substitution can cause changes in the structure and function of proteins. Mutations in these genes could be assumed to help in inhibiting the immune response of the person with the virus, causing increased fatality. The virus is known to activate pathogenic Th1 to secrete proinflammatory cytokines (Hussman 2020).

Next, two different Delta variants isolated in Indonesia were blasted. The previous blast was from May 2021, and the next two were EPI_ISL_6262561 and EPI_ISL_6827388, from August and November 2021 with a 3-month period, respectively. There were signature spike mutations in the S gene typical for the Delta and Delta Plus variants. From the BLAST results, the EPI_ISL_2537488.2 and EPI_ISL_6262561 isolates were shown to have an atypical V1264L mutation in the S gene, and in the EPI_ISL_6827388 isolate, there were S704L and P1263L mutations.

In 2020, the mutation S704L was geographically found in North America (Guruprasad 2021). P1263L was typically found in the USA, Scotland, and Europe (Pulakuntla et al. 2021), and V1264L in North America. Aside from the geographical locations these mutations are mostly found in, not much about the effect of these mutations has been covered in previous research. Although EPI_ISL_6827388 in the GISAID database was said to have an amino acid substitution A222V, which would indicate a Delta Plus variant, this mutation was not shown from the BLAST results. The presence of an A222V mutation represents increased virality (Yang 2021b), while P1263L

**TABLE 2** Comparison of signature Delta and Delta Plus variant spike mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Signature Delta and Delta Plus Variant (*) Spike Mutations (Kannan et al. 2021) | Present Mutations | Other Mutations |
|---|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | | T19R, L452R, T478K, D614G, P681R, D950N | V1264L |
| 2 | EPI_ISL_6262561 (August 2021) | T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, E484Q, D614G, P681R, and D950N | T19R, L452R, T478K, D614G, P681R, D950N | V1264L |
| 3 | EPI_ISL_6827388 (November 2021) | | L452R, T478K, D614G, P681R, and D950N | S704L, P1263L |

is a mutation that is not included in the SARS-CoV-2 structure and is at the near-end tail of the spike protein (Korber et al. 2020). As seen in Table 2, there was an uncommon substitution of V1264L in two of the Indonesian isolates. This mutation was suspected to drive the pandemic in Indonesia, which may have improved the performance of the spike protein because it aided in optimizing the function of the protein's cytoplasmic tail, and helped in expanding the variant's evolutionary cage (Yang 2021a). Another major spike mutation from the BLAST results that were not present in the Indonesian isolates was the E484Q mutation. The signature mutations mainly work in increasing the affinity of ACE2 which increases the transmissibility, pathogenicity, and risk of immune escape (Khateeb et al. 2021). E484Q in particular with L452R demonstrated a decrease in sensitivity to vaccine-elicited neutralizing antibodies (Ferreira et al. 2021). The absence of E484Q mutation may indicate that vaccination could be more effective in Indonesia to fight the Delta variant. The mutation D950N has also been stated to improve the dynamics of the spike protein (Plante et al. 2021a).

Recorded mutations in the M gene of any other SARS-CoV-2 virus are shown in Table 3. The mutation V10A generates an N-myristylation site and P132S generates a casein kinase II phosphorylation site (Jakhmola et al. 2021). M proteins, on the other hand, interact with the other proteins, and changes in PKC phosphorylation sites may change the virus' endocytosis and dynamic ruffling (Hui and Nayak 2002). This feature was shown in the respiratory syncytial virus (RSV) which had this characteristic, and this helped evasion into the phagolysosome pathway (Duncan et al. 2002).

Table 3 shows that the isolates obtained from Indonesia only had the common M gene mutation for the Delta variant which is the I82T mutation. I82T mutation in the M gene was proposed to improve biological fitness and change the uptake of glucose during viral replication (Khateeb et al. 2021).

Table 4 shows that there was no similarity between normally found nucleocapsid phosphoprotein mutations in SARS-CoV-2 in India and the chosen Delta ones from Indonesia, but has the proven highly prevalent missense mutation of R203M and D377Y, specifically for the Delta variant (Suratekar et al. 2022). Mutations in the N gene of the SARS-CoV-2 virus, specifically the R203M mutation in the Delta variant, had been shown to improve the mRNA packaging and replication which creates higher levels to 1000-fold of viral RNA in patients (Li et al. 2021). The presence of this mutation could explain the rocketing of the number of SARS-CoV-2 cases from May to November based on Figure 1 in Indonesia. The mutations D63G, R203M, G215C, D377Y were in the N protein, and due to the high immunogenicity and conservation of the N gene, N proteins with these mutations were available and used as a diagnostic tool (Acro Biosystems 2021).

Mutations in the ORF7a protein can cause reduced immunity because it had been found to bind to human monocytes which would reduce the antigen-presenting ability and can result in high expression of pro-inflammatory cytokines (Zhou et al. 2021). Tabel 5 showed a comparison

**TABLE 3** Comparison of signature Delta variant glycoprotein mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Recorded Delta Glycoprotein Mutations (No Variant Specified) (Jakhmola et al. 2021) | Signature Delta Glycoprotein Mutations (Suratekar et al. 2022) | Present Mutations |
|---|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | | | - |
| 2 | EPI_ISL_6262561 (August 2021) | D3G, V10A, V70F, H125Y, P123S, D209Y, T175M, K15R | D3G, I82T | I82T |
| 3 | EPI_ISL_6827388 (November 2021) | | | I82T |

**TABLE 4** Comparison of signature Delta variant nucleocapsid mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Recorded Delta Nucleocapsid Phosphoprotein Mutations in India (No Variant Specified) (Azad and Khan 2021) | Signature Delta Nucleocapsid Phosphoprotein Mutations (Syed et al. 2021) | Present Mutations |
|---|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | P6T, P13L, S33I, R92S, G120R, L139F, A152S, A156S, R191L, S194L, S202N, R203K, G204R, M234I, G236C, P302S, P344S, D348Y, T362I, T393I | R203M, D377Y | R203M, G215C, D377Y |
| 2 | EPI_ISL_6262561 (August 2021) | | | D63G, R203M, G215C, D377Y |
| 3 | EPI_ISL_6827388 (November 2021) | | | D63G, R203M, G215C, D377Y |

**TABLE 5** Comparison of signature Delta variant ORF7a mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Signature NS7a/ORF7a Mutations (Gupta et al. 2021) | Present Mutations |
|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | V82A, T120I | In ORF7a: V82A<br>In ORF7b: T40I |
| 2 | EPI_ISL_6262561 (August 2021) | | In ORF7a: V82A, T120I<br>In ORF7b, T40I |
| 3 | EPI_ISL_6827388 (November 2021) | | In ORF7b, T40I |

between signature Delta variant ORF7a mutations with chosen Indonesian isolates.

The S26L mutation is common in ORF3a protein and was analyzed to have effects on the stability of the protein (Azad and Khan 2021). This mutation was also found in chosen Indonesian isolates as shown in Table 6.

Table 7 shows all three of the isolates contain P4715L (NSP 12) mutation common in the B.1.617.2 lineage in the ORF1ab polyproteins of the SARS-CoV-2 virus and contain many mutations from some sub-lineages of the Delta variant based on the research by (Gupta et al. 2021). P4715L mutation is one of the four significant mutations which acts either a replicase or helicase, and play an important role in viral RNA synthesis (Banerjee et al. 2021). The three isolates contain other mutations that were not present and similar mutations as well. These other mutations could play an important role in improved virality due to the ORF1ab playing an essential role in the synthesis of the virus' RNA. P5041L mutation plays a role as helicase and other mutated amino acids could be assumed with roles, such as immune evasion, innate immunity interaction, and inflammasome interaction (Banoun 2021).

It can be observed as well from the analyses with BLAST, that the mutations in the E gene and other protein genes for the three Delta variant samples from Indonesian isolates were not commonly present.

The three Delta variants were aligned with MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/index.html) and the phylogenetic tree was observed using the PhyloIO_, a sub-menu of the online software as done in the research by (Ulfah and Helianti 2021) to view the grouping of the isolates more clearly as in the research by (Wruck and Adjaye 2021). It was shown that the outgroup was only the original Wuhan isolate with accession ID NC_045512_2 from NCBI, while all the other three Indonesian isolates from GISAID were similar and grouped in one clade albeit the scale bars showing some sequence divergence. Thus, the Delta variant isolates from Indonesia had varying sequence divergence when compared to the original Wuhan wild type isolate.

### *3.4. Comparison and analysis of Indonesian Alpha variants*

Supplementary Table 3 shows that there was a mutation on the S gene that codes for the spike protein of the Alpha variant, which is N (Asparagine) to Y (Tyrosine) at position 501 (N501Y) which made binding to ACE2 receptor stronger (Tian et al. 2021). The other substitutions were D614G, P681H, and T1238I. The mutation at position 614 for the substitution of aspartic acid to glycine (D614G) has been explained previously. The proline to histidine mutation at position 681 (P681H) was proven to not significantly affect viral entry or cell-to-cell spread (Lubinski et al. 2022). There was also a threonine to isoleucine substitution (T1238I), a substitution of a polar to non-polar amino acid at position 1238, which was found to cause the forming of an extra helix because of the polarity change (Rehman et al. 2020).

**TABLE 6** Comparison of signature Delta variant ORF3a mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Signature NS3/ORF3a Mutations (Gupta et al. 2021) | Present Mutations |
|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | | S26L |
| 2 | EPI_ISL_6262561 (August 2021) | S26L | S26L |
| 3 | EPI_ISL_6827388 (November 2021) | | S26L |

**TABLE 7** Comparison of signature Delta variant ORF1ab mutations with chosen Indonesian isolates.

| No. | Indonesian Isolate Delta Variant Accession ID from GISAID | Signature ORF1ab Mutations (Gupta et al. 2021) | Present Mutations | Other Mutations |
|---|---|---|---|---|
| 1 | EPI_ISL_2537488.2 (May 2021) | Sub-lineage I: A1306S, P2046L, P2287S, T3255I, T3446A, G5063S, P5401L, and A6319V<br>Sub-lineage II: P309L, A3209V, V3718A, G5063S, and P5401L<br>Sub-lineage III: A3209V, V3718A, T3750I, G5063S, and P5401L | Sub-lineage I: A1306S, P2046L, P2287S, T3255I, T3446A, G5063S, P5401L, A6319V<br>Common in B.1.617.2 lineage: P4715L | P380L, T1496I, V2930L |
| 2 | EPI_ISL_6262561 (August 2021) | Sub-lineage IV: P309L, D2980N, and F3138S<br>Common in B.1.617.2 lineage: P4715L | Sub-lineage I: A1306S, P2046L, T3255I, A6319V<br>Common in B.1.617.2 lineage: P4715L | P380L, P1921Q, T3646A, I3738T |
| 3 | EPI_ISL_6827388 (November 2021) | | Sub-lineage I: A1306S, P2046L, T3255I, A6319V, P2287S<br>Sub-lineage II and III: G5063S, P5401L<br>Common in B.1.617.2 lineage: P4715L | A11V, T1822I, S2114F, V2930L, T3646A |

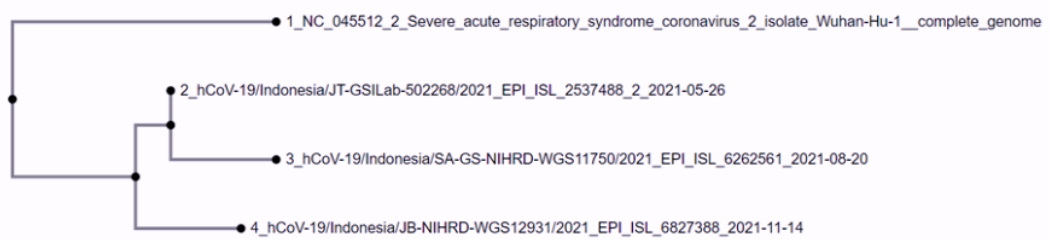


**FIGURE 5** Phylogenetic tree of chosen Delta variant SARS-CoV-2 Indonesian isolates with the NC_045512.2 as reference.

Supplementary Table 4 showed that the S gene that codes for D614G substitution affected the rise in infectivity and stability of virions, which results in a more vigorous entry of the virus into lung epithelial cells and causes viral replication enhancement (Plante et al. 2021a). Then there are other mutations R (Arginine) to M (Methionine),

G (Glycine) to C (Cysteine), and D (Aspartic Acid) to Y (Tyrosine).

The spike protein had been suspected to have more effects than its common signature. It was reported by a paper that the spike protein could change its shape to make it prone to bind to more cells, and some other papers show that it can harm endothelial cells by itself and can disturb the blood-brain barrier (Theoharides and Conti 2021). Due to the spike protein having a major role in the SARS-CoV-2 disease, for the Alpha and Beta Indonesian variants, the mutations in the S gene will be focused on to be analyzed.

Based on the analysis of Supplementary Tables 3 and 4, it can be concluded that some mutations in the Alpha variant are present as shown in Table 8.

Table 8 indicates that the Indonesian isolate contained the common H69del, N501Y, and A570D mutation, and instead of V70del, it had a nucleotide substitution of V70I. These mutations as mentioned were known to improve binding affinity to the ACE2 receptor and allow antibody escape. Infectivity is increased because of improved absorption of the cleaved spike into virions, which increases efficiency in virus entry (Meng et al. 2021). In addition, as previously mentioned, N501Y mutation displays an improved affinity of spike protein to ACE2 receptors, increasing viral attachment and entry into host cells (Aleem et al. 2021). Aside from that, all the present mutations in the S gene for the Indonesian Alpha variant combined have the possibility to increase transmission and infection severity (Mohammadi et al. 2021). However, not all signature mutations were present in the Indonesian isolates. The smaller number of Alpha variants, when compared to the Delta variant that had been collected in Indonesia, could be caused by this.

### 3.5. Comparison and analysis of Indonesian Beta variants

Based on the analysis of Supplementary Tables 5 and 6, it can be concluded that some mutations in the Beta variant are present as shown in Table 9.

The mutations from Table 9 combined can increase transmission and reinfection rates (Mohammadi et al. 2021). The mutations present in the Indonesian isolates were all included in the spike mutations of interest for the Beta variant that were proven to affect transmissibility, immunity, and severity (European Centre for Disease Prevention and Control 2021). It had been suggested that the mutations K417N and E484K may defeat the polyclonal antibody response, whereas E484K may facilitate escape from some antibodies (Harvey et al. 2021). The mutations N501Y and D614G had been discussed previously.

### 3.6. Effects, vaccination, and manifestations

The comorbidity diseases that can co-occur with SARS-CoV-2 include, ranging from highest to lowest proportion, hypertension, diabetes mellitus, cardiovascular diseases, chronic obstructive pulmonary diseases, respiratory problems, kidney diseases, asthma, and more (Karyono and Wicaksana 2021). As mentioned previously, one of the major point mutations in the Delta variant, the T478K, mutation in the S gene, aids the binding of the virus to human lung cells. Post-mortem tests were done previously by other research, and it was found that SARS-CoV-2 antigens damage pancreatic Beta cells of the pancreas which lessens the secretion of insulin (Müller et al. 2021). Thus, because the SARS-CoV-2 Delta variant was shown to weaken immunity to diabetes, and diabetic patients have a high risk of COVID-19 infection, vaccination is extremely recommended (Zhang et al. 2022).

In the context of vaccines, the Delta variant with its many mutations that have been covered has a significant reduction in the neutralization efficacy of sera from vaccines, similar to that of the Beta variant while the Alpha variant only has a modest reduction (Mohammadi et al. 2021). All these could explain how the Delta variant spread rapidly and fatally in Indonesia from May to August 2021, and why it remained the highest number of variants infecting residents from August to November 2021.

Some important mutations to note from the Discussion section and their clinical manifestations are shown in Table 10 with a focus on mutations on the spike protein.

The majority of symptomatic SARS-CoV-2 patients commonly present with fever, cough, and shortness of breath and less commonly, with a sore throat, anosmia, dysgeusia, anorexia, nausea, malaise, muscle pain, and diarrhea (Cascella et al. 2020). The SARS-CoV-2 variant of concern strain B.1.1.7, also known as the Alpha variant causes fever or chill, cough, shortness of breath, fatigue, muscle or body aches, headache, loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea, severe pneumonia (The Franciscan Missionaries of Our Lady Health Systeml 2021; Vassallo et al. 2021).

B.1.351 is another SARS-CoV-2 variant, also known as the Beta variant. There was no evidence that the symptoms of this variant differ from those of other variants. However, no large-scale studies have been conducted because the number of cases is small in comparison to other variants. Although the variant is more contagious than the original Wuhan virus, it does not appear to cause more se-

**TABLE 8** Comparison of signature Alpha variant spike mutations with chosen Indonesian isolates.

| Indonesian Isolate Alpha Variant Accession ID from GISAID | Signature Alpha Variant Mutations in Spike Protein (Rosa et al. 2021) | Present Mutations |
|---|---|---|
| EPI_ISL_3138832 | H69del (94%), V70del (95%), Y144del (94%), E484K (0.2%), S494P (0.3%), N501Y (97%), A570D (98%) | H69del, V70I, N501Y, A570D |

**TABLE 9** Comparison of signature Beta variant spike mutations with chosen Indonesian isolates.

| Indonesian Isolate Beta Variant Accession ID from GISAID | Signature Beta Variant Mutations in Spike Protein (Rosa et al. 2021; Mohammadi et al. 2021) | Present Mutations |
|---|---|---|
| | L18F,<br>D80A (87%),<br>D215G (85%),<br>L242- (78%),<br>L243- (77%),<br>R246I,<br>P384L (1.2%), | K417N, E484K, N501Y, D614G, A701V |
| EPI_ISL_2500469 | K417N (88%),<br>E484K (87%),<br>N501Y (87%),<br>N501Y (87%),<br>D614G,<br>A701V,<br>LAL 242-244 del | |

**TABLE 10** Biological manifestations of mutations in SARS-CoV-2 variants.

| No | Variant | Protein | Mutation | Biological Manifestation |
|---|---|---|---|---|
| 1 | Delta | S gene | V1264L | Helps in optimizing the function of the spike protein's cytoplasmic tail and expands the variant's evolutionary cage which increases the performance of the virus. |
| 2 | Delta | S gene | L452R | Improves infectivity by reducing the neutralizing activity of antibodies |
| 3 | Delta | S gene | T478K | Affects ACE2 binding |
| 4 | Delta | S gene | D614G | Improves infectivity and stability of virions causing more vigorous entry to host cells |
| 5 | Delta | S gene | P681R | Improves infectivity and transmissibility |
| 6 | Delta | S gene | D950N | Contribution in regulating dynamics of the spike protein |
| 7 | Delta | M gene | I82T | Increases uptake of glucose in viral replication and improves the biological life of the virus |
| 8 | Delta | N gene | R203M | Increases packaging of mRNA and replication |
| 9 | Delta | ORF3a gene | S26L | Affects the stability of protein |
| 10 | Alpha | S gene | H69del, N501Y, A570D, V70I | Increases transmission and infection severity, efficiency in virus entry, and binding affinity to ACE2 receptor for antibody escape |
| 11 | Beta | S gene | N501Y | Improves viral entry to cells |
| 12 | Beta | S gene | E484K | Aids in escape from antibodies |

vere disease (Gulland 2021).

Although not covered in this study, it is important to note the third variant of concern, the P.1 variant, which is the Gamma variant. This may occur usually with cold-like symptoms, but also with abdominal pain, diarrhea, nausea, and vomiting (Luna-Muschi et al. 2022). Hyposmia/anosmia and dysgeusia have also been reported to be more common in younger and female patients (Zahra et al. 2020; Lee et al. 2020).

A few reports suggested that the symptoms are different for patients infected with the Delta variant. The common symptoms for the Delta variant include coughing, effect on taste and smell, nausea, sore throat, tiredness, aches in muscles or joints, lung complications, headache, fever, and vomiting (CDC 2021).

The Delta variant has some differences where patients can have runny noses, sneezing, and persistent cough, with the normal symptoms of headache and fever. There is as well a difference in symptom severity for those fully vaccinated, single vaccinated, or unvaccinated. Fully vaccinated people who are infected with the Delta variant could have headaches, runny nose, sore throat, and loss of smell. Single-vaccinated people affected could have more symptoms including sore throat, sneezing, and persistent cough. Unvaccinated people are most severely affected that they can have fevers (Chowdhury et al. 2021).

The Alpha variant was known to cause many deaths due to severe pneumonia (Vassallo et al. 2021) and was

**TABLE 11** Clinical manifestations of mutations in SARS-CoV-2 variants.

| No | Variant | Clinical Manifestations |
|---|---|---|
| 1 | Aplha (B.1.1.7) | Fever, runny nose, shortness of breath, nausea or vomiting, coughing, muscle or body aches, headache, loss of taste or smell, sore throat, fatigue, severe pneumonia, diarrhea. |
| 2 | Beta (B.1.351) | No indication that the variant has different or various symptoms when compared to others. |
| 3 | Gamma (P.1) | Cold symptoms, abdominal pain, nausea/vomiting, hyposmia/dysosmia, dysgeusia. |
| 4 | Delta (B.1.617.2) | Symptoms are similar to patients with the common cold, e.g., fever, sore throat, rhinorrhea, and have less chance to lead to loss of smell when compared to other variants. |

known to have less effect on causing loss of smell (Chohan et al. 2021). Meanwhile, the Beta variant has been recorded to have similar symptoms to other variants with no difference due to the small number of cases (Gulland 2021).

The differences between the clinical manifestations of the researched variants for SARS-CoV-2 are shown in Table 11.

## 4. Conclusions

Most of the Indonesian isolates had mutations typical of that of variants found in the world, although there were some atypical ones, such as the mutation V1264L in the Delta variant that was suspected to worsen the pandemic in Indonesia. The Delta variant had the most mutations in the spike protein, which have been found to play essential roles in infectivity, vigorous entry into host cells, and more suspected major impacts when compared to the Alpha and Beta variants. This could explain why in the period of May to November 2021 in Indonesia, there was a rocketing cases for the Delta variant, unlike the other variants. As of November 2021, the number of SARS-CoV-2 cases in Indonesia was led by the Delta variant, with clinical manifestations of fever, runny nose, sore throat, and headache. The rapid growth of the Delta variant caused severe symptoms and hence contributed greatly to the adverse cases recorded in 2021.

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## Authors' contributions

KAA designed the study. ELL, TDFH analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript

## Competing interests

We declare that there are no competing interests.

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