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# S-D614G Mutation Reveals the Euro-America and East-Asia Origin SARS-CoV-2 Virus Spread in Indonesia

**Nina Bunga Anggraini[1], Dwi Listyorini[1,2*]**
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang
[2]Department of Biotechnology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang
Jln. Semarang 5, Malang, East Java 65145, Indonesia
*Corresponding Author:
e-mail: listyorini.aljabari@um.ac.id

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## ABSTRACT

COVID-19 is a pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. The first case was found in the city of Wuhan, Hubei province, China. The first case in Indonesia was reported in March 2020 and currently there are 0.5 million cases with a death rate of 3.1%. This rapid increase in cases is thought to due to presence of the mutant strain S-D614G, which causes a faster rate of infection and spread. The purpose of this study was to determine the presence of S-D614G mutations in Indonesian samples in order to find the origin of COVID-19 which was spread in Indonesia based on the *Spike* gene sequences and the *RdRp* genes from 25 countries, and one control sequence China/Wuhan-Hu-1 obtained from the NCBI and GISAID databases. Mutation analysis was carried out through multiple alignments using BioEdit software. Phylogenetic tree reconstruction using MEGA6 software with the Neighbor Joining method. This study found mutation of S-D614G in one Indonesian sample, namely the Indonesian/SBY9 sample along with 23 samples from Europe, America, and Africa. The phylogenetic tree reconstruction confirmed these findings; the mutated samples were closely related to samples from these continents, while the non-mutated Indonesian samples were closely related to sample from East Asia. These findings indicate that the origin of the SARS-CoV-2 virus in Indonesia possibly came from the East Asia cluster and the European-American cluster.

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## INTRODUCTION

COVID-19 is a pandemic caused by the Severe acute respiratory syndrome coronavirus (SARS-CoV-2) (Decaro & Lorusso, 2020; Gralinski & Menachery, 2020). This virus attacks human respiratory system (Rhotan *et al.*, 2020). Early 2020 this virus shocked the world with the surge of infected patients. The origin of this case began the discovery of pneumonia cluster cases with unclear etiology from Wuhan City, Hubei Province, China, according to the information released by World Health Organization (WHO) on December 31, 2019 (Shreen *et al.*, 2020). That case continued to develop until there are reports of fatality and cases reported outside China (Safrizal *et al.*, 2020). As of November 19, 2020 there were 56,564,475 cases worldwide with the number of deaths reaching 1,354,858 (Worldometer, 2020). The global COVID-19 outbreak is a pandemic that is very important to be taken seriously. Based on the predictive value set by the CDC around 20-60% of the population worldwide will be infected by this virus (Baud *et al.*, 2020).

Coronavirus was confirmed for the first time in Indonesia at March 2020 (Indonesian Ministry of Health, 2020). A patient was confirmed positive for

COVID-19 after attending an event in Jakarta where the patient had been in contact with a foreigner, the patient complained of fever, cough and blown (WHO, 2020). As of November 2020, there were 478,720 cases of COVID-19 in Indonesia and the death rate reached 15,530 or 3,2% of confirmed cases (Worldmeter.info, 2020; Satgas penanganan COVID-19, 2020).

Corona virus is transmitted by aerosol droplet or nasopharyngeal droplets of patient (Mao *et al.*, 2020). Aerosol or nasopharyngeal droplets are transmitted when people come into direct contact with patient (Safrizal *et al.*, 2020; Huang *et al.*, 2020). Several efforts to prevent the transmission rate of COVID-19 have been carried out, such as physical distancing, sels-quarantine, early detection, and basic protection in the form of wearing masks when traveling and lockdowns area (WHO, 2020; Susilo *et al.*, 2020).

SARS-CoV-2 virus is included in the subgenus Sarbecovirus, genus Betacoronavirus, and the Coronaviridae family (Khot & Nadkar, 2020) which has incubation symptoms similar to SARS-CoV 2016, there are fever, dry cough, blown, and various other symptoms different from each individual (Safrizal *et al.*, 2020; Gorbalenya *et al.*, 2020; Jin *et al.*, 2020). The incubation period for this virus is between 5-14 days (Jin *et al.*, 2020). The recovery rate from the COVID-19 reached 36% (Mahase *et al.*, 2020), even so this virus is an obstacle to human activities in various fields such as economy, development and education (Schleicher, 2020; Wren-Lewis, 2020).

SARS-CoV-2 virus has spread throughout the world along with the mobilization of its host cell (Zheng *et al.*, 2020). Differences in climatic conditions, environment and host cell antibodies will make the virus adapt or even mutate (Sanjuán & Domingo-Calap, 2016). Mutations that have an impact on the spread of SARS-CoV-2 are mutations in the 614th amino acid position of *Spike* protein or called S-D614G mutation, that is substitution of the aspartate to glycine (Laha *et al.*, 2020; Hu *et al.*, 2020). This mutation causes the virus become more pathogenic and spread faster (Korber *et al.*, 2020).

Research on the *Spike* protein is mostly carried out due to the important role of this protein in inserting the genetic material of the SARS-CoV-2 virus into host cells through the ACE2 receptor (Shang *et al.*, 2020; Tay *et al.*, 2020). According to Biswas & Majumder (2020) there are two type of SARS-CoV-2, there are type (O) and type (A2a). Type (O) is a type that does not have mutations in the *Spike* protein. Type A2a is a variant of SARS-CoV-2 that has a mutation in the amino acid S-D614G.

The crucial process after the insertion of the virus into host cell is the replication facilitated by the *RdRp* gene (Lung *et al.*, 2020). This gene produces the RNA polymerase enzyme which is needed in the replication process (Zhu *et al.*, 2020). The length of this gene is from 182 to 881 base pairs, that will be translated into protein with 60 to 269 amino acids long as orf1ab polyprotein (Parikesit & Nurdiansyah, 2020).

Phylogenetic tree reconstruction will provide information on the relationship, similarity based on genes and the origin of SARS-CoV-2 in Indonesia (Turista *et al.*, 2020). Information about the relationship and origin of SARS-CoV-2 in Indonesia can be a source to find out the type of SARS-CoV-2 in Indonesia. Therefore, the right steps can be found to overcome the COVID-19 pandemic in Indonesia. Hence, more in-depth research is needed the distribution, types of viruses and mutations that occur to be used as the basis for appropriate prevention and treatment efforts in the future. This research aimed to determine the presence of S-D614G mutations in Indonesian samples in order to find the origin of COVID-19 which was spread in Indonesia based on the Spike gene sequences and the RdRp genes from 25 countries, and one control sequence China/Wuhan-Hu-1 obtained from the NCBI and GISAID databases

## MATERIALS AND METHODS

This research was included in a qualitative descriptive study conducted in the Biotechnology room, O4 Building, Laboratorium Sentral Mineral dan Material Maju, Universitas Negeri Malang in June to August 2020. The samples of this study were 50 SARS-CoV-2 *Spike* and *RdRp* genes sequences from 25 countries obtained through data mining from the National Center for Biotechnology Information (NCBI) database and the Global Initative on Sharing All Influenza Data (GISAID). The control sequences used were RNA sequences of *Spike* and *RdRp* SARS-CoV-2 genes from Wuhan China (Wuhan-Hu-1).

The tools used are BioEdit for alignment sequences process and MEGA6 software for phylogenetic tree reconstruction using neighbour joining method. Data analysis was by observing the 1842th base to determine the presence of base substitution from Adenine (A) to Guanine (G). Analysis of the relationship and origin of the SARS-

CoV-2 viruses based on the formed phylogenetic tree.

## RESULTS AND DISCUSSION

Multiple alignment result of *Spike* gene using BioEdit software showed there was an 1842th base change in some samples from base A to G (Table 1). The multiple alignment result of *Spike* gene sequences using BioEdit software showed that there was mutation on 1842th base in several samples of the *Spike* gene, from base A to G (Table 1). This base substitution result in a change in the amino acid aspartate (GAT) to glycine (GGT) (Eaaswarkhanth *et al.*, 2020; Hu *et al.*, 2020).

**Table.1** Mutations A>G cause a change in the amino acid aspartate (S-D614) to glycine (S-G614).

| No | Sample Origin | Accession Number | A-G |
|---|---|---|---|
| 1 | Indonesia /Jakarta 1 | EPI_ISL_467370 | A |
| 2 | Indonesia/Jakarta 2 | EPI_ISL_437192 | A |
| 3 | Indonesia/Jakarta 3 | EPI_ISL_437191 | A |
| 4 | Indonesia/Jakarta 4 | EPI_ISL_437190 | A |
| 5 | Indonesia/Jakarta 5 | EPI_ISL_437189 | A |
| 6 | Indonesia/Jakarta 6 | EPI_ISL_435283 | A |
| 7 | Indonesia/Jakarta 7 | EPI_ISL_435282 | A |
| 8 | Indonesia/Jakarta 8 | EPI_ISL_435281 | A |
| 9 | Indonesia/Surabaya 9 | EPI_ISL_437188 | G |
| 10 | Indonesia/Surabaya 10 | EPI_ISL_437187 | A |
| 11 | Indonesia/Manado 11 | EPI_ISL_467375 | A |
| 12 | Indonesia/Samarinda 12 | EPI_ISL_467374 | A |
| 13 | Indonesia/Pasuruan 13 | EPI_ISL_458081 | A |
| 14 | China 1 | MT510727 | A |
| 15 | China/Guangzhou 2 | MT568634 | A |
| 16 | China/Guangzhou 3 | MT568638 | A |
| 17 | China/Guangzhou 4 | MT568641 | A |
| 18 | South Korea 1 | MT039890 | A |
| 19 | South Korea 2 | MT304474 | A |
| 20 | South Korea 3 | MT304475 | A |
| 21 | South Korea 4 | MT304476 | A |
| 22 | Japan 1 | LC556320 | G |
| 23 | Japan 2 | LC549340 | G |
| 24 | Taiwan | MT374104 | A |
| 25 | Hongkong | MT114412 | A |
| 26 | Malaysia | MT372481 | A |
| 27 | Australia/Northern terrritory | MT641648 | A |
| 28 | Australia/Victoria | MT451009 | G |
| 29 | India | MT415321 | G |
| 30 | Bangladesh | MT762396 | G |
| 31 | Saudi Arabia | MT755885 | G |
| 32 | Italy 1 | MT531537 | G |
| 33 | Italy/Lazio 2 | MT527178 | G |
| 34 | Italy/Lazio 3 | MT527184 | G |
| 35 | Spain | MT359866 | G |
| 36 | Netherlands | MT705206 | G |
| 37 | Germany | MT358643 | G |
| 38 | France | MT470150 | A |
| 39 | USA/Virginia | MT929083 | G |
| 40 | USA/North America | MT764170 | G |
| 41 | Brazil | MT738101 | G |
| 42 | Colombia | MT470219 | G |
| 43 | South Africa | MT324062 | G |
| 44 | Egypt 1 | MT627391 | G |
| 45 | Egypt 2 | MT510690 | G |
| 46 | Marocco | MT731292 | G |
| 47 | Guam | MT459985 | A |
| 48 | Jamaica | MT507793 | A |
| 49 | Serbia 1 | MT569470 | G |
| 50 | Serbia 2 | MT569471 | G |
| 51 | China/Wuhan-Hu-1 | NC_045512 | A |

Total samples that had mutations were 23 of the 50 samples used. The result of the alignment of 13 samples from Indonesia found 1 sample Indonesia/JKT9 that had mutation. Other samples that had mutations included 3 samples from Italy, two samples from America, Egypt, Japan and Serbia. One sample from Spain, Netherlands, South Africa, Morocco, Germany, France, Brazil, Colombia, India, Saudi Arabia, Bangladesh. The remaining 27 samples did not change base or were identical to the control sequence from China/Wuhan-Hu-1. Most of the samples that had mutations came from countries in Europe (Eaaswarkhanth *et al.* 2020) and America (Gudbjartsson *et al.*, 2020).

Mutations in viral RNA have a high rate and affect the process of spread and virulence (Hu *et al.*, 2020; Becerra-Flores & Cardozo, 2020). In the case of the S-D614G mutation, the substitution of aspartate to glycine causes the S-G614G strain virus to be more pathogenic and have higher transmission rate than the S-D614G strain (Becerra-Flores & Cardozo, 2020). This is due to the presence of single nucleotide deletion (delC) at a known variant site (rs35074065) in the cis-eQTL of TMPRSS2 which facilitates SARS-CoV-2 to be more easily inserting host cells (Bhattacharyya *et al.*, 2020). The *Spike* gene in the S-G614 strain divides faster due to the presence of new serine protease (elastase-2) cleavage site located near the S1-S2 junction of the *Spike* gene (Bhattacharyya *et al.*, 2020), this strain is also more stable than S-D614 so it is strongly suspected to have a more efficient transmission (Zhang *et al.*, 2020).

Until November 19, 2020 Indonesia was in the 19th position among countries with high cases of COVID-19 in the world with a total of 478,720 cases (Worldmeter, 2020; WHO, 2020; CSIS, 2020). East Java Province is the second highest number of COVID-19 cases in Indonesia after DKI Jakarta with total of 56,070 cases and highest fatality with a total of 4008 death (Satgas Penanganan COVID-19, 2020) although a mutant SARS-CoV-2 was found from East Java. It is suggested that there were some samples with mutation which were not yet uploaded to both NCBI GenBank and GISAID within the research sampling period. The discovery of the S-G614 variant virus from the city of Surabaya could be one of the causes of COVID-19 cases in Indonesia increase due to the high transmission rate of this strain (Bhattacharyya *et al.*, 2020). In addition, countries in Europe and America are in the row of countries with the most cases in the world (Bhattacharyya *et al.*, 2020; Worldometer., 2020) accompanied by many samples that have mutations in the amino acid 614 *Spike* gene on both continents.

Phylogenetic tree reconstruction based on the *Spike* gene form two large cluster, there are cluster A and cluster G. Most of the SARS-CoV-2 *Spike* gene sequences from Indonesia were in cluster A, but the Indonesian/SBY9 samples were in cluster G which was in one clade with Italy/LZ3 sample. The bootstrap value (54) revealed that the Indonesian/SBY9 sample has a close relationship to Italy/LZ3 sample. These results can be an indication that the Indonesian/SBY9 patient was in a close contact with a COVID-19 patient from Italy or the related patient was suspected of having a history of travel to Italy. This can occur due to the spread of the SARS-CoV-2 virus through aerosols or nasopharyngeal droplets which are transmitted when people have direct contact with infected persons (Safrizal *et al.*, 2020; Huang *et al.*, 2020).

The other samples from Indonesia were in cluster A. The sample Indonesia/JKT3 was in one branch with South Korea3 with a bootsrap value of 79. However, the Indonesia/JKT3 sample formed different branch along with Asian countries in which several small clades were formed. The samples of Indonesia/MND were in one branch with samples from China/GHZ2 and France with a bootsrap value of 85 and were in one clade with Jamaica and Taiwan. The sample of Indonesia/JKT6 was in one branch with South Korea2 with a bootsrap value of 80 and formed a small clade with the samples of Indonesia/JKT1 and Indonesia/SBY10. The samples of Indonesia/JKT7 and Indonesia/PSR13 were in one branch with a bootsrap value of 88 and formed a small clade with Indonesia/SMR12 and Australia/NT. The sample Indonesia/JKT5 was in one branch with China/GHZ3 with a bootsrap value of 76 and formed a small clade with China1 and China/Wuhan-Hu-1. The Indonesian/JKT2 sample was in one branch with the China/GHZ4 sample with a bootsrap value of 74 and formed a small clade with Indonesia/JKT4 and South Korea4. The sample Indonesia/JKT8 was in one branch with South Korea1 with bootsrap value of 87 and s in the same clade as Hongkong, Malaysia and Guam (Figure 1).

The results of the phylogenetic tree reconstruction also proved that the mutant samples formed separate clades from the non-mutant samples. Generally, samples of the *Spike* gene in cluster G (type A2a) were suggested came from European and American countries with type A2a since this type was commonly found in Europe and America which bear a non-synonymous S-D614G *Spike* gene mutation which making it easier for the virus to enter the lung cells of the host (Biswas *et al.*, 2020; Gudbjartsson, *et al.*, 2020). This is the reason why type A2a has the advantage of infecting and surviving which results in faster spread across geographic areas (Biswas *et al.*, 2020). One of the causes of mutations in viruses is the adaptation of a virus to geographic environmental conditions and the condition of host cell antibodies (Sanjuán & Domingo-Calap, 2016).

Although many samples of Indonesia were found in cluster A, Indonesian samples were not grouped in the same branch. This could be an indication that there are variations in the Indonesian *Spike* gene sequence. Most of the samples of Indonesian *Spike* genes are closely related to the samples from East Asia countries, especially China and South Korea. It can be assumed that the patient has had contact with foreigners from or had a history of travel to those two countries.

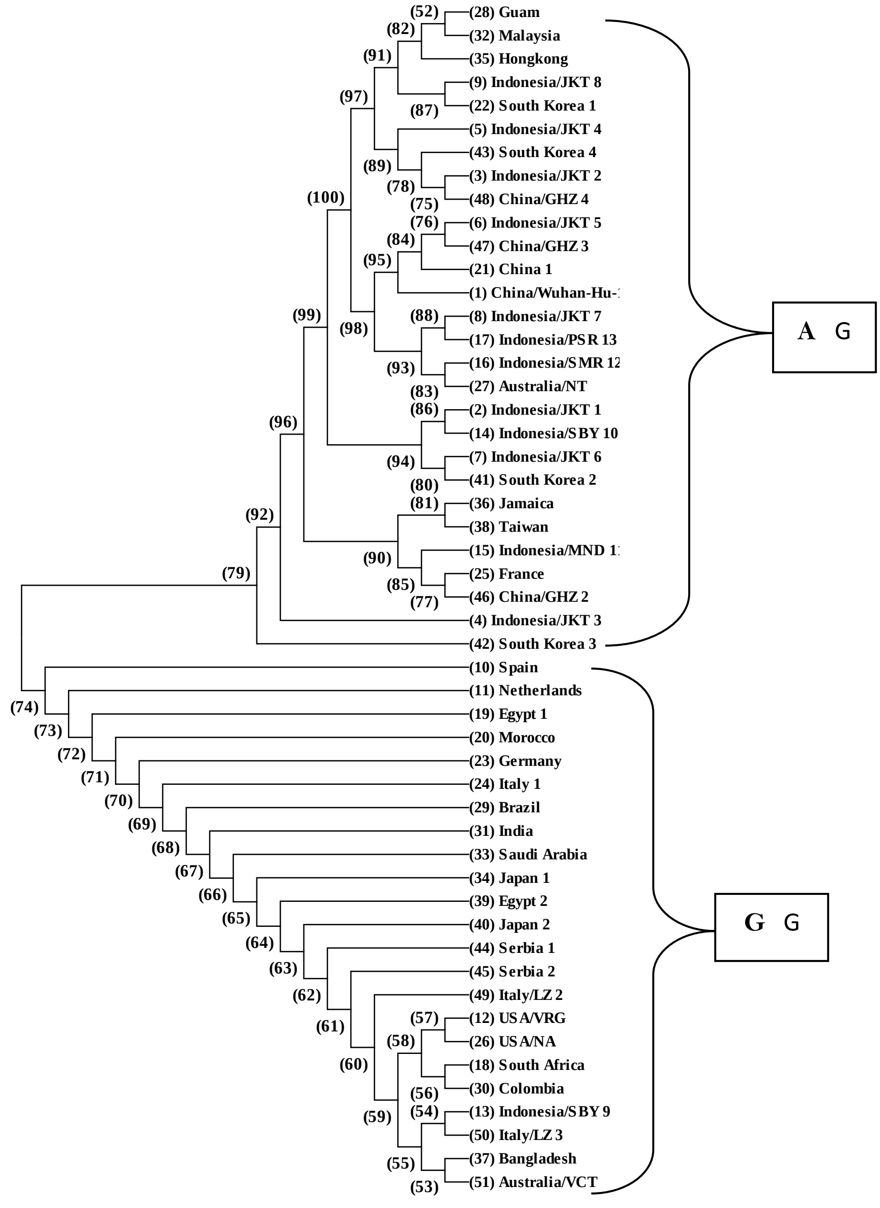

**Figure 1.** Phylogenetic tree reconstruction of *Spike* gene sequences using the Neighbour Joining method

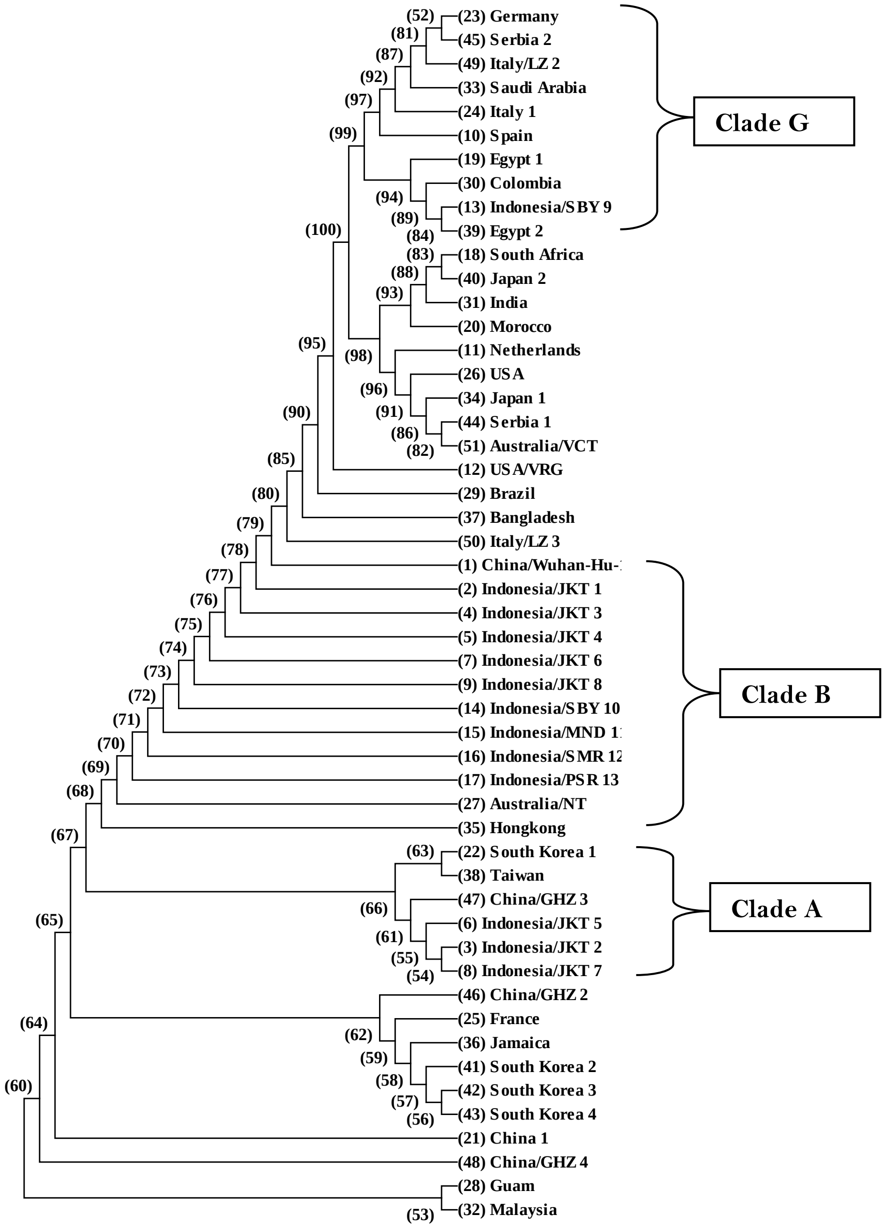

**Figure 2.** Phylogenetic tree reconstruction of *RdRp* gene sequences using the Neighbour Joining method

The result of phylogenetic tree reconstruction using the *RdRp* gene showed that Indonesian samples were in one large clade but separated in three different small clade (Figure 2). The non-mutant Indonesian samples on this phylogenetic tree are in the adjacent clade, namely clade A and clade B. In clade A the samples Indonesia/JKT2,

Indonesia/JKT5 and Indonesia/JKT7 are in one small clade with China/GHZ3 with bootsrap value 61, and formed a larger clade A with South Korea1 and Taiwan with bootsrap value 66.

Samples of Australia/NT, Hongkong and China-Wuhan-Hu-1 form clade B together with Indonesian samples including Indonesia/MND11, Indonesia/PSR13, Indonesia/SBY10, Indonesia/JKT8, Indonesia/JKT6, Indonesia/JKT4, Indonesia/JKT4, Indonesia/JKT3 and Indonesia/JKT1 with a bootsrap value of 68. In this clade, the Indonesian sample appears to form new sub-branches with varied bootstrapping values, thus indicating that there are variations between the *RdRp* gene sequences in the Indonesian sample. As well as having close similarities to Hongkong, Australia/NT and China-Wuhan-Hu-1 (Figure 2, Clade B).

In clade G, the sample Indonesia/SBY9 is in the same branch with Egypt2 with a value of 84 and is in a small clade with Colombia and Egypt1. The small clade was in one clade G together with samples from Spain, Italy/LZ2, Serbia and Germany with a value of 99. Samples in clade G were samples that had the S-D614G mutation or mutant strains.

The results of phylogenetic tree reconstruction using *Spike* and *RdRp* genes were found to be similar (Figure 1 & 2) where 12 samples of Indonesia are closely related to East Asian countries, while one sample Indonesia/SBY9, is closely related to countries from the continents of Europe, Africa and America. This indicates that the origins of the SARS-CoV-2 virus in Indonesia came from East Asia, the continent of Europe, America and Africa either through direct patient contact with foreigners from these countries or a history of travel to these countries.

The *Spike* gene and the *RdRp* gene are two crucial genes in the process of viral infection into host cells. The *Spike* gene acts as an intermediary for fusion between the virus and the cellular membrane of the host cell through endocytosis (Zhang *et al.*, 2020; Tay *et al.*, 2020), and the *RdRp* gene activates the RNA polymerase enzyme for the virus replication process in the host cell (Lung *et al.*, 2020). High variations through this research can cause obstacles for scientists to determine drugs based on the anti-sense silencing RNA (SiRNA) method (Parikesit & Nurdiansyah, 2020). Transmission of the SARS-CoV-2 virus through aerosols or nasopharyngeal droplets which is transmitted when people make direct contact with patient (Safrizal *et al.*, 2020; Huang *et al.*, 2020) accompanied by high mobilization and interaction between humans in their daily lives will also be an obstacle as well in reducing cases of COVID-19. Therefore, vaccines, physical distancing (Aminnejad & Alikhani, 2020), wearing masks (Howard *et al.*, 2021) and diligently washing hands are still the most appropriate steps to take for prevention of each individual in preventing the spread and transmission of COVID-19 (Bloomfield *et al.*, 2020).

## CONCLUSION

This study found a mutation of the *Spike* gene at amino acid14 in Indonesian/SBY9 sample originating from the city of Surabaya. The *Spike* gene sequences and the *RdRp* gene from 12 samples from Indonesia are closely related to countries from East Asia. One sample from Indonesia is closely related to countries from the European-American continent, Italy, more specifically.

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## REFERENCES

Aminnejad, R., Alikhani, R. (2020). Physical distancing or social distancing: that is the question Physical distancing or social distancing : that is the question. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie.* 67, 1457–1458. https://doi.org/10.1007/s12630-020-01697-2

Baud, D., X. Qi, X., Nielsen-Saines, K., Musso, D., Pomar, L., Favre, G. (2020). Real estimates of mortality following COVID-19 infection. *The Lancet Infectious Diseases.* 773. https://doi: 10.1016/S1473-3099(20)30195-X.

Becerra-Flores, M., Cardozo, T. (2020). SARS-CoV-2 viral *Spike* G614 mutation exhibits higher case fatality rate. *International Journal of Clinical Practice*, 74(8), 4–7. https://doi: 10.1111/ijcp.13525.

Bhattacharyya, C., Das, C., Ghosh, A., Singh A,K., Mukherjee, S., Majumder, P, P., Basu, A., Biswas, N,K. (2020). Global Spread of SARS-CoV-2 Subtype with *Spike* Protein Mutation D614G is Shaped by Human Genomic Variations that Regulate Expression of TMPRSS2 and MX1 Genes. *bioRxiv.* https://www.biorxiv.org/content/10.1101/2020.05.04.075911v1.full

Biswas, N.K., Majumder, P.P. (2020). Analysis of RNA Sequences of 3636 SARS-COV-2 Collected From 55 Countries Reveals Selective Sweep of One Virus Type. *Indian J Med Res.* 151(5), 450-458. https://doi: 10.4103/ijmr.IJMR_1125_20.

Bloomfield, S. F. Pharm, B., Boyle, C., Larson, E.L. (2007). The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers. *American Journal of Infection Control - Amer J Infect Control.*35. https://doi: 10.1016/j.ajic.2007.07.001.

CSIS Indonesia. (2020). Indeks intensitas Covid-19. https://covid19.csis.or.id (Accesed 6th of September 2020).

Decaro, N., Lorusso, A. (2020). Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Veterinary Microbiology.* 244.https://doi.org/10.1016/j.vetmic.2020.108693

Eaaswarkhanth, M., Al-Madhoun, A., Al-Mulla, F. (2020).

Could the D614G substitution in the SARS-CoV-2 *Spike* (S) protein be associated with higher COVID-19 mortality?. *International Journal of Infectious Diseases.* 96, 459–460. https://doi: 10.1016/j.ijid.2020.05.071.

Gorbalenya, A.E., Baker, S.C., Baric, R,S., Groot, R.J., Dosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., Leontovich, A.M., Neuman, B.W., Penzar, D., Perlman, S., Poon, L.L., Samborskiy, D.V., Sidorov, I.A., Sola, I., Ziebuhr, J. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology.* 5(4), 536–544. https://doi.org/10.1038/s41564-020-0695-z

Gralinski, L.E., Menachery, V.D. (2020). Return of the coronavirus: 2019-nCoV. *Viruses MDPI.* 12(2), 135. https://doi.org/10.3390/v12020135

Gudbjartsson, D.F., Helgason, A., Jonsson, H., Magnusson, O.T. (2020). Spread of SARS-CoV-2 in the Icelandic Population. The New England Journal of Medicine. 382, (2302-2315). http://doi: 10.1056/NEJMoa2006100

Hu, J. He, C.L., Gao, Q.Z., Cao, X., Long, Q., Deng, H., Huang, L., Chen, J., Wang, K., Tang, N., Huang, A. (2020). The D614G mutation of SARS-CoV-2 *Spike* protein enhances viral infectivity and decreases neutralization sensitivity to individual convalescent sera. *bioRxiv.* https://doi.org/10.1101/2020.06.20.161323.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 395(10223). https://doi.org/10.1016/S0140-6736(20)30183-5

Indonesian Ministry of Health. 2020. *Pedoman Pencegahan Dan Pengendalian Coronavirus Disesase (Covid-19).* Revisi Ke-5. pp. 21.

Jin, X., Lian, J.S., Hu, J.H., Gao, J., Zheng, L., Zhang, Y.M., Hao, S.R., Jia, H.Y., Cai, H., Zhang, X.L., Yu, G.D., Xu, K,J., Wang, X.Y., Gu, J.Q., Zhang, S.Y., Ye, C.Y., Jin, C.L., Lu, Y.F., Yu, X., Yu, X.P., Huang, J.R., Xu, K.L., Ni, Q., Yu, C.B., Zhu, B., Li, Y.T., Liu, J., Zhao, H., Zhang, X., Yu, L., Guo, Y.Z., Su, J.W., Tao, J.J., Lang, G.J., Wu, X.X., Wu, W.R., Qv, T.T., Xiang, D.R., Yi, P., Shi, D., Chen, Y., Ren, Y., Qiu, Y.Q., Li, L.J., Sheng, J., Yang, Y. (2020). Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut.* 69**,** 1002-1009. http://dx.doi.org/10.1136/gutjnl-2020-320926

Khot, W.Y., Nadkar, M.Y. (2020). The Novel Coronavirus Outbreak-A Global Threat. *Journal of Association of Physicians of India.* 68(3), 67-71.

Korber, B. Fischer, W.M., Gnanakaran, S., Yoon, H., Abfalterer, J.T.W., Giorgi, B.F.EE., Bhattacharya, T., Parker, M.D., Partridge, D.G., Evans, C.M., Freeman, T.M., de Silva, T.I., LaBranche, C.C., Monteifiori, D.C. (2020). *Spike* mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2. *bioRxiv.* https://doi.org/10.1101/2020.04.29.069054

Laha, S. Chakraborty, J., Das, S., Manna, S.K., Biswas, S., Chatterjee, R. (2020). Characterizations of SARS-CoV-2 mutational profile, *Spike* protein stability and viral transmission. *bioRxiv.* . https://doi.org/10.1101/2020.05.03.066266

Lung, J. Lin, Y., Yang, Y., Chou, Y., Shu, L., Cheng, Y., Liu, H., Wu, C. (2020). The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. *Journal of Medical Virology.* 92(6), 693–697. https://doi: 10.1002/jmv.25761.

Mahase, E. (2020). Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. *BMJ.* 368. https://doi: 10.1136/bmj.m641.

Mahase, E. (2020). Coronavirus: Wales tests 90% of suspected patients in their own home," *BMJ.* 368, 698. https://doi: 10.1136/bmj.m698.

Mao, L., Jin, H., Wang, M. (2020). Neurologic Manifestations of Hospitalized Patients with Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol.* 2020;77(6), 683–690. https://doi:10.1001/jamaneurol.2020.1127

Parikesit, A.A., Nurdiansyah, R. (2020). The predicted structure for the anti-sense SiRNA of the RNA polymerase enzyme (*RdRp*) Gene of the SARS-CoV-2. *Jurnal Ilmu-ilmu Hayati.* 19 (1), 97-108.

Rothan, H.A., Byrareddy, S.N. (2020). The Epidemology and Phatogenesis of Coronavirus Diseases (COVID-19) Outbreak. *Journal of Autoimunity.* 109, 1-4. https://doi: 10.1016/j.jaut.2020.102433.

Safrizal, Z.A., Putra, D.I., Sofyan, S., Bimo. (2020). *Pedoman Umum Menghadapi Pandemi COVID-19 Bagi Pemerintah Daerah.* Kementrian Dalam Negeri: Jakarta

Sanjuán, R., Domingo-Calap, P. (2016). Mechanisms of viral mutation. *Cell. Mol. Life Sciences.* 73(23), 4433–4448. https://doi: 10.1007/s00018-016-2299-6

Satgas Penanganan COVID-19. (2020). *Peta Sebaran Kasus Per Provinsi.* https://covid19.go.id/peta-sebaran. (Accesed 6th of September 2020).

Schleicher, A. (2020). *The Impact of Covid-19 on Education Insights From Education At a Glance* 2020. pp. 1–31.

Shang, J. Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., Li, F. (2020) "Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences May*

*2020.* 117 (21), 11727-11734. https://doi: 10.1073/pnas.2003138117

Susilo, A., Rumende, C.M., Pitoyo, C.W., Santoso, W.D., Yulianti, M., Herikurniawan., Sinto, R., Singh, G., Nainggolan, L., Nelwan, E.J., Chen, L.K., Widhani, A., Wijaya, E., Wicaksana, B., Maksum, M., Annisa, F., Jasirwan, C.O., Yunihastuti, E. (2020). Coronavirus Disease 2019: Tinjauan Literatur Terkini. *Jurnal Penyakit dalam Indonesia.* 7(1), 45-69. http://dx.doi.org/10.7454/jpdi.v7i1.415

Tay, M. Z. Poh, C.M., Renia, L., MacAry, P.A., Lisa, F.P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews Immunology.* 20(6), 363–374. https://doi: 10.1038/s41577-020-0311-8.

Turista, D. D. R., Islamy, A., Kharisma, V.D., Ansori, A.N.M. (2020). Distribution of COVID-19 and phylogenetic tree construction of sars-CoV-2 in Indonesia. *Journal of Pure and Applied Microbiology.* 14, 1035–1042. https://doi: 10.22207/JPAM.14.SPL1.42.

World Health Organization. Situation Report – 42 [Internet]. 2020 [updated 2020 March 02; cited 2020 March 15]. Available from: https://www.who.int/docs/default-source/coronavirus/situation-reports/20200302-sitrep-42-covid-19. pdf?sfvrsn=224c1add_2.

Worldometers. (2020) WorldoMeter: Real Time World Statistics. Current World Population. Worldo Meter. https://www.worldometers.info/coronavirus/. (Accessed 6th of September 2020).

Wren-Lewis, S. (2020) *The economic effects of a pandemic, Economics in the Time of COVID-19.* https://www.socialeurope.eu/the-economic-effects-of-a-pandemic

Zhang, L, Jackson, C.B, Mou H., Ojha, A., Peng, H., Quinlan, B.D., Rangarajan, E.S., Pan A., Vanderheiden, A., Suthar, M.S., Li, W., Izard, T., Rader, C., Farzan, M., Choe, H. (2020) SARS-CoV-2 *Spike*-protein D614G mutation increases virion *Spike* density and infectivity. *Nature Communication.* 11:6013 https://doi.org/10.1038/s41467-020-19808-4

Zheng, Y.Y., Ma, Y.T., Zhang, J.J., Xie, X. (2020). COVID-19 and the cardiovascular system. *Nature Reviews Cardiology.* 17(5), 259–260. https://doi.org/10.1038/s41569-020-0360-5

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., Tan, W. (2020) A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine* 382(8), 727–733. https://DOI: 10.1056/NEJMoa2001017

Zhu, W., Chen, C.Z., Gorshkov, K., Xu, M., Lo, D.C., Zheng, W. (2020). RNA-Dependent RNA Polymerase as a Target for COVID-19 Drug Discovery. *SAGE Journals.* 25(10), 1141-1151. https://doi: 10.1177/2472555220942123.