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Mini Review
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Effect of RBD Mutation (Y453F) in Spike Glycoprotein of SARS-CoV-2 on Neutralizing Antibody Affinity

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Abstract

Background: Infection with Receptor-Binding Domain (RBD) mutant (Y453F) of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from farmed minks is known to widely spread among humans.

Methods: We investigated the characteristics of SARS-CoV-2 RBD Y453F mutant using three-dimensional structural analysis. We investigated the effect of the RBD Y453F mutant of SARS-CoV-2 on neutralizing antibodies in serum derived from Coronavirus Disease 2019 (COVID-19) positive patients.

Results: Our studies suggest that virus variants with RBD Y453F mutation partially escaped detection by four neutralizing monoclonal antibodies and neutralizing antibodies in serum.

Conclusion: Consequently, raising concern that infection of SARS-CoV-2 mutants that cause serious symptoms in humans may spread globally.

Keywords: RBD Y453F mutant; Neutralizing antibody; SARS-CoV-2; COVID-19

Abbreviations: AIDS: Acquired Immunodeficiency Syndrome; COVID-19: Coronavirus Disease-2019; HIV: Human Immunodeficiency Virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; RBD: Receptor Binding Domain; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; UK: United Kingdom

Description

Mutations in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can jeopardize the efficacy of potential vaccines and therapeutics against coronavirus disease-2019 (COVID-19). Animals of the Mustelidae family, such as minks and ferrets can be infected with SARS-CoV-2 relatively easily compared with other mammals [1]. However, the reason why SARS-CoV-2 is extremely contagious to these animals remains to be elucidated. Nonetheless, it is clear that when several farmed minks kept in a high-density environment are infected with SARS-CoV-2, the virus proliferates in large numbers. Consequently, humans and minks may be at high risk of SARS-CoV-2 infection.

Natural selection "adaptation" in the coronavirus can occur during coronavirus amplification *in vivo* in farmed minks [2]. Natural selection in such viruses is observed bby the introduction of mutations in SARS-CoV-2 is not observed during the growth process in humans [2,3]. Infection with a mutant (Y453F) of SARS-CoV-2 from farmed minks is known to widely spread among humans 4 (Supplementary Figure 1). We investigated the virological characteristics of this SARS-CoV-2 mutant (Y453F) using three-dimensional protein structural analysis (Spanner program, MOE protein program and Cn3D macromolecular structure viewer) [4-6].

Literature Review

Analyzes the three-dimensional structure of the binding site between mink and human ACE2

SARS-CoV-2 mutant has an amino acid mutation Y453F in the sequence encoding spike glycoprotein [4]. This SARS-CoV-2 mutant has been detected in approximately 300 viral sequences isolated from the European and Dutch population as well as in minks.

Data on the three-dimensional structure of the receptor binding domain (RBD) of the spike glycoprotein of SARS-CoV-2 (PDB ENTITY SEQ 6VW1_1) was used in these studies [5]. Data (PDB: 6XC2, 6XC4, 7JMP, 7JMO, 6XKQ, and 6XKP) (Supplementary Figure 4) on the three-dimensional structure of six neutralizing antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, and CV07-270) (Supplementary Figure 4) that bind to the spike glycoprotein of SARS-CoV-2 was used in these studies [6].

Using the Spanner program, we predicted the three-dimensional structure of the SARS-CoV-2 spike glycoprotein Y453F mutant based on PDB (ENTITY SEQ 6VW1_1). We investigated the binding of the spike glycoprotein Y453F mutant of SARS-CoV-2 to human angiotensin-converting enzyme 2 (ACE2) and determined the affinity of the spike glycoprotein Y453F mutant of SARS-CoV-2 to six neutralizing monoclonal antibodies using the MOE program (three-dimensional protein structure modeling, protein docking analysis: MOLSIS Inc., Tokyo, Japan) and Cn3D macromolecular structure viewer.

Analyses of protein contact residues and protein buried surface areas Protein contact residues were analyzed using the LigPlot+

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program (v.1.4.5) (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). Protein buried surface areas were analysed using PDBePISA tool (http://pdbe.org/pisa/) and MOE project DB (MOLSIS Inc. Tokyo Japan). The modeling and Docking of the mink ACE2 protein and RBD in Spike Glycoprotein SARS-CoV-2 was analyzed by MOE project DB with previously posted ID PDB and protein ID (MOLSIS Inc. Tokyo Japan). The binding affinity between mink ACE2 and RDB in Spike Glycoprotein of SRARS-CoV-2 was analyzed by MOE project DB (MOLSIS Inc.).

Adsorption of RBD or RBD Y453F recombinant protein to the solid phase surface of the ELISA plate

HeLa cells were then transfected with pcMV3-2019-nCov-RBD-Flag tag expression vector (2 μg), or the pcMV3-2019-nCov-RBD Y453F-FLag tag expression vector (2 μg) (Sino Biological Inc. Beijing, China). The transfected cells with each 2019-nCov-RBD or 2019-nCov-RBD Y453F expression vector were incubated for an 48 h prior to harvesting. The RBD and the RBD Y453F recombinant proteins were purified using His tag column by standard procedure. Purified RBD or RBD Y453F recombinant protein was adsorbed on the solid phase surface of the ELISA plate (SMILON ELISA plate MS-3508F, Tokyo Japan).

Quantitative measurement of SARS-CoV-2 neutralizing antibody levels

The SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit (EpiGentek Group Inc., NY) contains all reagents necessary for quantitatively measuring SARS-CoV-2 neutralizing antibody level. In this assay, RBD or RBD Y453F of SARS-CoV-2 spike protein is stably pre-coated onto microplate wells. His-tagged ACE2 is bound to the coated spike protein in the presence or absence of neutralizing antibody contained in the sample. The amount of the bound ACE2, which is proportional to ACE2 inhibition intensity, is then recognized by the Neutralizing Detection Complex containing anti-His antibody and measured through an ELISA-like reaction by reading the absorbance in a microplate spectrophotometer at a wavelength of 450 nm. The neutralizing antibody level is inversely proportional to the optical density intensity measured. The higher the neutralizing antibody is, the lower the OD intensity is. Quantitative measurements of SARS-CoV-2 neutralizing antibody levels were performed according to the manufacturer's procedure.

Qualitative assay: Neutralizing inhibition ≥ 20% indicates Positive and SARS-CoV-2 neutralizing antibody detected; <20% indicates Negative and No detectable SARS-CoV-2 neutralizing antibody. The detail of the manufacturer's procedure is indicated in the supplementary materials. Serum from 10 normal (negative for COVID-19) patients and 20 COVID-19 patients with varying immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody levels. COVID-19 status was confirmed with RT-PCR, antigen, and/or antibody serology tests. 25 μL per sample. All serum samples were provided from RayBiotech (RayBiotech Life GA).

Details of materials and methods are described in the materials and methods section of supplementary materials.

Results

Studies by the Spanner program revealed that the Y453F mutation did not affect the three-dimensional structure of conventional SARS-CoV-2 spike glycoproteins (Supplementary Figure 2). From the present study, it was clarified that the binding property of the spike glycoprotein Y453F mutant to human ACE2 was slightly weaker than that of the conventional SARS-CoV-2 spike glycoprotein (Figure 1A). This was due to the replacement of Tyr at position 453 by Phe, which was unable to form a hydrogen bond with His at position 34 in human ACE2.

The present study revealed that the affinity between the spike glycoprotein Y453F mutant and four of the six monoclonal antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270) examined was clearly weak compared with the conventional SARS-CoV-2 spike glycoprotein (Figure 1B, Table 1, Supplementary Figure 3A-3F, Supplementary Figure 4).

Binding energy between RBD and Human ACE2				**Effect of mutation on antibody**
RBD	**RBD (Y453F)**			
-12.39 Kcal/mol	-10.27 Kcal/mol			Slightly lower
Neutralizing antibody	**Binding energy between RBD and antibodies**			**Effect of mutation on antibody**
	RBD	**RBD (Y453F)**		
CC12.1	-14.65 Kcal/mol	-8.91 Kcal/mol		Low affinity
CC12.3	-5.85 Kcal/mol	-1.36 Kcal/mol		Low affinity
COVA2-39	not calculated	not calculated		NA
COVA2-04	-12.4 Kcal/mol	-3.42 Kcal/mol		Low affinity
CV07-250	-1.09 Kcal/mol	-0.23 Kcal/mol		Low affinity
CV07-270	not calculated	not calculated		NA
Patient Serums	**Number of samples with affinity for RBD**			**Effect of mutation on antibody**
	RBD	**RBD (Y453F)**	**Both**	
Positive 21 samples	14 samples	2 samples	2 samples	Low affinity
Negative 10 samples	0 sample	0 sample	0 sample	NA

Table 1: Affinity of RBD or RBD Y453F in spike glycoprotein of SARS-CoV-2 with neutralizing antibodies or serum from each patient. The binding of RBD Y453F mutant in the spike glycoprotein of SARS-CoV-2 to human ACE2 and the affinity of RBD Y453F mutant in the spike glycoprotein of SARS-CoV-2 to six neutralizing monoclonal antibodies were investigated using the MOE program (three-dimensional protein structure modeling, protein docking analysis: MOLSIS Inc., Tokyo, Japan) and Cn3D macromolecular structure viewer. Binding energy calculated by the MOE program is shown in the table. Quantitative measurement of SARS-CoV-2 neutralizing antibody levels is examined by using SeroFlash ™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit (EpiGentek Group Inc. NY). The strong affinity for RBD was shown in IgG in the serum of 14 of the 21 COVID-19-positive patients. However, no affinity for RBD Y453F was shown in the serum IgG of 19 of the 21 COVID-19-positive patients. Weak affinity for both RBD and RBD Y453F was shown in IgG in the serum of 2 of the 21 COVID-19-positive patients. No affinity for RBD or RBD Y453F was shown in IgG in the serum of all 10 COVID-19 negative subjects. Detailed experimental results are indicated in Supplementary Table 1.

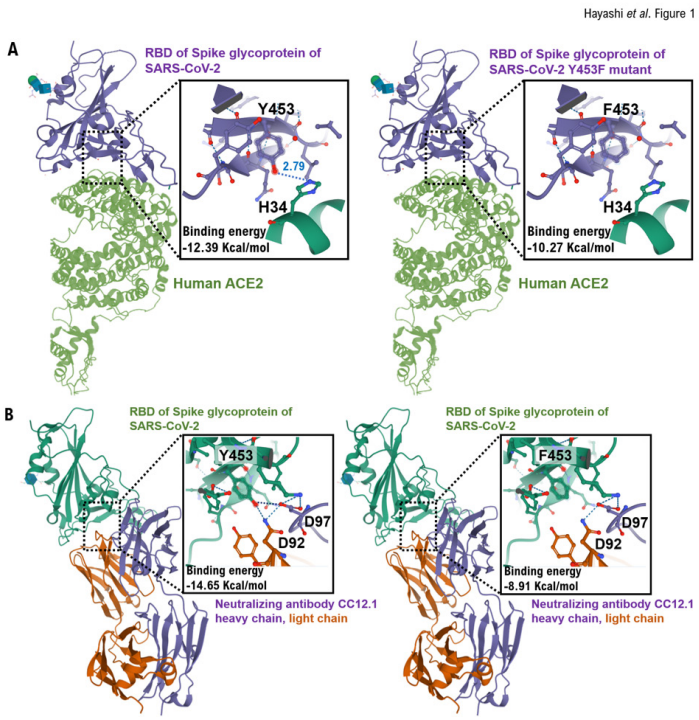

Figure 1: Interactions between the RBD and RBD Y453F mutant in the spike glycoprotein of SARS-CoV-2 and heavy chain of neutralizing monoclonal antibody (CC12.2). (A) The interaction between Angiotensin-converting enzyme 2 (ACE2) (green) and residues of the conventional receptor binding domain (RBD) or RBD Y453F mutant (purple) is shown using the three-dimensional structure model. It is speculated that the Y453 amino acid residue of the conventional RBD is hydrogen-bonded to the H34 amino acid residue of human ACE2. However, the binding between the F453 amino acid residue of the RBD mutant and the H34 amino acid residue of human ACE2 is presumed to be slightly weak. From these results, the affinity between the spike glycoprotein of the RBD Y453F mutant and human ACE2 is presumed to be slightly weak compared with the conventional RBD (B) The interaction between the heavy chain (purple) and light chain (brown) of neutralizing monoclonal antibody CC12.1 and residues of the conventional RBD or RBD Y453F mutant (green) is shown using the three-dimensional structure model. It is speculated that the Y453 amino acid residue of the conventional RBD is hydrogen-bonded to the D92 amino acid residue of the light chain and D97 amino acid residue of the heavy chain of neutralizing monoclonal antibody CC12.1. However, the binding between the F453 amino acid residue of the RBD mutant and the D92 and D97 amino acid residues of neutralizing monoclonal antibody CC12.1 is presumed to be weak. From these results, the affinity between the spike glycoprotein of the RBD Y453F mutant and neutralizing monoclonal antibody CC12.1 is presumed to be low compared with the conventional RBD. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in Supplementary data.

Therefore, we investigated the affinity of IgG in the serum of COVID-19-positive patients and IgG in the serum of healthy subjects for RBD or RBD Y453F. The affinity of IgG in the serum of 21 COVID-19-positive patients and IgG in the serum of 10 COVID-19 negative subjects with RBD or RBD Y453F was examined by Enzyme-Linked Immuno Sorbent Assay (ELISA) (SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit: EpiGentek Group Inc. NY). As a result, a strong affinity for RBD was shown in IgG in the serum of 14 of the 21 COVID-19-positive patients (Table 1, Supplementary Table 1). However, no affinity for RBD Y453F was shown in the serum IgG of 19 of the 21 COVID-19-positive patients (Table 1). Affinity for both RBD and RBD Y453F was shown in IgG in the serum of 2 of the 21 COVID-19-positive patients (Table 1, Supplementary Table 1). No affinity for RBD or RBD Y453F was shown in IgG in the sera of all 10 COVID-19 negative subjects (Table 1, Supplementary Table 1). From these results, the mutation of tyrosine at amino acid residue 453 of RBD to phenylalanine eliminated the inhibitory effect of the neutralizing antibody on binding between ACE2 and RBD.

It is considered that the affinity between the appropriate amino acid residues in the variable region of the antibody and the spike glycoprotein Y453F mutant was diminished owing to weak recognition of the monoclonal antibody to spike glycoproteins.

Discussion

To the best of our knowledge, data on all SARS-CoV-2 mutants have not been published to date. Therefore, it is unclear whether SARS-CoV-2 mutants detected in people working on mink farms are actually derived from the farmed minks. However, in the present study the subspecies of SARS-CoV-2 derived from farmed minks has been observed in the group of infected people, and the virus mutants that were inherited by infected individuals (Supplementary Figure 5).

Mutations in SARS-CoV-2 that lead to the generation of SARS-CoV-2 subspecies, have made humans and animals susceptible to infection through easy propagation in the host, thereby making it difficult to identify the effects of therapeutic agents or vaccines for COVID-19. Moreover, the spread of SARS-CoV-2 variants mediated by millions of infected farmed mink is uncontrolled; consequently, raising a concern that infection of SARS-CoV-2 mutants that cause serious symptoms in humans may spread globally.

As of January 2021, the number of peoples infected with SARS-CoV-2N501F subspecies, which are believed to have occurred in the United Kingdom (UK), South Africa and Brazil, has increased significantly in the UK and European countries. In recent studies, in comparison with previous SARS-CoV-2 variant, infectivity of SARS-CoV-2N501Y subspecies is found to be at approximately up to 1.7 from 1.4 times [7].

In addition, SARS-CoV-2N501F subspecies has a property of easily infect children. Currently, the question is whether the SARS-CoV-2N501Y subspecies is resistant to the COVID-19 vaccine inoculated in the UK and the United States. Pfizer Inc. (NY, USA) and BioNTech SE (Mainz, Germany), which created the COVID-19 vaccine (BNT162b2), have shown the possibility of immediate production of mRNA that should correspond to the SARS-CoV-2 mutations. On January 8, 2021, Pfizer and BioNTech SE published the efficacy of the COVID-19 vaccine against both SARS-CoV-2 subspecies from the UK and South Africa based on the results of Phase I clinical trials [8]. However, many people around the world are worried about the effectiveness of the COVID-19 vaccine against these SARS-CoV-2 variants i.e., SARS-CoV-2 Y453F subspecies or SARS-CoV-2N501Y subspecies [3,4,9].

Mankind has suffered in the therapy of acquired immunodeficiency syndrome (AIDS) to correspond to the speed of human immunodeficiency virus (HIV) mutations [10]. To date, no treatment has been established with drugs that directly inhibit the SARS-CoV-2 life cycle. In the fight between mankind and viruses so far, mankind has not stopped the mutation of viruses. More than 50 countries and territories around the world have strict restrictions on entry from the UK, South Africa and Brazil [11]. First, people around the world should reaffirm the importance of wearing masks, hand hygiene, and social distance.

Footnote

All authors are receiving medical ethics education. In addition, this study has been approved as a clinical medical study at each medical facility. The human serum samples used in this study were purchased from RayBiotecn life, and therefore Informed consent from the patient is not required.

Data Sharing

Data are available on various websites and have also been made publicly available (more information can be found in the first paragraph of the Results section).

Disclosure

The authors declare no potential conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Author Contributions

T.H. performed most of the experiments and coordinated the project. T.H. and N.Y. conceived the study and wrote the manuscript. N.Y. and I.K. provided with information on clinical medicine and oversaw the entire study.

Transparency Document

The authors declare no potential conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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