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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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EFFECT OF RBD MUTATIONS IN SPIKE GLYCOPROTEIN OF SARS-COV-2 ON NEUTRALIZING IGG AFFINITY

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Abstract.

Background: Certain mutant strains of SARS-CoV-2 are known to spread widely among humans, including the receptor binding domain (RBD) mutant, Y453F, from farmed minks, and the RBD mutant, N501Y, a mutation common to three major SARS-CoV-2 subvariants (B.1.1.7, B.1.351, and B.1.1.248) and omicron type SARS-CoV-2 BQ.1.1 and XBB.1.16 subvariants.

Methods: We investigated the characteristics of the RBD mutants, Y453F and N501Y, using three-dimensional structural analysis. We also investigated the effect of Y453F, N501Y or the mutants of RBD of omicron type SARS-CoV-2 BQ.1.1 and XBB.1.16 subvariants on neutralizing antibodies in serum derived from individuals including children (aged 5–11 years) inoculated with mRNA based COVID-19 vaccine (BNT162b2: Pfizer/BioNTech) or COVID-19-positive patients or children (aged 5–11 years) after vaccination with BNT162b2.

Results: Our results suggest that SARS-CoV-2 subspecies with the RBD mutations Y453F or N501Y partially escaped detection by 4 neutralizing monoclonal antibodies and 21 neutralizing antibodies in serums derived from COVID-19-positive patients. The significantly low antibody titer of children against Omicron type SARS-CoV-2 BQ.1.1 subvariant and XBB.1.16 subvariant in Japan.

Conclusions: Infection with SARS-CoV-2 subspecies that causes serious symptoms in humans may spread globally. In particular, since the antibody titer against the omicron type is low in children (aged 5–11 years) who have been vaccinated with conventional vaccines, therefore it is important for children to receive vaccines specific for the omicron type.

Key words. 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.

Introduction.

Mutations in the SARS-CoV-2 virus can jeopardize the efficacy of potential vaccines and therapeutics against COVID-19. Mustelidae animals (e.g., minks and ferrets) can be infected with SARS-CoV-2 relatively easily compared with other mammals [1] however, it has not yet been elucidated why SARS-CoV-2 is extremely contagious among these animals. Nonetheless, it is clear that when several farmed minks kept in a high-density environments 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 virus amplification *in vivo* in farmed minks [2]. Natural selection in such viruses is observed by the introduction of mutations in SARS-CoV-2 that are not observed during the growth process in humans [2,3]. Infections with the mutant strain Y453F (from farmed minks) or N501Y (a mutation common to three major subspecies of SARS-CoV-2: B.1.1.7, B.1.351, and B.1.1.248) or omicron type SRAS-CoV-2 BQ.1.1 subvariant and XBB.1.16 subvariant, which is the current epidemic strain, are known to widely spread among humans [4-6].

In this study, we investigate the virological characteristics of these two-receptor binding domain (RBD) mutants using three-dimensional protein structural analysis [7,8]. We also investigated the affinity of IgG for the conventional RBD and the mutant RBDs, Y453F and N501Y, in serum obtained from 41 COVID-19-positive patients and 20 COVID-19-negative patients or serum collected from children aged 5 to 11 years. The findings indicate that SARS-CoV-2 infection with Y453F or N501Y mutations in the spike glycoprotein may escape the antiviral effect of neutralizing antibodies or COVID-19 vaccination.

Now, the omicron type SARS-CoV-2 B.1.1.529 variant evolved into several subvariants, three of which (BA.1, BA.2, and BA.5) became globally dominant. Currently, the prevalence of omicron type SARS-CoV-2 BQ.1 subvariants (a BA.5 variant), its sub lineage BQ.1.1, and XBB.1.16 sub lineage, which is a recombinant of two different BA.2 subvariants, are increasing rapidly in the Europe, America, Southeast Asia, and elsewhere. BQ.1.1 sub lineage and XBB.1.16 sub lineage possess substitutions relative to BA.5 and BA.2, respectively, in the receptor-binding domain of their spike glycoprotein, which is the major target for vaccines and therapeutic monoclonal antibodies (mAbs) for COVID-19 [9]. Therefore, we, the medical staff, collected blood from children aged 5 to 11 years who had been inoculated with BNT162b2, and examined neutralizing antibody titers against Omicron type SARS-CoV-2 variants. In particular, since the antibody titer against the omicron type is low in children (aged 5–11 years) who have been vaccinated with conventional vaccines, it is important for children to receive vaccines specific for the omicron type.

Materials and Methods.

Analysis of the three-dimensional structures of the binding sites of mink and human angiotensin-converting enzyme 2 (ACE2)

Some subspecies of SARS-CoV-2 have the amino acid mutations, Y453F or N501Y, in the sequence that encodes the

spike glycoprotein [4,7,8]. These SARS-CoV-2 mutations have been detected in approximately 300 viral sequences isolated from European and Dutch populations, as well as in minks. We used the data on the three-dimensional structure of the RBD of the spike glycoprotein of SARS-CoV-2 (Protein Data Bank (PDB) ENTITY SEQ 6VW1_1) [7] and the data (PDB: 6XC2, 6XC4, 7JMP, 7JMO, 6XKQ, 6XKP, and 6XGD) for the three-dimensional structure of six neutralizing antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270, and REGN-COV2) that bind to the spike glycoprotein of SARS-CoV-2 [8].

Using the Spanner program, we predicted the three-dimensional structure of the SARS-CoV-2 spike glycoprotein Y453F mutant based on PDB data (ENTITY SEQ 6VW1_1). We investigated the binding of the spike glycoprotein mutant Y453F to human angiotensin-converting enzyme 2 (ACE2) and determined the affinity of the spike glycoprotein mutants, Y453F and N501Y, 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.

Protein contact residues and buried surface areas were analyzed using the LigPlot+ program (v.1.4.5) (https://www.ebi.ac.uk/thornton-srv/software/ LigPlus/). Protein buried surface areas were analyzed using the PDBePISA tool (http://pdbe.org/pisa/) and MOE project DB (MOLSIS Inc. Tokyo Japan). The modeling and docking of mink ACE2 and RBD in the spike glycoprotein of 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 the SARS-CoV-2 spike glycoprotein RBD was analyzed using MOE project DB (MOLSIS Inc.).

Adsorption of conventional RBD, Y453F and N501Y recombinant proteins to the solid phase surface of the ELISA plate

HeLa cells were transfected with either pcMV3-2019-nCov-RBD-Flag tag expression vector (2 μg), pcMV3-2019-nCov-RBD Y453F-Flag tag expression vector (2 μg), or pcMV3-2019-nCov-RBD N501Y-Flag tag expression vector (2 μg) (Sino Biological Inc. Beijing, China). The cells transfected with 2019-nCov-RBD, 2019-nCov-RBD Y453F, or 2019-nCov-RBD N501Y expression vectors were incubated for 48 h prior to harvesting. The conventional RBD, Y453F, and N501Y recombinant proteins were purified using a His-tag column following the standard procedure. Purified conventional RBD, Y453F, or N501Y recombinant proteins were adsorbed on the solid phase surface of the ELISA plate (SMILON ELISA plate MS-3508F, Tokyo Japan).

Quantitative measurement of SARS-CoV-2 neutralizing antibodies

The SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit (EpiGentek Group Inc., NY) contains all the reagents necessary for quantitatively measuring the level of SARS-CoV-2 neutralizing antibodies. In this assay, conventional RBD, Y453F, or N501Y spike proteins were stably pre-coated onto microplate wells. His-tagged ACE2 binds to the coated spike protein in the presence or absence of neutralizing antibodies in the sample. The amount of the bound ACE2, which is proportional to ACE2 inhibition intensity, is then recognized by the neutralizing detection complex, which contains anti-His antibodies and is measured through an ELISA-like reaction, where the absorbance is read by a microplate spectrophotometer at a wavelength of 450 nm. The neutralizing antibody level is inversely proportional to the optical density intensity measured, i.e., the higher the level of neutralizing antibody, the lower the OD intensity. Quantitative measurements of SARS-CoV-2 neutralizing antibody levels were performed according to the manufacturer's procedure.

Qualitative measurement of SARS-CoV-2 neutralizing antibodies

Serum samples (25 μL) from 20 COVID-19-negative patients and 41 COVID-19-positive patients with varying immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody levels were used for this assay. COVID-19 status was confirmed by RT-PCR, antigen, and/or antibody serology tests. All serum samples were provided by RayBiotech (RayBiotech Life, GA). The details of the materials and methods used are described in the supplementary materials. A neutralizing inhibition score of ≥ 20% was used to indicate a positive result and detection of SARS-CoV-2 neutralizing antibody; a score under 20% was used to indicate a negative result and no detectable SARS-CoV-2 neutralizing antibody.

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

Spanner is a structural homology modeling pipeline that threads a query amino-acid sequence onto a template protein structure. Spanner is unique in that it handles gaps by spanning the region of interest using fragments of known structures.

To create a model, you must provide a template structure, as well as an alignment of the query sequence you wish to model onto the template sequence. The spanner will replace mismatched residues, and fill any gaps caused by insertions or deletions.

For users that are unable to create an alignment a method for building a model starting only from sequence is also available. During this process a template search is conducted, and an alignment is built dynamically using FORTE before being passed through to the main part of the pipeline.

Spanner consists of several modules written in the Go programming language. For Spanner jobs which build a model only from sequence, the first step is a search of the PDB for possible templates using BLAST. These possible templates are then aligned and scored with FORTE.

The next step involves defining the start and end points of fragments corresponding to insertions or deletions. The start and end points are referred to as anchors because they must be equivalent in both the template and any candidate fragment. The margin parameter determines how far from the edge of a gap the fragment begins or ends. For example, a margin of 0 would mean that the anchors begins at the very edge of a gap. This is usually not a good idea, and the default margin is set to 1.

A representative set of protein chains was prepared using CD-HIT at 100% sequence identity.3 All continuous fragments were then extracted from this set of chains and stored in a relational

database, indexed by the internal coordinates of the fragment endpoints. A separate database is prepared for each fragment length. Currently, fragments of length 8-40, including the 8 anchor residues, are stored in the database.

Cn3D ("see in 3D") is a helper application for your web browser that allows researcher to view 3-dimensional structures from NCBI's Entrez Structure database. Cn3D is provided for Windows and Macintosh and can be compiled on Unix. Cn3D simultaneously displays structure, sequence, and alignment, and now has powerful annotation and alignment editing features. *(For those who prefer to view 3D structures on the web, without the need to install a separate application, iCn3D ("I see in 3D") is available as of April 2016).*

Web-based Structure VieweriCn3D ("I see in 3D"), released in April 2016, provides interactive views of three-dimensional macromolecular structures on the web.

There is no need to install a separate application in order to use iCn3D; you just need to use a web browser that supports WebGL.

iCn3D also allows you to customize the display of a structure and generate a URL that allows you to share the link, and to incorporate iCn3D into your own web pages.

New Features in Cn3D 4.3.1: View superpositions of structures that have similar molecular complexes, as identified by the newly released VAST+ (an enhanced version of the existing Vector Alignment Search Tool). The VAST+ help document provides additional details about the tool and examples of how it can be used to learn more about proteins.

Cn3D 4.3.1 uses the MIME type: application/vnd.ncbi.cn3d.

Analyses of protein contact residues and protein buried surface areas Protein contact residues were analyzed using the LigPlot^{+} 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.).

Cells

Vero E6-TMPRSS2-T2A-ACE2 cells (provided by Dr. Barney Graham, NIAID Vaccine Research Center) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Calf Serum (FCS), 100 U/mL penicillin–streptomycin, and 10 μg/mL puromycin. VeroE6/TMPRSS2 (JCRB 1819) cells were propagated in the presence of 1 mg/ml geneticin (G418; Invivogen) and 5 μg/ml plasmocin prophylactic (Invivogen) in DMEM containing 10% FCS. Vero E6-TMPRSS2-T2A-ACE2 and VeroE6/TMPRSS2 cells were maintained at 37°C with 5% CO2. The cells were regularly tested for mycoplasma contamination by using PCR and confirmed to be mycoplasma-free.

Viruses

hCoV-19/Japan/TY41-796/2022 (Omicron BQ.1.1)1, hCoV-19/Japan/TY41-795/2022 (Omicron XBB.1.16), hCoV-19/Japan/UT-NCD1288-2N/2022 (Omicron BA.2)2, hCoV-19/Japan/TY41-702/2022 (Omicron BA.5)3, and SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo were propagated in VeroE6/TMPRSS2 cells.

All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3 (BSL3) containment laboratories at the Kyoto University and the National Institute of Infectious Diseases, Japan, which are approved for such use by the Ministry of Agriculture, Forestry, and Fisheries, Japan.

Clinical specimens

After informed consent was obtained, plasma specimens were collected from COVID-19 convalescent individuals and vaccinees. The research protocol was approved by the Research Ethics Review Committee of the Institute of Medical Science of the Kyoto University (approval numbers: 2019–71–0201 and 2020-74-0226).

Focus reduction neutralisation test

Neutralisation activities of plasma were determined by using a focus reduction neutralisation test as previously described.4 After the plasma samples were incubated at 56°C for 1 h, the samples were serially diluted five-fold with DMEM containing 2% FCS in 96-well plates and mixed with 100–400 focus-forming units (FFU) of virus/well, followed by incubation at room temperature for 1 h. The plasma-virus mixture (50 μl) was then inoculated onto Vero E6-TMPRSS2-T2A-ACE2 cells in 96-well plates in duplicate and incubated for 1 h at 37°C. An equal volume of 1.5% Methyl Cellulose 400 (FUJIFILM Wako Pure Chemical Corporation) in culture medium was then added to each well. The cells were incubated for 14–16 h at 37°C and then fixed with formalin. After the formalin was removed, the cells were immuno stained with a mouse monoclonal antibody against SARS-CoV1/2 nucleoprotein [clone 1C7C7 (Sigma-Aldrich)], followed by a horseradish peroxidase-labeled goat anti-mouse immunoglobulin (SeraCare Life Sciences or Jackson ImmunoResearch Laboratories Inc.). The infected cells were stained with TrueBlue Substrate (SeraCare Life Sciences) and then washed with distilled water. After cell drying, the focus numbers were quantified by using an ImmunoSpot S6 Analyzer, ImmunoCapture software, and BioSpot software (Cellular Technology). The results are expressed as the 50% focus reduction neutralisation titre (FRNT50). The FRNT50 values were calculated by using GraphPad Prism (GraphPad Software).

Results.

Structural analysis:

The results from the Spanner analysis revealed that the RBD mutants Y453F and N501Y did not affect the three-dimensional structure of conventional SARS-CoV-2 spike glycoproteins. The results clarified that the binding property of the Y453F mutant spike glycoprotein to human ACE2 was slightly weaker than that of the conventional SARS-CoV-2 spike glycoprotein (Figure 1A, Table 1). Conversely, the binding property of the N501Y mutant spike glycoprotein to human ACE2 was stronger than that of the conventional RBD (Figure 1B, Table 1). The slightly weaker affinity observed in Y453F was due to the

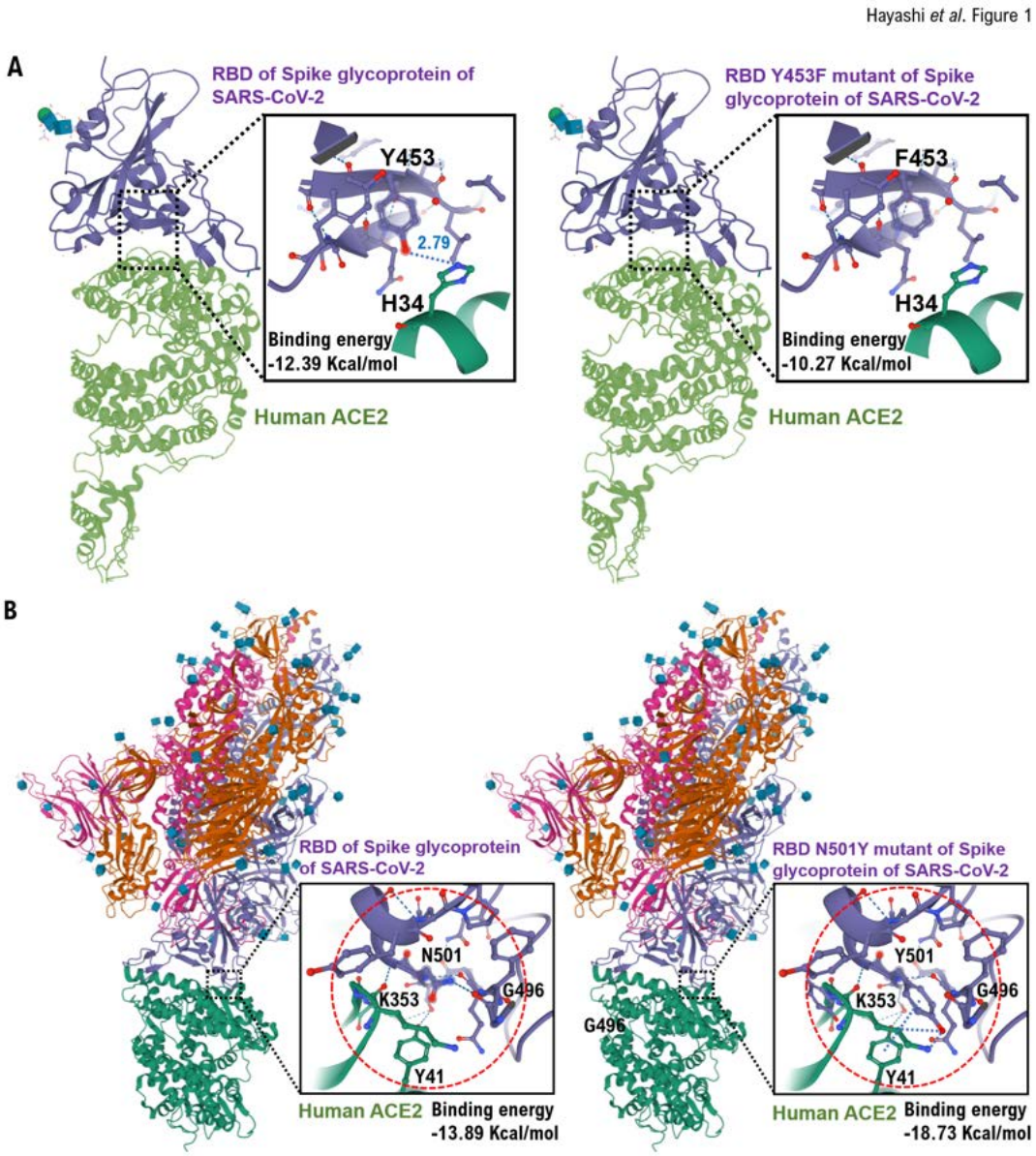

Figure 1. Interactions between human ACE2 and the SARS-CoV-2 spike glycoprotein receptor binding domain (RBD) mutants, Y453F and N501Y.

(A) The interaction between ACE2 (green) and residues of the conventional RBD and the Y453F variant (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 weak. From these results, the affinity between the spike glycoprotein RBD mutant Y453F and human ACE2 is presumed to be slightly weaker compared with the conventional RBD. (B) The interaction between ACE2 (green) and residues of the conventional RBD and the RBD mutant N501Y (purple) is shown using the three-dimensional structure model. It is speculated that the N501 amino acid residue of the conventional RBD is hydrogen-bonded to the Y41 and K353 amino acid residues of human ACE2. However, the binding between the Y501 amino acid residue of the RBD mutant and the Y41 and K353 amino acid residue of human ACE2 is presumed to be strong. From these results, the affinity between the RBD mutant (N501Y) of the spike glycoprotein and human ACE2 is presumed to be stronger compared with the conventional RBD. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in the supplementary data.

replacement of Tyr at position 453 by Phe, which was unable to form a hydrogen bond with His at position 34 in human ACE2 (Figure 1A). The strong affinity observed in the N501Y mutant was due to the replacement of Asn at position 501 by Tyr, which was able to form a hydrogen bond with Lys at position 353 in human ACE2, and to create a benzene ring interaction with Tyr at position 41 in human ACE2 (Figure 1B).

The results of the structural analysis reveal that the affinity between the Y453F spike glycoprotein and four of the six examined monoclonal antibodies (CC12.1, CC12.3, COVA2-39, COVA2-04, CV07-250, CV07-270) was clearly weak compared with the conventional RBD (Figure 2A, Table 1,). The results also demonstrated that the affinity between the N501Y spike glycoprotein and four of the six examined monoclonal

Table 1. Affinity of conventional RBD, Y453F, and N501Y with neutralizing antibodies or serum from each patient.
The binding of Y453F or N501Y to human ACE2 and to six neutralizing monoclonal antibodies were investigated using the MOE program 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 was examined using a SeroFlash™ SARS-CoV-2 Neutralizing Antibody Assay Fast Kit (EpiGentek Group Inc. NY). A strong affinity for the conventional RBD was shown in the serum IgG of 29 out of 41 COVID-19-positive patients. However, no affinity for the RBD mutant, Y453F, was shown in the serum IgG of 34 out of 41 COVID-19-positive patients. Weak affinity for both conventional RBD and N501Y was shown in the serum IgG of 7 out of 41 COVID-19-positive patients. However, no affinity for N501Y was shown in the serum IgG of 33 out of 41 COVID-19-positive patients. Various affinity for both conventional RBD and N501Y was shown in the serum IgG of 8 out of 41 COVID-19-positive. No affinity for conventional RBD, Y453F, or N501Y was shown in the serum IgG of all 20 COVID-19-negative subjects. Detailed experimental results are indicated in Supplementary Table 1.

Binding energy between RBD and human ACE2				**Effect of mutation on antibody**		
RBD (Y453)	RBD (Y453F)	RBD N501	RBD (N501Y)	Y453F	N501Y	
-12.39 Kcal/mol	-10.27 Kcal/mol	-13.89 Kcal/mol	-18.73 Kcal/mol	Slightly lower	Significantly Higher	
Neutralizing antibody	**Binding energy between RBD and antibodies**				**Effect of mutation on antibody**	
	RBD (Y453)	**RBD (Y453F)**	**RBD (N501)**	**RBD (N501Y)**	**Y453F**	**N501Y**
CC12.1	-14.65 Kcal/mol	-8.91 Kcal/mol	-15.49 Kcal/mol	-13.72 Kcal/mol	Low affinity	Low affinity
CC12.3	-5.85 Kcal/mol	-1.36 Kcal/mol	-5.41 Kcal/mol	-13.84 Kcal/mol	Low affinity	High affinity
COVA2-39	not calculated	not calculated	not calculated	not calculated	NA	NA
COVA2-04	-12.4 Kcal/mol	-3.42 Kcal/mol	-14.75 Kcal/mol	-13.18 Kcal/mol	Low affinity	Low affinity
CV07-250	-1.09 Kcal/mol	-0.23 Kcal/mol	-8.69 Kcal/mol	-5.97 Kcal/mol	Low affinity	Low affinity
CV07-270	not calculated	not calculated	not calculated	not calculated	NA	NA
REGN-COV2	-13.78 Kcal/mol	-10.54 Kcal/mol	-1.69 Kcal/mol	-10.23 Kcal/mol	Low affinity	High affinity
Patient Serums	**Number of samples with affinity for RBD**				**Effect of mutation on antibody**	
	RBD	**RBD (Y453F)**	**RBD (N501Y)**	**Both**	**Y453F**	**N501Y**
Positive 41 samples	14 samples	3 samples	4 samples	2 samples	Low affinity	Different effects
Negative 20 samples	0 sample	0 sample	0 sample	0 sample	NA	NA

antibodies was clearly weak compared with the conventional SARS-CoV-2 spike glycoprotein RBD (Figure 2B, Table 1).

Quantitative measurement of SARS-CoV-2 neutralizing antibodies

On September 28, 2020, the US pharmaceutical manufacturer, Regeneron Pharmaceuticals, announced the production of the antibody cocktail therapy, REGN-COV2, which combined two neutralizing antibodies, casirivimab and imdevimab, for the treatment and prevention of COVID-19 [10] (Figure 3A). On January 11, 2021, Regeneron Pharmaceuticals stated the likelihood of the antiviral effectiveness of REGN-COV2 on SARS-CoV-2 subspecies [11]. Therefore, we investigated the affinity of REGN-COV2 to the spike glycoproteins of Y453F and N501Y. We found that the affinity of REGN-COV2 (6XGD) to Y453F was weaker than that to the conventional RBD (Figure 3B, Table 1). However, the affinity of REGN-COV2 to N501Y was strong compared to the conventional RBD (Figure 3B). Therefore, the antiviral effect of REGN-COV2 as a neutralizing antibody may be maintained even against RBD mutant strains of SARS-CoV-2.

Qualitative measurement of SARS-CoV-2 neutralizing antibodies

Next, we investigated the affinity of serum IgG to the conventional RBD, Y453F, and N501Y in COVID-19-positive patients and healthy subjects. A strong affinity for conventional RBD was shown for the serum IgG of 29 of the 41 COVID-19-positive patients (Table 1). Moderate affinity for conventional RBD, Y453F, and N501Y was shown in the serum IgG of four of the COVID-19-positive patients (Table 1). No affinity for conventional RBD, Y453F, or N501Y was shown in the serum IgG of the COVID-19-negative subjects (Table 1).

From the results, we concluded that the mutation of tyrosine at amino acid residue 453 to phenylalanine or the mutation of asparagine at amino acid residue 501 to tyrosine eliminated the inhibitory effects of the neutralizing antibody on binding between ACE2 and the RBD of the SARS-CoV-2 spike glycoprotein. It is possible that the affinity between the appropriate amino acid residues in the variable region of the antibody and the RBD of Y453F or N501Y was diminished owing to weak recognition of the monoclonal antibody to SARS-CoV-2 spike glycoproteins.

Antibody titer of children against Omicron type SARS-CoV-2 BQ.1.1 subvariant and XBB.1.16 subvariant

To verify the antiviral effect of the mRNA-based COVID-19 vaccine () against the Omicron type SARS-CoV-2 BQ.1.1 subvariant and the XBB.1.16 subvariant, our medical team investigated the neutralizing activity of antibodies* in plasma of BNT162b2 vaccinated individuals against BQ.1.1 subvariant and XBB.1.16 subvariant isolated from Omicron type SARS-CoV-2 infected patients. As a result, compared with the neutralizing activity of plasma antibodies against the conventional strain, BA.2 subvariant, or BA.5 subvariant, the neutralizing activity of plasma antibodies against BQ.1.1 subvariant and XBB.1.16 subvariant was significantly turned out to be 21.06-fold and 22.33-fold low (Figure 4A).

Furthermore, our research team examined the neutralizing activity of antibodies in the plasma of patients infected with the BA.2 subvariant after the third dose of BNT162b2 (i.e., the BA.2 variant breakthrough infection) against the BQ.1.1 subvariant and the XBB.1.16 subvariant. As a result, compared with the neutralizing activity of plasma antibodies against the conventional strain, BA.2 subvariant, or BA.5 subvariant,

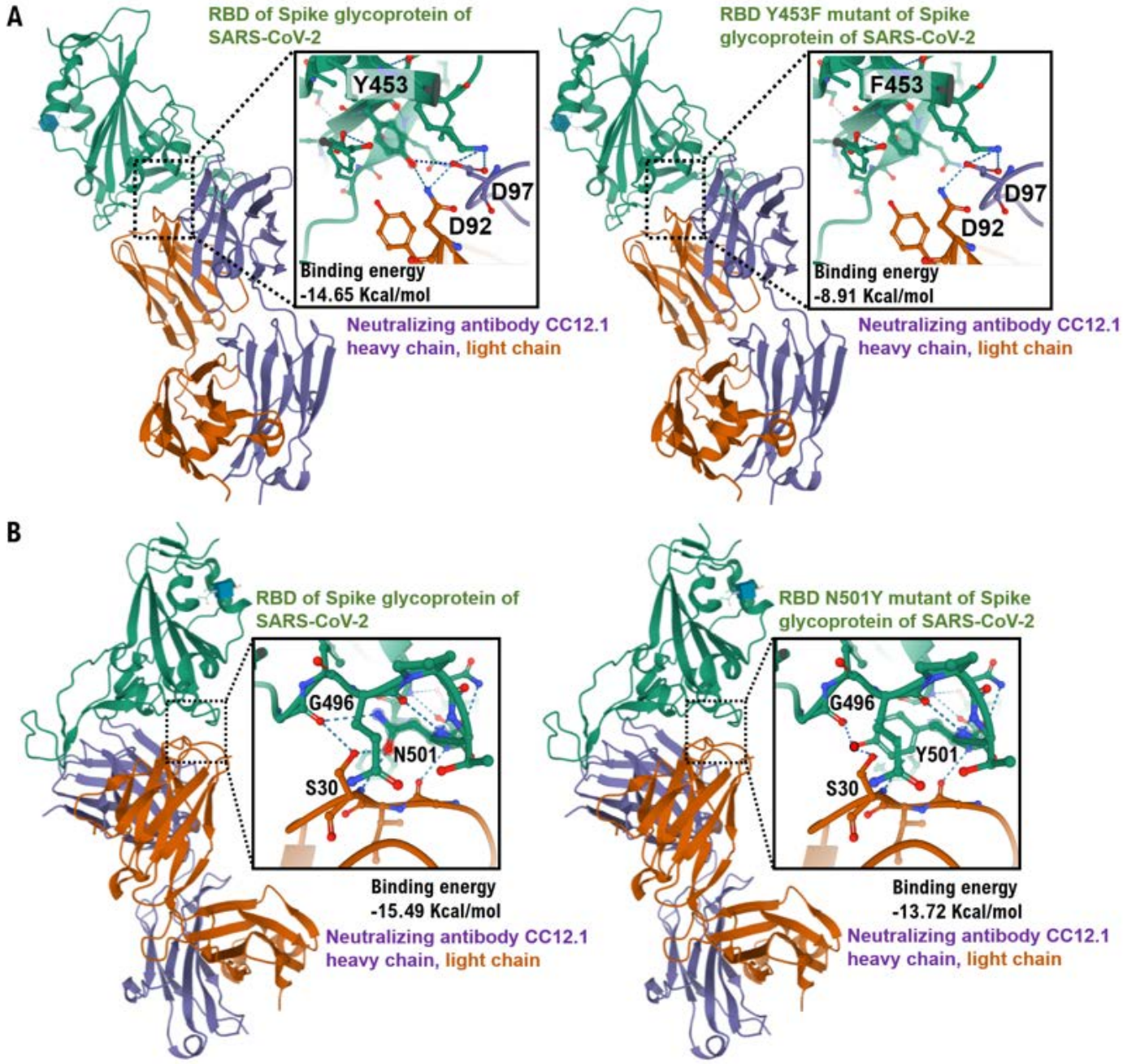

Figure 2. Interactions between the heavy chain of the neutralizing monoclonal antibody, CC12.2 and the SARS-CoV-2 spike glycoprotein RBD mutants, Y453F and N501Y.

(A) The interaction between the heavy chain (purple) and light chain (brown) of neutralizing monoclonal antibody CC12.1 and residues of the conventional RBD or Y453F (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 to the D97 amino acid residue of the heavy chain of CC12.1. However, the binding between the F453 amino acid residue of the RBD mutant and the D92 and D97 amino acid residues of CC12.1 is presumed to be weak. From these results, the affinity between the spike glycoprotein of Y453F and CC12.1 is presumed to be lower compared with the conventional RBD. (B) The interaction between the heavy chain (purple) and light chain (brown) of CC12.1 and the residues of the conventional RBD or N501Y (green) is shown using the three-dimensional structure model. It is speculated that the N501 amino acid residue of the conventional RBD is hydrogen-bonded to the S30 amino acid residue of the light chain of CC12.1. However, the binding between the Y501 amino acid residue of the RBD mutant and the S30 amino acid residue of CC12.1 is presumed to be weak. From these results, the affinity between the RBD of N501Y and CC12.1 is presumed to be slightly lower compared with the conventional RBD. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in the supplementary data.

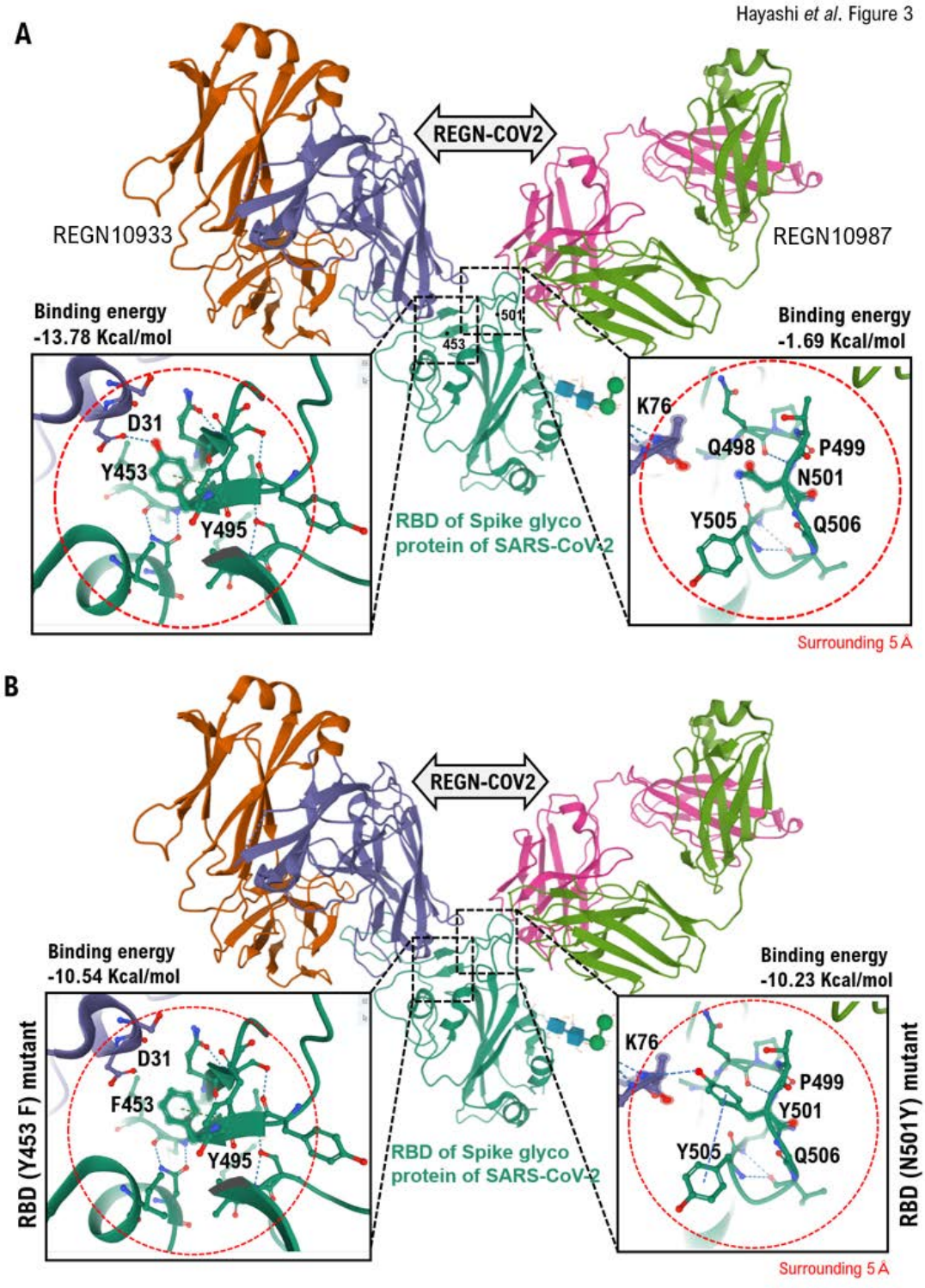

Figure 3. Interactions between the antibody cocktail therapy REGN-COV2 (casirivimab REGN10933 and imdevimab REGN10987; 6XGD) and Y453F and N501Y.

(A) The interaction between the heavy chains (purple, light green) and light chains (brown, pink) of casirivimab and imdevimab and residues of the conventional RBD (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 D31 amino acid residue of the light chain of casirivimab. However, the binding between the N501 amino acid residue of the conventional RBD and the amino acid residues of imdevimab is presumed to be weak. (B) The interactions between the heavy chain (purple) and light chain (brown) of casirivimab and imdevimab and residues of Y543F and N501Y (green) are shown using the three-dimensional structure model. The binding between the F453 amino acid residue of Y453F and the amino acid residue of the light chain of casirivimab is presumed to be weak. However, it is speculated that the Y501 amino acid residue of N501Y is hydrogen-bonded to the K76 amino acid residue of the light chain of imdevimab. The three-dimensional structure models are shown by Cn3D macromolecular structure viewer. Detailed experimental results are indicated in the supplementary data.

Figure 4

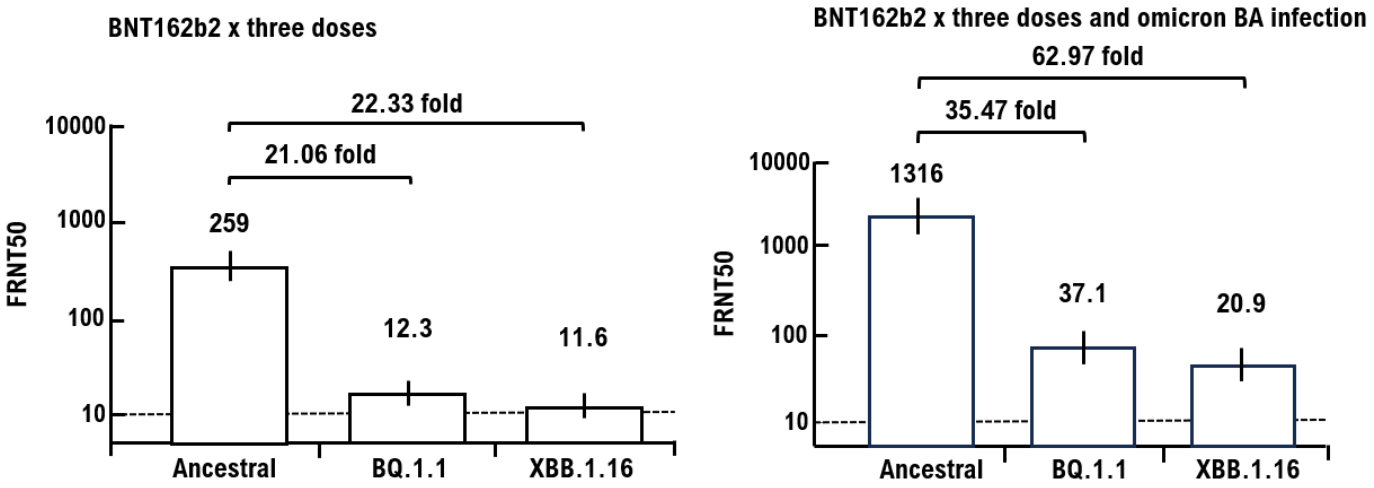

Figure 4. Antibody responses to SARS-CoV-2 omicron subvariants

(A) Neutralizing antibody titers of human plasma obtained from individuals immunized with a third dose of BNT162b2. Samples were collected 180–189 days after the third immunization (n=35). (B) Neutralizing antibody titers of human plasma obtained from individuals who were infected with omicron BA.2 after three doses of BNT162b2. Samples were collected 29–89 days after symptom onset (n=21). Each dot represents data from one individual. The lower limit of detection (value=10) is indicated by the horizontal dashed line. Samples under the detection limit (<10-fold dilution) were assigned an 50% focus reduction neutralization titer (FRNT50) value of 10 and are represented by X. Geometric mean titers are shown. FRNT50=50% focus reduction neutralization titer. Ancestral; the conventional strain, BA.2 subvariant, or BA.5 subvariant.

the neutralizing activity of these plasma antibodies against BQ.1.1 subvariant and XBB.1.16 subvariant was found to be significantly 35.47-fold and 62.97-fold lower (Figure 4B). However, most of the antibodies in plasma were found to have neutralizing activity against BQ.1.1 subvariant and XBB.1.16 subvariant, albeit at a significant low level.

*Clinical studies used antibodies in plasma from children (aged 5–11 years, n=35) 1–2 months after the third dose of BNT162b2 and the antibodies in plasma from children infected with the BA.2 subvariant (aged 5–11 years, n=21) after the third dose of BNT162b2 (the BA.2 subvariant breakthrough infection).

Discussion.

COVID-19 is an emerging infectious disease caused by infection with SARS-CoV-2, a type of coronavirus. In December 2019, the world's first case of severe COVID-19 was reported in Wuhan, China. COVID-19 is still a global epidemic. To the best of our knowledge, detailed data for SARS-CoV-2 mutants have not yet been published. Therefore, it is unclear whether the SARS-CoV-2 mutants detected in people working on mink farms are actually derived from farmed minks. However, in the present study, the subspecies of SARS-CoV-2 derived from farmed minks or humans was observed in a group of infected people and those that were inherited by infected individuals. Mutations in SARS-CoV-2 that have led 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 subspecies mediated by millions of infected farmed mink is uncontrolled, raising a concern that infection by SARS-CoV-2 subspecies that cause serious symptoms in humans may spread globally.

As of January 2021, the number of people infected with SARS-CoV-2 N501F subspecies, which are believed to have occurred in the United Kingdom, South Africa, and Brazil, has increased significantly in the UK and other European countries. Recent studies have shown that the infectivity of the N501Y variant is approximately 1.4–1.7 times that of previous strains of SARS-CoV-2 [12]. In addition, N501F variants have the property of easily infecting children. Currently, the question is whether N501Y variants are resistant to the COVID-19 vaccines which has been distributed in the UK, the US, and other countries. Pfizer, who created the first-to-be-approved COVID-19 vaccine (known as BNT162b2), has demonstrated the possibility of the immediate production of mRNA that should correspond to SARS-CoV-2 mutations. On January 8, 2021, Pfizer and BioNTech reported the efficacy of the COVID-19 vaccine against the UK and South Africa SARS-CoV-2 variants based on the results of phase I clinical trials [13]. On January 11, 2021, Regeneron Pharmaceuticals announced the antiviral effectiveness of the antibody cocktail therapy, REGN-COV2, against SARS-CoV-2 subspecies. However, concerns remain regarding the effectiveness of the COVID-19 vaccine against these SARS CoV-2 variants [3,4,14-16].

Based on textbooks, it is known that viruses gradually mutate in the process of repeating proliferation and infection. SARS-CoV-2 is also repeating mutations little by little, and so the infectivity of SARS-CoV-2 to humans and other mammals is changing. Most mutations have little effect on the characteristics of SARS-CoV-2, but some mutations may affect SARS-CoV-2 infectivity/transmissibility, risk of severe disease, efficacy of vaccines/therapeutic drugs, diagnosis. With the advancement of science and technology, novel vaccines against COVID-19 are also being rapidly developed, contributing to the prevention

of severe cases of COVID-19. From the results of this clinical study, it was revealed that in children aged 5-11 years who received BNT162b2, the neutralizing antibody titers against the Omicron type SARS-CoV-2 variants were significantly lower than those against the Wuhan type ARS-CoV-2 variants and the Delta type SARS-CoV-2 variants. However, elevated neutralizing antibody titers against Omicron type SARS-CoV-2 variants were also observed in children aged 5–11 years who received BNT162b2. Therefore, for children aged 5–11 years with underlying medical conditions who are at high risk of severe COVID-19, from the viewpoint of preventing the severity of COVID-19, vaccination with mRNA-based COVID-19 vaccine is recommended regardless of age.

Mankind has struggled to develop therapies against AIDS to correspond to the speed of mutations that arise in the human immunodeficiency virus (HIV) [17]. In the fight between humans and viruses, mankind has never been able to prevent the mutation of viruses. To date, more than 50 countries and territories around the world have imposed strict restrictions on entry from the UK, South Africa, and Brazil [18]. First, people around the world should reaffirm the importance of wearing masks, hand hygiene, and social distancing.

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

Ethics statement.

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization of Japan (Meguro, Tokyo, Japan) and the Central Ethics Review Board of Kyoto University (Kyoto, Kyoto, Japan). The approved number for this study is 50-201504. In order to carry out this research, the authors attended a research ethics education course (e-APRIN) conducted by Association for the Promotion of Research Integrity (APRIN; Shinjuku, Tokyo, Japan). The approved numbers of e-APRIN are AP0000151756, AP0000151757, AP0000151758, AP0000151769.

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 information on clinical medicine and oversaw the entire study.

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