May 25, 2020

Dear Editors,

Please find our attached manuscript entitled “Genetic variants that influence SARS-CoV-2 receptor *TMPRSS2* expression among population cohorts from multiple continents,” which we are submitting for consideration for publication as Original Research article in ***Biochemical and Biophysical Research Communications*** (**BBRC-20-4164**). We are thankful for your kind encouragement regarding to our manuscript. Herewith we are sending our revised manuscript in accordance with the comments given by the reviewers. The revised parts of the manuscript are highlighted as yellow color. Finally, we would like to thank you once again for providing us the opportunity to improve our manuscript. We hope very much these revisions are adequate for publication.

Sincerely yours,

Wei-Chiao Chang (D.Phil.; Oxon)
Professor, Department of Clinical Pharmacy,
Taipei Medical University, Taiwan
250 Wu-Hsing Street, Taipei 110, Taiwan

Reviewer Comment:

The authors examined expression profiles of *TMPRSS2* supporting SARS-CoV-2 infection. They found four *TMPRSS2* variants affecting *TMPRSS2* expression and obtained data indicating higher frequencies of *TMPRSS2*-upregulating variants in European and American populations than in Asian populations. Accumulation of these data would contribute to our understanding of the factors affecting SARS-CoV-2 epidemics. Unfortunately, however, there is no description on the numbers of samples or data they examined at all. So, it is highly recommended to revise the manuscript by describing "n" (the number of samples) in Figures and Tables and resubmit it to BBRC. We would be pleased to review the revised manuscript.

Answer:

Thank you so much for your comments. We have revised the manuscript according to your suggestions. As shown in Table 1 and Figure 3, the expression of *TMPRSS2* gene are retrieved from The Genotype-Tissue Expression (GTEx) portal database which contains 515 samples. [line 140-141, page 4]. Furthermore, the genetic data for general Europe, Africa, America, East Asia, Southeast Asia. Japan, China (Han) and Taiwan population were retrieved through the Ensemble Database (Table 2). The total number of samples for each region contains 503 individuals (European), 661 individuals (African), 347 (American), 504 individuals (East Asian), 489 individuals (Southeast Asian), 103 individuals (Han China), 104 individuals (Japanese) and 1517 individuals (Taiwanese). [line 155-158, page 4 and Table 2]

Fwd: BBRC-20-4164: Decision

From: Wei-Chiao Chang (weichiao.chang@gmail.com)

To: lalu_irham@yahoo.com

Date: Friday, May 22, 2020, 01:55 AM PDT

---------- Forwarded message ---------
寄件者： **BBRC (ELS)** <em@editorialmanager.com>
Date: 2020年5月22日 週五 下午4:27
Subject: BBRC-20-4164: Decision
To: Wei-Chiao Chang <weichiao.chang@gmail.com>

Manuscript No.: BBRC-20-4164
Title: Genetic variants that influence SARS-CoV-2 receptor TMPRSS2 expression among population cohorts from multiple continents
Journal Title: Biochemical and Biophysical Research Communications
Corresponding Author: Dr Wei-Chiao Chang
All Authors: Ialu Muhammad Irham, M.Sc; Wan-Hsuan Chou, M.Sc; Marcus J. Calkins, PhD; Wirawan Adikusuma, M.Sc; Shie-Liang Hsieh, PhD; Wei-Chiao Chang
Submit Date: May 12 2020 01:41AM

Dear Dr Chang:

After a careful review of your manuscript, I am afraid that we are not able to accept it for publication in BBRC in its current form, but would like to recommend you to resubmit your revised manuscript according to the following comment.

The authors examined expression profiles of TMPRSS2 supporting SARS-CoV-2 infection. They found four TMPRSS2 variants affecting TMPRSS2 expression and obtained data indicating higher frequencies of TMPRSS2-upregulating variants in European and American populations than in Asian populations. Accumulation of these data would contribute to our understanding of the factors affecting SARS-CoV-2 epidemics. Unfortunately, however, there is no description on the numbers of samples or data they examined at all. So, it is highly recommended to revise the manuscript by describing "n" (the number of samples) in Figures and Tables and resubmit it to BBRC. We would be pleased to review the revised manuscript.

I strongly encourage you to significantly revise your manuscript based on the above commentary. The concerns of the reviewer should be addressed in the cover letter and changes in the manuscript must be identified by page and paragraph, and noted by underlining or italics in the text. Please submit your manuscript as a new submission and note that it will be assigned to the same editor.

With kind regards,

Tetsuro Matano, PhD, MD
Editor
Biochemical and Biophysical Research Communications

Note: While submitting the revised manuscript, please double check the author names provided in the submission so that authorship related changes are made in the revision stage. If your manuscript is accepted, any authorship change will involve approval from co-authors and respective editor handling the submission and this may cause a significant delay in publishing your manuscript.

If you do not wish to revise your manuscript to meet the requests of the editor, Elsevier provides an Article Transfer Service from Biochemical and Biophysical Research Communications to alternative Elsevier journals that may have a more appropriate scope for your article: Biochemistry and Biophysics Reports and Heliyon.

To accept the transfer offer and select your preferred journal, please click: Agree to Transfer
To decline the transfer offer, please click: Decline to Transfer

The offer is valid for 30 days.

This service gives you the option to have all of your manuscript files and details transferred, which removes the need for you to resubmit and reformat your manuscript and saves valuable time and effort during the submission process.

Biochemistry and Biophysics Reports and Heliyon are open-access, online-only, peer-reviewed journals from Elsevier. The primary criteria for acceptance are that the work is original, scientifically and technically sound and provides valuable knowledge to life sciences research.

The Article Transfer Service aims to speed up the publication of promising papers. To help with that process, any editor and reviewer comments are transferred with your manuscript - if they are available for your submission, you will find them listed at the end of this letter. Please note that your acceptance of a transfer offer does not guarantee that your paper will be published in the journal you have selected.

To learn more about the Article Transfer Service, please visit www.elsevier.com/authors/article-transfer-service

---

Data in Brief (optional):

We invite you to convert your supplementary data (or a part of it) into an additional journal publication in Data in Brief, a multi-disciplinary open access journal. Data in Brief articles are a fantastic way to describe supplementary data and associated metadata, or full raw datasets deposited in an external repository, which are otherwise unnoticed. A Data in Brief article (which will be reviewed, formatted, indexed, and given a DOI) will make your data easier to find, reproduce, and cite.

You can submit to Data in Brief via the Biochemical and Biophysical Research Communications submission system when you upload your revised Biochemical and Biophysical Research Communications manuscript. To do so, complete the template and follow the co-submission instructions found here: www.elsevier.com/dib-template. If your Biochemical and Biophysical Research Communications manuscript is accepted, your Data in Brief submission will automatically be transferred to Data in Brief for editorial review and publication.

Open Access fees apply. Please contact the Data in Brief editorial office at dib-me@elsevier.com or visit the Data in Brief homepage (www.journals.elsevier.com/data-in-brief/) for further information.

MethodsX (optional)

If you have customized (a) research method(s) for the project presented in your Biochemical and Biophysical Research Communications article, you are invited to submit this part of your work as MethodsX article alongside your revised research article. MethodsX is an independent journal that publishes the work you have done to develop research methods to your specific needs or setting. This is an opportunity to get full credit for the time and money you may have spent on developing research methods, and to increase the visibility and impact of your work. Open access fees apply.

More information about submission, and example MethodsX articles, can be found here: http://www.sciencedirect.com/science/journal/22150161, or contact the MethodsX team at methodsx@elsevier.com.

Fwd: BBRC-20-4565: Final Decision

From: Wei-Chiao Chang (weichiao.chang@gmail.com)

To: lalu_irham@yahoo.com; adikusuma28@gmail.com

Date: Thursday, May 28, 2020, 09:13 PM PDT

This letter might be helpful for scholarship application

---------- Forwarded message ---------
寄件者： **BBRC (ELS)** <em@editorialmanager.com>
Date: 2020年5月26日 週二 上午8:46
Subject: BBRC-20-4565: Final Decision
To: Wei-Chiao Chang <weichiao.chang@gmail.com>

Ms. No.: BBRC-20-4565
Title: Genetic variants that influence SARS-CoV-2 receptor TMPRSS2 expression among population cohorts from multiple continents
Corresponding Author: Dr Wei-Chiao Chang
Authors: Ialu Muhammad Irham, M.Sc; Wan-Hsuan Chou, M.Sc; Marcus J. Calkins, PhD; Wirawan Adikusuma; Shie-Liang Hsieh, PhD

Dear Dr Chang,

We are pleased to inform you that your manuscript referenced above has been accepted for publication in Biochemical and Biophysical Research Communications.

Many thanks for submitting your fine paper to Biochemical and Biophysical Research Communications.

With kind regards,

Tetsuro Matano, PhD, MD
Editor
Biochemical and Biophysical Research Communications

Please note: Your accepted manuscript will now be transferred to our production department and work will begin on creation of the proof. If we need any additional information to create the proof, we will let you know. If not, you will be contacted again in a few days with a request to approve the proof and to complete a number of online forms that are required for publication.

Fwd: Proofs of [YBBRC_44510]

From: Wei-Chiao Chang (weichiao.chang@gmail.com)

To: ocean.chou@tmu.edu.tw; lalu_irham@yahoo.com

Date: Sunday, June 7, 2020, 04:29 PM PDT

---------- Forwarded message ---------
寄件者： <corrections.esch@elsevier.tnq.co.in>
Date: 2020年6月7日 週日 下午5:51
Subject: Proofs of [YBBRC_44510]
To: <weichiao.chang@gmail.com>

PLEASE DO NOT ALTER THE SUBJECT LINE OF THIS E-MAIL ON REPLY

Dear Dr. Wei-Chiao Chang,

Thank you for publishing with Biochemical and Biophysical Research Communications. We are pleased to inform you that the proof for your upcoming publication is ready for review via the link below. You will find instructions on the start page on how to make corrections directly on-screen or through PDF.

https://elsevier.proofcentral.com/en-us/landing-page.html?token=7ac2d2e706754f8fae578005bd0814

Please open this hyperlink using one of the following browser versions:

- Google Chrome 50+
- Mozilla Firefox 45+
- Mac OS Safari 10+

(Note: Microsoft Edge not supported at the moment)

We ask you to check that you are satisfied with the accuracy of the copy-editing, and with the completeness and correctness of the text, tables and figures. To assist you with this, copy-editing changes have been highlighted.

You can save and return to your article at any time during the correction process. Once you make corrections and hit the SUBMIT button you can no longer make further corrections. If you require co-authors to also review the proof, note that only one person may be working on the proof in the system at a time. Please make sure to only hit the SUBMIT button once all reviews are complete. When multiple authors are expected to make corrections, we recommend you to make use of the "Collaboration" feature.

We will do everything possible to get your article published quickly and accurately. The sooner we hear from you, the sooner your corrected article will be published online. You can expect your corrected proof to appear online within a week after we receive your corrections.

We very much look forward to your response.

Yours sincerely,

Elsevier

E-mail: corrections.esch@elsevier.tnq.co.in

For further assistance, please visit our customer support site at http://support.elsevier.com. Here you can search for solutions on a range of topics. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Fwd: Publication of your article [YBBRC_44510] in Biochemical and Biophysical Research Communications is on hold due to file problems

From: Wei-Chiao Chang (weichiao.chang@gmail.com)

To: lalu_irham@yahoo.com

Date: Monday, June 1, 2020, 04:30 AM PDT

---------- Forwarded message ---------
寄件者： <A.Achuthan@elsevier.com>
Date: 2020年6月1日 週一 下午7:19
Subject: Publication of your article [YBBRC_44510] in Biochemical and Biophysical Research Communications is on hold due to file problems
To: <weichiao.chang@gmail.com>

Our reference: YBBRC 44510
Article reference: YBBRC_BBRC-20-4565
Article title: Genetic variants that influence SARS-CoV-2 receptor TMPRSS2 expression among population cohorts from multiple continents
To be published in: Biochemical and Biophysical Research Communications
---

Dear Dr. Chang,

Congratulations on having your article accepted.

We have now received your manuscript in production and would like to begin the typesetting process.

Unfortunately we have encountered a problem with the electronic files you provided and cannot process your article further until the following issues are resolved:

* Q1. We have received designator(HASH SYMBOL) for the authors(Lalu Muhammad Irham) AND (Wan-Hsuan Chou), but corresponding foot note is missing, kindly check and advise. Q2. We have received citation for figure(Figure 5) in page no 4, but the corresponding figure is not supplied, kindly check and provide figure with caption.

We would be grateful if you could kindly address the problem as quickly as possible, ideally within 48 hours, by replying to this message.

Further information on acceptable file formats can be found at http://www.elsevier.com/guidepublication.

Please quote the reference for your article, YBBRC 44510, in all of your messages to us.

Thank you for your help with this issue; I look forward to hearing from you soon.

Kind regards,

A Achuthan
Data Administrator
Elsevier
E-Mail: A.Achuthan@elsevier.com

HAVE QUESTIONS OR NEED ASSISTANCE?

For further assistance, please visit our Customer Support site, where you can search for solutions on a range of topics,

such as Open Access or payment queries, and find answers to frequently asked questions. You can also talk to our customer support team by phone 24 hours a day from Monday-Friday and 24/7 by live chat and email.

Get started here: http://service.elsevier.com/app/home/supporthub/publishing

---

Proofs of BBRC manuscript

From: Wei-Chiao Chang (weichiao.chang@gmail.com)

To: lalu_irham@yahoo.com; lalu.irham@pharm.uad.ac.id; adikusuma28@gmail.com; marcus.calkins@gmail.com; slhsieh@gate.sinica.edu.tw; ocean.chou@tmu.edu.tw

Date: Monday, June 8, 2020, 06:08 PM PDT

Dear all,

I am sending you our manuscript accepted in BBRC.
Please have a look and return it to me if there are errors/typos .
Thank you so much for your help.

Best regards,

wei chiao

張偉嶠 特聘教授/副院長
臺北學大學 藥學院
Wei-Chiao Chang (D.Phil.; Oxon)
Distinguished Professor/Vice Dean, School of Pharmacy
Taipei Medical University, Taiwan
Tel: 886-2-27361661 ext.6187
http://twsnp.tmu.edu.tw/

pagination_YBBRC_44510 (proof_20200609).pdf
959.2kB

Re: Proofs of BBRC manuscript

From: slhsieh (slhsieh@gate.sinica.edu.tw)

To: weichiao.chang@gmail.com

Cc: lalu_irham@yahoo.com; lalu.irham@pharm.uad.ac.id; adikusuma28@gmail.com; marcus.calkins@gmail.com; ocean.chou@tmu.edu.tw

Date: Monday, June 8, 2020, 07:00 PM PDT

Wei-chiao

Great job. Congratulation.

Shie-Liang Edmond Hsieh, MD, D.Phil.

Distinguished Research Fellow
Genomics Research Center
Academia Sinica
128 Academia Road, Section 2, Nankang, Taipei 115, Taiwan
E-mail: slhsieh@gate.sinica.edu.tw
TEL: 886-2-27871245;
FAX: 886-2-27898811

> On Jun 9, 2020, at 9:07 AM, Wei-Chiao Chang <weichiao.chang@gmail.com> wrote:
>
> Dear all,
>
> I am sending you our manuscript accepted in BBRC.
> Please have a look and return it to me if there are errors/typos .
> Thank you so much for your help.
>
> Best regards,
>
> wei chiao
>
> ---
> 張偉嶠 特聘教授/副院長
> 臺北學大學 藥學院
> Wei-Chiao Chang (D.Phil.; Oxon)
> Distinguished Professor/Vice Dean, School of Pharmacy
> Taipei Medical University, Taiwan
> Tel: 886-2-27361661 ext.6187
> http://twsnp.tmu.edu.tw/
> <pagination_YBBRC_44510 (proof_20200609).pdf>

Biochemical and Biophysical Research Communications 529 (2020) 263–269

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Genetic variants that influence SARS-CoV-2 receptor *TMPRSS2* expression among population cohorts from multiple continents

Lalu Muhammad Irham [a, c, 1], Wan-Hsuan Chou [a, b, 1], Marcus J. Calkins [d], Wirawan Adikusuma [a], Shie-Liang Hsieh [e], Wei-Chiao Chang [a, b, f, g, *]

[a] Department of Clinical Pharmacy, School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, 11031, Taiwan
[b] Master Program for Clinical Pharmacogenomics and Pharmacoproteomics, School of Pharmacy, Taipei Medical University, Taipei, 11031, Taiwan
[c] Faculty of Pharmacy, University of Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[d] Institute of Cellular and Organismic Biology, Academia Sincia, Taipei, 11529, Taiwan
[e] Genomics Research Center, Academia Sinica, Taipei, 11529, Taiwan
[f] Integrative Research Center for Critical Care, Wan Fang Hospital, Taipei Medical University-Taipei, 11696, Taiwan
[g] Department of Medical Research, Shuang Ho Hospital, Taipei Medical University, New Taipei City, 23561, Taiwan

ARTICLE INFO

Article history:
Received 25 May 2020
Accepted 25 May 2020
Available online 8 June 2020

Keywords:
SARS-CoV-2
COVID-19
TMPRSS2
Variant gene

ABSTRACT

The World Health Organization recently announced that pandemic status has been achieved for coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Exponential increases in patient numbers have been reported around the world, along with proportional increases in the number of COVID-19-related deaths. The SARS-CoV-2 infection rate in a population is expected to be influenced by social practices, availability of vaccines or prophylactics, and the prevalence of susceptibility genes in the population. Previous work revealed that cellular uptake of SARS-CoV-2 requires Angiotensin Converting Enzyme 2 (ACE-2) and a cellular protease. The spike (S) protein on SARS-CoV-2 binds ACE-2, which functions as an entry receptor. Following receptor binding, transmembrane protease serine 2 (encoded by *TMPRSS2*) primes the S protein to allow cellular uptake. Therefore, individual expression of *TMPRSS2* may be a crucial determinant of SARS-CoV-2 infection susceptibility. Here, we utilized multiple large genome databases, including the GTEx portal, SNP nexus, and Ensembl genome project, to identify gene expression profiles for *TMPRSS2* and its important expression quantitative trait loci. Our results show that four variants (rs464397, rs469390, rs2070788 and rs383510) affect expression of *TMPRSS2* in lung tissue. The allele frequency of each variant was then assessed in regional populations, including African, American, European, and three Asian cohorts (China, Japan and Taiwan). Interestingly, our data shows that *TMPRSS2*-upregulating variants are at higher frequencies in European and American populations than in the Asian populations, which implies that these populations might be relatively susceptible to SARS-CoV-2 infection.

1. Introduction

The new infectious respiratory disease, coronavirus disease 2019 (COVID-19), first emerged in Wuhan, China according to the World Health Organization (WHO) [1]. The initial cluster of infections was linked to a seafood market in Wuhan, where animal contact led to transfer of the virus to humans and then ultimately to human-to-human transmission [2]. In February of this year, the International Committee on Taxonomy of Viruses named the virus that causes COVID-19 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3] based on its close homology with SARS-CoV and its pathological sequelae that often include respiratory conditions, such as severe pneumonia [4]. The continuous increase of patients suffering from SARS-CoV-2 infection and COVID-19 across all inhabited continents (i.e., Asia, Australia, Europe, the Americas, and Africa) eventually led the WHO to declare this disease a pandemic. Strikingly, the last updated data from the coronavirus worldometer website (https://www.worldometers.info/coronavirus/) on May 07, 2020, 02:48 GMT, showed that the total

* Corresponding author. Department of Clinical Pharmacy, School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, 11031, Taiwan.
E-mail address: wcc@tmu.edu.tw (W.-C. Chang).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.bbrc.2020.05.179
0006-291X/

number of confirmed COVID-19 cases in more than 210 countries was 3.822.860 while the reported deaths were 265.076. In order to slow the spread of virus and to keep the number of critical patients within the capacity of hospitals, nearly all developed nations have instituted tight restrictions on movement of their residents, which has come at an extreme economic cost. While these social policies led to a reported net decrease in the number of active cases within China in late March, the spread of disease was still rampant in other countries, highlighting the dynamic nature of the pandemic and the major challenge of suppressing spread of SARS-CoV-2 transmission simultaneously around the world.

Mitigation of disease spread would almost certainly be aided by effective vaccines or prophylactic agents that target SARS-CoV-2 cellular uptake. However, vaccines require a long development time and clinical trials for potential SARS-CoV-2 uptake inhibitors are ongoing. Recent reports have indicated that infection of lung epithelial cells with SARS-CoV-2 requires angiotensin converting enzyme 2 (ACE-2) and transmembrane protease serine 2 (encoded by *TMPRSS2*) [5]. For successful infection to occur, coronaviruses must utilize a protease from the host cell to activate the viral spike (S) protein, and *TMPRSS2* was recently reported to be an essential host factor in airway epithelial cells [6] that allows entry into the cells [5,7,8]. It is still unclear, however, whether and how *TMPRSS2* allelic variants in the lung might affect protease expression. Upregulation of *TMPRSS2*, would be expected to enhance S protein processing, and therefore, susceptibility to SARS-CoV-2 infection and COVID-19 pathogenesis. Moreover, a population with a large proportion of susceptibility gene carriers would be expected to be more vulnerable to the disease. Up to now, no susceptibility markers for COVID-19 have been identified, and no lung-associated variants in *TMPRSS2* have been reported. Here, we used various databases to assess the tissue expression profiles of *TMPRSS2* and occurrence of genetic variants in populations from several continents. The results will allow future studies assessing whether these variants may be associated with different risks of SARS-CoV-2 infection and susceptibilities to COVID-19.

2. Methods

In order to evaluate the relationship between genetic variants and gene expression profiles, expression quantitative trait loci (eQTL) were examined using the GTEx portal database (http://www.gtexportal.org/home/), which compiles the expression of genes in a variety of tissues. The genetic variants of *TMPRSS2* in human lung tissue were obtained from the GTEx portal database and confirmed with the Ensembl Genome Browser (https://www.ensembl.org/index.html). The allele frequencies among populations of European, African, American, East Asian, Southeast Asian. Japanese, and Han Chinese were extracted from the Ensembl Genome Browser (http://www.ensembl.org/Homo_sapiens/Variation), while the allele frequency in the Taiwan population was obtained from the Taiwan Biobank website (https://taiwanview.twbiobank.org.tw/index). Annotations, such as location or type of variant, were retrieved from the Ensembl Genome Browser (https://www.ensembl.org/index.html). Variants function was evaluated using the SNP nexus database (https://www.snp-nexus.org). SNPs were prioritized based on PubMed text mining to check whether the genetic variants of interest showed any correlation in the clinical significance.

3. Results

3.1. TMPRSS2 gene expression in various tissues

In order to evaluate *TMPRSS2* expression in human tissues, we examined eQTLs using the GTEx portal database (http://www.gtexportal.org/home/), which contains gene expression levels in a variety of tissues. The eQTL annotations comprise the most

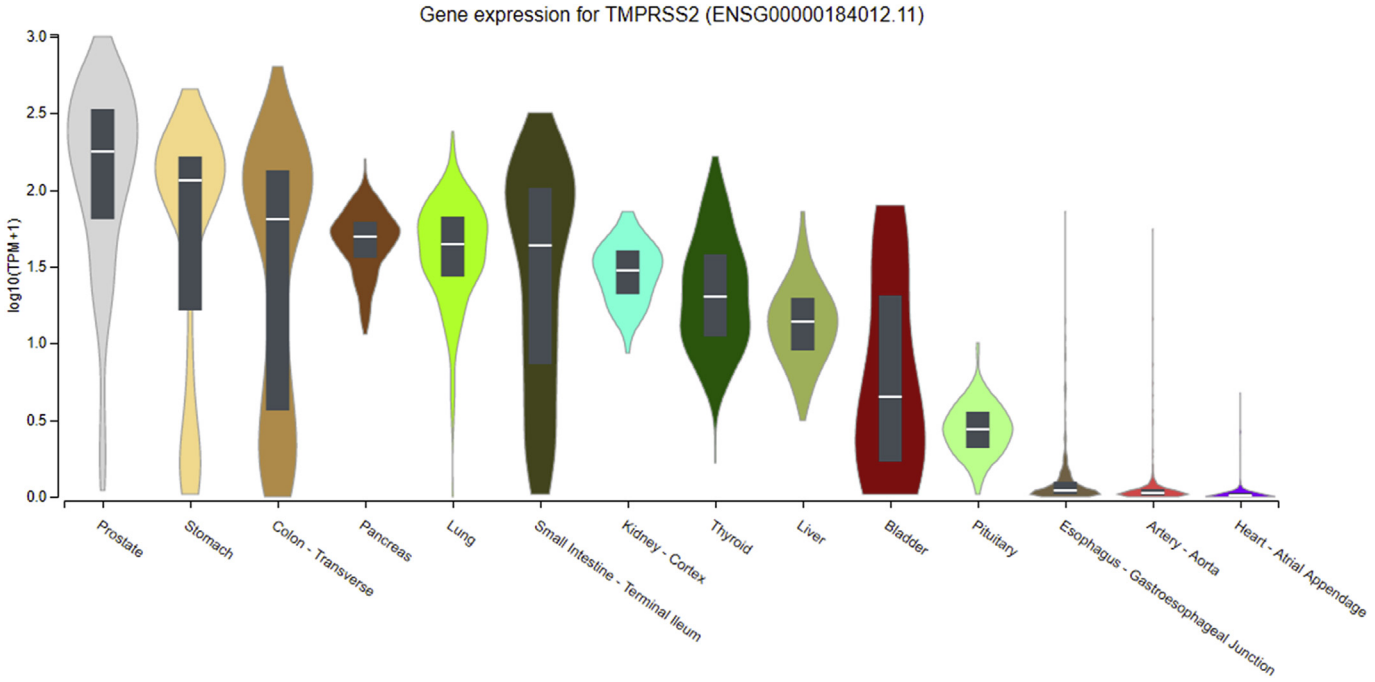

Fig. 1. Expression of transmembrane serine protease 2 (*TMPRSS2*) in different human tissues according to the GTEx database project (https://www.gtexportal.org/home).

apparent functional consequences of genetic variations [9], and the database revealed that *TMPRSS2* displays high expression in the prostate, colon, stomach, pancreas and lung (Fig. 1).

3.2. Important eQTLs for TMPRSS2 in the lung

To identify eQTLs associated with *TMPRSS2* expression in lung, the GTEx portal database was utilized. We identified a total of 203 eQTLs for *TMPRSS2* in all tissues. Among them, 136 variants indicated predominant effects on *TMPRSS2* expression in lung, while 56 eQTLs were relevant to testis, nine to prostate, one to ovary, and one to thyroid (**Additional File S1**). Next, we further examined the 136 eQTLs in lung using the SNP nexus database and PubMed text mining to determine whether the genetic variants might have the clinical significance. Among the 136 lung-associated variants, rs469390 was confirmed as a missense mutation, and three others, rs2070788, rs383510 and rs464397, were previously reported to have clinical significance in infectious disease. Cheng at al., revealed that two genetic variants rs2070788 and rs383510 were significantly associated with the susceptibility to influenza [10], while rs464397 was reported to associate with poor immune response in patients co-infected with HIV and HCV [11]. Therefore, our subsequent analyses were focused on these four SNPs. Three of them (rs2070788, rs383510 and rs464397) are located at the intronic region and one (rs469390) is located at the exon region, as depicted in Fig. 2.

3.3. Association between TMPRSS2 expression and the four eQTLs

We next tested potential associations between the four identified variants (rs464397, rs469390, rs2070788 and rs383510) and *TMPRSS2* expression in lung. Using the publicly available GTEx Portal (http://www.gtexportal.org/home/), we obtained the tissue expression of *TMPRSS2* across different genotypes in each eQTL from 515 samples. As shown in Table 1 and Fig. 3, the TT genotype of rs464397 was associated with higher expression of *TMPRSS2* and *MX1* in the lung compared to the CT and CC genotypes. Furthermore, the AA genotype of rs469390 was associated with higher expression of *TMPRSS2* and *MX1* in the lung compared to the AG and GG genotypes, and the rs2070788 GG genotype had higher expression of *TMPRSS2* and *MX1* in the lung compared to the AG and AA genotypes. Lastly, the rs383510 TT genotype was associated with higher expression of *TMPRSS2* in the lung compared to the CT and CC genotypes.

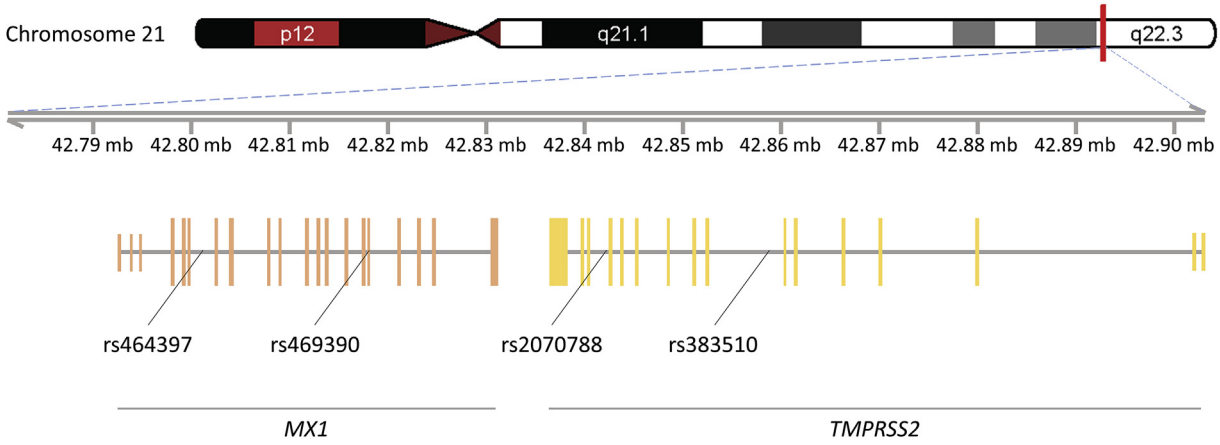

Fig. 2. The location of selected variants (rs469390, rs2070788, rs383510 and rs464397) on chromosome 21.

Table 1
Cis-expression of quantitative trait loci results for the transmembrane serine protease 2 (*TMPRSS2*) SNPs from genotype-tissue expression database.

SNP ID	Gencode ID (ENSG00000-)	Gene symbol	*p*-value	Effect size	Tissue	Actions
rs464397	184012.11	*TMPRSS2*	0.000010	−0.08	**Lung**	TT > TC > CC
	157601.13	*MX1*	2.1e-13	−0.31	Thyroid	TT > TC > CC
rs469390	184012.11	*TMPRSS2*	0.0000068	0.091	**Lung**	AA > AG > GG
	157601.13	*MX1*	1.40E-13	0.32	Thyroid	AA > AG > GG
	157601.13	*MX1*	4.50E-12	0.27	Nerve - Tibial	AA > AG > GG
	157601.13	*MX1*	9.00E-08	0.25	**Lung**	AA > AG > GG
	157601.13	*MX1*	5.60E-07	0.2	Artery - Tibial	AA > AG > GG
	157601.13	*MX1*	8.60E-07	0.21	Breast - Mammary Tissue	AA > AG > GG
	157601.13	*MX1*	1.1E-06	0.18	Muscle - Skeletal	AA > AG > GG
	157601.13	*MX1*	1.5E-06	0.2	Skin - Not Sun Exposed (Suprapubic)	AA > AG > GG
	157601.13	*MX1*	3.3E-06	0.18	Esophagus - Mucosa	AA > AG > GG
	157601.13	*MX1*	3.8E-06	0.19	Esophagus - Muscularis	AA > AG > GG
	157601.13	*MX1*	4.8E-06	0.21	Testis	AA > AG > GG
	157601.13	*MX1*	0.000013	0.22	Colon - Transverse	AA > AG > GG
	157601.13	*MX1*	0.000014	0.24	Esophagus - Gastroesophageal Junction	AA > AG > GG
	157601.13	*MX1*	0.000023	0.16	Skin - Sun Exposed (Lower leg)	AA > AG > GG
	157601.13	*MX1*	0.000043	0.21	Artery - Aorta	AA > AG > GG
rs2070788	184012.11	*TMPRSS2*	8.9e-9	−0.11	**Lung**	GG > AG > AA
	157601.13	*MX1*	0.000012	−0.18	Thyroid	GG > AG > AA
rs383510	184012.11	*TMPRSS2*	1.2e-8	−0.11	**Lung**	TT > TC > CC

Source: Expression Quantitative trail loci (eQTL) obtained from https://gtexportal.org/home.

3.4. Allele frequencies of the candidate variants in different populations

Once we had identified the four candidate *TMPRSS2* expression-associated variants, we were interested to know the allele frequencies in different populations. As shown in Table 2, the allele frequencies for the four variants were evaluated in different populations from Europe, Africa, America, East Asia, Southeast Asia, Japan, China (Han) and Taiwan. Samples from each region contains 503 individuals (European), 661 individuals (African), 347 individuals (American), 504 individuals (East Asian), 489 individuals (Southeast Asian). Among the East Asians, the subpopulations include103 Han Chinese and 104 Japanese. Furthermore, allele frequancies in a group of 1517 individuals from Taiwan Biobank were also assessed. Allele frequencies in European, African, American, East Asian, and Southeast Asian populations were extracted from the Ensembl Genome Browser (http://www.ensembl.org/Homo_sapiens/Variation). The Taiwan Biobank was queried using the Taiwan View website (https://taiwanview.twbiobank.org.tw/index) to examine the Taiwanese population. The allele frequencies across different populations for each *TMPRSS2*-upregulating variants are shown in Table 2 and Fig. 4. Strikingly, the frequency of rs464397 T allele, which is associated with higher *TMPRSS2* expression in lung, is much lower in the Asian (East Asian and Southeast Asian) populations compared to European, African and American populations. Cohorts from China, Japan and Taiwan exhibited the lowest T allele frequencies of rs464397 (~1%). In addition, the frequencies of rs383510 T allele and rs469390 A allele,

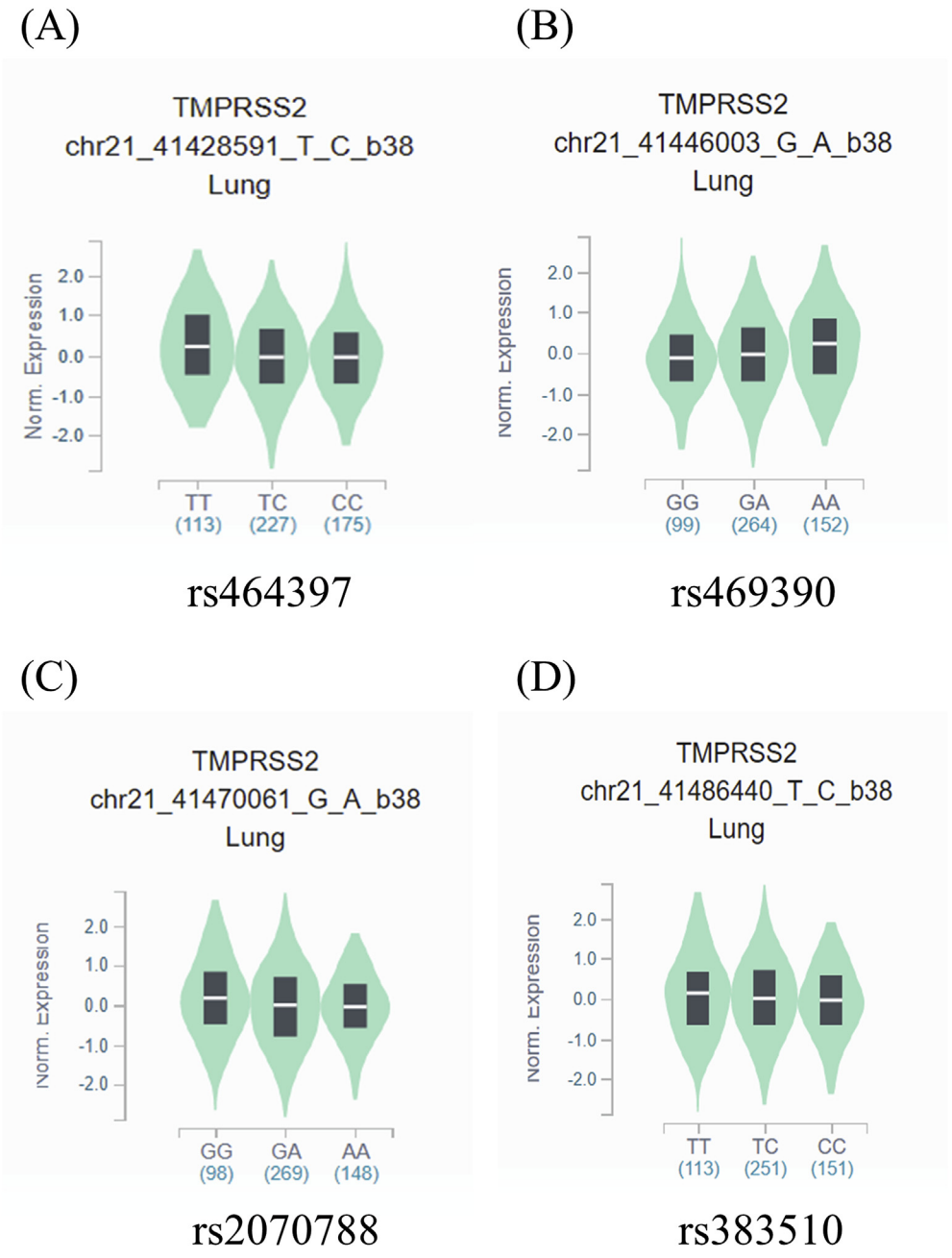

Fig. 3. *Cis*-expression of quantitative trait loci results for transmembrane serine protease 2 (*TMPRSS2*) in lung tissue. Plots show the expression of *TMPRSS2* for each genotype of the four SNPs: rs464397 (A), rs469390 (B), rs2070788 (C) and rs383510 (D).

Table 2
Allele frequencies for transmembrane serine protease 2 (*TMPRSS2*) SNPs examined in this study.

SNP	Position (hg19) (bp)	Gene	Location	Amino Acid Change	Allele		Allele Frequencies(N)							
					Ref	Eff*	EUR (503)	AFR (661)	AMR (347)	EAS (504)	SAS (489)	CHB (103)	JPT (104)	TWB (1517)
rs464397	Chr 21:42800518	*MX1*	Intron	–	C	T	0.48	0.14	0.32	0.01	0.25	0.01	0.01	0.01
rs469390	Chr 21:42817930	*MX1*	Exon	Val/Ile	G	A	0.59	0.44	0.45	0.28	0.55	0.21	0.20	0.26
rs2070788	Chr 21:42841988	*TMPRSS2*	Intron	–	A	G	0.46	0.27	0.49	0.36	0.47	0.28	0.30	0.34
rs383510	Chr 21:42858367	*TMPRSS2*	Intron	–	C	T	0.48	0.33	0.38	0.36	0.46	0.27	0.30	0.34

EUR, European; AFR, African; AMR, American; EAS, East Asian; SAS, Southeast Asian. JPT, Japanese in Tokyo, CHB, Han Chinese and TWB, Taiwan Biobank. N; total number of samples, Ref, Reference and Eff, Effect allele of EUR, AFR, AMR, EAS, SAS, CHB, JPT were extracted from the Ensembl,org (http://www.ensembl.org/Homo_sapiens/Variation). TWB were obtained from the Taiwan View website (http://taiwanview.twbiobank.org.tw/index). * Effect allele is defined as the allele associated with higher expression *TMPRSS2* in lung.

which are also associated with higher *TMPRSS2* expression in lung, are lower in East Asian populations compared to European and American populations (Fig. 4). The respective G and A allele frequencies for rs2070788 and rs383510 also appeared to have lower prevalence in East Asia (rs2070788, 36% and rs383510, 36%), China (rs2070788, 28% and rs383510, 27%), Japan (rs2070788, 30% and rs383510, 30%) and Taiwan (rs2070788, 34% and rs383510, 34%) compared to the European population (rs2070788, 46% and rs383510, 48%) and American (rs2070788, 49% and rs383510, 38%) (Table 2). Taken together, the allele frequencies of variants rs464397, rs469390, rs2070788 and rs383510 would suggest that expression of *TMPRSS2* may be lower in East Asian populations compared to European and American populations.

4. Discussion

In this work, we examined the lung tissue expression of the *TMPRSS2* gene, as ACE2 and *TMPRSS2* encode necessary components for cell entry of SARS-CoV [12] and SARS-CoV-2 [13]. Coronavirus infection requires a host cell protease to activate the viral S protein and allow entry into airway epithelial cells. One such protease-encoding gene, *TMPRSS2*, was recently reported to be a host factor essential for viral uptake [6]. Furthermore, the gene product of *TMPRSS2* is known to activate coronavirus S proteins and allow entry of SARS-CoV and MERS-CoV [5,14]. In vitro and in vivo studies of *TMPRSS2* revealed its role in viral replication and pathogenicity of influenza A(H7N9), and A(H1N1) pdm09 [15,16] as well. However, the variants that associate to *TMPRSS2* expression have not been reported. Since COVID-19 has already had serious impacts on the world, examining the distribution of *TMPRSS2* variants may be an important pursuit, which allows us to understand infection susceptibilities in different populations. While the demographic characteristics associated with individual susceptibility to SARS-CoV-2 remain unknown, it will be interesting to study how variants in the protease and in ACE2 [17] affect viral transmission.

We utilized publicly available databases, such as GTEx portal, SNP nexus, and Ensembl to examine the genetic variants associated with *TMPRSS2* expression in lung tissue, the major site of infection for SARS-CoV-2 [4,18,19]. Notably, the *TMPRSS2* acts as a host protease in the human respiratory tract and gastrointestinal tract [20], which can explain many of the symptoms of COVID-19, such as cough, sore throat, rhinorrhea, and shortness of breath [21,22].

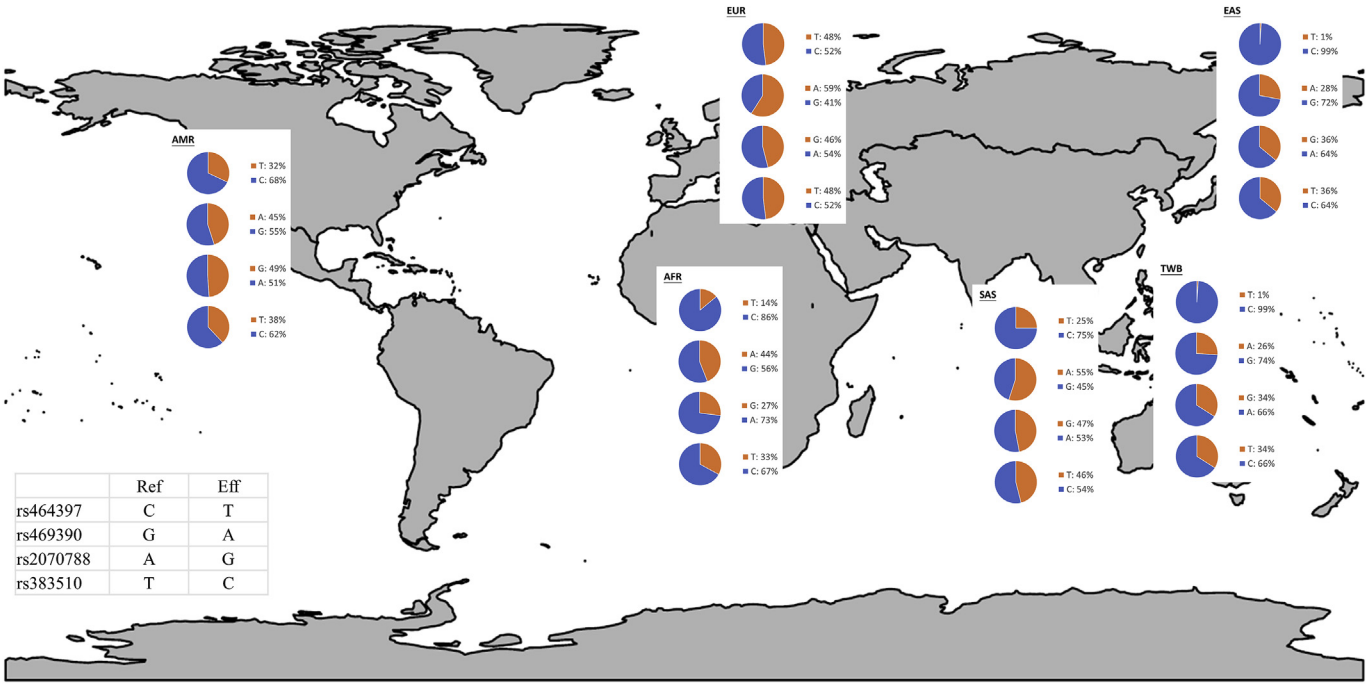

Fig. 4. The distribution of allele frequencies that influences *TMPRSS2* expression among different populations. Pie charts show the distributions of four variants (rs464397, rs469390, rs2070788 and rs383510) in various populations. EUR, European; AFR, African; AMR, American; EAS, East Asian; SAS, Southeast Asian and TWB, Taiwan Biobank. The alleles associated with higher expression of *TMPRSS2* in lung tissue are shown in orange in the pie charts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Moreover, high SARS-CoV-2 viral loads were identified in respiratory tract specimens obtained from 18 patients in Zhuhai, Guangdong, China [23], further suggesting that respiratory tract tissue is a major target for the new virus. In the present study, we found *TMPRSS2* is highly expressed in the lung. The expression of *TMPRSS2* might be expected to influence the severity of phenotype, and in support of this idea, Yoshikawa et al. found that a lack of *TMPRSS2* in the airways reduces the severity of lung pathology after infection by SARS-CoV and MERS-CoV [14].

In this study, we found that rs469390, rs2070788, rs383510 and rs464397 are associated with differential expression of *TMPRSS2*. Furthermore, rs469390 encodes a missense mutation and the AA genotype had highest expression of *TMPRSS2* in lung, with heterozygous AG carriers showing intermediate expression and the homozygous GG genotype with lowest expression (Fig. 3). According to this finding, rs469390 has potential to be associated with susceptibility to COVID-19. We suspect that the high expression AA genotype of rs469390 may be associated with higher susceptibility to the disease. Furthermore, the A allele was present at a low frequency in Asian populations, as compared to the European and American populations (Fig. 4). In addition, the rs464397 and rs383510 homozygous TT genotypes had the highest expression in lung, compared to the heterozygous CT (intermediate expression) and homozygous CC genotype (lowest expression). The T alleles of rs464397 and rs383510 exhibited lower frequency in East Asian populations compared to European and American populations, suggesting that a relatively high percentage of Europeans and Americans may have upregulated *TMPRSS2* expression associated with these genotypes. We also found the rs2070788 with GG genotype had the highest expression in lung compared to heterozygous AG and AA genotypes. This finding was consistent with a previous study that showed rs2070788 carriers with GG genotype have higher risk for severe A (H1N1) pdm09 influenza and avian human A (H7N9) influenza [10]. The G allele frequency for rs2070788 appeared to be lower in Asian populations compared to the European and American populations as well. Overall, the frequencies of variant alleles (rs464397, rs469390, rs2070788 and rs383510) associated with high *TMPRSS2* expression in lung seemed to be lower in East Asian populations compared to European, African and American populations. Thus, the European, African and American populations may have more individuals with elevated *TMPRSS2* expression, which might lead to higher susceptibility to COVID-19.

5. Conclusion

Our identification of four genetic variants with higher *TMPRSS2* expression in lung has implications for SARS-CoV-2 susceptibility in individuals and populations. We found that rs464397, rs469390, rs2070788 and rs383510 all have effects on expression of *TMPRSS2* and occur at different frequencies in various populations. Thus, the allele frequency of each variant may be an important consideration when predicting the susceptibility of populations to coronaviruses. Based on these results, future studies may examine these four variants in COVID-19 patients and assess whether any are associated with disease susceptibility and severity.

Contributions

LMI, WHC and WCC conceived and designed the study and performed all data analyses. LMI, WHC, WA, MJC, WCC and SLH interpreted the results. LMI, WHC, MJC and WCC wrote the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors disclose no conflict.

Acknowledgements

This work was supported by the grants from Ministry of Science and Technology, Taiwan (105-2628-B-038-001-MY4; 108-2314-B-038-027) and Taipei Medical University (106-5807-001-400; Yusuke Nakamura Chair Professorship, 2020). Part of the data were extracted from the Genotype-Tissue Expression (GTEx) Portal on 03/20/2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.05.179.

Abbreviations

ACE-2	Angiotensin Converting Enzyme 2
COVID-19	Corona virus disease 2019
CADD	Combined Annotation Dependent Depletion
e-QTL	Expression quantitative trait loci
ICTV	International Committee on Taxonomy of Viruses
WHO	World Health Organization
MERS	Middle East respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TMPRSS2	Transmembrane protease serine *2*

References

[1] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.

[2] J.F. Chan, S. Yuan, K.H. Kok, K.K. To, H. Chu, J. Yang, F. Xing, J. Liu, C.C. Yip, R.W. Poon, H.W. Tsoi, S.K. Lo, K.H. Chan, V.K. Poon, W.M. Chan, J.D. Ip, J.P. Cai, V.C. Cheng, H. Chen, C.K. Hui, K.Y. Yuen, A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, Lancet 395 (2020) 514–523.

[3] W.H. Organization, Naming the Coronavirus Disease (COVID-19) and the Virus that Causes it, 2020.

[4] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A Novel Coronavirus from Patients with Pneumonia in China, 2019, 382, 2020, pp. 727–733.

[5] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, S. Pohlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2) (2020) 271–280, https://doi.org/10.1016/j.cell.2020.02.052.

[6] M. Laporte, L. Naesens, Airway proteases: an emerging drug target for influenza and other respiratory virus infections, Curr. Opin. Virol. 24 (2017) 16–24.

[7] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (2003) 450–454.

[8] I. Glowacka, S. Bertram, M.A. Muller, P. Allen, E. Soilleux, S. Pfefferle, I. Steffen, T.S. Tsegaye, Y. He, K. Gnirss, D. Niemeyer, H. Schneider, C. Drosten, S. Pohlmann, Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response, J. Virol. 85 (2011) 4122–4134.

[9] V. Emilsson, G. Thorleifsson, B. Zhang, A.S. Leonardson, F. Zink, J. Zhu, S. Carlson, A. Helgason, G.B. Walters, S. Gunnarsdottir, M. Mouy, V. Steinthorsdottir, G.H. Eiriksdottir, G. Bjornsdottir, I. Reynisdottir, D. Gudbjartsson, A. Helgadottir, A. Jonasdottir, A. Jonasdottir, U. Styrkarsdottir, S. Gretarsdottir, K.P. Magnusson, H. Stefansson, R. Fossdal, K. Kristjansson, H.G. Gislason, T. Stefansson, B.G. Leifsson, U. Thorsteinsdottir, J.R. Lamb, J.R. Gulcher, M.L. Reitman, A. Kong, E.E. Schadt, K. Stefansson, Genetics of gene expression and its effect on disease, Nature 452 (2008) 423–428.

[10] Z. Cheng, J. Zhou, K.K. To, H. Chu, C. Li, D. Wang, D. Yang, S. Zheng, K. Hao, Y. Bossé, M. Obeidat, C.A. Brandsma, Y.Q. Song, Y. Chen, B.J. Zheng, L. Li, K.Y. Yuen, Identification of TMPRSS2 as a susceptibility gene for severe 2009

pandemic A(H1N1) influenza and A(H7N9) influenza, J. Infect. Dis. 212 (2015) 1214–1221.
[11] M. García-Álvarez, J. Berenguer, M.A. Jiménez-Sousa, D. Pineda-Tenor, T. Aldámiz-Echevarria, F. Tejerina, C. Diez, S. Vázquez-Morón, S. Resino, Mx1, OAS1 and OAS2 polymorphisms are associated with the severity of liver disease in HIV/HCV-coinfected patients: a cross-sectional study, Sci. Rep. 7 (2017) 41516.
[12] I. Glowacka, S. Bertram, M.A. Müller, P. Allen, E. Soilleux, S. Pfefferle, I. Steffen, T.S. Tsegaye, Y. He, K. Gnirss, D. Niemeyer, H. Schneider, C. Drosten, S. Pöhlmann, Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response, J. Virol. 85 (2011) 4122–4134.
[13] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Müller, C. Drosten, S. Pöhlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2) (2020) 271–280, https://doi.org/10.1016/j.cell.2020.02.052.
[14] N. Iwata-Yoshikawa, T. Okamura, Y. Shimizu, H. Hasegawa, M. Takeda, N. Nagata, TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection, J. Virol. 93 (2019).
[15] C. Tarnow, G. Engels, A. Arendt, F. Schwalm, H. Sediri, A. Preuss, P.S. Nelson, W. Garten, H.D. Klenk, G. Gabriel, E. Böttcher-Friebertshäuser, TMPRSS2 is a host factor that is essential for pneumotropism and pathogenicity of H7N9 influenza A virus in mice, J. Virol. 88 (2014) 4744–4751.
[16] K. Sakai, Y. Ami, M. Tahara, T. Kubota, M. Anraku, M. Abe, N. Nakajima, T. Sekizuka, K. Shirato, Y. Suzaki, A. Ainai, Y. Nakatsu, K. Kanou, K. Nakamura, T. Suzuki, K. Komase, E. Nobusawa, K. Maenaka, M. Kuroda, H. Hasegawa, Y. Kawaoka, M. Tashiro, M. Takeda, The host protease TMPRSS2 plays a major role in in vivo replication of emerging H7N9 and seasonal influenza viruses, J. Virol. 88 (2014) 5608–5616.
[17] Y. Zhao, Z. Zhao, Y. Wang, Y. Zhou, Y. Ma, W. Zuo, Single-cell RNA Expression Profiling of ACE2, the Putative Receptor of Wuhan 2019-nCov, 2020, 2020.2001.2026.919985.
[18] J. Gu, E. Gong, B. Zhang, J. Zheng, Z. Gao, Y. Zhong, W. Zou, J. Zhan, S. Wang, Z. Xie, H. Zhuang, B. Wu, H. Zhong, H. Shao, W. Fang, D. Gao, F. Pei, X. Li, Z. He, D. Xu, X. Shi, V.M. Anderson, A.S. Leong, Multiple organ infection and the pathogenesis of SARS, J. Exp. Med. 202 (2005) 415–424.
[19] S. Matsuyama, N. Nao, K. Shirato, M. Kawase, S. Saito, I. Takayama, N. Nagata, T. Sekizuka, H. Katoh, F. Kato, M. Sakata, M. Tahara, S. Kutsuna, N. Ohmagari, M. Kuroda, T. Suzuki, T. Kageyama, M. Takeda, Enhanced Isolation of SARS-CoV-2 by TMPRSS2-Expressing Cells, 2020, 202002589.
[20] S. Bertram, A. Heurich, H. Lavender, S. Gierer, S. Danisch, P. Perin, J.M. Lucas, P.S. Nelson, S. Pöhlmann, E.J. Soilleux, Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts, PloS One 7 (2012), e35876.
[21] I. Ghinai, T.D. McPherson, J.C. Hunter, H.L. Kirking, D. Christiansen, K. Joshi, R. Rubin, S. Morales-Estrada, S.R. Black, M. Pacilli, M.J. Fricchione, R.K. Chugh, K.A. Walblay, N.S. Ahmed, W.C. Stoecker, N.F. Hasan, D.P. Burdsall, H.E. Reese, M. Wallace, C. Wang, D. Moeller, J. Korpics, S.A. Novosad, I. Benowitz, M.W. Jacobs, V.S. Dasari, M.T. Patel, J. Kauerauf, E.M. Charles, N.O. Ezike, V. Chu, C.M. Midgley, M.A. Rolfes, S.I. Gerber, X. Lu, S. Lindstrom, J.R. Verani, J.E. Layden, First Known Person-To-Person Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in the USA, Lancet, London, England), 2020.
[22] Y.-C. Liu, C.-H. Liao, C.-F. Chang, C.-C. Chou, Y.-R. Lin, A Locally Transmitted Case of SARS-CoV-2 Infection in Taiwan, 382, 2020, pp. 1070–1072.
[23] L. Zou, F. Ruan, M. Huang, L. Liang, H. Huang, Z. Hong, J. Yu, M. Kang, Y. Song, J. Xia, Q. Guo, T. Song, J. He, H.-L. Yen, M. Peiris, J. Wu, SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients, 382, 2020, pp. 1177–1179.