




ACE2 and TMPRSS2 Potential Involvement in Genetic Susceptibility to SARS-COV-2 in Cancer Patients

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic. One open question is whether genetics could influence the severity of symptoms. Considering the limited data on cancer patients, we analyzed public data repositories limited to investigate angiotensin-converting enzyme 2 (ACE2) and the transmembrane serine protease 2 (TMPRSS2) expressions and genetic variants to identify the basis of individual susceptibility to SARS-CoV-2.

Gene expression and variant data were retrieved from Tissue Cancer Genome Atlas, Genotype-Tissue Expression, and gnomAD. Differences in gene expression were tested with Mann-Whitney U-test. Allele frequencies of germline variants were explored in different ethnicities, with a special focus on ACE2 variants located in the binding site to SARS-CoV-2 spike protein.

The analysis of ACE2 and TMPRSS2 expressions in healthy tissues showed a higher expression in the age class 20 to 59 years (false discovery rate [FDR] < 0.0001) regardless of gender. ACE2 and TMPRSS2 were more expressed in tumors from males than females (both FDR < 0.0001) and, opposite to the regulation in tissues from healthy individuals, more expressed in elderly patients (FDR = 0.005; FDR < 0.0001, respectively). ACE2 and TMPRSS2 expressions were higher in cancers of elderly patients compared with healthy individuals (FDR < 0.0001). Variants were present at low frequency (range 0% to 3%) and among those with the highest frequency, the variant S19P belongs to the SARS-CoV-2 spike protein binding site and it was exclusively present in Africans with a frequency of 0.2%.

The mechanisms of ACE2 and TMPRSS2 regulation could be targeted for preventive and therapeutic purposes in the whole population and especially in cancer patients.

Further studies are needed to show a direct correlation of ACE2 and TMPRSS2 expressions in cancer patients and the incidence of COVID-19.

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Keywords

ACE2, TMPRSS2, SARS-CoV-2, individual susceptibility

Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory disease that outbreaked first in the city of Wuhan (China) in December 2019 and has reached a global pandemic dimension as declared by the World Health Organization on March 11, 2020, globally affecting about 3 million people as of April 27 this year¹⁻³. This respiratory disease is caused by a novel RNA coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), characterized by rapid and efficient human-to-human transmission in the population that is devoid of preexisting immunity and left without efficacious treatments or vaccines. Given the COVID-19 coronavirus emergency, a special focus is needed on the impact of this rapidly spreading viral infection on cancer patients. Patients with cancer are more vulnerable to infections than other individuals because malignancy and anticancer therapy result in an immunosuppressive status and more likely to have higher morbidity and mortality than the general population³. For example, patients with lung cancer usually have compromised lung function with associated dyspnea, cough, and polypnea. They might be at higher risk of severe forms of SARS-CoV-2 infection due to decreased pulmonary function³. In the small case series of 18 Chinese patients considered, those with cancer were older and more likely to have a history of smoking than patients without cancer. However, no significant differences in sex, other baseline symptoms, and other comorbidities were found except older age, which was the only risk factor for severe events². An Italian study assessing the case fatality of COVID-19 found that among 355 patients who died and underwent detailed chart review, 72 (20.3%) had active cancer⁴. Some recent evidences suggest that gender⁵, smoke⁵, physical exercise⁶, previous vaccinations⁷, blood type⁸, human leukocyte antigen (HLA) variants⁹, and alterations in the genes involved in the pathogenetic mechanism of SARS-CoV-2 like the angiotensin-converting enzyme 2 (ACE2) and the transmembrane serine protease 2 (TMPRSS2), can affect individual susceptibility to SARS-CoV-2 infection¹⁰. SARS-CoV cell entry relies on the binding of the spike protein S1 to ACE2 receptor¹¹⁻¹³ and on protein priming by host's serine protease TMPRSS2, which entails S protein cleavage at the S1/S2 and the S2' site leading to the fusion of the viral envelope with cell membranes by the S2 subunit¹⁴. Analysis of the receptor-binding motif, a portion of the receptor-binding domain that takes contact with ACE2^{12,15,16}, revealed that most amino acid residues essential for ACE2 binding by SARS-CoV S were conserved in SARS-CoV-2 S. In contrast, most of these residues were absent from S proteins of SARS-CoV previously found not to use ACE2 for entry¹⁷⁻¹⁹. Among the many mysteries

about COVID-19, one is why it hits some people harder than others. In recent weeks, researchers have begun asking whether genetics could influence the severity of symptoms. In this context, oncologic patients require even more attention. Recently, COVID-19 and Cancer Consortium, a collaborative effort aimed to learn more about the impact of the new coronavirus on people affected by hematologic and solid malignancies, has been launched. In order to protect cancer patients against COVID-19 infection, some treatments can be envisioned (Fig. 1). Active vaccination is the gold standard but anti-COVID-19 vaccines efficacy is still under investigation. Passive vaccination can be elicited by hyperimmune plasma or by neutralizing antibodies administration thereby hindering SARS-CoV-2 spike protein interaction with ACE2. ACE2 and SARS-CoV-2 interaction can be altered also by targeting the process of protein terminal glycosylation in the endoplasmic reticulum for both ACE2 and the spike protein in infected cells (i.e., by hydroxychloroquine and/or by ammonium chloride [NH₄Cl])^{20,21}. Moreover, TMPRSS2 can be targeted both by drugs that inhibit its enzymatic activity (i.e., camostat mesilate) or by treatments that inhibit its expression (i.e., antiandrogen therapies)²² (Fig. 1).

The knowledge of the genetic variability in the cancer population could be useful to identify new mechanisms of protection against infection. Considering the poor and discordant data on the genetic characteristics that can affect SARS-CoV-2 susceptibility in cancer patients, we retrieved available data from public repositories and aimed to investigate:

- ACE2 and TMPRSS2 differences in gene expression in healthy tissues were analyzed according to age and gender, whereas germline variants frequencies for ACE2 and TMPRSS2 for ethnicity to identify possible genetic predispositions or resistance determinants to SARS-CoV-2 infection.
- ACE2 and TMPRSS2 somatic variant frequencies and differences in their expression between different tumor types as compared with their tissue of origin to test if specific cancer types may provide an additional replication site for SARS-CoV-2 during infection in those patients.

Methods

The data were downloaded from the Tissue Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) portal²³ (dbGaP accession number phs000424.v8.p2 on 04/10/2020), for tumor and healthy samples, respectively. In the TCGA portal, we selected PanCancer Atlas studies,

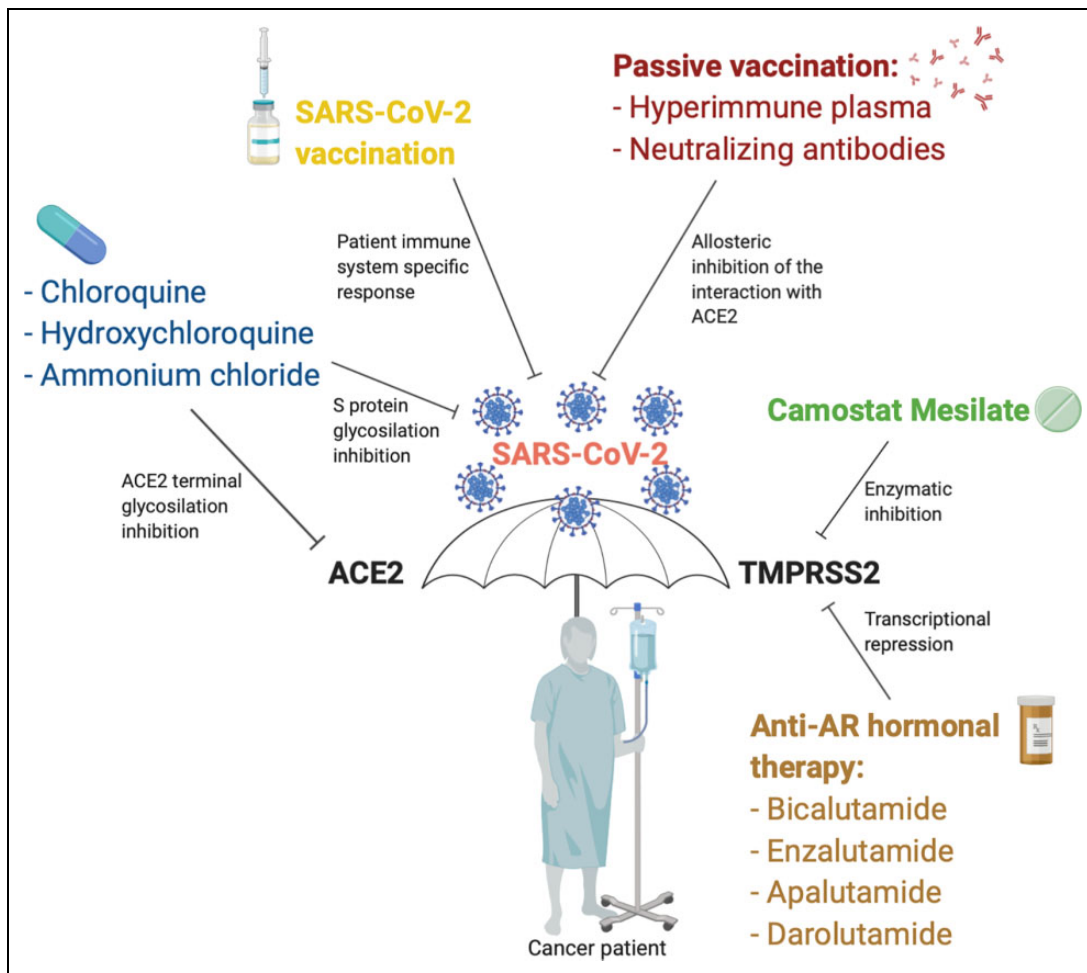


Fig. 1. Schematic representation of the possible treatments to protect a cancer patient from severe acute respiratory syndrome coronavirus 2 infection.

including 35 cancer types. In this dataset, we focused on the expression data of ACE2 and TMPRSS2 in 10,953 oncologic patients. From the GTEx portal, we analyzed the expression of 22,951 tissue samples from 980 healthy donors and related clinical data available. Gene expression data were downloaded as transcripts per million (TPM) and transformed to $\log_2(\text{TPM} + 1)$ before analyses. Given the strong asymmetry of data distribution, even after log transformation, the difference in expression among groups was tested with Mann-Whitney U-test (SciPy v1.4.1); resulting P values were adjusted for multiple testing using Benjamini-Hochberg procedure (statsmodels v0.11.0)^{24,25}, resulting in FDR values. Reflecting epidemiological evidence on COVID-19 mortality in Italy (<https://www.statista.com/statistics/1106372/coronavirus-death-rate-by-age-group-italy/>), the variable age was grouped into two categories: under (20 to 59) and over 60 years (60 to 69), labeled, respectively, as “young” and “elder” in the plots and tables.

Data on DNA variants in healthy populations were downloaded from gnomAD v3 in the form of 2 Variant Call

Format files, one for chromosome X and one for chromosome 21. Variants overlapping with ACE2 and TMPRSS2 were extracted using bedtools v2.29.2 and then annotated with ANNOVAR (v2016-02-01)²⁶. For somatic variants, we explored TCGA mutation data from 35 tumor types and extracted only variants affecting ACE2 and TMPRSS2. For ACE2, variants were deemed as possibly affecting function if (1) were frameshift INDELs or (2) single nucleotide variants located in residues involved in binding to SARS-CoV-2 spike protein¹⁰.

Results

ACE2 and TMPRSS2 Expression Analysis in Healthy Tissues from GTEx Portal and Cancers from TCGA Dataset

The analysis of healthy tissues from the GTEx portal revealed that the testis, small intestine, kidney, thyroid, and heart express the highest amount of ACE2 gene in the human

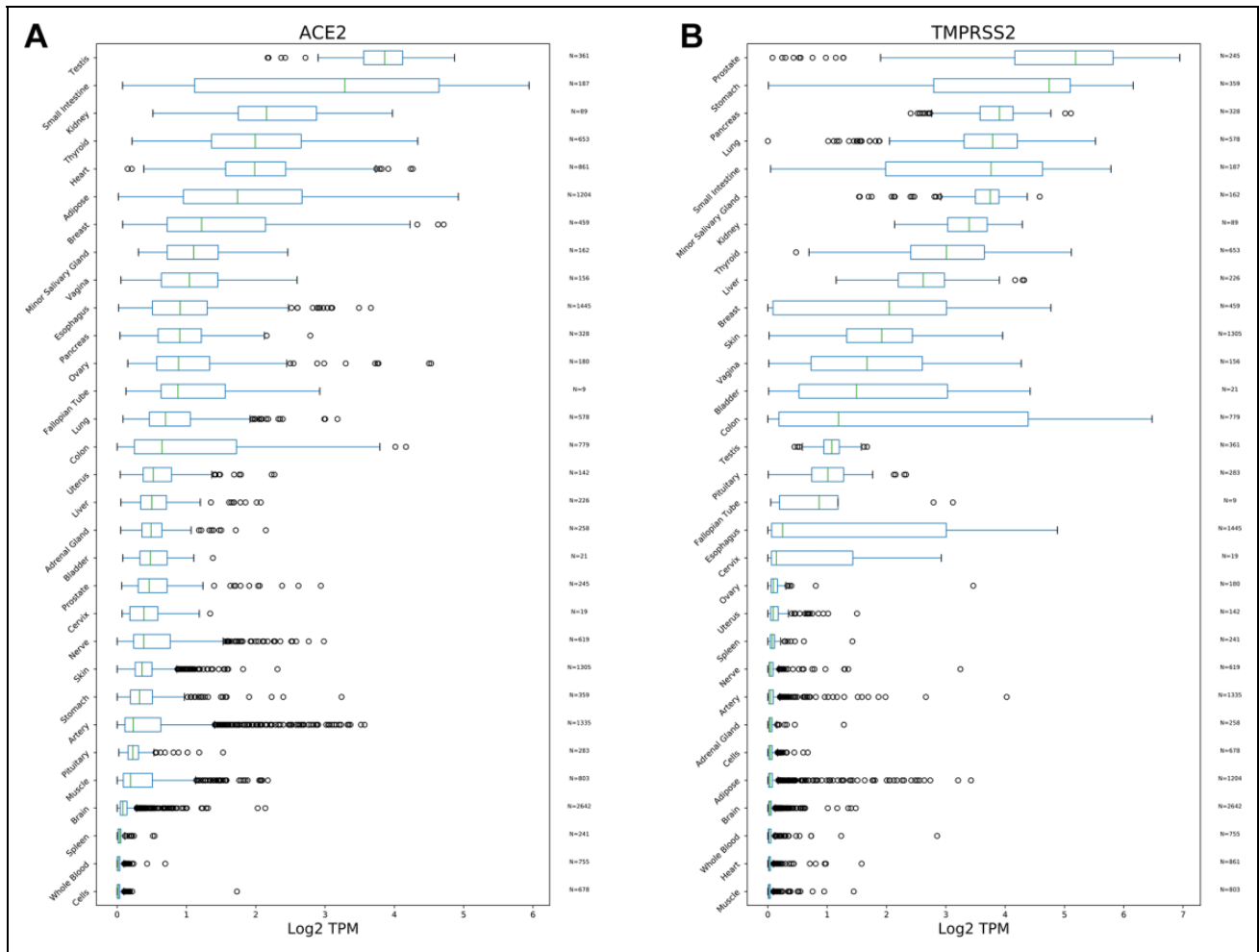


Fig. 2. (A) ACE2 and (B) TMPRSS2 expression in healthy tissues included in the Genotype Tissue Expression portal. ACE2, angiotensin-converting enzyme 2 (ACE2); TMPRSS2: transmembrane serine protease.

body (Fig. 2A). Surprisingly, ACE2 expression was low in lung tissue. As for the expression of TMPRSS2 in healthy tissues, the highest levels were observed in the prostate, stomach, pancreas, lung, small intestine, and salivary glands. The highest variability in TMPRSS2 expression was seen in colon tissue, even if the median expression value was quite low (Fig. 2B).

The analysis of the TCGA dataset revealed that the highest levels of ACE2 expression are in the renal carcinoma, colorectal adenocarcinoma, and in the undifferentiated stomach adenocarcinoma (Fig. 3A). Conversely, leukemias, mature B cell neoplasms, melanoma, and pleural mesothelioma showed the lowest ACE2 expression (Fig. 3A). Interestingly, the tumors with the highest levels of TMPRSS2 expression were prostate adenocarcinoma, followed by colorectal adenocarcinoma and esophagogastric adenocarcinoma (Fig. 3B). TMPRSS2 was almost absent in the adrenocortical carcinoma, pheochromocytoma, leukemia, and B-cell neoplasm (Fig. 3B).

The analysis of ACE2 and TMPRSS2 expressions in healthy tissues showed a higher expression in the age class 20 to 59 years (false discovery rate [FDR] < 0.0001), regardless of gender (Fig. 4A). ACE2 expression analyzed in healthy tissues was higher in younger individuals considering nerve, colon, blood, and salivary gland tissues. Data suggested that females' blood, adipocytes, brain, and heart have a higher expression of ACE2 than males. On the contrary, ACE2 expression was higher in males' esophagus and breast. Expression of TMPRSS2 was higher in the colon and in the lung of younger subjects, in females' breast and males' pituitary glands.

ACE2 and TMPRSS2 are more expressed in males considering the tumor dataset (both FDR < 0.0001) and more expressed in older patients (FDR = 0.005; FDR < 0.0001, respectively) (Fig. 4B). In contrast, a lower expression of ACE2 was seen in males with head and neck (FDR = 0.005), renal clear cell (FDR = 0.02), and bladder cancers (FDR = 0.02). The analysis of TMPRSS2 expression on the basis of tumor type suggested that males have higher

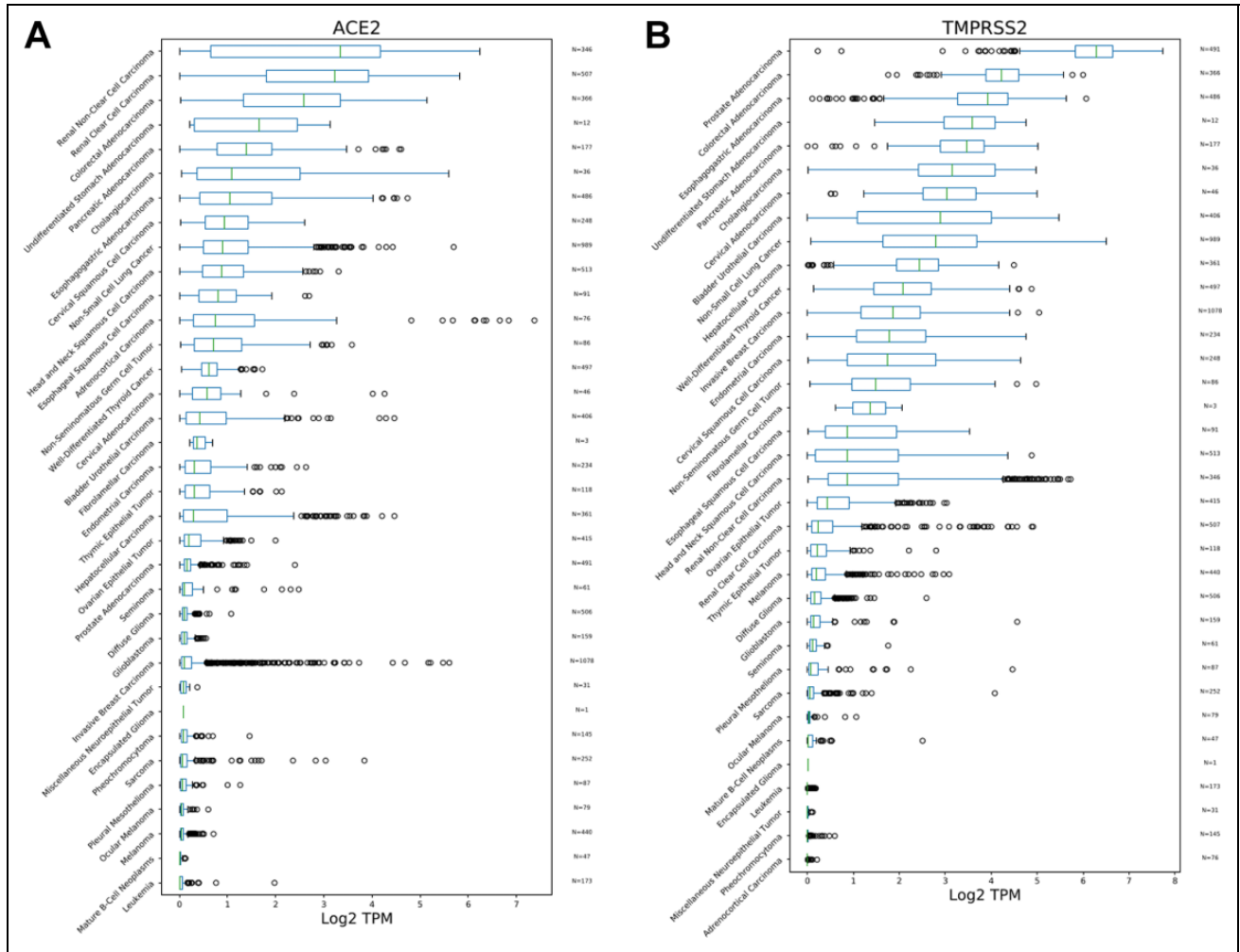


Fig. 3. (A) ACE2 and (B) TMPRSS2 expression among cancer types of Tissue Cancer Genome Atlas. ACE2, angiotensin-converting enzyme 2 (ACE2); TMPRSS2: transmembrane serine protease 2.

expression in head and neck cancers ($P = 0.013$, $FDR = 0.09$). In contrast, a lower expression was seen in males than in females with nonsmall cell lung cancer ($FDR < 0.0001$). Prostate adenocarcinoma of younger patients expressed slightly more TMPRSS2 than the elderly with $FDR = 0.026$.

Comparison of Expression Data Between GTEx and TCGA Datasets

Sex, age, and tissue categories in the GTEx dataset were compared with their corresponding category in the TCGA dataset (i.e., healthy young males to TCGA young males).

Elderly cancer patients had higher expression of both genes ($FDR < 0.0001$) (Fig. 5). TMPRSS2 was more expressed in cancer patients for all age and sex categories considered as compared with healthy subjects (Fig. 5). In particular, ACE2 and TMPRSS2 expressions were higher in elderly males with cancers compared with healthy individuals ($FDR < 0.0001$) (Fig. 5).

ACE2 expression was significantly higher as compared with the healthy tissue for the following cancers: adrenal gland, brain, colon, kidney, lung, pancreas, stomach. A significant decrease of ACE2 expression was seen in the breast, liver, ovarian, prostate, skin, testis, thyroid, and uterus cancers compared with the healthy tissue (Fig. 6). TMPRSS2 expression was significantly higher in tumor tissue of bladder, brain, colon, esophagus, ovary, prostate, and uterus cancers as compared with healthy tissues. Conversely, TMPRSS2 expression was significantly lower in tumor tissue of the adrenal gland, kidney, liver, lung, pancreas, skin, stomach, testis, and thyroid compared with the respective healthy tissue (Fig. 6). Although there were few samples of healthy bladder tissue.

ACE2 and TMPRSS2 Variant Analysis

The following analysis focused on the examination of ACE2 and TMPRSS2 germline variants in the healthy population

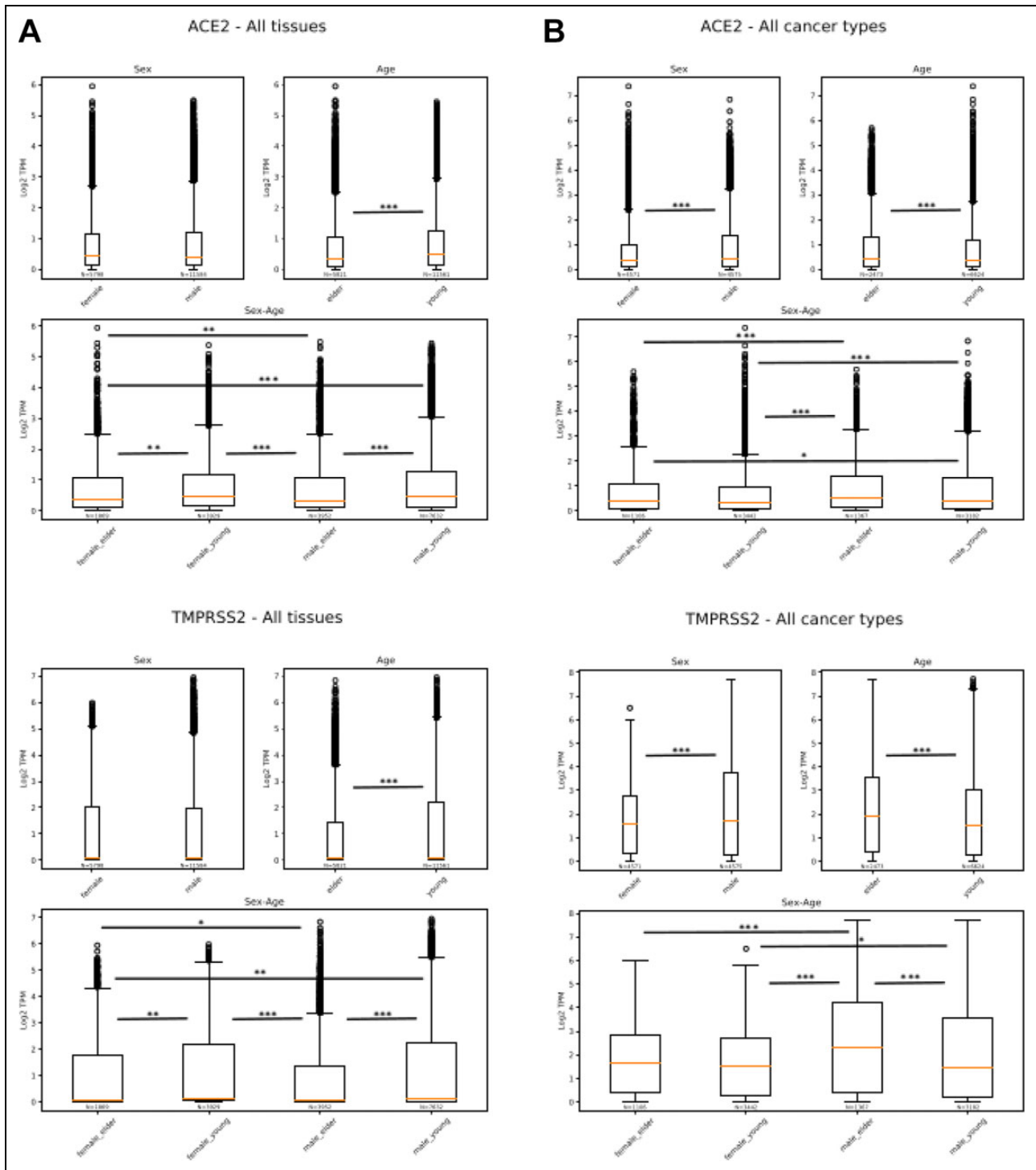


Fig. 4. ACE2 and TMPRSS2 expressions according to sex and age categories, in (A) healthy donor tissues from Genotype Tissue Expression portal and (B) Tissue Cancer Genome Atlas cancers. *FDR < 0.05 ** FDR < 0.01 *** FDR < 0.001. ACE2: angiotensin-converting enzyme 2 (ACE2); FDR: false discovery rate; TMPRSS2: transmembrane serine protease 2.

considering nonsynonymous variants, insertions, and deletions that cause amino acids to change along the whole coding sequence. This analysis regarding ACE2 identified 178 variants in nine ethnic groups (Fig. 7A). Variants were present at low frequency (range 0% to 3%) among the different

populations and did not differ significantly between genders (Fig. 7A). Most of these variants had very low frequency (<0.2%); in particular, out of 178 variants identified, 10 for females and 11 for males had considerably higher frequency compared with the others (Fig. 7A). In particular, we

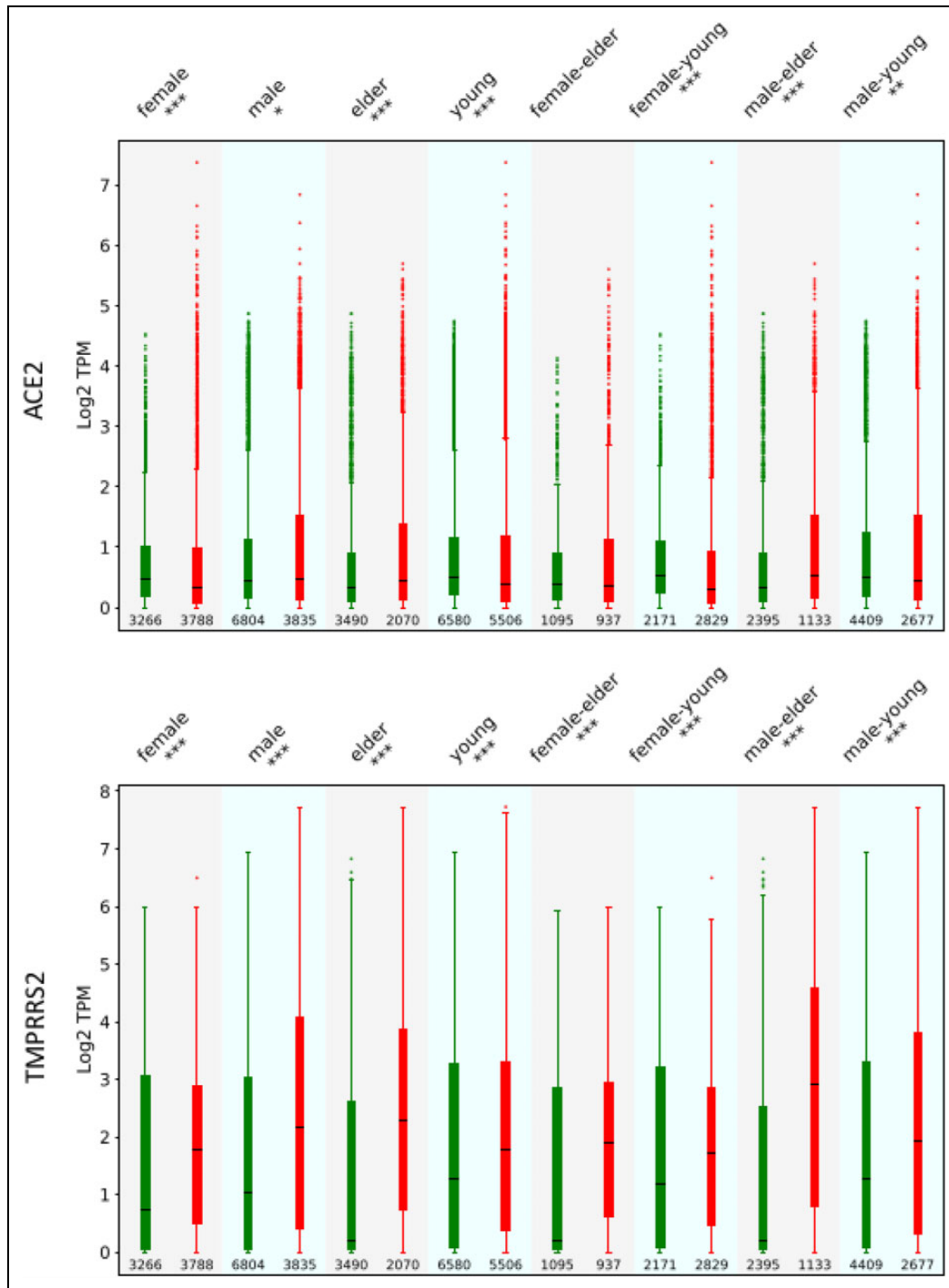


Fig. 5. Comparison of ACE2 and TMPRSS2 expressions between healthy donor tissues (displayed in green boxplots) and Tissue Cancer Genome Atlas cancers (displayed in red boxplots) according to sex and age categories. *FDR < 0.05; **FDR < 0.01; ***FDR < 0.001. ACE2: angiotensin-converting enzyme 2 (ACE2); FDR: false discovery rate; TMPRSS2: transmembrane serine protease 2.

analyzed the variants in the region involved in the interaction with SARS-CoV-2 spike protein¹⁰ and identified possibly functional modifications (see section Methods) that may hinder viral docking and entry into target cells in the different ethnic groups. Interestingly, among those with the highest frequency, the variant S19P belongs to the

SARS-CoV-2 spike protein binding site and it was exclusively present in Africans with a frequency of 0.2% (Fig. 7A). In Finnish male, the E37 K variant was found with a frequency of 0.05%. According to a recent study, both mutations may affect the interaction with the virus to a certain extent²⁷.

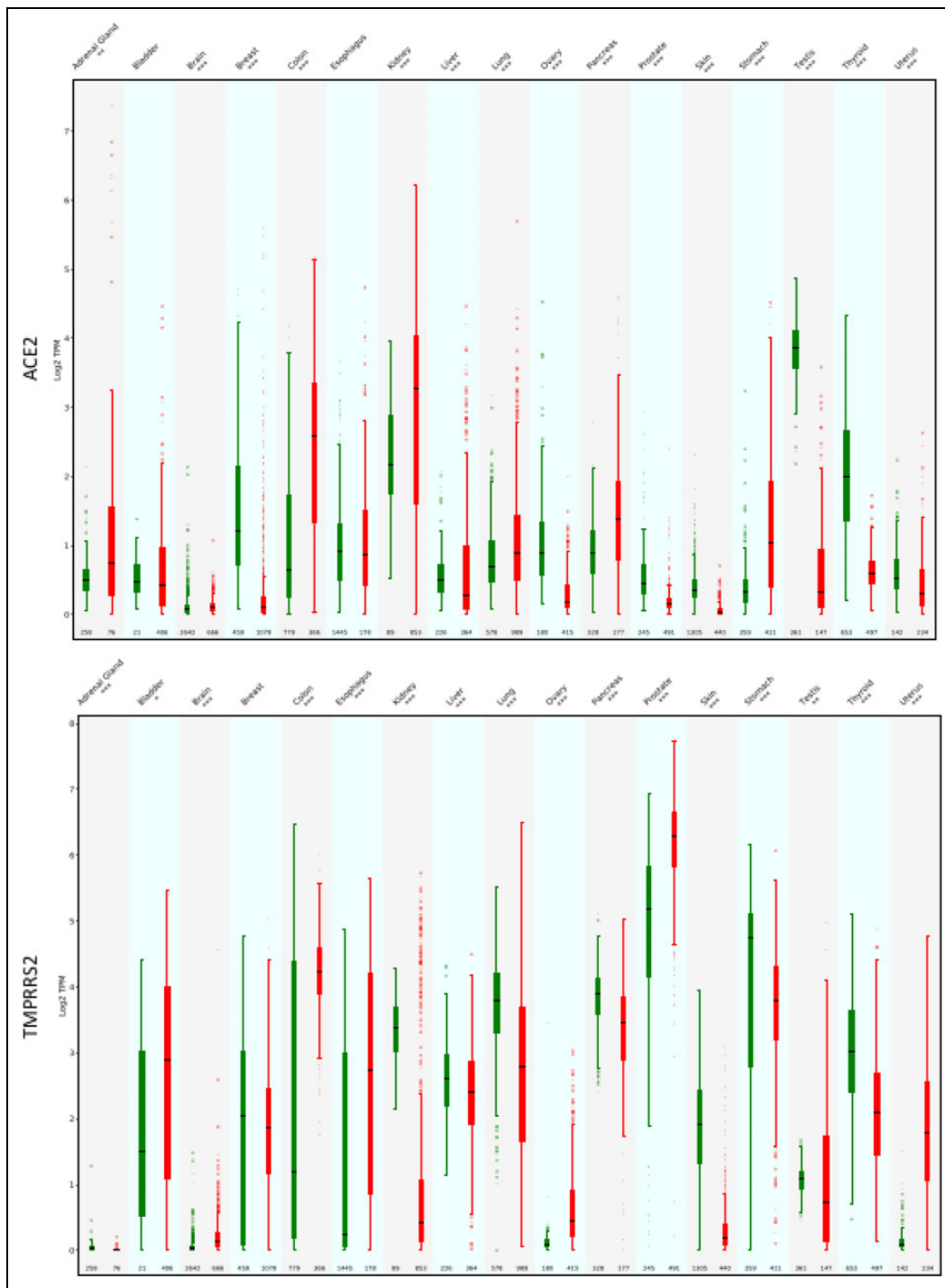


Fig. 6. Comparison of ACE2 and TMPRSS2 expressions between healthy donor tissues (displayed in green boxplots) and Tissue Cancer Genome Atlas cancers (displayed in red boxplots) in the different tissues. *FDR < 0.05; **FDR < 0.01; ***FDR < 0.001. ACE2: angiotensin-converting enzyme 2 (ACE2); FDR: false discovery rate; TMPRSS2: transmembrane serine protease 2.

We identified 261 TMPRSS2 variants across the ethnic groups with frequencies of up to 50% in African and Asian populations (Fig. 7B). No differences in their frequency between males and females were observed. None of the residues of TMPRSS2 active sites (H²⁹⁶, D³⁴⁵, D⁴³⁵, and S⁴⁴¹) have been found altered in the considered populations.

The mutation analysis of the 35 cancer types included in the TCGA project showed that ACE2 was frequently altered in the different tumor types (18/35; 51.4%), while it showed alterations in only 1.0% of all TCGA patients. In particular, endometrial carcinoma showed the highest proportion of patients with ACE2 somatic mutations

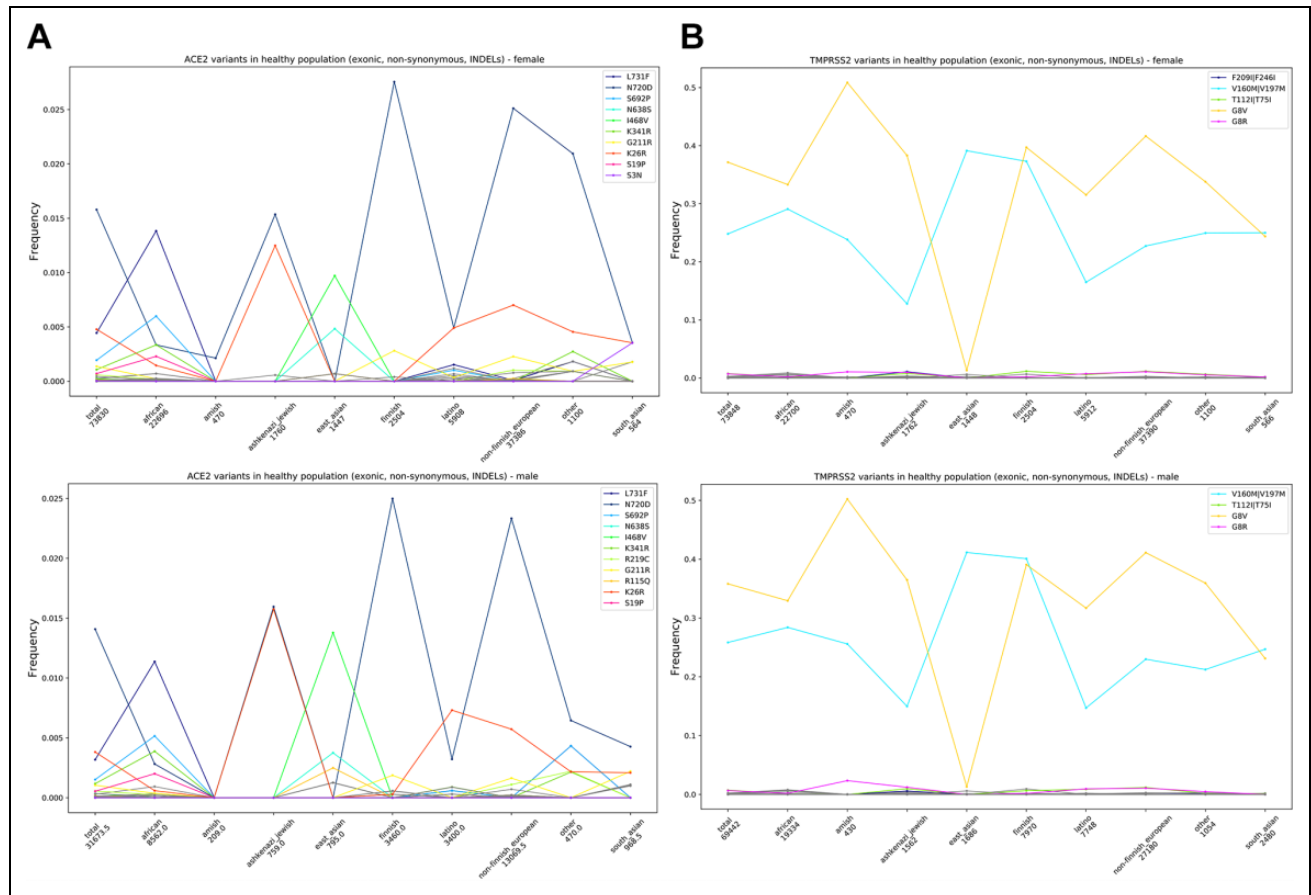


Fig. 7. (A) ACE2 and (B) TMPRSS2 variant frequencies in the healthy population considering non-synonymous variants, insertion, and deletions, in females (upper side) and males (lower side). Variants with the frequency above the threshold 0.2% for ACE2 and 1% for TMPRSS2 are highlighted with a color; the others are displayed in grey. ACE2: angiotensin-converting enzyme 2 (ACE2); TMPRSS2: transmembrane serine protease 2.

(6.0%), followed by melanoma (3.2%) and colorectal adenocarcinoma (2.2%). For what concerns the analysis of TMPRSS2, 14/35 (40%) cancer types presented mutations on this gene and 0.7% of patients had at least one mutation. Endometrial carcinoma (5.8% of patients with mutations), melanoma (5.4%), and colorectal adenocarcinoma (3.5%) were the cancer types with the highest percentage of mutations, and, surprisingly, they were the same cancer types with the highest percentage of ACE2 mutations.

Discussion

Considering that the COVID-19 emergency is affecting the worldwide population, there is an urgent need to identify among the vulnerable subjects the weakest ones. Special attention should be paid to oncologic patients that are at an advanced age and are commonly affected by comorbidities. Treatment with single or combined chemotherapy, radiotherapy, or more recently immunotherapy may affect the immune status and cause adverse events.

Therefore, we focused our research limited on the study of ACE2 and TMPRSS2 gene expression variants and from healthy donors and cancer patients using public datasets, in order to potentially identify the individuals at a higher risk of SARS-CoV-2 infection. We supposed that the patients with a higher expression of ACE2 and TMPRSS2 could be more vulnerable because viral entry into host cells is facilitated given that a higher proportion of cells are expressing ACE2 and TMPRSS2²⁸. It is reasonable to think that tumor patients whose cancers show high expression levels of ACE2 and TMPRSS2 may provide additional target cells for SARS-CoV-2 replication.

The overall pooled prevalence of cancer in patients with COVID-19 in a recent meta-analysis was 2%; however, current evidence on the association between cancer and SARS-CoV-2 infection remains inconclusive²⁹. Results from studies on the genetics of COVID-19 susceptibility and severity are beginning to trickle in, evaluating if variants in the HLA genes or ABO blood type might play a role, but the findings are all preliminary and require follow-up with larger datasets⁸. With this retrospective study, we investigated

ACE2 and TMPRSS2 as susceptibility factors to SARS-CoV-2 infection. In contrast to epidemiological evidences of higher mortality in elders, we found a higher expression of ACE2 and TMPRSS2 in healthy tissues of young individuals. In contrast, regarding the tumor dataset, ACE2 and TMPRSS2 were highly expressed in males with tumors with respect to females and in elderly patients suggesting they might be even more at risk and predisposed to higher chances of severe adverse events.

Viral effects on humans are different, probably due to the heterogeneous individual susceptibility and to the different viral spreading and host immune response. Although the lungs are ground zero, symptoms can extend up to many organs, including the small intestine and kidneys probably due to high ACE2 and TMPRSS2 expressions. We also compared our expression data with the mRNA and protein expression levels reported on both the Tissue and Pathology Atlas databases of The Human Protein Atlas. The comparison showed that the data were concordant, and in particular, renal, colorectal, and stomach cancers had the highest levels of ACE2 protein expression, coherent with their own mRNA expression, while lung cancers had low or no detectable protein and mRNA expressions.

However, it is not clear if the gravity of symptoms is proportional to the expression of key viral entry enzymes in specific organs or even due to the patient's immune response or both. According to literature data, we observed that the highest expression values of ACE2 and TMPRSS2 were in the small intestine and this could explain the intestinal symptoms in COVID-19 patients. The fecal-oral transmission of SARS-CoV-2 has not been demonstrated yet. However, if confirmed, the virus could reach intestinal cells expressing ACE2 and TMPRSS2. These data might be relevant in light of the clinical manifestations of the disease. It is indeed believed that multiorgan failure in the advanced stages of COVID-19 is the result of an uncontrolled proliferation and activation of the immune system with a supra-physiological release of IL-1, IL-6, and TNF- α resulting in witnessing a cytokine release syndrome. In addition, COVID-19 patients present with diarrhea in 2% to 50% of cases³⁰, might show cardiac complications up to fibrillations, myocarditis, and heart arrest³¹, and are likely related to high ACE2 expression in the intestine and the heart, respectively. Importantly, patients with diarrhea remain positive for SARS-CoV-2 in the feces up to 12 days after disease onset, with a considerable fraction of individuals still negative if tested in the respiratory tract³². Hence, it is not surprising that traces of viral RNA have been detected in wastewater and sewers in several countries, and that disease spreading can be monitored through the analysis of wastewater³³. Our understanding of SARS-CoV-2 transmission is still evolving and the fecal-oral route cannot be per se ruled out, also considering the extreme stability of the virus. Increasing evidence instead indicates a possible fecal-oral COVID-19 transmission³⁴, defining a particularly high risk for populations in countries where hygienic

measures and the availability of clean drinking water are inadequate. In these areas of the globe, specific measures should be considered.

Retrospective and prospective analyses of ACE2 and TMPRSS2 in COVID-19 patients with severe symptoms such as stroke and interstitial pneumonia have to be performed to study the correlation of their expression in specific organs.

Surprisingly and slightly different from what reported from others³⁵, despite the high variability observed, we found only moderate median values in terms of expression of ACE2 in lungs considering both healthy and tumor tissues that anyway presented a higher expression, while TMPRSS2 was more expressed in healthy lung tissue as compared with the tumor tissue.

However, we observed a significantly higher ACE2 expression in cancers of the adrenal gland, brain, colon, kidney, lung, pancreas, and stomach as compared with the relative healthy tissue, resulting in a likely higher susceptibility of these tumors to SARS-CoV-2 infection. However, data on the higher susceptibility to the virus for lung cancer patients are discordant and not related only to ACE2 expression^{29,35}. In addition, the high expression of TMPRSS2 in the healthy prostatic tissue and ACE2 in healthy testis is not surprising and could explain the higher incidence of COVID-19 in male subjects. Recently, impaired testosterone production from testes in males has been reported³⁶, coherently with the corresponding tissues showing the highest ACE2 expression. In fact, androgens and androgen receptor (AR) have been known to affect ACE2 and TMPRSS2 expressions^{37,38}. This implies that patients with prostate cancer that are treated with anti-AR therapy could be less susceptible to viral infection. The use of anti-AR compounds could also be used as a therapeutic strategy and preventive option in these patients to avoid viral entry^{38,39}. In the future, other cancer types expressing AR, besides prostate cancer that can be treated with anti-AR compounds as a therapeutic option, would benefit from this type of treatment and could be less susceptible to SARS-CoV-2 infection and likely to its most severe effects.

Previous studies have discovered gene variants that can alter a person's chances of contracting an infectious disease. An example is a mutation in the CCR5 gene that offers protection against HIV infection⁴⁰. Similar results in the context of SARS-CoV-2 infection are lacking. Our findings show some differences in terms of frequency of ACE2 and TMPRSS2 variants among different populations. In particular, the ACE2 variants S19P, exclusively present in Africans, and the E37 K in Finnish males, even if with a low frequency, could be protective factors to SARS-CoV-2¹⁰. It should be mentioned that the presence of genetic variants does not always affect gene expression and that ACE2 and TMPRSS2 overexpression could occur in response to other mechanisms, such as alterations in other genes. Moreover, other studies are needed to demonstrate a direct correlation of ACE2 and TMPRSS2 genes and the incidence of COVID-

19 in cancer patients as well as the role of other factors involved in SARS-CoV-2 entry.

Conclusions

We are aware that this study is limited by the lack of statistics on oncologic patients affected by SARS-CoV-2, due to its retrospective nature. However, our results, derived from a systematic analysis of online public data repositories, try to explain different individual susceptibility to SARS-CoV-2 among the healthy and oncologic population. Our descriptive study supports hypotheses that merit further investigations with a future impact on patient management decisions. In conclusion, we have analyzed ACE2 and TMPRSS2 expressions and their variants, as potential factors of susceptibility to SARS-CoV-2. The analysis of these genes and the mechanisms involved in their regulation could be used for both preventive and therapeutic purposes in the whole population, paying particular attention to cancer patients.

Additional Information

All the data are available on request.

Authors' Contributions

GM conceived the study. SR, MM, and SB wrote the article. MT, EF, DA, AL, FF, FP, MMT, CCPV, and VS performed the data collection. SR, SB, MM, MT, EF, and DA analyzed the data. FN contributed to the design of the figures. All the authors approved and revised the manuscript.

Ethical Approval

The study was reviewed and approved by the Medical Scientific Committee of Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS (code: L2P2320) and by Comitato Etico della Romagna, C.E.ROM (Approval number IRST100.51).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Medical Scientific Committee of Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS (code: L2P2320) and by Comitato Etico della Romagna, C.E.ROM (Approval number IRST100.51).

Statement of Informed Consent

Informed consent for patient information to be published in this article was not obtained because our study was carried out on public data repositories and does not include any individual person's data in any form.


Declaration of Conflicting Interests


The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: MG has conflicts of interest with Novartis, BMS, Roche, Pfizer, ARIAD, and MSD. All other authors declare no conflicts of interest.

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