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# *TMPRSS2* and COVID-19: Serendipity or Opportunity for Intervention?

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**Summary:** *TMPRSS2* is both the most frequently altered gene in primary prostate cancer and a critical factor enabling cellular infection by coronaviruses, including SARS-CoV-2. The modulation of its expression by sex steroids could contribute to the male predominance of severe infections, and given that *TMPRSS2* has no known indispensable functions, and inhibitors are available, it is an appealing target for prevention or treatment of respiratory viral infections.

## INTRODUCTION

The global COVID-19 pandemic, caused by the SARS-CoV-2 virus, has led to more than 1,400,000 diagnosed cases and more than 80,000 reported deaths as of April 8, 2020. These epidemiologic statistics represent the tip of the iceberg, given ongoing significant transmission, high rates of subclinical infection, insufficient testing in multiple communities, and potential differences in attribution of cause of death in infected patients.

The global research community has coalesced on multiple fronts to understand the mechanisms of infection and the heterogeneity in the virulence of SARS-CoV-2, as well as the constellation of symptoms and risk factors for subsequent mortality. One key discovery in understanding the mechanism of SARS-CoV-2 infection involves the role of the transmembrane serine protease 2 (TMPRSS2), a cell-surface protein that is expressed by epithelial cells of specific tissues including those in the aerodigestive tract. As one of the serendipities of science, many of the insights related to TMPRSS2 have come from cancer research. This overview summarizes the history of the connection of TMPRSS2 with coronaviruses as well as influenza viruses, provides insights derived from cancer research, and integrates what is known (and not known) concerning the potential roles of TMPRSS2 as a target for intervention in or prevention of COVID-19.

## TMPRSS2 AND RESPIRATORY VIRUSES

Coronaviruses as well as influenza viruses critically depend on TMPRSS2 for viral entry and spread in the host. As a first step enabling host-cell entry, the viral hemagglutinin protein attaches to angiotensin-converting enzyme 2 (ACE2), encoded by the *ACE2* gene, that is expressed on respiratory epithelial cells. In a second step, hemagglutinin is cleaved to activate internalization of the virus. This second step is dependent on proteases on the host cell, particularly TMPRSS2 (1). Moreover, not only SARS-CoV-2 but also other types of coronaviruses and influenza viruses depend on TMPRSS2 for viral activation and cell entry, including SARS-CoV, the agent responsible for the 2003 SARS outbreak, as well as influenza H1N1, the agent responsible for the 1918 and 2009 influenza pandemics (2–4). These examples highlight the central and conserved role of TMPRSS2 in the pathogenesis of the illnesses caused by coronaviruses and influenza viruses.

In an *in vitro* study using cell lines and primary pulmonary cells, an inhibitor of the protease activity of TMPRSS2, camostat mesylate, partially inhibited the entry of SARS-CoV-2 into these lung epithelial cells (4). In a *Tmprss2* knockout model, mice infected with the H1N1 influenza virus showed minimal initial infection and had a considerably attenuated disease course with protection from lung pathology, weight loss, and mortality compared with wild-type control mice (5). Given its central role in initiating SARS-CoV-2 and other respiratory viral infections, modulating TMPRSS2 expression or activity is hypothesized to represent a promising candidate for potential interventions against COVID-19.

## *TMPRSS2*, A KEY REGULATOR IN PROSTATE CANCER

*TMPRSS2* was first identified in prostate cancer shortly after the gene had been originally cloned. Prostate cancer cell lines strongly upregulated *TMPRSS2* expression in response to androgens (6). TMPRSS2 is expressed on the luminal side of the prostate epithelium, and its expression is increased in prostate cancer tissue compared with noncancerous prostate tissue (7). Notably, the *TMPRSS2* gene is a partner in one of the most common gene fusion events in solid tumors: somatic gene rearrangements involving *TMPRSS2* with a member of the ETS family of oncogenic transcription factors, most commonly *ERG*. This fusion occurs in approximately 50% of primary prostate cancers among men of European ancestry. Although *ERG* is not normally regulated

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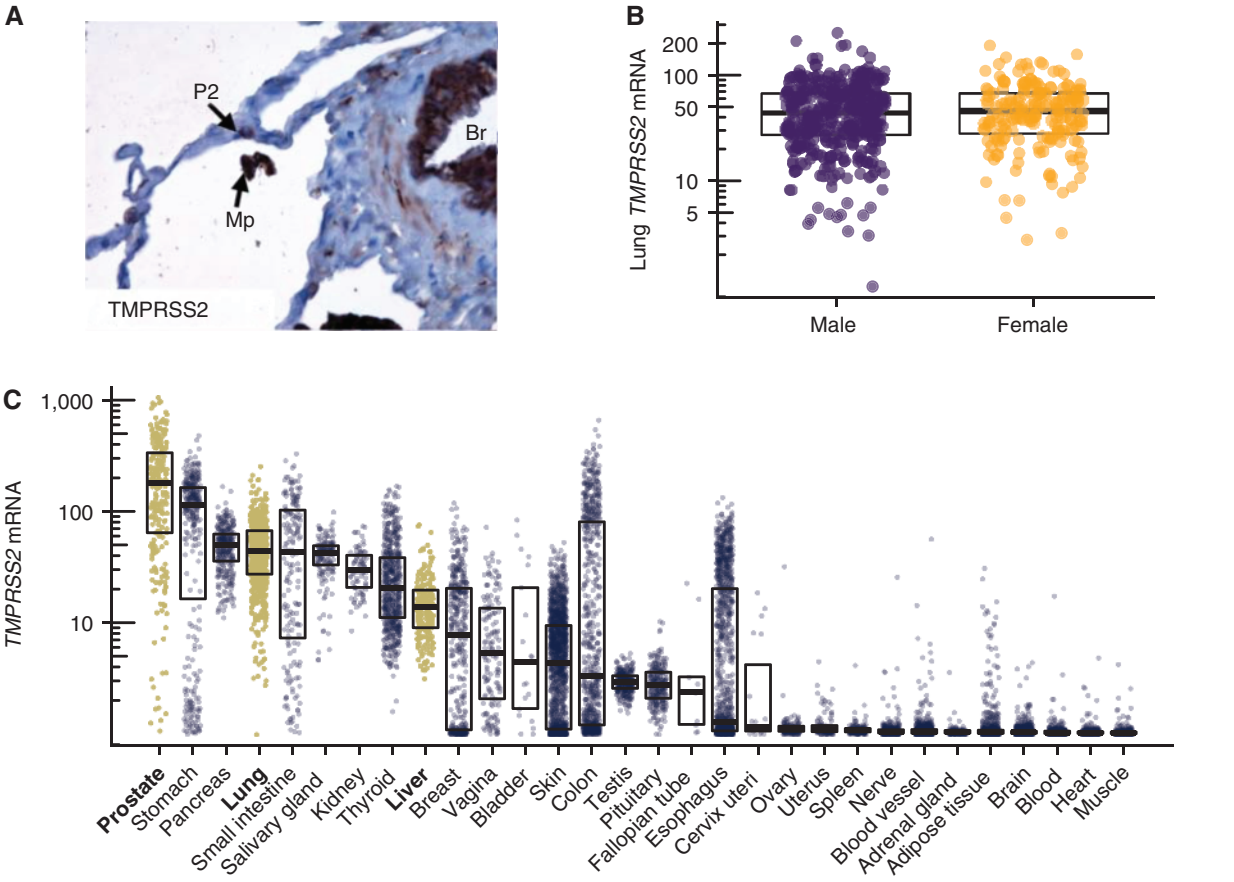


**Figure 1.** *TMPRSS2* mRNA and TMPRSS2 protein expression in humans. **A,** TMPRSS2 lung expression is localized to type 2 alveolar cells (P2), alveolar macrophages (Mp) and bronchial epithelial cells (Br). **B,** *TMPRSS2* expression in lung tissue is similar in men and women. **C,** *TMPRSS2* is expressed in several human tissues. Levels in prostate, lung, and liver are highlighted. **A** is from ref. 12, reused under the CC-BY license. Data for **B** and **C** are from the Genotype-Tissue Expression (GTEx) project, version 8, showing $\log_{10}$ of RNA transcripts per million (TPM) + 1; boxes span interquartile ranges, thick lines indicate medians.

by androgen, the gene fusion juxtaposes the androgen receptor regulatory elements of *TMPRSS2* with the *ERG* gene. The *ERG* gene is consequently controlled by androgen receptor signaling and expressed highly in prostate cancers harboring the *TMPRSS2–ERG* fusion.

Intriguingly, the prevalence of the *TMPRSS2–ERG* fusion is lower in the prostate tumors of both black and Asian men. The relevance of this to the current COVID-19 pandemic is unclear. *TMPRSS2–ERG* fusion–positive prostate cancers also have a distinct set of risk factors related to hormonal signaling. For example, men with higher genetically determined transcriptional activity of the androgen receptor have a higher risk of *TMPRSS2–ERG* fusion–positive prostate cancer but not of fusion-negative prostate cancer (8). Moreover, in mice, prostate tumors arising in the presence of *Tmprss2* have a higher capacity to metastasize (9). In men, tumors with the *TMPRSS2–ERG* fusion have higher insulin/insulin-like growth factor signaling, and *TMPRSS2–ERG* may modify how hormonal risk factors such as obesity influence the risk of metastasis (10). Together with the observation that *TMPRSS2–ERG* fusion–positive and fusion–negative prostate cancers may have distinct germline genetic risk factors (11), these findings from cancer research suggest potential drivers of differential *TMPRSS2* expression.

## TMPRSS2 AND SUSCEPTIBILITY TO CORONAVIRUS AND INFLUENZA INFECTIONS

Understanding how TMPRSS2 protein expression in the lung varies in the population could reveal important insights into differential susceptibility to influenza and coronavirus infections. Immunohistochemistry studies, with limited sample size, suggest that the TMPRSS2 protein is more heavily expressed in bronchial epithelial cells than in surfactant-producing type II alveolar cells and alveolar macrophages, and that there is no expression in type I alveolar cells that form the respiratory surface (Fig. 1A; ref. 12).

Determinants of TMRPSS2 protein expression in the lung could overlap with risk factors of *TMPRSS2–ERG* fusion–positive prostate cancer, some of which are modifiable and may merit testing in trials as a means of reducing risk of respiratory viral infections—as well as of prostate cancers harboring the *TMPRSS2–ERG* fusion. It is important that studies also be conducted in lung tissue from individuals not affected by a respiratory virus, given that viral infection may in turn alter TMPRSS2 expression, just as SARS-CoV does with the ACE2 receptor (13).

Importantly, such insights can indeed help us better understand the risk of respiratory viral infections. For example,

patients who carried a single-nucleotide polymorphism associated with higher *TMPRSS2* expression were more susceptible to influenza virus infection in two separate patient cohorts (14).

## DOES TMPRSS2 EXPLAIN THE MALE PREDOMINANCE IN DEATHS FROM COVID-19?

There are suggestions from epidemiologic studies across diverse countries including China, Italy, and the United States that the incidence and severity of diagnosed COVID-19 as well as other TMPRSS2-dependent viral infections such as influenza may be higher in men than women. One explanation underlying these potential differences is cigarette smoking, which is generally more common among men, and to date the epidemiologic investigations of COVID-19 have not sufficiently accounted for tobacco use in sex comparisons. Any sex-specific differences in the overall incidence should also take into account possible differences in laboratory testing for SARS-CoV-2. Still, the presence of *TMPRSS2–ERG* in prostate cancer as well as the strong regulation of *TMPRSS2* by androgens have raised the hypothesis that the male predominance in the COVID-19 pandemic could partially be explained by TMPRSS2.

Interestingly, at least on an mRNA level, constitutive expression of *TMPRSS2* in lung tissue does not appear to differ between men and women (Fig. 1B). Yet, there is a wide variation among both sexes in terms of mRNA expression levels. Low levels of androgens present in women may suffice to sustain TMPRSS2 expression. In addition, *TMPRSS2* (and tumors with the *TMPRSS2–ERG* fusion) may be responsive to estrogen signaling (9, 15). It is tempting to speculate that androgen receptor–inhibitory therapies might reduce susceptibility to COVID-19 pulmonary symptoms and mortality.

## TARGETING TMPRSS2 EXPRESSION

An important open question is to what degree susceptibility to viral infection could potentially be reduced by inhibiting androgen signaling. A subsequent question is to what extent TMPRSS2 protein expression in the lung or other sites of viral entry is regulated by androgen signaling.

Studies thus far suggest that androgen receptors are expressed in the epithelium of the respiratory tract in mice and humans, especially in type 2 pneumocytes and in bronchial epithelial cells. Androgen administration to a lung adenocarcinoma cell line upregulated the *TMPRSS2* transcript more than two-fold, accompanied by an androgen-dependent loading of the androgen receptor protein onto the *TMPRSS2* enhancer (16). However, whether antagonists of androgen receptor signaling can in turn abrogate TMPRSS2 expression in this cell line, or in noncancerous human respiratory epithelium, in not known. Observational studies that assess TMPRSS2 protein expression in (nontumor) lung tissue from men who have been treated for prostate cancer with androgen receptor signaling inhibitors, compared with those with normal androgen levels, could shed further light on this question.

If indeed observational studies further corroborate androgen receptor signaling inhibition as a viable strategy, then several therapeutics that effectively repress androgen receptor signaling activity could be rapidly repurposed to determine benefit in patients with COVID-19 infection. These medications have been used in the treatment of prostate cancer for decades. Commercially available antiandrogens such as enzalutamide, apalutamide, or darolutamide, or chemical gonadal ablation, could potentially downregulate expression of TMPRSS2, thereby attenuating symptom severity in patients who have contracted the SARS-CoV-2 virus. Such a trial could be conducted rapidly, given the immediate availability of these agents as well as their known safety profile in both men and women. Further, if TMPRSS2 is negatively regulated via estrogen receptor activity, then similar studies of estrogen receptor–modulating drugs could be considered.

## TARGETING TMPRSS2 PROTEASE ACTIVITY

Besides the theoretical potential for androgen receptor–targeted therapies to modulate TMPRSS2 expression, an alternative strategy involves directly impairing the protease activity of TMPRSS2. Although not developed specifically for targeting TMPRSS2, the protease inhibitors camostat, nafamostat, and aerosolized aprotinin have been shown to attenuate TMPRSS2 protease activity and are approved for unrelated indications in specific countries. At least one phase I–II clinical trial of camostat for COVID-19 (ClinicalTrials.gov; NCT04321096) has been recruiting since April 6, 2020. Whether camostat leads to clinical toxicity by inhibiting trypsin-like proteases other than TMPRSS2 remains to be seen.

Additional discoveries in cancer research point to other potentially promising TMPRSS2 antagonists. Preclinical studies suggested that TMPRSS2 promoted the metastatic spread of prostate cancer. A large-scale chemical library screen designed to select compounds capable of blocking TMPRSS2 activity identified bromhexine as a potent and TMPRSS2-specific protease inhibitor. Systemic administration of bromhexine reduced the frequency of prostate cancer metastases with no evidence of systemic toxicity (9).

Bromhexine has been in clinical use for decades as a mucolytic agent and expectorant. However, on a cautionary note, bromhexine's long track record should not be taken as evidence of its efficacy or safety at dosing schedules needed to sufficiently inhibit TMPRSS2 in patients, given the paucity of modern randomized controlled trials. Indeed, *TMPRSS2* mRNA and protein are expressed in several tissues other than lung and prostate (refs. 7, 12; Fig. 1C), perhaps contributing one explanation for nonrespiratory symptoms seen with influenza and coronaviruses. That TMPRSS2 is expressed in liver tissue, for example, merits attention in clinical trials of TMPRSS2 inhibitors, as the functional role of TMPRSS2 in the normal physiology of various cells and tissues is not completely understood. In the normal prostate, TMPRSS2 contributes to proteolytic cascades that result in the activation of prostate-specific antigen, itself a protease involved in ejaculate production (9).

Notably, TMPRSS2 does not appear to play an essential role in any organ, as other proteases may provide a degree of redundancy. A compelling argument that TMPRSS2 inhibition may have few on-target side effects again comes from the *Tmprss2* knockout mouse model in cancer research, where *Tmprss2* appeared entirely dispensable for normal development, growth, and organ function (17).

## CONCLUSIONS

Taken together, cancer-focused studies have contributed critical elements to our understanding of TMPRSS2, a key factor in the pathogenesis of coronavirus and influenza infections. Cancer epidemiology studies point to potential explanations for differential susceptibility to such infections through androgen regulation and differential TMPRSS2 expression. It remains to be explored in well-designed observational studies whether TMPRSS2 polymorphisms or other determinants of differential expression contribute to viral susceptibility, disease progression, and mortality. Approaches that merit further study and consideration for clinical testing could be the modulation of TMPRSS2 expression indirectly via androgen pathway blockade, or direct inhibition of TMPRSS2 function using protease inhibitors. As with all other potential novel treatments for COVID-19, such as those targeted at the ACE2 receptor, the existing data on TMPRSS2-targeted treatments can be summarized only as promising hypotheses. They require further nonrandomized studies and eventually rigorous testing for efficacy and safety by randomized controlled trials in humans.

### Disclosure of Potential Conflicts of Interest

E.S. Antonarakis is a paid consultant/advisor to Janssen, Astellas, Sanofi, Dendreon, Pfizer, Amgen, AstraZeneca, Bristol-Myers Squibb, Clovis, and Merck; has received research funding to his institution from Janssen, Johnson & Johnson, Sanofi, Dendreon, Genentech, Novartis, Tokai, Bristol-Myers Squibb, AstraZeneca, Clovis, and Merck; and is the co-inventor of a biomarker technology that has been licensed to Qiagen. P.S. Nelson is an advisory board member for Astellas and Janssen and has received other remuneration from Veneble/Fitzpatrick. P.W. Kantoff is a consultant at Bavarian Nordic, DRGT, GE Health Care, Janssen, OncoCellMDX, Progenity, SEER Biosciences, and Tarveda, is a board member at Context, is a DSMB board member at Genentech/Roche and Merck, and has ownership interest (including patents) in Context, DRGT, Placon, and SEER Biosciences. No potential conflicts of interest were disclosed by the other authors.

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