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Article – Human and Animal Health

The Important Role of TMPRSS2 Gene in Covid-19 and Prostate Cancer: In Silico Approach

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HIGHLIGHTS

- The main characteristic of SARS-CoV, MERS-CoV and SARS-CoV-2 is that air passes through the upper respiratory tract
- The tissue of the upper respiratory tract can express both ACE2 and TMPRSS2.
- AR is abundantly expressed in lungs.
- TMPRSS2, ACE2, and AR connection explains why high expression of AR gene is present in the male lung.

Abstract: Some studies have discovered a connection between prostate cancer and COVID-19. In this study, we evaluated the link between prostate cancer and COVID-19, contributing to elucidate the connection between *TMPRSS2* and *ACE2*. We discovered 209 number of variants in *TMPRSS2* gene, and 110 variants represent EA populations and 99 of them represent AA populations. Moreover, we found 23 suspected missense and 3 unknown variants. Then, linked genes to *TMPRSS2* and *ACE2* were found in our study. We investigated the expression level of *TMPRSS2* and the results showed that it was very high in the prostate, colon, lung, kidney, and saliva-secreting gland. Also, the important role of the *AR* gene was revealed in addition to other oncogenes and tumor suppressor genes for prostate cancer by KEGG Pathway analysis. In conclusion, these results can highlight several molecular mechanisms of SARS-CoV-2, and also *TMPRSS2*, *ACE2*, and *AR* connection explains the high expression level of *AR* gene found in the male lung.

Keywords: COVID-19; ACE2; Prostate cancer; AR; TMPRSS2.

INTRODUCTION

Prostate cancer is one of the most common types of cancer in men that cause death all over the world [1,2]. According to the American Cancer Society, the number of newly detected prostate cancer patients has increased by approximately 30% since 2020 and researchers have thought that there will be more than 34,000 prostate cancer-linked deaths in this year [3]. Although the cause of prostate cancer is not known exactly, advanced age, ethnicity, genetic factors, and family history are thought to be the leading factors for prostate cancer [4,5]. According to the latest studies, the *TMPRSS2* gene is identified as a prostate-specific and androgen-responsive gene that encodes transmembrane serine protease 2 and it plays an important role in the development of prostate cancer, as it is expressed in large amounts on prostate cancer and contains androgen response elements [6,7]. *TMPRSS2* is known to be expressed on the luminal side of the prostate epithelium, and when the expression of the *TMPRSS2* on the normal tissue is compared with the cancerous prostate epithelium, the cancerous tissue of the prostate shows higher expression than the normal tissue [8]. *TMPRSS2*, a component of the serine protease family, is participating in a range of pathophysiological events, as well as enabling the entrance of viruses such as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) through cleaving and stimulating viral envelope glycoproteins, or proteolytically cleaving the ACE2 (angiotensin-converting enzyme 2) receptor for viral entry. [8]. Many studies have shown that in vitro activation of viral spikes might be mediated by other proteases such as TMPRSS4, TMPRSS11A, TMPRSS11D, TMPRSS11E1, and TMPRSS2 activity, and it is considered to have a role in cell entry and viral pathogenesis [9]. Although the presence of *TMPRSS-ERG* fusion gene (transmembrane protease serine2:v-ets erythroblastosis virus *E26* oncogene homolog) has been detected in 40-80% of human prostate cancer, its role is not defined clearly [10]. Tomlins and his colleagues [11] identified that overexpression of *ERG* has been seen in 55% of prostate cancer cases. *ERG* is an oncogene that plays a regulator role in cell proliferation, angiogenesis, inflammation, and apoptosis [12]. The *TMPRSS2:ERG* gene fusion is a genomic alteration in human malignancy [13]. By interrupting the androgen receptor (AR) lineage-specific differentiation pathway of the prostate, *TMPRSS2:ERG* fusion might promote carcinogenesis. The gene fusion can suppress AR signaling, resulting in a restrictive pressure that contributes to recurring cancers with an increase in AR [14].

As reported by the study indicating *ACE2* and *TMPRSS2* expression levels and their distribution across cell types in lung tissue and cells derived from subsegmental bronchial branches by single nuclei and single-cell RNA sequencing, respectively, and this study has revealed that *TMPRSS2* is abundantly expressed in lung tissue and cells derived from subsegmental bronchial branches but *ACE2* is highly expressed in a transient secretory cell type [9]. Another study has been evaluated the effectiveness of two different inhibitors of TMPRSS2 which are nafamostat and camostat in the treatment of COVID-19 [15]. There is an interesting relationship between the *TMPRSS2* gene and the COVID-19 which has affected many people recently. It is a fact that *TMPRSS2* leading viral entry within the lung cells promotes the infection by other viruses. A study has demonstrated that *TMPRSS2* knockout mice infected with the H1N1 influenza virus did not cause serious respiratory diseases compared to wild-type mice [16].

According to the World Health Organization Coronavirus disease 2019 (COVID-19) Weekly Report – 11 May 2021, the total number of confirmed cases reached approximately 157 million, with over 3 million deaths in the world [17]. Scientists have continued to research why the most severe course of the disease is associated with older age in males. Some studies have shown that the relationship between high androgen levels and *TMPRSS2* expression in prostate cancer may also be related to COVID-19 in which the prevalence is found to be higher in men [18,19]. In this study, we investigated the link between prostate cancer and COVID-19, contributing to elucidate the relationship between *TMPRSS2* and *ACE2*.

MATERIAL AND METHODS

Finding of the TMPRSS2 gene dataset collection

Databases of Online Mendelian Inheritance in Man (OMIM) [20] and Entrez Gene on National Center for Biological Information (NCBI) [21] were used to obtained information on *TMPRSS2*. The Single Nucleotide Polymorphism (SNPs) information (Protein accession number and SNP ID) of the *TMPRSS2* gene was acquired from the NCBI dbSNP [22], and SWISS Prot databases [23].

Analysis of Functional and Physical Genes Similar to ACE2 and TMPRSS2

STRING database was used to show the protein associations network. Moreover, GeneMania was employed to exhibit the relationship between the known gene and other genes. Analysis of functional and physical similar genes was found by the GeneMania program to understand the relationship between *ACE2* and *TMPRSS*. Results obtained by this database was verified with STRING database [24].

Finding of Suspicious SNPs in TMPRSS2 and Prediction of the Expression level of TMPRSS2 gene

Exome Variant Server (EVS) was used to detect *the* number of variations of *TMPRSS2* gene and its frequency to find suspicious SNPs. In this study, probable damaging SNPs were identified for *TMPRSS2* gene and allele frequency were calculated with this database [25]. Determining of nonsynonymous variants was found by SIFT algorithm [26] and defining damaging effects of the nonsynonymous variant was found by Polyphen-2. Then all data was verified with each other [27]. Analyzing the expression level of a gene is important and it establishes a link between genes and diseases. The expression level of the *TMPRSS2* gene was found by UniProt, and BioXpress databases. Then, gene expression, exon expression, and junction expression of the gene were verified with GTex portal, and Ensembl Genome Browser [28-30].

Pathway and Diseases Analysis of TMPRSS2

KEGG is a database which is used to find the methodical searching of gene functions. KEGG database was employed to process of mapping of the *TMPRSS2* gene and we have shown the importance of *TMPRSS2* gene in prostate cancer. The database is correlated to genomic information with other advanced classified functional parts [30]. Our results were compared to each other and validation was done by STRING. Additionally, diseases and *TMPRSS2* gene association was found by the DISEASES resource database [32].

RESULTS

GeneMANIA helps to investigate many large, biological datasets to discover associated genes. This program analyzes protein-protein, protein-DNA and genetic interactions, pathways, reactions, gene and protein expression data, protein domains and phenotypic screening profiles. We found that TMPRSS2 interacted with A*CE2, ACE, AGT, PDE9A, SLC44A4, RASEF, TRPM4, SLC10A2, SLC37A1,* and *CAT* genes shown in Figure 1. Secondly, we searched genetic variants identified in patients using a chromosome position by Exome Variant Server for and we found 209 variants in the *TMPRSS2* gene, and 110 variants represent EA populations and 99 of them represent AA populations. Moreover, we found 23 suspected missense and 3 unknown variants indicated in Table 1. Molecular interactions, reactions and relations were mapped for *TMPRSS2* and other connected genes by KEGG pathway analysis. The mechanism of the *AR* gene in connection with *TMPRSS2* was highlighted in the context of reported effects on prostate cancer demonstrated in Figure 2. Analyzing the expression level of the *TMPRSS* gene is significant and we have shown the expression level of *TMPRSS2* in different tissues in Figure 3 and Figure 4 by UniProt, BioXpress databases, and Expression Atlas. Then, we analyzed the relationship between *TMPRSS2* gene and diseases by DISEASES resource database in Table 2. According to our results, *TMPRSS2* can play role in prostate cancer, COVID-19, carcinoma, breast cancer, middle east respiratory syndrome, severe acute respiratory syndrome, lung cancer, sarcoma, leukemia, influenza.

Analysis of Functional and Physical Genes Similar to *ACE2* and *TMPRSS2*

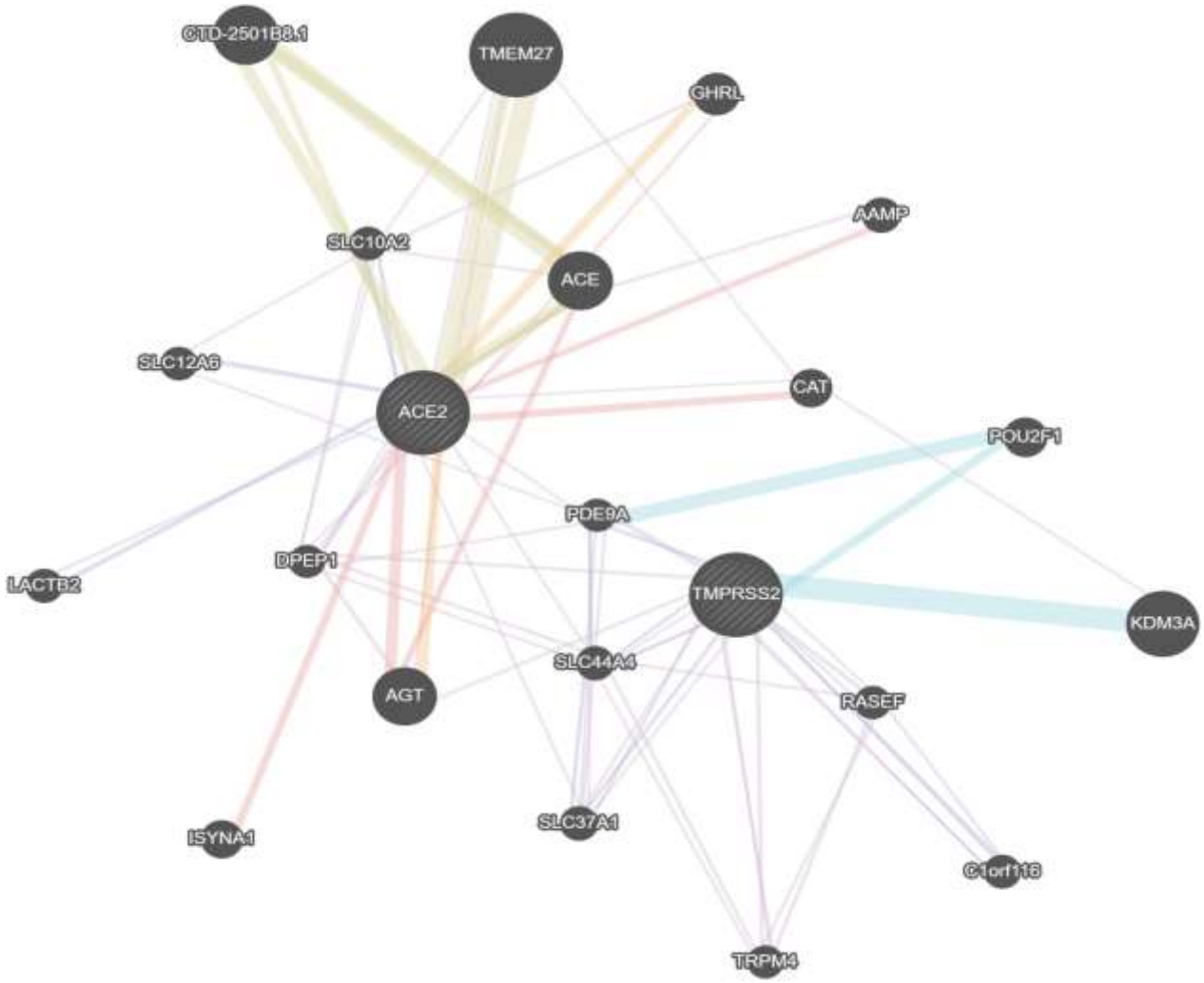

Figure 1. The figure obtained from the GeneMania database shows the relationship between TMPRSS2 and other genes. Co-expression (lilac), co-localization (blue), physical interaction (pink), and shared protein domains (brown) have been shown in different colors.

Finding of Suspicious SNPs in *TMPRSS2*

Table 1. The predicted number of variations in TMPRSS2 was indicated and suspected SNPs detected on TMPRSS2 were listed in addition to genotypes, functions and PolyPhen2 scores to predict probable damages.

rs ID	Alleles	EA Genotype	AA Genotype	GVS Function	PolyPhen2 (Class: Score)
rs368268847	C>A	A=0/C=8600	A=1/C=4405	missense	probably-damaging:0.999
rs145171279	G>C	C=1/G=8599	C=0/G=4406	missense	probably-damaging:1.0
rs373952557	T>G	G=1/T=8597	G=0/T=4404	missense	possibly-damaging:0.692
rs370043174	T>C	C=1/T=8599	C=0/T=4404	missense	probably-damaging:0.964
rs144046631	T>C	C=0/T=8600	C=2/T=4402	missense	probably-damaging:0.997
rs146654734	G>C	C=2/G=8552	C=0/G=4354	missense	probably-damaging:0.998
rs372563970	C>T	T=0/C=8554	T=1/C=4353	Missense	probably-damaging:0.999
rs142446494	C>T	T=2/C=8554	T=0/C=4362	missense	possibly-damaging:0.64
rs140547429	C>T	T=0/C=8560	T=1/C=4351	missense	possibly-damaging:0.812
rs150445636	G>A	A=1/G=8561	A=1/G=4359	missense	probably-damaging:0.999
rs143049780	A>G	G=1/A=8557	G=0/A=4364	missense	probably-damaging:0.998
rs148049486	A>G	G=1/A=8599	G=0/A=4406	missense	possibly-damaging:0.82
rs150554820	A>T	T=2/A=8598	T=0/A=4406	missense	possibly-damaging:0.935
rs139926880	A>C	C=0/A=8600	C=1/A=4405	missense	probably-damaging:0.992
rs139092674	C>T	T=1/C=8599	T=0/C=4406	missense	probably-damaging:0.966
rs373847134	T>A	A=1/T=8599	A=0/T=4406	missense	probably-damaging:0.993
rs12329760	C>T	T=1860/C=6740	T=1279/C=3127	missense	probably-damaging:0.999
rs147711290	A>T	T=0/A=8600	T=31/A=4375	missense	probably-damaging:0.999
rs114363287	C>T	T=1/C=8599	T=29/C=4377	missense	possibly-damaging:0.641
rs377060358	G>A	A=2/G=8598	A=0/G=4406	missense	possibly-damaging:0.673
rs138651919	G>A	A=5/G=8595	A=0/G=4406	missense	probably-damaging:0.998
rs371006143	G>T	T=1/G=8599	T=0/G=4406	missense	probably-damaging:0.968
rs375223866	C>T	T=2/C=8598	T=0/C=4406	missense	probably-damaging:0.994
rs386416	G>C	C=6201/G=2399	C=2472/G=1932	intron	unknown
rs422471	C>T	T=6204/C=2396	T=2475/C=1931	intron	unknown
rs17854725	A>G	G=4660/A=3900	G=1714/A=2650	Coding-synomous	unknown

Pathway Analysis of Prostate Cancer

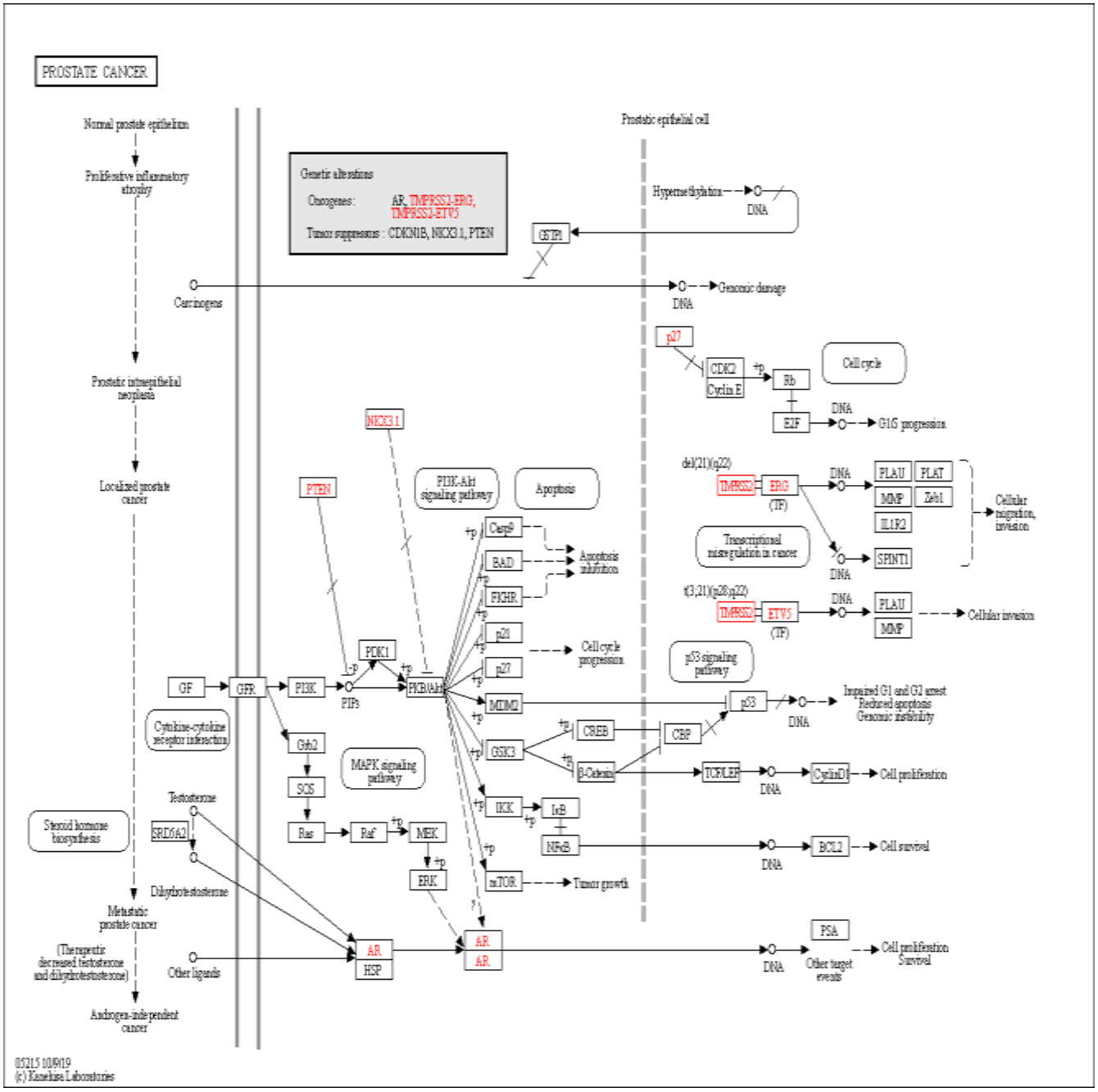

Figure 2. KEGG pathway analysis was used to identify prostate cancer mechanisms for TMPRSS2 in the form of a schematic diagram. It shows the metabolic and signaling pathways, pathways involved in different cellular processes and structures, and perturbed pathways linked to prostate cancer. The important role of the AR gene was shown together with other oncogenes and tumor suppressor genes (highlighted in red) to indicate the connection and function in the prostate cancer pathway.

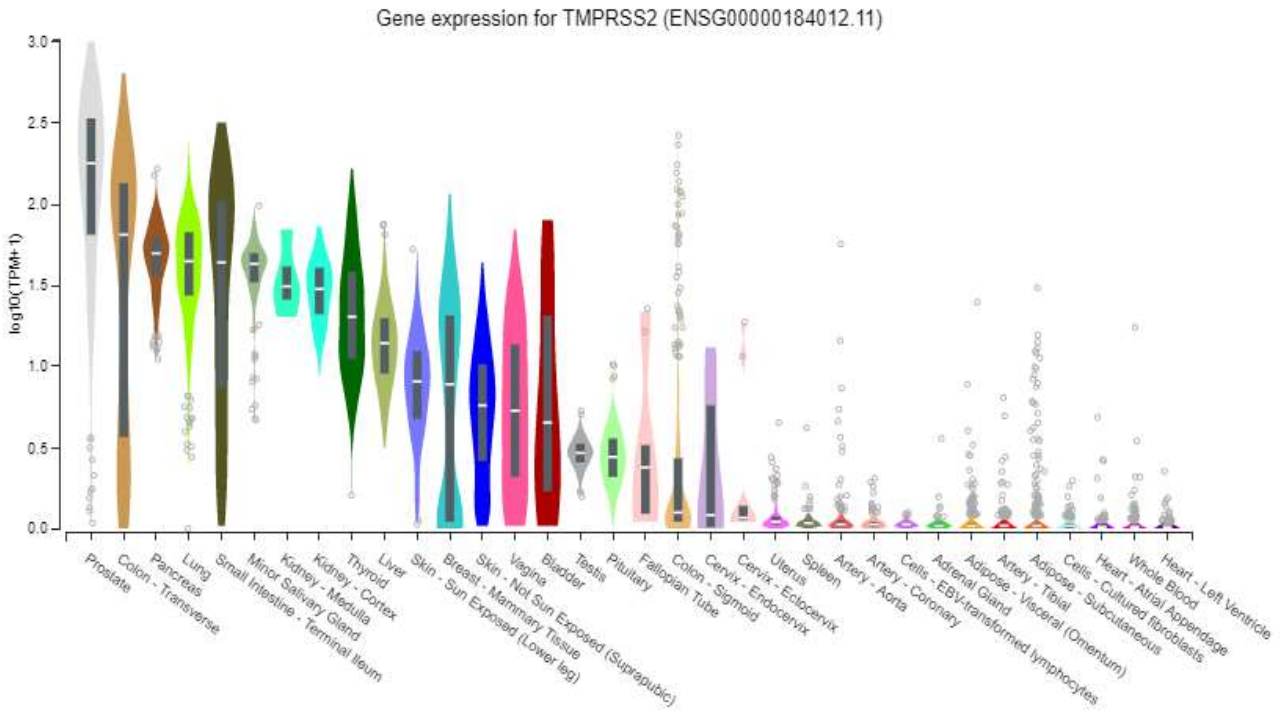

Figure 3. This figure shows differential expression analysis for the TMPRSS2 gene in a logarithmic scale. Values of gene expression were indicated in transcripts per million. The points displayed as outliers shows that they are below or above 1.5 times the interquartile range.

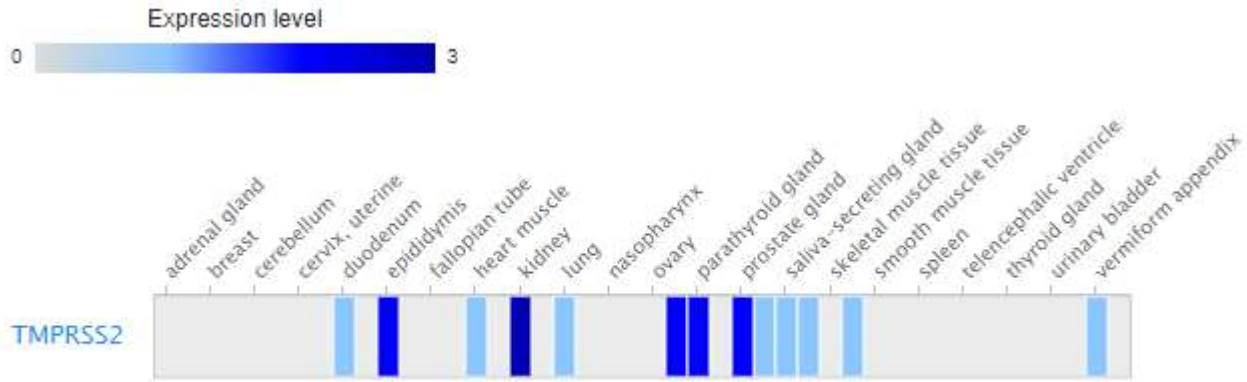

Figure 4. Expression level analysis of *TMPRSS2* was found by Expression Atlas.

Table 2. Reported diseases and TMPRSS2 gene associations were indicated with Z score and confidence score obtained from the DISEASES database. Prostate cancer has the highest Z score followed by COVID-19.

Name	Z Score	Confidence
Prostate cancer	7.0	****
COVID-19	6.3	****
Carcinoma	5.2	***
Breast Cancer	4.5	***
Middle East Respitatory Syndrome	4.4	***
Severe Acute Respiratory Syndrome	4.4	***
Lung Cancer	4.3	***
Sarcoma	4.3	***
Leukemia	4.2	***
Influenza	4.2	***

Z score indicates the density of people of the same age, sex, and genetic background.
Confidence score indicates a comparable value for different types and sources of evidence.

DISCUSSION

Recently, studies have demonstrated that prostate cancer is linked with COVID-19 [33]. Especially, based on a study, prostate cancer patients who received androgen therapy had a lower risk for SARS-COV-2 infection compared to patients who did not (receive androgen deprivation therapy) [20]. The comprehensive mechanism of prostate cancer treatment is also important in understanding the protective effects against COVID-19 [34].

In this study, we investigated the link between prostate cancer and COVID-19, contributing to elucidate the relationship between *TMPRSS2* and *ACE2*. We found that *TMPRSS2* interacted with *ACE2, ACE, AGT, PDE9A, SLC44A4, RASEF, TRPM4, SLC10A2, SLC37A1,* and *CAT* genes illustrated in Figure 1. Our study has shown that *SLC44A, PDE9A, SLC37A1* have a relationship with *ACE2*, and they are co-expressed with *TMPRSS2*. That may be because of their genetically similar expression patterns. The *PDE9A* gene is phosphodiesterase specific [19], which has a special role in signal transduction, and it regulates the intracellular concentration of cyclic nucleotides. As stated in a study, *PDE9A* is used as a marker for detecting, determining, diagnosing prostate cancer [35,36]. *SLC37A1* is a solute carrier protein that it has pointed out as a potential candidate for the carrying of *G3P* in the endoplasmic reticulum and/or mitochondria [37]. Co-expression of genes represents a relationship in transcription levels, and co-expression can be used to understand the connection of proteins, metabolites, or a combination of transcripts, with other genes in the biological process [38,39]. This study has revealed that *TMPRSS2* is linked with *ACE2* and both two genes have a co-expression profile with *SLC37A1*, *PDE9A*, and *SLC44A* genes. There is currently no study in the literature that summarizes this situation. In the future perhaps this study will contribute to why COVID-19 is seen more in men over the age of 65 and defining gene co-expression networks could help to understand the mechanism of COVID-19.

SNPs are one of the most important types of genetic variations in the human genome and SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are related to genetic susceptibility to cancer and alterations in gene expression and their consequence on cancer susceptibility differ depending on the location of the SNP [40]. Hariom and his colleagues have thought that investigating the genetic variants and expression of *ACE2* and *TMPRSS2* genes, in a population can support the genetic marker for susceptibility or resistance against the coronavirus infection, which could be advantageous for defining the susceptible population groups for targeted interventions [41]. Thus; we aimed to find variants in the *TMPRSS2* gene in this study and we discovered 209 variants in the *TMPRSS2* gene, and 110 variants represent EA populations and 99 of them represent AA populations. Moreover, we found 23 suspected missense and 3 unknown variants indicated in Table 1. We assumed that these mutations can be significant for pulmonary expression of the *TMPRSS2*. We are the first group who is defining variants on *TMPRSS2* gene. We have thought that this information can contribute to other studies.

ACE2 gene encodes a cell receptor, and it permits the viral S protein to attach to the cell. On the other hand, the *TMPRSS2* gene encodes a serine protease which locates on the cell membrane as a fusion protein and facilitates the host cell membrane and viral membrane to fuse during the attachment with spike protein [44]. Latest studies have highlighted that roles of *ACE2* and *TMPRSS2* genes have been identified in SARS-CoV and MERS-CoV infections [42-44]. Therefore, describing the molecular mechanisms regulating the expression of TMPRSS2 and ACE2 probably clarifies the variation in SARS-CoV-2 infection-related death in men [15]. A study has indicated that finding details of ACE2-Spike protein and TMPRSS2-Spike protein interactions might further highlight future research on COVID-19 [45]. We evaluated the *TMPRSS2* mechanism through the prostate cancer pathway and interpreted its relationship with the *ACE2* gene (Figure 2). According to the results, the expression level of *TMPRSS2* is very high in the prostate, colon, lung, kidney, and saliva secreting glands (Figure 3, Figure 4). Therefore, we have seen that AR is a marker for the phenotype of prostate cancer cells. Generally, AR is activated by an androgen ligand and it is a DNA-binding transcription factor, and it has a critical role in the development of male sexual phenotype [46]. AR is abundantly expressed in the lungs and high expression of AR is seen in males compared with females. According to the results, we assumed that our study supports significant information regarding the probable connection between *TMPRSS2* and *ACE2* co-expression and gender differences in COVID-19. We predict special information concerning *TMPRSS2* and *ACE2* expression in patients experiencing various clinical manifestations might propose new directions for present and future research on COVID-19.

In this study, when the mechanism of SARS-CoV-2 was analyzed, we have seen that *ACE2* and *TMPRSS2* use the same pathway to attach to human cells. The main infection route of the SARS-CoV, MERS-CoV, and SARS-CoV-2 is that air passes through the upper respiratory tract [47,48]. The tissue of the upper respiratory tract can express both *ACE2* and *TMPRSS2*. Thus, it can be a potential target for the entry

of these viruses into human cells. COVID-19 is a disease that has never been seen in humans before, but there are no validated drugs at this time, and the most significant public health remedy will be an efficient vaccine. The male predisposition of SARS-CoV-2 and the androgen-dependent expression of *TMPRSS2* suggest that, as with SARS-CoV and influenza virus, targeting *TMPRSS2* may be a novel choice for treating COVID-19 [9, 49]. Male pneumocytes I/II had higher *TMPRSS2* and *ACE2* co-expression than female pneumocytes I/II, despite no differences between men and women in *TMPRSS2* distribution in human lung tissue. That insight highlights a significant problem with the COVID-19 pandemic's epidemiology of sex differences [50, 51].

In conclusion, *TMPRSS2*, *ACE2*, and AR connections explain why the high expression of the *AR* gene is present in the male lung, and it can contribute to solving a part of the mechanism of SARS-CoV-2. Detailed information on *TMPRSS2* and *ACE2* expression in patients with various clinical indications, we assume, may point to new possibilities for current and future COVID-19 study. This study provides important knowledge to the scientific world. On the other hand, the biggest limitation is that this study can only be evaluated as a prediction study. It should be supported by the laboratory work and will also shed a light on these studies.

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