Vol.63: e20200304, 2020
http://dx.doi.org/10.1590/1678-4324-2020200304
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

Role of *ACE2* Gene Expression in Renin Angiotensin System and Its Importance in Covid-19: In Silico Approach

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Received: 2020.05.16; Accepted: 2020.07.27.

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HIGHLIGHTS

- *ACE2* is also expressed in testis, heart, thyroid, colon, esophagus, breast, pancreas, lung, liver.
- Under expression of *ACE2* can cause prostate cancer and head and neck cancer.
- *ACE2* is over-expressed in the small intestine.
- Suggesting that over expression of *ACE2* in small intesine can cause symptoms of in COVID-19.

Abstract: We aimed to analyze the expression profile of *ACE2* and similar genes with *ACE2*, predict the number of variations in *ACE2*, detect the suspected SNPs on *ACE2* gene, and perform the pathway analysis of renin-angiotensin system (RAS) and protein absorption-digestion. Moreover, we have predicted the gene-related diseases with *ACE2*. STRING was used to analyze functionally similar genes with *ACE2*. Exome Variant Server, SIFT, Polyphen2 were used to predict the number of variations in *ACE2* and detect the suspected SNPs on *ACE2*. KEGG database and STRING were used to draw pathway of *ACE2*. Then, DISEASES resource, FitSNPs, UniProt, BioXpress, IGV Browser, Ensembl Genome Browser, and

UCSC Genome Browser were used to predict the *ACE2* gene-related diseases and expression profile in human normal and cancer tissues. We have shown that expression of *ACE2* was correlated with *AGT, REN, AGTR1, AGRT2, MME2, DPP4, PRCP, MEP1A, XPNPEP2, MEP1B* and *ACE2* is expressed in testis, kidney, heart, thyroid, colon, esophagus, breast, minor salivary gland, pancreas, lung, liver, bladder, cervix, and muscle tissues. We found 99 variations in *ACE2* gene, in which no previous study has been performed. In the future, this *in silico* analysis should be combined with other pieces of evidence including experimental data to assign function.

Keywords: COVID-19; *ACE2*; Sars-CoV-2; small intestine; kidney failure; testis; heart; renin angiotensin system.

INTRODUCTION

According to the World Health Organization Coronavirus disease 2019 (COVID-19) Situation Report - 116, the total number of confirmed cases hit almost 4.3 million in the world by May 15th, 2020 [1]. The severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) is a positive-strand RNA virus that caused 297,119 deaths over the world which makes this disease quite serious at the global level [2], [3]. SARS-CoV-2 infection is a pandemic that has continued to cause serious death in the world but still, no drug or vaccine was found to completely eliminate SARS-CoV-2 [4–6].

Angiotensin-converting enzyme II (ACE2) is well-known as a host receptor for SARS-COV-2 in human so studies in recent days have shown that *ACE2* has been the center of interest by researchers for SARS-CoV-2 [3], [7], [8]. *ACE2* is composed of three parts which are distinct signal peptide, a single metalloprotease active site, and a transmembrane domain. [9] *ACE* is one of the significant parts of the Renin-Angiotensin System (RAS) that is a monomeric, membrane-bound, zinc- and chloride-dependent peptidyl dipeptidase. [10] Moreover, it catalyzes the translation of the decapeptide angiotensin I to the octapeptide angiotensin II, by eliminating a carboxy-terminal dipeptide. RAS plays a role as a homeostatic regulator of vascular function. [11] The expression and distribution of the *ACE2* can have an important point at the probable infection mechanism of SARS-CoV-2. Recent studies have shown that the expression of *ACE2* is fundamentally in lung [12], intestine [13], kidney [14], and heart [15]. A group of researchers investigated RNA expression profile of *ACE2* at single-cell [7] and they have reported that high-level expression of *ACE2* gene has been found on type II alveolar cells (AT2) [7,16,17], together with several genes promoting reproduction and transmission of the virus. Esophagus upper and stratified epithelial cells [18], absorptive enterocytes from ileum and colon [7], cholangiocytes [18], myocardial cells, kidney proximal tubule cells, and bladder urothelial cells [17] are other types of cells which have found to be positive for the high level of *ACE2* expression.

Another study has also shown that attenuated expression of *ACE2* is correlated with cardiovascular diseases [19]. In the light of these findings, we thought that some organs with the high number of *ACE2*-expressing cells are directly linked to the probable high risk for SARS-CoV-2 infection. Discoveries about *ACE2* have the greatest value for therapeutic approaches for the current infection because of acting *ACE2* as a host receptor for SARS-CoV-2. Prediction of the expression profile of *ACE2* on normal human tissues, similar genes to *ACE2*, *ACE2* related pathways and physiological features of *ACE2* is significant for developing anti-*ACE2* antibodies or blocking SARS-CoV-2 to prevent binding the virus to the receptor [20]. In this study, we aimed to analyze the expression profile of *ACE2* and similar genes to *ACE2*, predict the number of variations in *ACE2* gene and detect the suspected SNPs on *ACE2* gene, perform pathway analysis of the renin-angiotensin system, protein absorption and digestion. Also, the purpose is to predict the connection between *ACE2* and gene-related diseases.

MATERIAL AND METHODS

Collection of the ACE2 gene dataset

Information on *ACE2* was collected by Online Mendelian Inheritance in Man (OMIM) [21] and Entrez Gene on National Center for Biological Information (NCBI) web site [22]. The Single Nucleotide Polymorphism (SNPs) information (Protein accession number and SNP ID) of the *ACE2* gene was retrieved from the NCBI dbSNP [23], and SWISS Prot databases [24].

Analyzing of Functionally Similar Genes with ACE2

STRING is a functional protein associations network database that shows the relationship between the input gene and other genes. Protein-protein interactions, co-expression, co-localization and genetic interactions of *ACE2* gene with other genes were shown by STRING [9–12,25].

Prediction of Number of Variation in ACE2 and Detection of Suspected SNPs on ACE2

National Heart, Lung, and Blood Institute (NHLBI) has supported the Exome Variant Server. Exome Variant Server was used to determine the number of variations according to the European African population (EA) and American African Population (AA) alleles [13,14]. In our study, suspected SNPs were identified for *ACE2* gene and allele frequency were evaluated with this database [26]. SIFT algorithm uses to find a nonsynonymous variant [7,15,27] and Polyphen-2 algorithm uses to predict damaging effects of nonsynonymous variant and it predicts benign or possibly damaging of variants of genes [16,17,28]. Consequently, our findings were confirmed with each other.

Pathway Analysis of ACE2

KEGG is a database that is used to determine the methodical searching of gene functions. It is related to genomic information with other advanced classify functional parts [18,29,30]. We used the KEGG database for mapping of Renin-Angiotensin System. The other database was employed for mapping system and pathway analysis is the Reactome Pathway Database. The results were compared to each other and validation was done by STRING.

Prediction of ACE2 Gene-Related Diseases and Expression Profile in Human Normal and Cancer Tissues

DISEASES resource was used to predict gene-related diseases [31]. Then our results were compared with FitSNPs, UniProt, BioXpress [32–34]. FitSNPs depends on human microarray data that also shows gene-related diseases. Gene/miRNA expression relationship with cancer was investigated by BioXpress. SwissProt and The Hive Lab also was used to analyze for expression profile in human normal tissues and human cancer tissues. IGV Browser, Ensembl Genome Browser, and UCSC Genome Browser were used to find gene expression, exon expression, and junction expression [35–37]. Thus, all results confirmed with each other and the GTEx portal [38].

RESULTS

Analyzing of Functionally Similar Genes with ACE2

We analyzed that *ACE2* relationship with ten different genes (Figure 1).

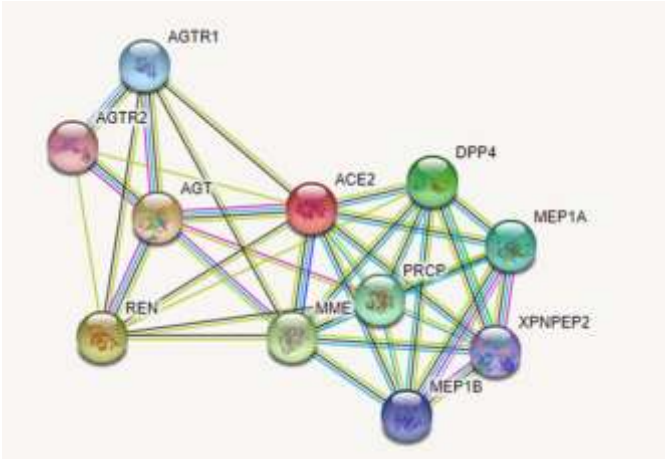

Figure 1. The picture shows the predicted functional partners of *ACE2* gene. This figure has shown that predicted interactions [gene neighborhood (green), gene fusions (red), gene co-occurrence (blue)] and co-expression network (black), experimentally determined (purple) between *ACE2* gene and its related genes.

Predicting the Number of Variations in ACE2 and Detection of Suspected SNPs on ACE2

Some of these genes play key rol in RAS. Then we showed that suspected variations in *ACE2* gene with three different programme (in Table 1, Table 2).

Table 1. This table shows the suspected variations of *ACE2* gene, that our findings were compared to SIFT, Exome Variant Server and Polyphen2.

	rs ID	Alleles	EA Genotype	AA Genotype	GVS Function	PolyPhen2 (Class:Score)
1	rs140016715	G>A	AA=0/AG=0/GG=2428/G=1872	AA=0/AG=1/GG=1631/G=571	Missense	probably-damaging:1.0
2	rs147311723	G>A	AA=0/AG=0/A=0/GG=2428/G=1872	AA=0/AG=62/A=6/GG=1570/G=565	Missense	probably-damaging:0.995
3	rs139980377	C>G	GG=0/GC=0/CC=2428/C=1872	GG=0/GC=1/CC=1631/C=571	Missense	probably-damaging:1.0
4	rs144869363	G>A	AA=0/AG=0/A=0/GG=2428/G=1872	AA=0/AG=0/A=1/GG=1632/G=570	Missense	probably-damaging:0.975
5	rs370187012	C>T	TT=0/TC=1/CC=2427/C=1872	TT=0/TC=0/CC=1632/C=571	Missense	probably-damaging:1.0
6	rs149039346	A>G	GG=0/GA=0/G=0/AA=2428/A=1872	GG=0/GA=13/G=6/AA=1619/A=565	Missense	possibly-damaging:0.774
7	rs140312271	C>G	GG=0/GC=0/G=1/CC=2428/C=1871	GG=0/GC=0/G=0/CC=1632/C=571	Missense	possibly-damaging:0.774
8	rs148036434	G>C	CC=0/CG=2/GG=2426/G=1872	CC=0/CG=0/GG=1632/G=571	Missense	probably-damaging:1.0
9	rs375352455	G>A	AA=0/AG=0/A=1/GG=2428/G=1871	AA=0/AG=0/A=0/GG=1632/G=571	Missense	probably-damaging:1.0
10	rs373025684	G>C	CC=0/CG=1/GG=2427/G=1872	CC=0/CG=0/GG=1632/G=571	Missense	probably-damaging:0.997
11	rs142984500	T>C	CC=0/CT=2/TT=2426/T=1872	CC=0/CT=0/TT=1632/T=571	Missense	probably-damaging:1.0

Table 2. Continued from Table 1

	rs ID	Alleles	EA Genotype	AA Genotype	GVS Function	PolyPhen2 (Class:Score)
12	rs370610075	C>A	AA=0/AC=1/CC=2427/C=1872	AA=0/AC=0/CC=1632/C=571	Missense	probably-damaging:1.0
13	rs372272603	G>A	AA=0/AG=2/A=1/GG=2426/G=1871	AA=0/AG=1/A=0/GG=1631/G=571	Missense	probably-damaging:1.0
14	rs148771870	C>T	TT=0/TC=12/T=2/CC=2416/C=1870	TT=0/TC=0/T=0/CC=1632/C=571	Missense	possibly-damaging:0.551
15	rs143158922	T>G	GG=0/GT=0/TT=2428/T=1872	GG=0/GT=3/TT=1629/T=571	Missense	possibly-damaging:0.488
16	rs146676783	C>T	TT=0/TC=0/CC=2428/C=1872	TT=0/TC=3/CC=1629/C=571	Missense	possibly-damaging:0.712
17	rs73635825	A>G	GG=0/GA=0/G=0/AA=2428/A=1872	GG=0/GA=14/G=1/AA=1618/A=570	Missense	possibly-damaging:0.767
18	rs373153165	C>T	TT=0/TC=0/CC=2428/C=1872	TT=0/TC=1/CC=1631/C=571	Missense	benign:0.102
19	rs147487891	G>A	AA=0/AG=1/GG=2427/G=1872	AA=0/AG=0/GG=1632/G=571	Missense	benign:0.002
20	rs375252585	T>C	CC=0/CT=0/C=1/TT=2428/T=1871	CC=0/CT=0/C=0/TT=1632/T=571	Missense	Unknown

Pathway Analysis of ACE2

Then the importance of *ACE2* gene (Figure 2) and protein secretion and absorption pathways throughout the digestion system (Figure 3) were described by KEGG pathway.

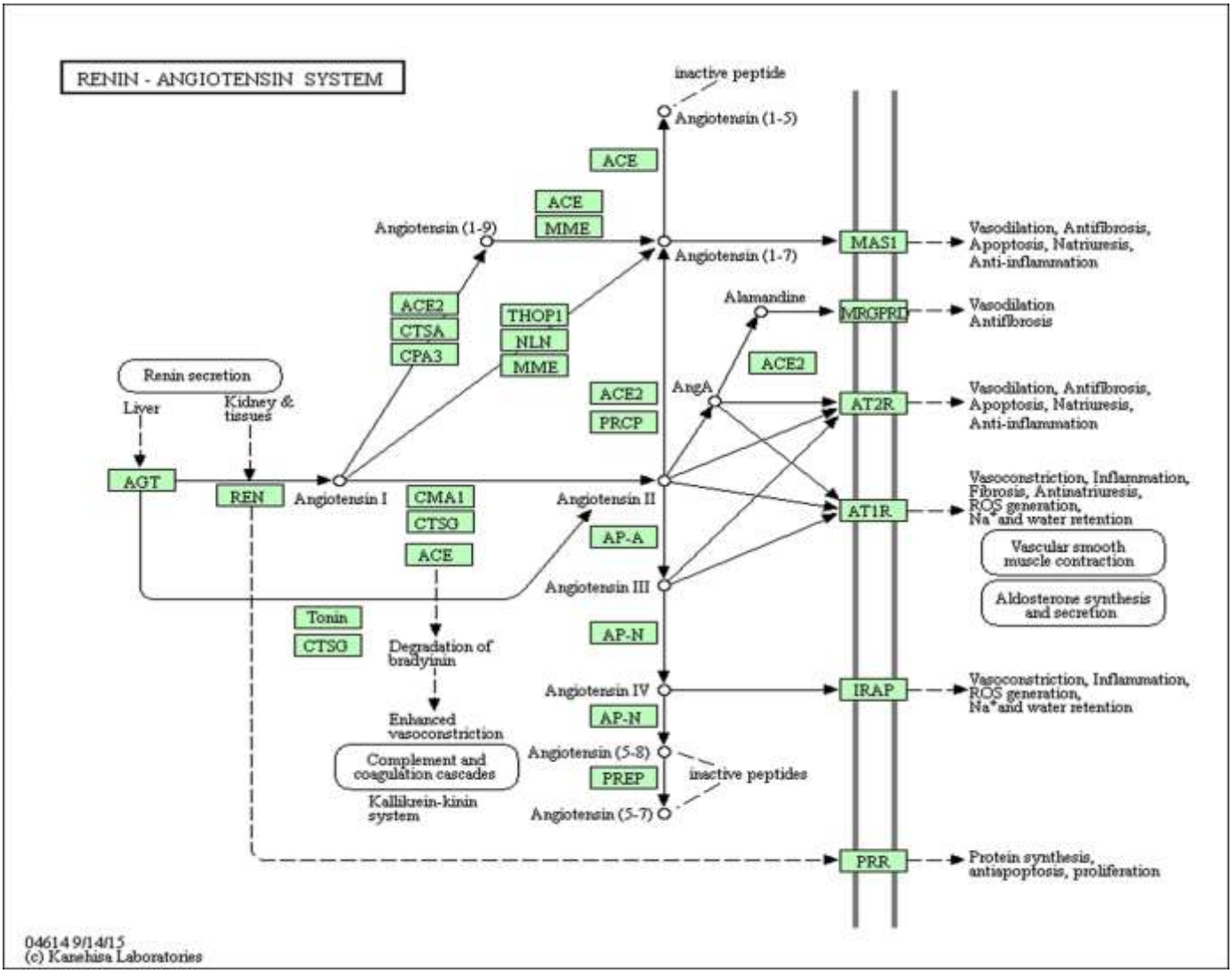

Figure 2. KEGG pathway analysis was used to describe the Renin-angiotensin system. The signaling pathways and their physiological relevance are shown such as *(ACE)/Ang II/AT1R* and *AT2R* axis, *ACE2/angiotensin-(1-7)/Mas* and *Ang IV/IRAP* pathway, and *Ang III*, *Ang A*, and alamandine axis.

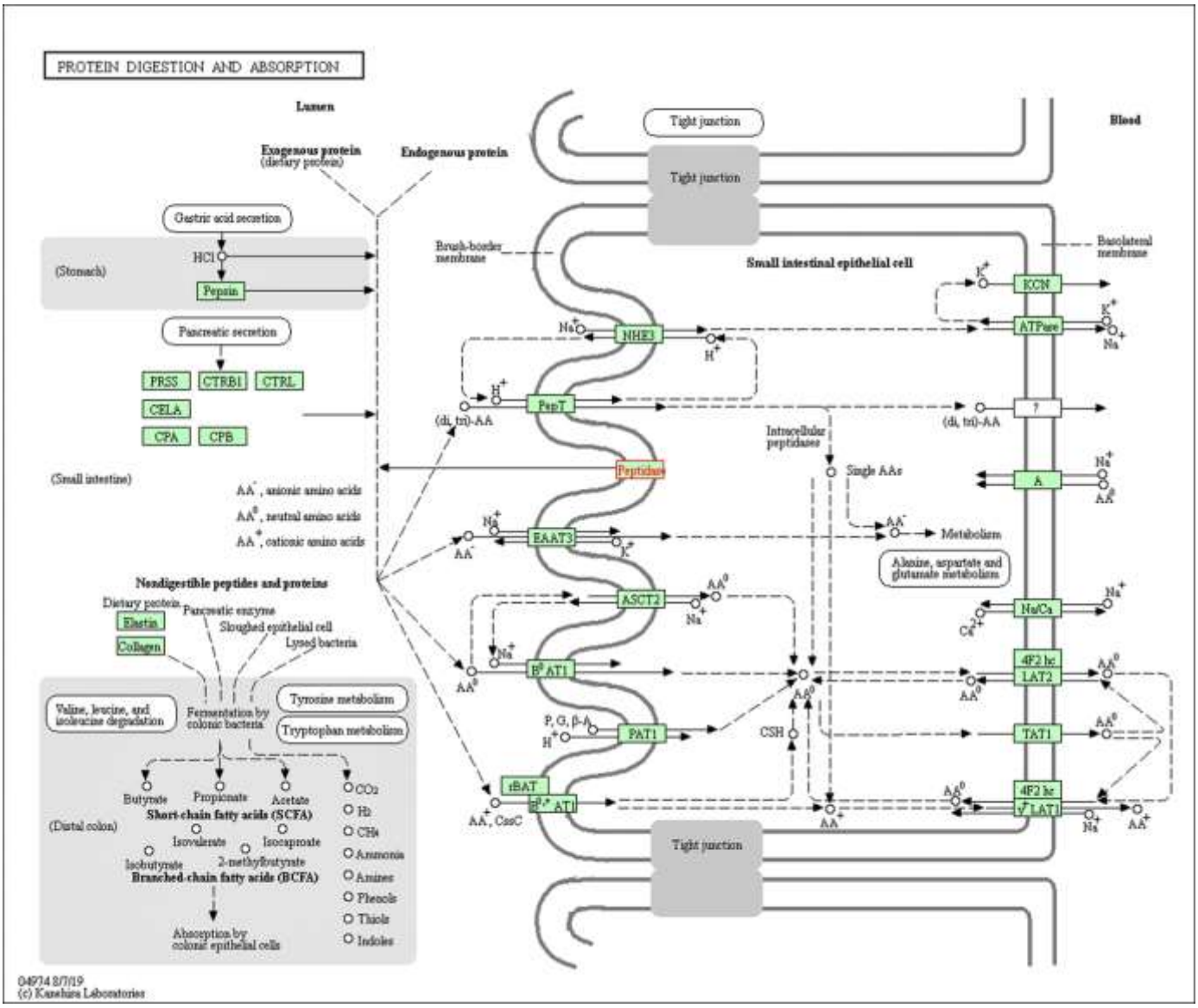

Figure 3. KEGG analysis shows protein secretion and absorption pathways throughout the digestion system.

Prediction of ACE2 Gene-Related Diseases and Expression Profile in Human Normal and Cancer Tissues

BioXpress has shown that *ACE2* gene had over-expression in kidney cancer (P-Value: 0.014, log2FC: 0.71) and esophageal cancer (P-Value: 0.012, log2FC: 1.61) and it had under expression in prostate cancer (P-Value 0.001, log2FC: -0.77) and head and neck cancer (P-Value 0.02, log2FC: -0.56).

When we predicted of *ACE2* Gene-Related Diseases and Expression Profile in Human Normal and Cancer Tissues, we have shown that *ACE2* gene had over-expression in kidney cancer, esophageal cancer and it had under expression in prostate cancer and head and neck cancer. And we had seen that *ACE2* gene were mostly expressed in testis, small intestine, kidney, heart, and adipose tissues (Figure 4, 5 and 6).

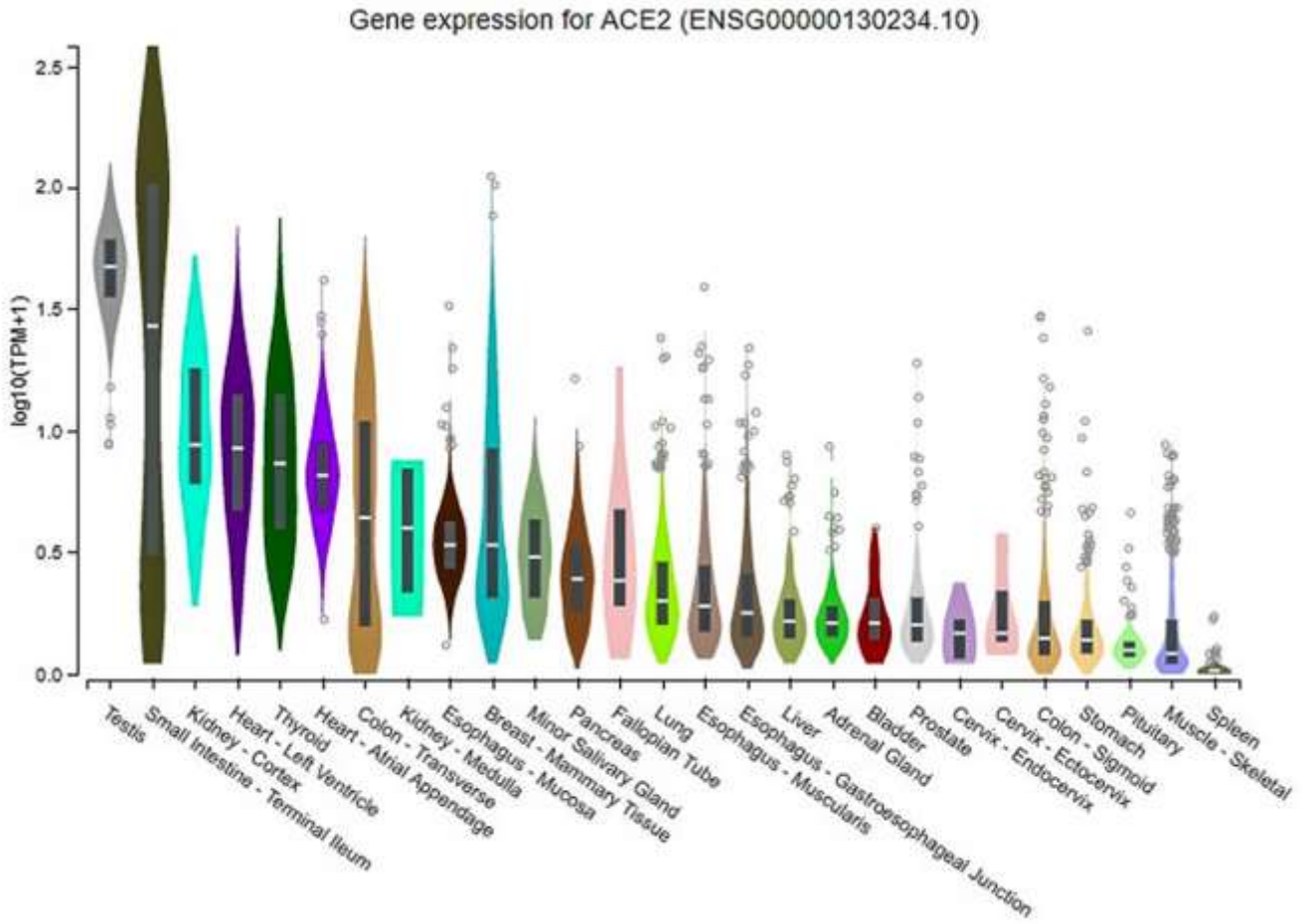

Figure 4. Gene expression profile for *ACE2* in a broad range of tissues.

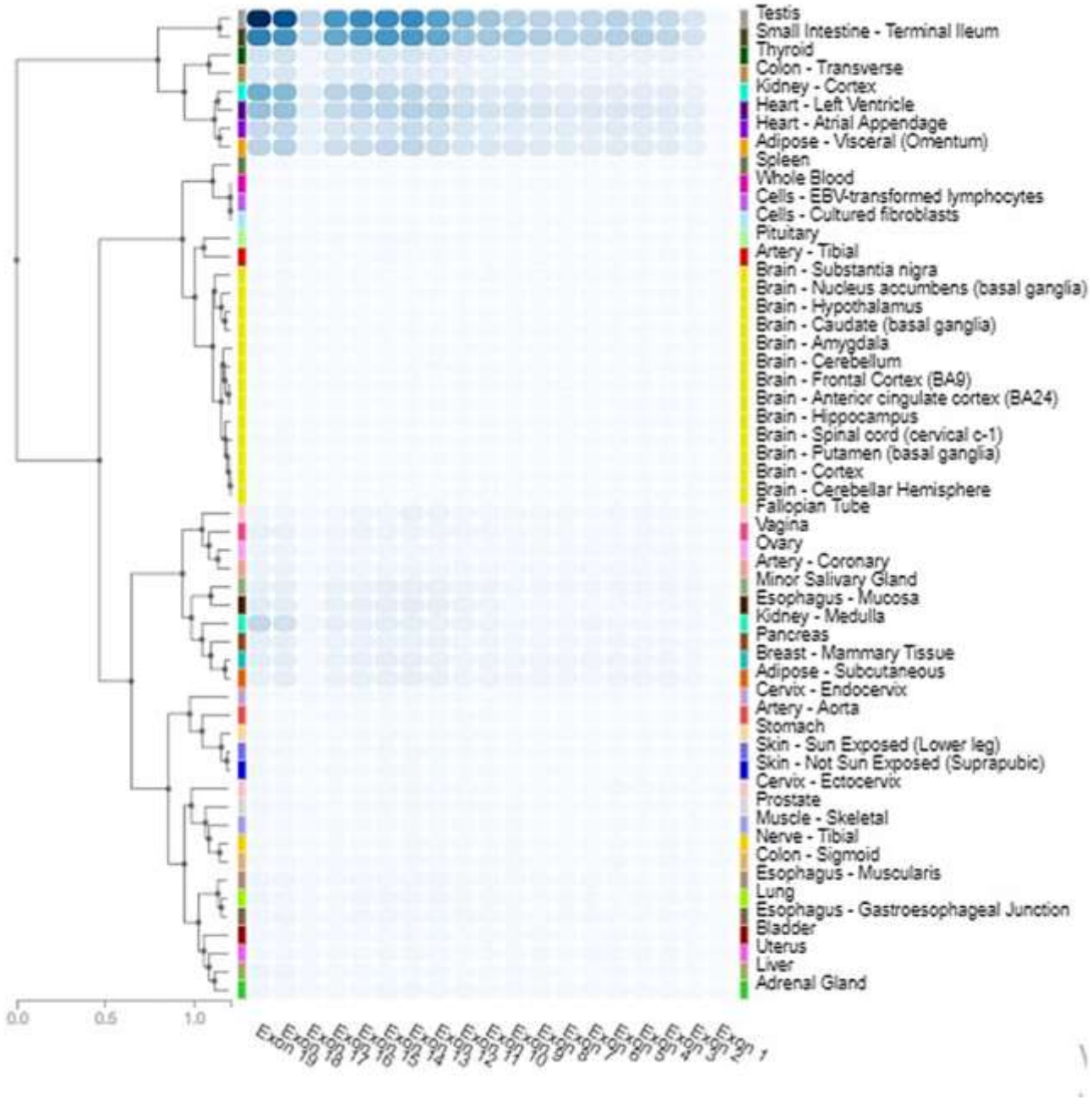

Figure 5. The exon expression analysis of *ACE2* was performed. The most related tissues were detected as testis, small intestine, kidney, heart, and adipose tissues.

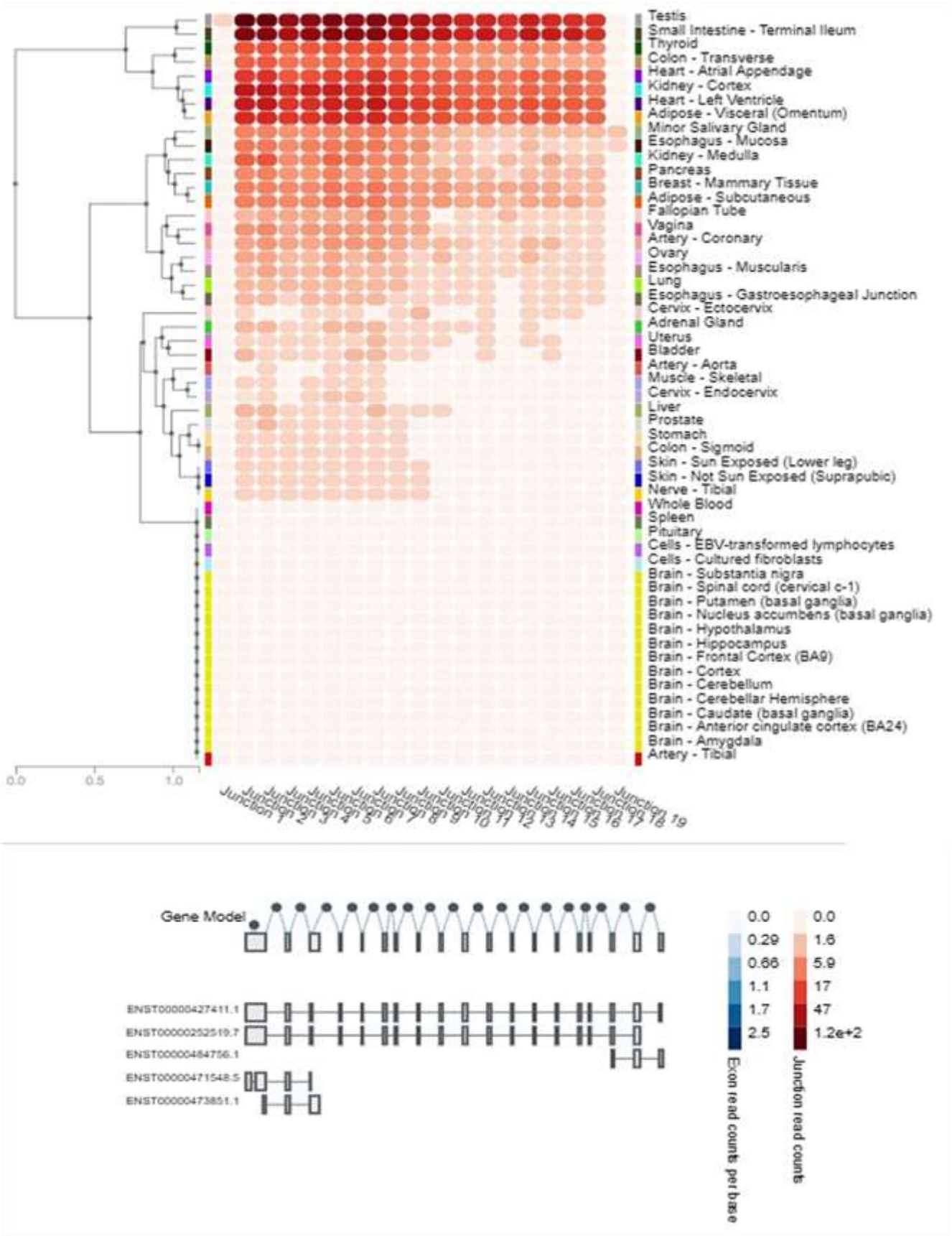

Figure 6. The junction expression analysis of *ACE2* was performed. Consistently, the most related tissues were detected as testis, small intestine, kidney, heart, and adipose tissues. FitSNPs database has shown that left ventricular hypertrophy, hypertension, blood pressure, arterial was associated with *ACE2*.

Finally we analyzed *ACE2* with gene-related diseases and these diseases showed in Table 3.

Table 3. The prediction of *ACE2* with gene-related diseases. Confidence was shown by number of stars.

Name	Z-Score	Confidence
Severe acute respiratory syndrome	6.8	****
Hypertension	6.2	****
Kidney disease	5.2	***
2019 Novel Coronavirus (2019-nCoV)	5.2	***
Diabetes mellitus	5.1	***
Hartnup disease	4.9	***
Heart disease	4.7	***
Coronary artery disease	4.7	***
Cerebrovascular disease	4.5	***
Atherosclerosis	4.3	***
Pneumonia	4.1	***

DISCUSSION

One of the type I integral glycoprotein is *ACE2* that has a role in RAS. RAS is responsible for organizing physiological processes in the cell which are cycle progression, survival, and apoptosis [39] and with RAS activation, it can initiate various pathological processes such as hypertension [40], heart failure [41] and kidney disease [42]. Thus; we analyzed functionally similar genes with *ACE2*. We found that *ACE2* was correlated with *Angiotensinogen (AGT), Renin (REN),* Angiotensin II Receptor Type 1 (*AGTR1),* Angiotensin II Receptor Type 1 (*AGRT2), NADP Malic Enzyme (MME2), Dipeptidyl Peptidase 4 (DPP4),* Prolylcarboxypeptidase *(PRCP),* Meprin A Subunit Alpha *(MEP1A),* X-prolyl Aminopeptidase 2 (*XPNPEP2),* Meprin A Subunit Beta (*MEP1B)* (Figure1). Especially *AGT, AGTR1, AGTR2,* and *REN* have a key role in the RAS such as sodium homeostasis, fluid balance and blood pressure [43], [44] although, all constituents of the RAS have expressed in a lot of tumor tissues [45]. Thus; Cambell and coauthors have reported that *ACE, AGT, AGTR1*, and *AGTR2* genes can promote renal function variation [45]. MME encodes neutral endopeptidase and *PRCP* encodes prolyl carboxypeptidase, and they are also under-expressed in tumor tissue, although they are highly expressed in normal lung tissues [46]. *DPP4* is also known as *CD26*, was associated as a cellular [47] and functional receptor [48] for MERS-CoV. Moreover, we thought that the important point in the function of these macromolecules were mostly peptidase activities. According to our results; we also think that *DPP2* and *ACE2* work together in the pathogenesis of 2019-nCoV infection as it is a direct functional partner. Last studies have shown that *ACE2* variations can be a probable candidate in many diseases such as cardiovascular diseases [49], 2019-nCoV infection [50], essential hypertension [51] in different populations. We found that there were a total number of 99 variants in *ACE2* gene that 53 of them belong to European American populations and 46 of them belong to African American populations. There were 17 suspected variants, 53 unknown variants, 29 benign variants in *ACE2* gene and exome variant server has shown that these mutations were possibly damaging (Table.1).

We analyzed the pathway of RAS and the protein digestion and absorption by KEGG analysis (Figure 2 and Figure 3). Blood pressure regulation is implemented by RAS which also employes in the regulation of electrolyte and fluid balance in the body. The enzyme-substrate mechanism starts with the renin enzyme by cleaving Agt into decapeptide angiotensin I. *ACE* takes place to form *Angiotensin II (AngII)* from decapeptide angiotensin I. *AngII* further employes on the activation of the main receptor responsible for the regulation of *AngII* function in kidney, *AT1 (AT1R)*. Therefore, RAS signaling pathways including *(ACE)/Ang II/AT1R*, and *AT2R* pathway, and *ACE2/angiotensin-(1-7)/Mas* and *Ang IV/IRAP* pathway have been shown in Figure 2.

Protein digestion route through the digestive system with digestive enzymes results in the generation of some amino acids and relatively small peptides. Amino acids transportation to intestinal epithelial cells by amino acids transporters is performed according to the pH level of amino acids. Moreover, *PEP1* is a

transporter that employes in the transportation of small peptides into the enterocytes where peptides hydrolyzation takes place. Amino acids released after hydrolyzation are transported to the bloodstream by amino acid transporters. (Figure 3).

We investigated the *ACE2* gene-related diseases and expression profiles in human normal and cancer tissues. BioXpress has shown that *ACE2* gene have over-expression in kidney cancer (P-Value: 0.014, log2FC: 0.71) and esophageal cancer (P-Value: 0.012, log2FC: 1.61) and it have under expression in prostate cancer (P-Value 0.001, log2FC: -0.77) head and neck cancer (P-Value 0.02, log2FC: -0.56). After COVID-19, maybe over-expression of *ACE2* can trigger some serious diseases and these can cause damage to kidney or esophageal tissues and promote cancer. In addition to this, underexpression of *ACE2* can cause prostate cancer and head and neck cancer. One of the most common symptoms is a severe sore throat, headache, nasal congestion, runny nose and maybe in the future recovered cancer patient may get serious illnesses again, after COVID-19. When we analyzed *ACE2* in human normal tissues, we saw that *ACE2* has mostly expressed in the small intestine. *ACE2* is over-expressed in the small intestine, suggesting that it may cause symptoms of diarrhea and abdominal pain in COVID-19. We found that *ACE2* is also expressed in testis, kidney, heart, thyroid, colon, esophagus, breast, minor salivary gland, pancreas, lung, liver, bladder, cervix and muscle (Figure4). On the other hand, the exon expression of *ACE2* analysis was described and it emphasizes far genomic variations related to splicing regulation. Respectively testis, small intestine, kidney, heart, and adipose tissues correlate with exon expression of *ACE2*. Another interesting point is the expression of *ACE2* in testis. This may be due to the aldosterone synthesis and secretion property of RAS. According to KEGG analysis; RAS has a role in vasodilation, anti-fibrosis, apoptosis, natriuresis, anti-inflammation, aldosterone synthesis, and secretion. We confirmed that our results were consistent with each other.

CONCLUSION

The role of *ACE2* is important in preventing, treating or developing drugs for COVID19. Defining why certain diseases are a high-risk group will help to clarify the pathogenesis of the disease. In the future, this *in silico* analysis should be combined with other pieces of evidence including experimental data in order to assign function.

Funding: Not applicable. The study is self-funded by the authors.
Conflict of interest: The authors declare that they have no conflict of interest.

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