 doi:10.20944/preprints202003.0295.v1

Short Report

DNA Methylation Analysis of the COVID-19 host cell receptor, Angiotensin I Converting Enzyme 2 gene *(ACE2)* in the Respiratory System Reveal Age and Gender Differences

AUTHORS:

[1]Michael J. Corley and [1]Lishomwa C. Ndhlovu*

AFFILIATION:

[1]Division of Infectious Diseases, Department of Medicine, Weill Cornell Medical College, New York, NY, USA.

**Corresponding Author*

Correspondence:

Lishomwa C. Ndhlovu MD, PhD,

Division of Infectious Diseases, Department of Medicine,

Weill Cornell Medical College,

413E 69th St, New York, NY, USA.

Email: lndhlovu@med.cornell.edu

Keywords:

Wuhan 2019-nCoV, ACE2, DNA methylation, epigenetics, profiling, lung tissue, age, gender, COVID-19, coronavirus

CONFLICT OF INTEREST: Authors declare no conflict of interest.

 doi:10.20944/preprints202003.0295.v1

ABSTRACT:

Background: Coronavirus disease 2019 (COVID-19) has emerged as a global threat to human health and disease risk increases with advancing age. The regulation of the *ACE2* gene that codes for COVID-19 host receptor ACE2 has been shown to be under epigenetic regulation. Here, we examined whether intensive DNA methylation profiling of the *ACE2* gene differed by human host tissue and cell type, gender, and age.

Results: Accessing four public datasets, we observed unique human cell-type-specific *ACE2* DNA methylation patterns. In human lung tissues, gender differences in DNA methylation at 2 sites related to the *ACE2* gene were identified. Further, in freshly isolated airway epithelial cells, DNA methylation near the transcription start site of the *ACE2* gene associated with biological age.

Conclusion: Epigenetic profiling of host tissue may permit discovery of age and gender-related potential risk factors for COVID-19. How perturbations in *ACE2* methylation relate to clinical severity across the ages and gender needs to be determined to guide screening tools and potential epigenetic modification targeting to alleviate COVID-19 morbidity in the elderly.

 doi:10.20944/preprints202003.0295.v1

INTRODUCTION:

An outbreak of COVID-2019 emerged in Wuhan, China in December 2019 as a highly contagious coronavirus[1] capable of human infection and transmission [2,3], and possible mortality following infection similar to the SARS coronavirus [4]. As of March 11, 2020, COVID-19 case fatality rate globally is estimated by the WHO at 3.48% based on 109,578 confirmed cases and 3,809 deaths and this infection has evolved into a pandemic[5]. In the United States (US) as of March 16, 2020 a total of 3,487 cases have been reported with total 68 deaths across 49 states including the District of Columbia as a multipronged surveillance and containment strategy has been initiated across the country with a reported mean incubation period of 6.4 days[6]. Recent analysis of epidemiological data suggests that as many as 86% of all COVID-19 infections are undocumented and are contributing to the rapid worldwide increase in cases[7].

Clinical research has described COVID-19 as an acute respiratory tract infection with varied severity of symptoms including fever onset by dry cough. Clinical treatment of patients infect with COVID-19 has evolving management guidelines of the disease[2,3,8,9]. Epidemiology research has documented the spread and fatality rates for the virus[10], virology research has characterized the genomic features and evolution of COVID-2019[11–14], and vaccine research has started development and testing of candidate vaccines[15,16]. The global spread of the virus and diversity of human host capable of infection highlight the importance of understanding host biological differences related to COVID-2019. There remain numerous key questions on biological factors that contribute to varying host responses and clinical outcomes. Data from other human

 doi:10.20944/preprints202003.0295.v1

pathogenic coronaviruses SARS-CoV and MERS-CoV[17–19] has provided some insight into potential biological factors underlying varied host responses and clinical outcomes. Sex-based differences in susceptibility to SARS-CoV infection have been reported in mice that parallel those observed in patients and this work identified estrogen receptor signaling as critical for protection in females[20]. Initial reports suggest sex and age-related differences in COVID-2019 that warrant further investigation[17,21]. Research is needed to understand specific sex-related biological features that underlie clinical severity and treatment strategies for COVID-2019 in men and women.

Structural analyses have revealed that receptor angiotensin-converting enzyme 2 (ACE2) as a host receptor permitting cell entry and viral infectivity for COVID-2019[22,23]. Notably, ACE2 was also identified as a host receptor for other coronaviruses including SARS-CoV and NL63[24]. ACE2 has been well studied as a central regulator of blood pressure in the renin-angiotensin-aldosterone system[25]. Studies have shown that ACE2 protein and mRNA expression occurs in a variety of human tissues including lung, liver, stomach, ileum, colon, and kidney[26,27]. Recent single cell analyses of normal human tissues have shown that *ACE2* is expressed in cells of the respiratory and digestive system suggesting the lung and gut body compartments as routes for COVID-2019 infection, viral replication, and viral shedding[28–30]. Epigenetics research has suggested that the *ACE2* gene that codes for ACE2 may be transcriptionally regulated by DNA methylation[31], which is a covalent chemical modification of host DNA. Moreover, *ACE2* is located on the X chromosome[32] raising the possibility of gender differences in susceptibility and progression of COVID-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

2019[17,19,21]. Thus far, no study has examined the epigenetic landscape of *ACE2* and whether it differs by age or gender.

In this study, we accessed four available genome-wide DNA methylation human datasets to examine whether DNA methylation profiling related to the *ACE2* gene differed by host tissue/cell type, sex, and varied by biological age to begin to understand and address the hypothesis of whether epigenetic footprints related to *ACE2* impact susceptibility risk for COVID-19, disease progression, mortality and morbidity.

RESULTS AND DISCUSSION:

We examined evidence for varied DNA methylation patterns related to the *ACE2* gene in human lung, gut, liver, pancreas, brain, and blood by accessing a subset of available raw genome-wide DNA methylation array data[33] (GEO accession: GSE122126). We analyzed the Illumina MethylationEPIC DNA methylation data using the Chip Analysis Methylation Pipeline[34] and observed that DNA methylation levels at loci related to the *ACE2* gene of various human tissue cell types showed that DNA methylation was varied across tissue cell types (**Fig. 1**). Notably, DNA methylation across three CpGs (cg04013915, cg08559914, cg03536816) assayed for the *ACE2* gene was lowest in lung epithelial cells compared to the other tissue cell types (**Fig. 1**)., suggesting transcription and expression to be highest in the lung/respiratory system compartment which supports emerging single cell RNA-seq analyses of normal human lung/respiratory system datasets[28–30]. Average total DNA methylation at all probes

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

related to the *ACE2* gene was hypermethylated in cortical neurons and leukocytes compared to other cell types examined, suggesting excluded *ACE2* transcription and protein expression in these cell types reported previously [26,27].

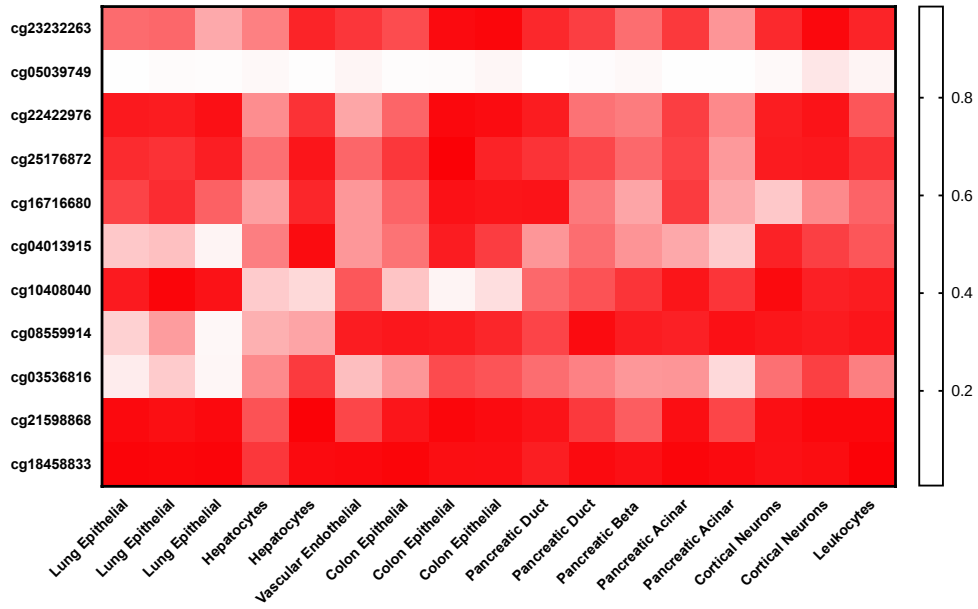

Figure 1. Distinct tissue cell type DNA methylation profile related to *ACE2*. DNA methylation of 11 CpGs surveyed by the Illumina Infinium MethylationEPIC array related to *ACE2* gene from lung epithelial, hepatocytes, colon epithelial, pancreatic duct, pancreatic beta, pancreatic acinar, cortical neurons, and leukocytes is shown from GEO accession: GSE122126. DNA methylation level is shown for each CpG as ranging from 0 to 1.0 (methylation normalized beta-value).

Since lung epithelial cells were observed to have a hypomethylation at the *ACE2* gene, we sought to further examine DNA methylation levels in human lung tissues. We accessed available genome-wide DNA methylation Illumina HumanMethylation450 data from human lung tissues containing samples from both males and females and including data from smokers and patients with chronic obstructive pulmonary disease[35]. Analysis

 doi:10.20944/preprints202003.0295.v1

of DNA methylation at two CpG sites related to the *ACE2* gene showed that females were significantly hypomethylated compared to males ($P = 0.0001$) (**Fig. 2)**. Additionally, DNA methylation trended towards being hyper-methylation in lung disease conditions including smoking and COPD compared to normal lung although not statistically significant due to limited sample size ($P = 0.18$). Notably, the *ACE2* gene is located on the X chromosome raising the possibility of methylation differences due to X chromosome activation[36]. Of note, the genome-wide Illumina HumanMethylation450 array measures DNA methylation in both X chromosomes of the females. These initial gender-associated differences in DNA methylation related to the *ACE2* gene in lung tissues warrant further investigation, especially in the context of *ACE2* gene and protein expression and COVID-19 severity. To examine the variability in DNA methylation levels related to *ACE2* in males and females, we accessed Illumina HumanMethylation450 DNA methylation data from 244 fresh human lung tissues[37]. We observed that DNA methylation at a CpG in the dataset related to the *ACE2* gene showed a large degree of variability in both men and women suggesting DNA methylation of *ACE2* varies by individual (**Fig. 3)**. Of note, this dataset did not have metadata for age and the differences in DNA methylation related to *ACE2* may be reflective of cell type differences in the lung tissues.

These gender-related differences in *ACE2* DNA methylation observed in the respiratory system support findings that indicate that Angiotensin II metabolism varies by gender and may relate to hormonal differences or genetic differences in chromosome dosage[38]. Our DNA methylation data contrast a recent preprint study that reanalyzed five bulk transcriptome datasets of normal lung tissue and two single cell transcriptome

 doi:10.20944/preprints202003.0295.v1

dataset reported no significant differences in *ACE2* between racial groups, age groups, or gender groups[30]. A major limitation of this study was the sample size and multiple comparisons as well as the focus only on *ACE2* transcription. Notably, the *ACE2* transcription preprint study did report higher expression in Asian current smokers compared to non-smokers supporting our observations of a trend in differences in *ACE2* in lung of male smokers. Smoking dramatically impacts the epigenome and will be a key environmental factor to examine in future epigenetic studies of COVID-19[39,40]. Additional research will need to determine whether protein, gene transcription, or epigenetic landscape of *ACE2* is most relevant to COVID-19 infection risk, disease severity, and transmission.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

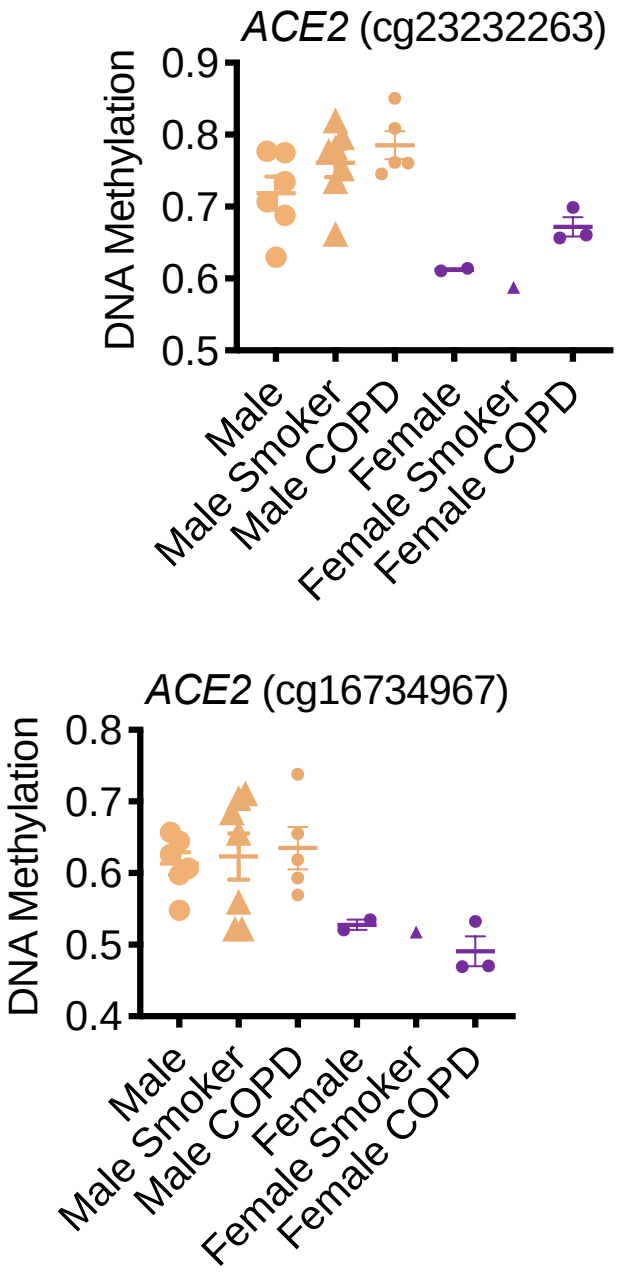

Figure 2. DNA methylation at two CpG loci related to *ACE2* gene in human lung tissues of smokers and patients with chronic obstructive pulmonary disease (COPD) by gender from GEO accession: GSM2430978. DNA methylation level is shown for each CpG as ranging from 0 to 1.0 (methylation normalized beta-value).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

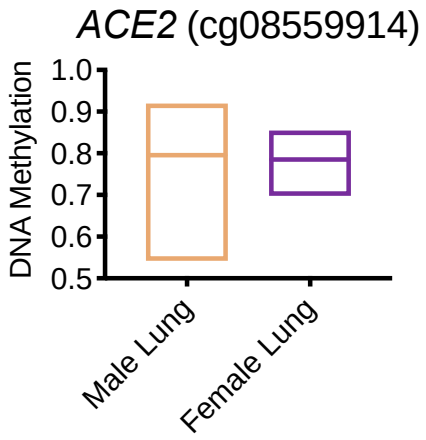

Figure 3. DNA methylation variability related to *ACE2* gene in human lung tissues men and women from GEO accession: GSE52401. DNA methylation level is shown line at median with minimum and maximum (methylation normalized beta-value).

Next, based on epigenetics work showing DNA methylation sites associated with longitudinal lung function decline and aging in humans[41] and given reports out China highlighting the increased risk of COVID-19 severity in older individuals[3] due to a myriad of potential biological factors, we sought to test our hypothesis that DNA methylation related to the ACE2 gene associated with age in the respiratory system. Therefore, we obtained genome-wide DNA methylation array data from freshly isolated airway epithelial cells of non-asthmatics of varying biological ages and evaluated whether the methylation levels for CpGs related to the *ACE2* gene related to biological age. We found that one CpG (cg08559914) near the transcription start site of the *ACE2* gene significantly associated with biological age in airway epithelial cells (r=-0.59, *P*= 0.001; **Fig. 4**). This finding was notable given that the Illumina DNA methylation array data analyzed for *ACE2* gene did not measure DNA methylation at every CpG but only 7 CpG sites. Whole genome bisulfite sequencing will provide further insight into whether there are additional DNA methylation sites related to the *ACE2* gene that dynamically

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

associate with age. These findings suggest and age-related change in the epigenetic regulation of *ACE2* in the respiratory system exist and are relevant to studies of ACE2 and COVID-19 in elderly. Our findings complement emerging single cell transcriptional profiling data that is suggesting that nasal goblet/secretory and ciliated cells of the respiratory system show the highest *ACE2* transcription[29] and highlight the need for age-related single transcriptomic and epigenetic profiling studies of the respiratory system in COVID-19. Whether this dramatic relationship between ACE2 DNA methylation and aging is mediated by other aging-related complications or is a major factor contributing to older individuals being more susceptible or younger individuals being less susceptible to severe COVID-19 infection needs to be studied.

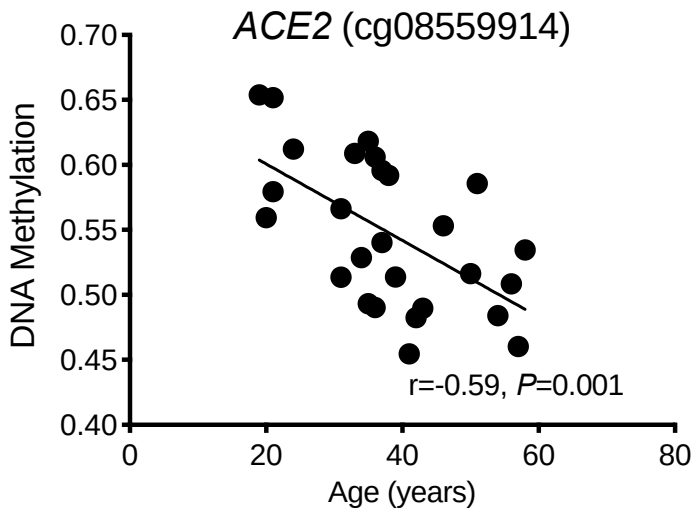

Figure 4. DNA methylation at a CpG loci in the TSS200 region related to *ACE2* gene in airway epithelial cells harvested via bronchial brushing from non-asthmatic females from GEO accession: GSE85566) significantly associates with biological age (r=-0.59, *P* = 0.001)

 doi:10.20944/preprints202003.0295.v1

We also examined public available data from ENCODE for RNA-Seq from human Lung donors including a 3 year old male, 30 year old female, and fetal samples[42,43]. Interestingly, high levels of transcription were observed in young male lung compared to fetal lung and female lung (**Fig. 5)**. Moreover, we observed in a publicly available ENCODE Hi-C dataset for IMR90 cells[42] used to examine genome-wide chromatin organization that chromatin interaction contacts occurred near the site we observed DNA methylation differences for the *ACE2* gene, suggesting a potential spatiotemporal gene expression program for *ACE2* mediated by DNA methylation. Additional single cell 3D chromatin research in normal respiratory system and during COVID-19 infection will need to examine whether a promoter-enhancer interaction for *ACE2* exist and modulates differential host and respiratory system cell type expression patterns.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 March 2020 doi:10.20944/preprints202003.0295.v1

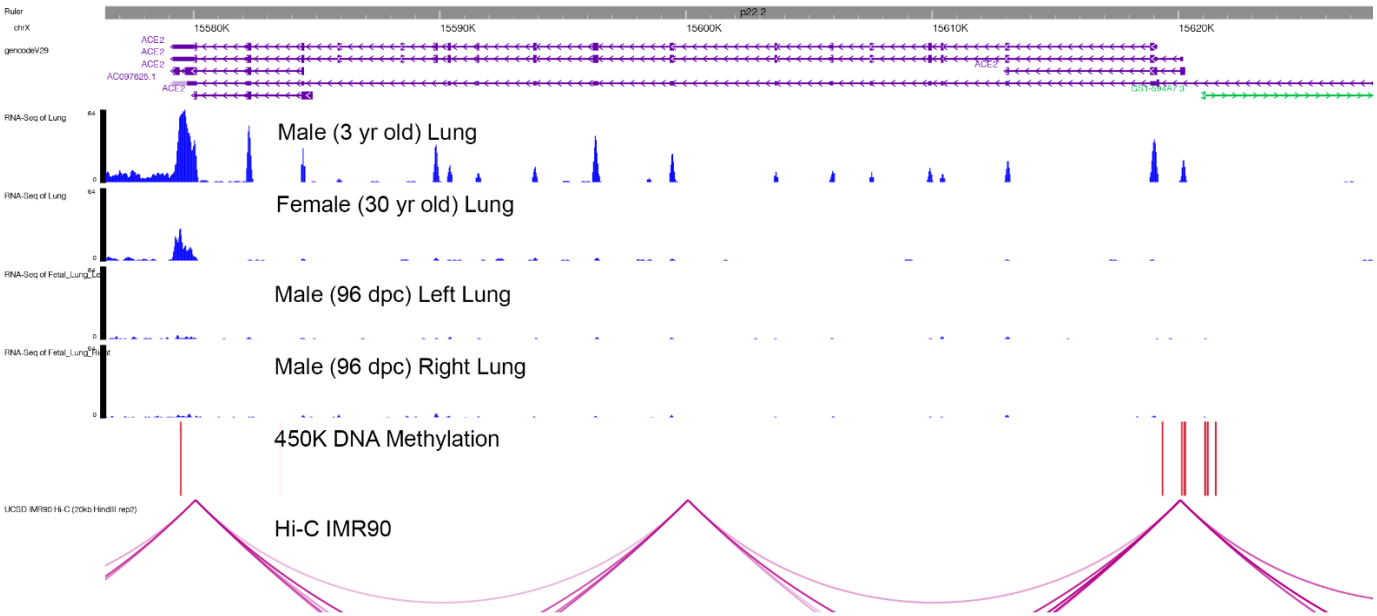

Figure 5. Scaled gene view from WashU Epigenome Browser[43] **of *ACE2* human gene. *ACE2* gene track showed in purple from gencodeV29. RNA-Seq tracks from human lung and fetal lung obtained from ENCODE**[42] **shown in blue. CpG methylation data of the seven 450K array probes over the *ACE2* gene shown as blue lines at each CpG. Hi-C genome-wide chromosome organization data from IMR90 cells shown with view of interaction contacts in purple 20kb resolution.**

 doi:10.20944/preprints202003.0295.v1

In this study, we observed DNA methylation levels associated with *ACE2* gene varied by tissue type, differed by gender and disease state in lung, and associated with biological age in airway epithelial cells. Notably, the significant relationship between DNA methylation of *ACE2* and biological age suggest age-dependent differences in host response may be mediated by the dysregulation of *ACE2* and increased expression of ACE2 with aging. While our DNA methylation findings related to the *ACE2* gene are compelling, ACE2 is dynamically regulated in the body transcriptionally, posttranscriptionally, and posttranslationally[44]. Further work will need to determine whether other pre-existing health conditions such as diabetes and hypertension or medications being taken such as ACE inhibitors and angiotensin II type-I receptor blockers lead the dysregulation of ACE2 and increased severe outcomes of COVID-19 infection[45]. This notion has recently received attention and controversy and deserves further study[46]. Additionally, the use of ACE inhibitors as therapeutics to prevent COVID-19 viral entry also warrants further investigation[47].

In summary, our work focused on studying available epigenetic DNA methylation data to begin to characterize and focus on the tissue/cell type specific epigenetic landscape of the COVID-19 host receptor gene *ACE2*. Future work should examine varying host epigenetic landscapes during COVID-19 infection to understand whether COVID-2019 antagonizes antigen presentation through epigenetic modulation similar to MERS-CoV [48]. This information will be vital to vaccine development and epigenetic therapeutics for COVID-19. Another compelling question is whether the DNA

 doi:10.20944/preprints202003.0295.v1

methylation profile of *ACE2* in COVID-19 viral replication body sites associates with "super spreaders" and enhanced transmissivity.

METHODS

Public dataset acquisition and processing.

Public DNA methylation datasets were downloaded from the Gene Expression Omnibus website. DNA methylation data for human isolated cell populations from tissues was obtained from (GEO: GSE122126). DNA methylation data for airway epithelial cells used to examine the relationship with age was obtained from (GEO: GSE85568). DNA methylation data for lungs tissues of smokers and patients with COPD by gender was obtained from (GEO: GSE92511). DNA methylation data for normal lungs tissues by gender was obtained from (GEO: GSE52401). RAW IDAT files were loaded into ChAMP pipeline, preprocessed, and normalized[34]. We loaded RNA-Seq and Hi-C datasets from the ENCODE portal[49] (https://www.encodeproject.org/) into the WashU Epigenome Browser. Statistical tests were conducted in Graphpad Prism 8 for DNA methylation data related to the *ACE2* gene.

 doi:10.20944/preprints202003.0295.v1

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to acknowledge the authors that deposited raw DNA methylation data for analysis relevant to *ACE2*. Public access to all formats of data relevant to COVID-19 will aid in overcoming the pandemic.

REFERENCES

1. Fehr AR, Perlman S. Coronaviruses: An Overview of Their Replication and Pathogenesis. In: Maier HJ, Bickerton E, Britton P, editors. Coronaviruses: Methods and Protocols. New York, NY: Springer New York; 2015. p. 1–23.

2. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–3.

3. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med [Internet]. 2020; Available from: http://dx.doi.org/10.1056/NEJMoa2002032

4. Yang Y, Peng F, Wang R, Guan K, Jiang T, Xu G, et al. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun. 2020;102434.

5. Roser M, Ritchie H, Ortiz-Ospina E. Coronavirus Disease (COVID-19) – Statistics and Research. Our World in Data [Internet]. 2020 [cited 2020 Mar 17]; Available from: https://ourworldindata.org/coronavirus

6. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020. Euro Surveill [Internet]. 2020;25. Available from: http://dx.doi.org/10.2807/1560-7917.ES.2020.25.5.2000062

7. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2). Science [Internet]. American Association for the Advancement of Science; 2020 [cited 2020 Mar 16]; Available from: https://science.sciencemag.org/content/early/2020/03/13/science.abb3221.full

8. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis [Internet]. 2020; Available from: http://dx.doi.org/10.1016/S1473-3099(20)30086-4

9. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered,

retrospective, observational study. Lancet Respir Med [Internet]. 2020; Available from: http://dx.doi.org/10.1016/S2213-2600(20)30079-5

10. Ghinai I, McPherson TD, Hunter JC, Kirking HL, Christiansen D, Joshi K, et al. First known person-to-person transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the USA. Lancet [Internet]. Elsevier; 2020; Available from: https://doi.org/10.1016/S0140-6736(20)30607-3

11. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, et al. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient [Internet]. bioRxiv. 2020 [cited 2020 Mar 16]. p. 2020.03.02.972935. Available from: https://www.biorxiv.org/content/10.1101/2020.03.02.972935v2

12. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Hu Y, et al. Complete genome characterisation of a novel coronavirus associated with severe human respiratory disease in Wuhan, China [Internet]. bioRxiv. 2020 [cited 2020 Mar 16]. p. 2020.01.24.919183. Available from: https://www.biorxiv.org/content/10.1101/2020.01.24.919183v2

13. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395:565–74.

14. Kim D, Lee J-Y, Yang J-S, Kim JW, Narry Kim V, Chang H. The architecture of SARS-CoV-2 transcriptome [Internet]. bioRxiv. 2020 [cited 2020 Mar 14]. p. 2020.03.12.988865. Available from: https://www.biorxiv.org/content/10.1101/2020.03.12.988865v1

15. Ahmed SF, Quadeer AA, McKay MR. Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies. Viruses [Internet]. 2020;12. Available from: http://dx.doi.org/10.3390/v12030254

16. Feng Y, Qiu M, Zou S, Li Y, Luo K, Chen R, et al. Multi-epitope vaccine design using an immunoinformatics approach for 2019 novel coronavirus in China (SARS-CoV-2) [Internet]. bioRxiv. 2020 [cited 2020 Mar 16]. p. 2020.03.03.962332. Available from: https://www.biorxiv.org/content/10.1101/2020.03.03.962332v1

17. Jin J-M, Bai P, He W, Wu F, Liu X-F, Han D-M, et al. Gender differences in patients with COVID-19: Focus on severity and mortality. medRxiv. Cold Spring Harbor Laboratory Press; 2020;2020.02.23.20026864.

18. Jansen A, Chiew M, Konings F, Lee C-K, Ailan L, on behalf the World Health Organization Regional Office for the Western Pacific MERS Event Management Team. Sex matters - a preliminary analysis of Middle East respiratory syndrome in the Republic of Korea, 2015. Western Pac Surveill Response J. 2015;6:68–71.

19. Karlberg J, Chong DSY, Lai WYY. Do men have a higher case fatality rate of severe acute respiratory syndrome than women do? Am J Epidemiol. 2004;159:229–31.

20. Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-Based Differences in Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Infection. J Immunol. 2017;198:4046–53.

 doi:10.20944/preprints202003.0295.v1

21. Cai H. Sex difference and smoking predisposition in patients with COVID-19. Lancet Respir Med [Internet]. 2020; Available from: http://dx.doi.org/10.1016/S2213-2600(20)30117-X

22. Jaimes JA, Andre NM, Millet JK, Whittaker GR. Structural modeling of 2019-novel coronavirus (nCoV) spike protein reveals a proteolytically-sensitive activation loop as a distinguishing feature compared to SARS-CoV and related SARS-like coronaviruses [Internet]. arXiv [q-bio.BM]. 2020. Available from: http://arxiv.org/abs/2002.06196

23. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol [Internet]. 2020; Available from: http://dx.doi.org/10.1128/JVI.00127-20

24. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pöhlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci U S A. 2005;102:7988–93.

25. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res. 2000;87:E1-9.

26. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004;203:631–7.

27. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett. 2002;532:107–10.

28. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov [Internet]. bioRxiv. 2020 [cited 2020 Mar 16]. p. 2020.01.26.919985. Available from: https://www.biorxiv.org/content/10.1101/2020.01.26.919985v1

29. Sungnak W, Huang N, Bécavin C, Berg M, HCA Lung Biological Network. SARS-CoV-2 Entry Genes Are Most Highly Expressed in Nasal Goblet and Ciliated Cells within Human Airways [Internet]. arXiv [q-bio.CB]. 2020. Available from: http://arxiv.org/abs/2003.06122

30. Cai G. Bulk and Single-Cell Transcriptomics Identify Tobacco-Use Disparity in Lung Gene Expression of ACE2, the Receptor of 2019-nCov [Internet]. LIFE SCIENCES. 2020. Available from: https://www.preprints.org/manuscript/202002.0051/v2

31. Zill P, Baghai TC, Schüle C, Born C, Früstück C, Büttner A, et al. DNA methylation analysis of the angiotensin converting enzyme (ACE) gene in major depression. PLoS One. 2012;7:e40479.

32. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem. 2000;275:33238–43.

 doi:10.20944/preprints202003.0295.v1

33. Moss J, Magenheim J, Neiman D, Zemmour H, Loyfer N, Korach A, et al. Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. Nat Commun. 2018;9:5068.

34. Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, et al. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Bioinformatics. 2017;33:3982–4.

35. Sundar IK, Yin Q, Baier BS, Yan L, Mazur W, Li D, et al. DNA methylation profiling in peripheral lung tissues of smokers and patients with COPD. Clin Epigenetics. 2017;9:38.

36. Cotton AM, Price EM, Jones MJ, Balaton BP, Kobor MS, Brown CJ. Landscape of DNA methylation on the X chromosome reflects CpG density, functional chromatin state and X-chromosome inactivation. Hum Mol Genet. 2015;24:1528–39.

37. Shi J, Marconett CN, Duan J, Hyland PL, Li P, Wang Z, et al. Characterizing the genetic basis of methylome diversity in histologically normal human lung tissue. Nat Commun. 2014;5:3365.

38. White MC, Fleeman R, Arnold AC. Sex differences in the metabolic effects of the renin-angiotensin system. Biol Sex Differ. 2019;10:31.

39. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, et al. DNA Methylation in Newborns and Maternal Smoking in Pregnancy: Genome-wide Consortium Meta-analysis. Am J Hum Genet. 2016;98:680–96.

40. Stueve TR, Li W-Q, Shi J, Marconett CN, Zhang T, Yang C, et al. Epigenome-wide analysis of DNA methylation in lung tissue shows concordance with blood studies and identifies tobacco smoke-inducible enhancers. Hum Mol Genet. 2017;26:3014–27.

41. Carmona JJ, Barfield RT, Panni T, Nwanaji-Enwerem JC, Just AC, Hutchinson JN, et al. Metastable DNA methylation sites associated with longitudinal lung function decline and aging in humans: an epigenome-wide study in the NAS and KORA cohorts. Epigenetics. 2018;13:1039–55.

42. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489:57–74.

43. Li D, Hsu S, Purushotham D, Sears RL, Wang T. WashU Epigenome Browser update 2019. Nucleic Acids Res. 2019;47:W158–65.

44. Turner AJ. Chapter 25 - ACE2 Cell Biology, Regulation, and Physiological Functions. In: Unger T, Steckelings UM, dos Santos RAS, editors. The Protective Arm of the Renin Angiotensin System (RAS). Boston: Academic Press; 2015. p. 185–9.

45. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med [Internet]. 2020; Available from: http://dx.doi.org/10.1016/S2213-2600(20)30116-8

46. Hiremath S. The Coronavirus Conundrum: ACE2 and Hypertension Edition — NephJC [Internet]. NephJC. NephJC; 2020 [cited 2020 Mar 16]. Available from: http://www.nephjc.com/news/covidace2

47. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. Drug Dev Res [Internet]. 2020; Available from: http://dx.doi.org/10.1002/ddr.21656

48. Menachery VD, Schäfer A, Burnum-Johnson KE, Mitchell HD, Eisfeld AJ, Walters KB, et al. MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. Proc Natl Acad Sci U S A. 2018;115:E1012–21.

49. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res. 2018;46:D794–801.