Research Article

Expression Level Analysis of *ACE2* Receptor Gene in African-American and Non-African-American COVID-19 Patients

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ABSTRACT

The COVID-19 pandemic caused by SARS-CoV-2 has spread rapidly across the continents. While the incidence of COVID-19 has been reported to be higher among African-American individuals, the rate of mortality has been lower compared to that of non-African-Americans. *ACE2* is involved in COVID-19 as SARS-CoV-2 uses the *ACE2* enzyme to enter host cells. Although the difference in COVID-19 incidence can be explained by many factors such as low accessibility of health insurance among the African-American community, little is known about *ACE2* expression in African-American COVID-19 patients compared to non-African-American COVID-19 patients. The variable expression of genes can contribute to this observed phenomenon.

In this study, transcriptomes from African-American and non-African-American COVID-19 patients were retrieved from the sequence read archive and analyzed for *ACE2* gene expression. HISAT2 was used to align the reads to the human reference genome, and HTseq-count was used to get raw gene counts. EdgeR was utilized for differential gene expression analysis, and enrichR was employed for gene enrichment analysis.

The datasets included 14 and 33 transcriptome sequences from COVID-19 patients of African-American and non-African-American descent, respectively. There were 24,092 differentially expressed genes, with 7,718 upregulated (log fold change > 1 and FDR 0.05) and 16,374 downregulated (log fold change -1 and FDR 0.05). The *ACE2* mRNA level was found to be considerably downregulated in the African-American cohort (p-value = 0.0242, p-adjusted value = 0.038).

The downregulation of *ACE2* in the African-American cohort could indicate a correlation to the low COVID-19 severity observed among the African-American community.

Keywords: *ACE2* gene; SARS-CoV-2; Transcriptomics; African-American; Non-African-American

Abbreviations: *ACE2*: Angiotensin-Converting Enzyme 2; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SRA: Sequence Read Archive; Ang-I: Angiotensin I; *TGFB1*: Transforming Growth Factor Beta-1; *APOL1*: Apolipoprotein L1; NLRP3: Nucleotide binding domain Leucine Rich containing family, Pyrin domain containing protein 3; STING1: Stimulator of Interferon Response CGAMP Interactor 1.

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a viral ailment caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Wuhan city, China was the first place where the virus was detected, before it spread to other countries throughout the world, causing a global pandemic. Fever, dry cough, and malaise are common COVID-19 symptoms. Difficulty breathing, chest pain as well as Acute Respiratory Distress Syndrome (ARDS) have been reported in severe cases [1].

As of August 1, 2020, the WHO recorded about 18 million

COVID-19 cases and 600,000 deaths globally [2]. The total number of COVID-19 cases in the European region and United States were about 3 million and 4 million confirmed cases, out of a population of 741 million and 327 million respectively [3]. The rate of mortality among the African-American community was reported to be 25 percent lower than white and native Americans while Asian Americans had the lowest rates [4]. Areas with a large African-American population however, reported that COVID-19 patients had increased in-hospital mortality, caused by more comorbid conditions being present [5]. Older residents, people in dense housing such as the projects and African-Americans with heart disease and lacking health insurance had a doubled chance of dying from COVID-19 [6,7]. Patient outcomes were improved by starting dialysis early and using *ACE* inhibitors prior to admission.

Asia with about 60% of the world population recorded about 28.6% of cases and 22.7% of deaths (303 deaths/million) as of the 1st of April 2022 [8]. According to the same report by worldometer, North America (4.7% of world population) appears to have been the worst hit with 19.7% and 23.4% of cases and deaths respectively (3,914 deaths/million). This is closely followed by Europe (9.6% of the world population) with 36.7% of cases and 28.7% of global cases and deaths respectively (2,374 deaths/million). Study reports have shown that next-generation sequences can be used to screen for disorders in newborns [9], for the study of pathogen evolution using African SARS-CoV-2 sequences[10], prostate cancer biomarker discovery [11], malaria/CoVID-19 biomarker discovery [12], epilepsy biomarker discovery [13], SARS-CoV-2 variants classification [14], HIV-1 evolution in sub-Saharan Africa [15], analysis of RNA-seq and ChIP-seq data [16], and Ebola virus comparative genomics [17]. We will be using next-generation sequencing data for our investigation in this study.

Zeberg and his colleagues showed that the major genetic risk to COVID-19 is caused by a 50-kilobase pair genomic segment inherited from the Neanderthals, which is present in 50% and 16% of people in south Asia and Europe respectively [18]. Gene allele distribution could also explain some of the differences in COVID-19 infection incidence among people in different geographic regions [3]. The authors however suggest that due to a lack of sufficient data, more genetic investigations are needed to corroborate this notion.

Angiotensin Converting Enzyme 2 (*ACE2*) hydrolyzes Ang-II to produce Ang- (1-7) - a vasodilator and anti-inflammatory agent, by. *ACE2* promotes a protective anti-inflammatory response in the lung by lowering oedema, vascular permeability, and pulmonary cell damage. *ACE2* promotes a protective anti-inflammatory response in the lung by lowering oedema, pulmonary cell damage and vascular permeability [19-21]. When the expression of *ACE2* receptors is reduced, its substrate Ang-II, accumulates leading to increased vascular permeability and pulmonary oedema [3].

While *ACE* supports vasoconstriction and inflammation through Ang-II induction, *ACE2* promotes vasodilation and anti-inflammatory responses through Ang-II breakdown. Thus, *ACE* and *ACE2* play opposite roles in the generation and degradation of Ang-II and RAS tissue inflammatory balance, and *ACE/ACE2* imbalance is believed to play a role in COVID-19 pathogenesis [22].

Genetics is believed to have a substantial control on plasma *ACE* levels [23]. There is a genetic deletion/insertion polymorphism of *ACE* enzyme, which is linked to half of the variation in the total plasma concentration of *ACE*. The *ACE* I/D polymorphism of the Alu repeat in intron 16, consists of either an Insertion (I) allele or a Deletion (D) allele of Alu repeat, leading to three possible genotypes: II (homozygous insertion), ID (heterozygous - insertion of Alu repeat in one allele and deletion of the Alu repeat in the other allele) and DD (homozygous deletion). Individuals with D/D genotype were reported to have significantly higher serum *ACE* concentrations compared to the I/D and I/I genotypes [24]. The dominant DD allele is associated with elevated levels of serum and tissue *ACE*, and subsequently increase in Ang-II levels [3].

Delanghe and his colleagues showed that variation in the distribution of D/I genotype was associated with prevalence of COVID-19 in 25 different European countries, where countries with higher *ACE* D allele frequency had lower prevalence of COVID-19 [25]. Similarly, nations with high COVID-19 prevalence, such as China and Korea, which were both heavily affected by the virus in the outset, have low *ACE* D allele frequencies [26].

SARS-CoV-2 virus enters host cells *via* the *ACE2* receptor [27]. *ACE2* receptor is a type I transmembrane amino-peptidase found on cell surfaces of the gut, heart, lungs, and many other organs, and also found in solution in blood plasma and urine [3,28]. The COVID-19 viral envelope's "Spike" (S) protein binds to *ACE2* receptors on nasopharyngeal mucosa and alveolar pneumocytes [27,29,30].

ACE2 expression is regulated by serum level of Ang-II through the AT1R, as Ang-II induces *ACE* in a dose dependent manner and decreases *ACE2* expression; Since *ACE* induces the production of Ang-II, high levels of Ang-II could therefore reduce *ACE2* expression and activity [21]. Therefore, in *ACE*-DD homozygous individuals with increased *ACE* activities and Ang-II level, *ACE2* expression might be down-regulated [22]. Furthermore, populations with this DD polymorphism may have reduced *ACE2* expression which may play a protective role against COVID-19.

ACE2 genes contain 18 exons and maps on Xp22. Genetic variations in *ACE2* gene have been proposed to also affect the COVID-19 viral spike protein interaction with the *ACE2* receptor [31]. Several *ACE2* gene variations can affect interaction with spike protein, protein stability, or change in ligand receptor affinity [32]. Therefore it can be inferred that genetic variations in the *ACE2* gene may influence the individual's susceptibility or resistance to SARS-CoV-2 [33]. Search for variants that show a correlation with clinical COVID-19 severity may explain the broad range of SARS-CoV-2 infection outcome. COVID-19. COVID-19 symptoms and outcomes might be associated with the pattern and level of human *ACE2* enzyme expression in different tissues [34]. Also, circulating *ACE2* might inhibit SARS-COV-2 entry into pulmonary alveoli and COVID-19 infection severity correlating with soluble/membrane bound ratio of *ACE2* [35].

ACE2 polymorphism, which reduces *ACE2* receptor expression, is consequently expected to be associated with increased Ang-II levels. Population distribution of these alleles may also explain the distribution of COVID-19. However, many other studies have not been able to find this association. For example, a study of three *ACE2* polymorphisms (rs2106809, rs4646155, and rs879922) in hypertensive patients did not show any significant relationship with circulating levels of *ACE2* [36]. Also, an Italian study did not find a strong association between COVID-19 disease severity and *ACE2* variants [37]. Furthermore, the SARS-CoV-2 virus may have an impact on the expression of *ACE2* receptors, where it is downregulated in infected cells [38]. Also, the function of the *ACE2* is likely impaired during the initial SARS-CoV-2 virus binding from *ACE2* steric domain hindrance or *ACE2* downregulation.

In the light of the potential role of *ACE2* and Ang-II in the SARS-CoV-2 pathogenesis the quantification of *ACE2* and Ang-II should be part of the COVID-19 patients' biological assessment and monitoring [39]. In this study, we compared the *ACE2* gene expression level in patients of African-American descent with COVID-19, to that of non-African-American COVID-19 patients.

MATERIALS AND METHODS

Data acquisition

Transcriptomic data of both African-American and Non-African-American populations was downloaded from NCBI. The reads were downloaded *via* their accession numbers from the command line using Fasterq-dump (https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software). The non-African-American population was obtained from bioproject PRJNA743862 ; GEO: GSE179448 while the African-American project was obtained from bioproject PRJNA679264; GEO: GSE161731. The African-American dataset included patients with COVID-19 as well as seasonal COVID.

Quality control

FastQC version 0.11.3 was used to verify the quality of raw sequencing reads, and subsequently trimming to eliminate adapter sequences was done Cutadapt [40,41].

Alignment to the human reference genome

After passing the quality check, the reads were aligned to the human reference genome (hg38) using Hisat2 [42]. Samtools v1.3.1 was used to convert the aligned reads' SAM file to a BAM file, which was then sorted [43].

Read counts

HTSeq-count was used to count reads associated with each human gene [44].

Normalization

EdgeR was used to normalize library sizes using Trimmed Mean

of M-values (TMM) by determining the scaling factor to obtain effective library sizes. Counts were then adjusted by effective library sizes to create counts per million (cpm) values [45].

Differential gene expression analysis

EdgeR was used to perform differential expression analysis according to established principles. Genes having corrected P-values of 0.05 and absolute fold increases of 1.5 were considered differentially expressed. Differentially expressed candidate genes were evaluated between African-American and non-African-American populations. Enrichr was used to perform Gene Ontology (GO) and KEGG pathway enrichment analysis of differentially expressed genes [46]. The data analysis workflow is shown in Figure 1 below.

RESULTS

Quality control

The datasets included 14 and 33 transcriptomic sequences of African-American and non-African-American COVID-19 patients, respectively. The sequences were of high quality, all the sequences scored a Phred score of above 30 after trimming of the adaptor sequence.

Identification of differentially expressed genes in the African-American population

The raw gene count matrix consisting of a total of 39,590 genes generated in the gene count step of our pipeline, was used in EdgeR to analyze differential expression levels of the genes. Differential expression analysis revealed 7,718 out of 24,092 genes were significantly upregulated (log fold change>1 and FDR< 0.05) while 16,374 genes were significantly downregulated (log fold change<-1 and FDR<0.05) between African-American and non-African-American populations (controls). We discovered that the mRNA level of *ACE2* was significantly downregulated in the African-American cohort. (p value=0.0242, p-adjusted value= 0.038) (Figures 2 and 3).
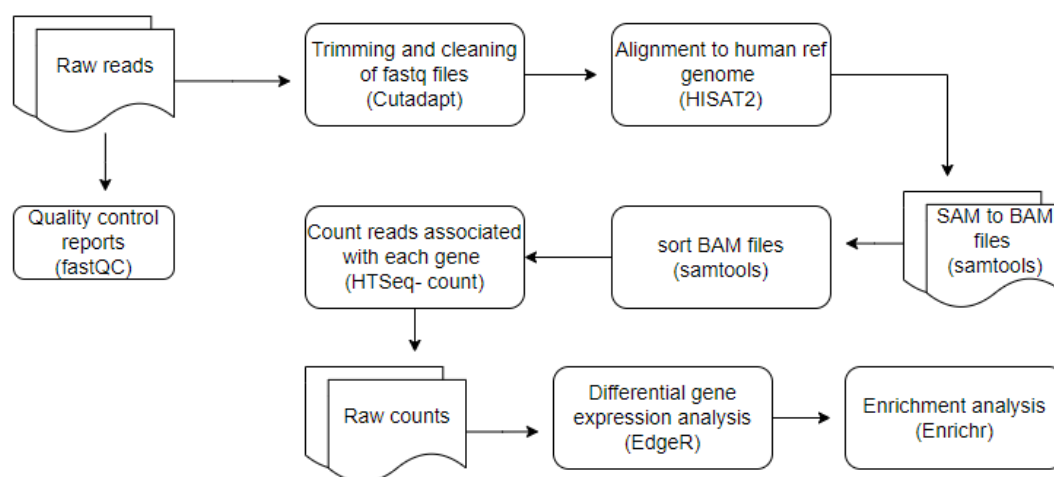

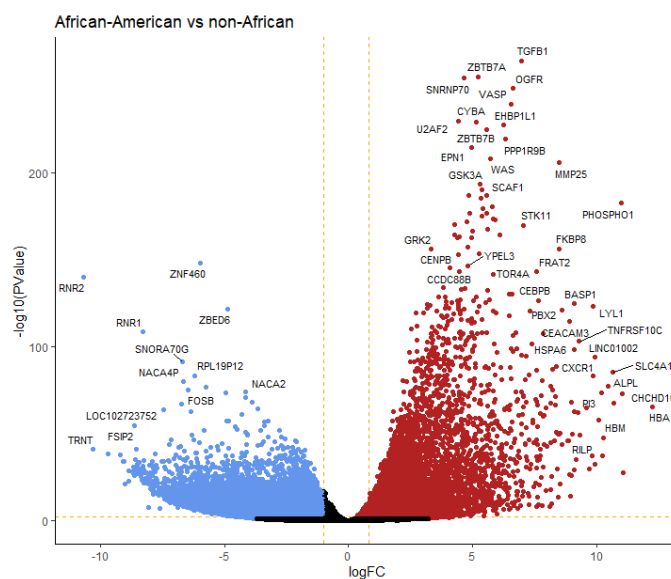**Figure 1:** Materials and methods used in data analysis.

Figure 2: Volcano plot for differentially expressed genes between African- American COVID-19 patient's *vs* non-African-American COVID-19 patients as controls. Scattered dots represent genes; the x-axis is the log fold change for the ratio of African-American *vs* non-African-American COVID-19 patients, whereas the y-axis is the -log 10 (P Value) where P is the probability that a gene has statistical significance in its differential expression. **Note:** (■): Genes significantly upregulated; (■): Genes significantly suppressed; (■): Genes with non-significant changes between the conditions.
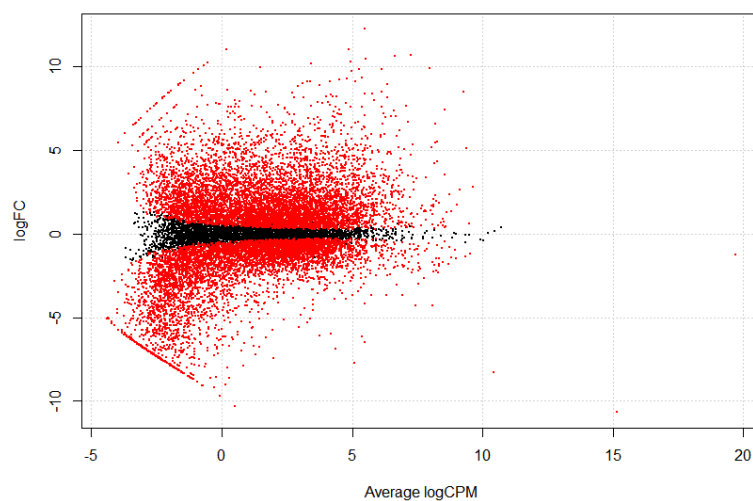

Figure 3: MA plot for transcript distribution determined by EdgeR. **Note:** (■): Differentially expressed transcripts (P-adjusted<0.05).

The top 10 most significantly upregulated genes include *TGFB1, ZBTB7A, SNRNP70, OGFR, VASP, U2AF2, CYBA, EHBP1L1, ZBTB7B*, and *PPP1R9B* with a P-adjusted value<0.05 (Table 1). The top 10 most significantly down regulated genes include *ZNF460, RNR2, ZBED6, RNR1, SNORA70G, RPL19P12, NACA4P, RPL23P8, FOSB, NACA2* with a P-adjusted value<0.05 (Table 2).

An unsupervised hierarchical analysis revealed that sets of genes with comparable expression characteristics clustered together, as expected. Groups of genes are systematically, either up-regulated (red) or down-regulated (green) in African-Americans versus non-African-Americans (Figure 4).

Functional analysis of significantly upregulated genes

Enrichment analysis was performed on significantly over-expressed genes to verify the molecular functions, biological processes, cellular components, and pathways associated with the differentially expressed genes. The fifteen most significant molecular functions, biological processes, cellular components, and ten most significant pathways associated with the differentially expressed genes are represented according to Gene Ontology (GO) biological process, Gene Ontology (GO) molecular function, Gene Ontology (GO) cellular component, and KEGG pathway analysis based on p value (Figures 5A- 5D).
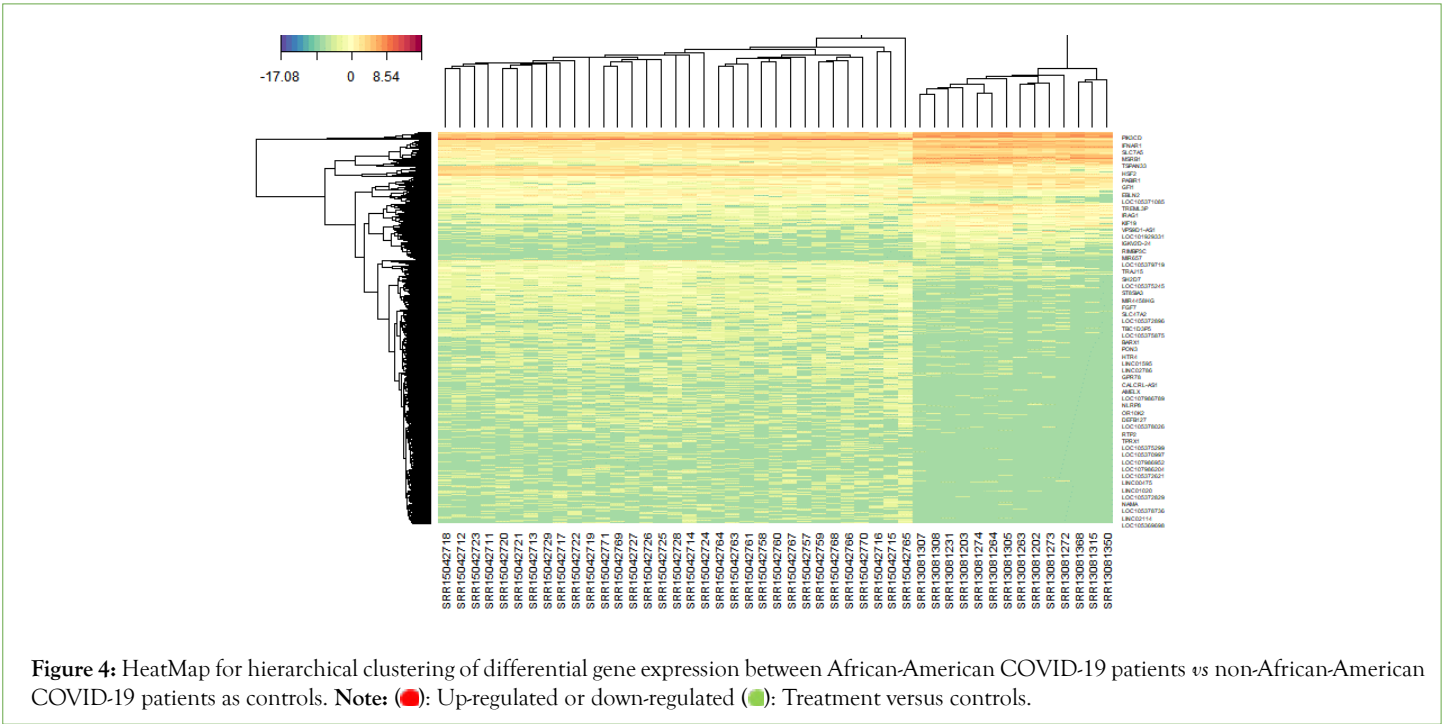
Table 1: Top 10 significantly upregulated genes between African-American COVID-19 patients *vs* non-African-American COVID-19 patients

Gene	Annotation*	Log Fold Change	P-Value	FDR P-Value
TGFB1	Transforming Growth Factor Beta 1	6.947	4.91E-265	1.83E-260
ZBTB7A	Zinc Finger and BTB Domain Containing 7A	5.201	6.88E-256	1.28E-251
SNRNP70	Small Nuclear Ribonucleoprotein U1 Subunit 70	4.643	6.98E-255	8.66E-251
OGFR	Opioid Growth Factor Receptor	6.609	5.98E-249	5.57E-245
VASP	Vasodilator Stimulated Phosphoprotein	6.547	5.25E-240	3.91E-236
U2AF2	U2 Small Nuclear RNA Auxiliary Factor 2	4.408	1.64E-230	1.02E-226
CYBA	Cytochrome B-245 Alpha Chain	5.159	5.82E-230	3.10E-226
EHBP1L1	EH Domain Binding Protein 1 Like 1	6.234	4.15E-228	1.93E-224
ZBTB7B	Zinc Finger and BTB Domain Containing 7B	5.573	1.44E-225	5.96E-222
PPP1R9B	Protein Phosphatase 1 Regulatory Subunit 9B	6.335	8.13E-220	3.03E-216

Table 2: Top 10 significantly downregulated genes between African-American COVID-19 patients *vs* non-African-American COVID-19 patient

Gene	Annotation*	Log Fold Change	P-Value	FDR P-Value
ZNF460	Zinc Finger Protein 460	-5.95926	7.16E-149	6.35E-146
RNR2	RNA, Ribosomal 45S Cluster 2	-10.6667	1.08E-140	8.35E-138
ZBED6	Zinc Finger BED-Type Containing 6	-4.86911	1.47E-122	7.02E-120
RNR1	RNA, Ribosomal 45S Cluster 1	-8.296	2.07E-109	6.75E-107
SNORA70G	Small Nucleolar RNA, H/ACA Box 70G	-6.69691	4.49E-92	8.25E-90
RPL19P12	Ribosomal Protein L19 Pseudogene 12	-6.19293	3.18E-84	4.60E-82
NACA4P	NACA family member 4, Pseudogene	-6.63674	1.10E-80	1.42E-78
RPL23P8	Ribosomal Protein L23 Pseudogene 8	-5.74711	9.70E-78	1.17E-75
FOSB	FosB Proto-Oncogene, AP-1 Transcription Factor Subunit	-6.47326	3.58E-76	4.19E-74
NACA2	Nascent Polypeptide Associated Complex Subunit Alpha 2	-4.14031	9.03E-75	1.02E-72

Note: * - Gene name in NCBI database; FDR – False Detection Rate corrected; ** - transcriptional changes of individual genes quantified in African-American COVID-19 patients compared to non-African-American COVID-19 patients as controls. The fold-changes are computed ratios of gene expression in African-American COVID-19 patients relative to similar expressions in the respective non-African-American COVID-19 patients. Consequently, positive and negative values indicate respective induced or suppressed genes relative to control.
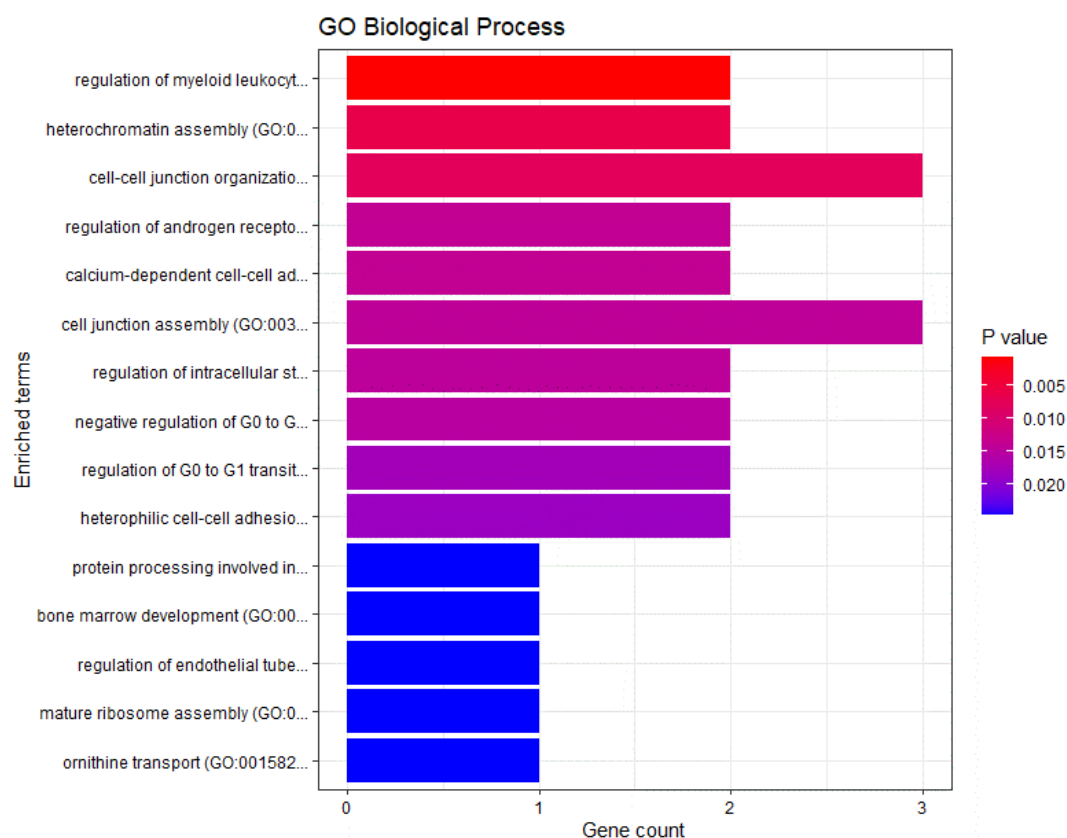

Figure 4: HeatMap for hierarchical clustering of differential gene expression between African-American COVID-19 patients *vs* non-African-American COVID-19 patients as controls. **Note:** (🔴): Up-regulated or down-regulated (🟢): Treatment versus controls.

Figure 5A: GO biological process. **Note:** P value - (■): 0.005; (■): 0.010; (■): 0.015; (■): 0.020
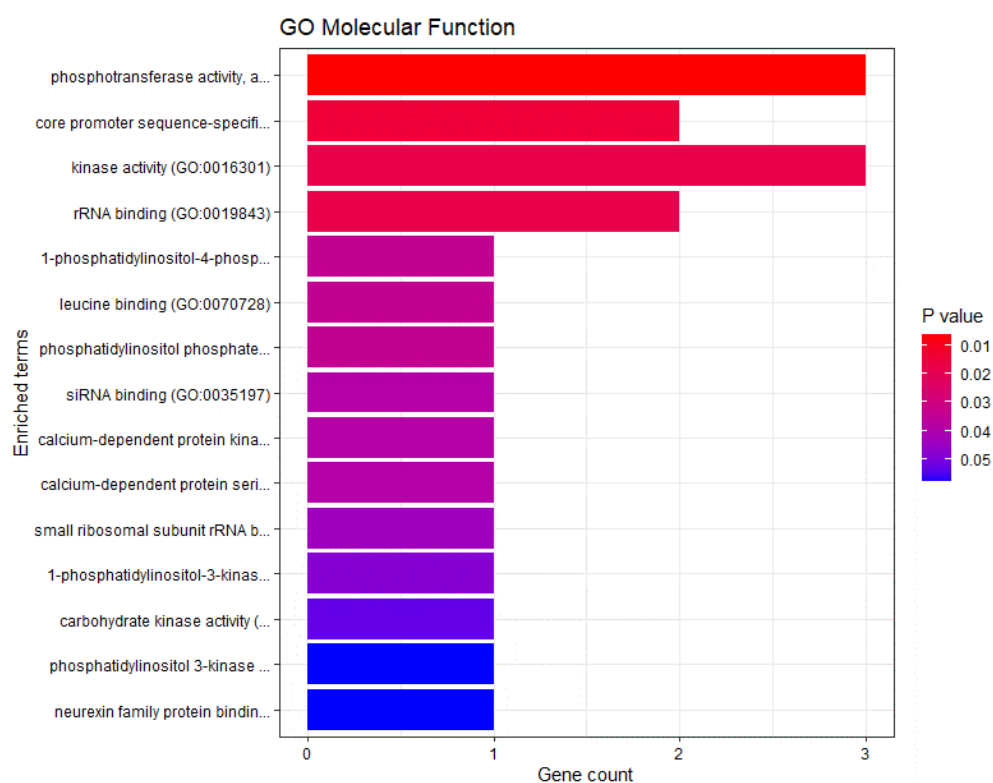

Figure 5B: GO molecular function. **Note:** P value (■): 0.01, (■): 0.02, (■): 0.03, (■): 0.04, (■):0.05
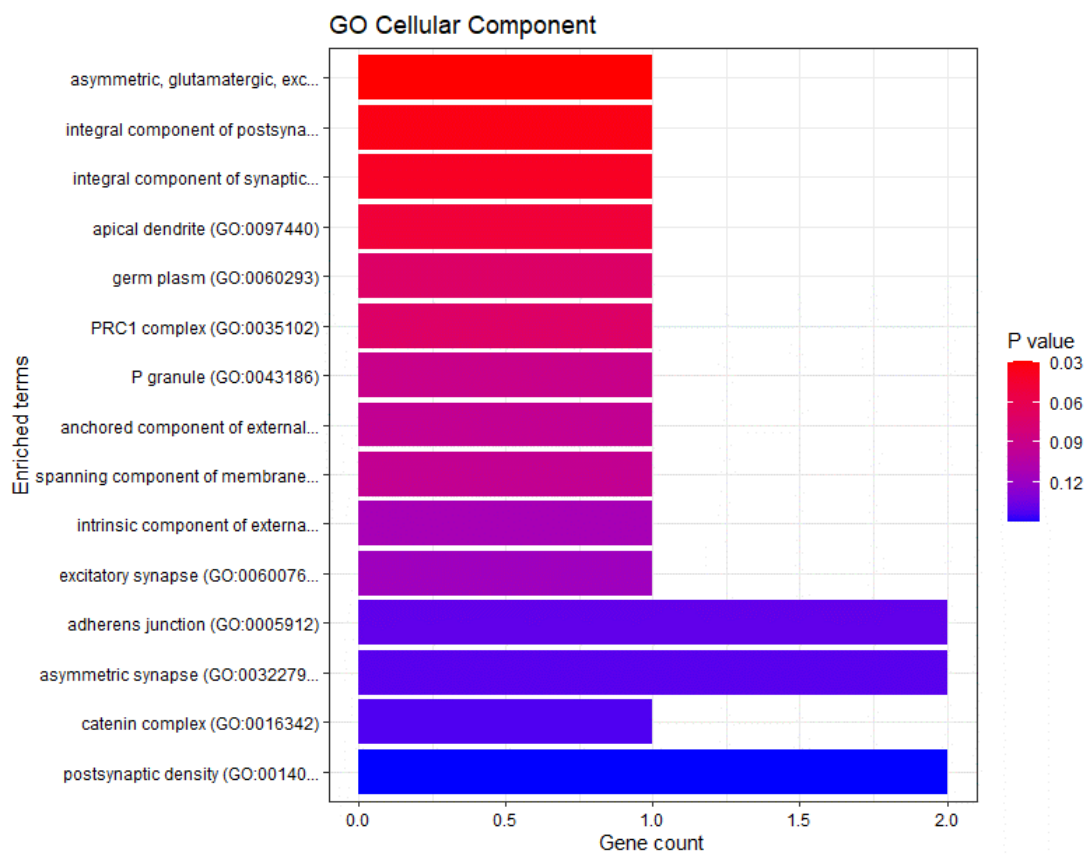
Figure 5C: GO cellular component. **Note:** P value (■): 0.03, (■): 0.06, (■): 0.09, (■): 0.012
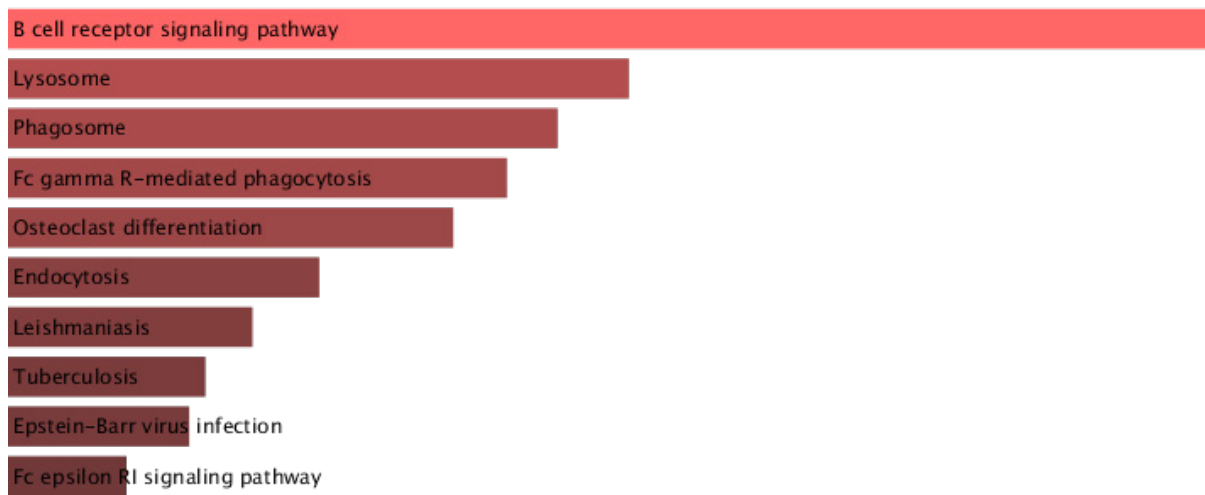

Figure 5D: KEGG pathways. **Note:** *ENRICHR Gene Ontology and KEGG pathways analysis of significantly over-expressed genes of the African-American population.

Significantly enriched KEGG pathways

Tuberculosis, Epstein- Barr virus infection, Influenza A, PD-L1 expression and PD-1 checkpoint pathway in cancer, B cell receptor signaling pathway, T cell receptor signaling pathways were observed to be significantly enriched in the upregulated genes, among other pathways with multiple overlap genes (Table 3, Figure 5E).
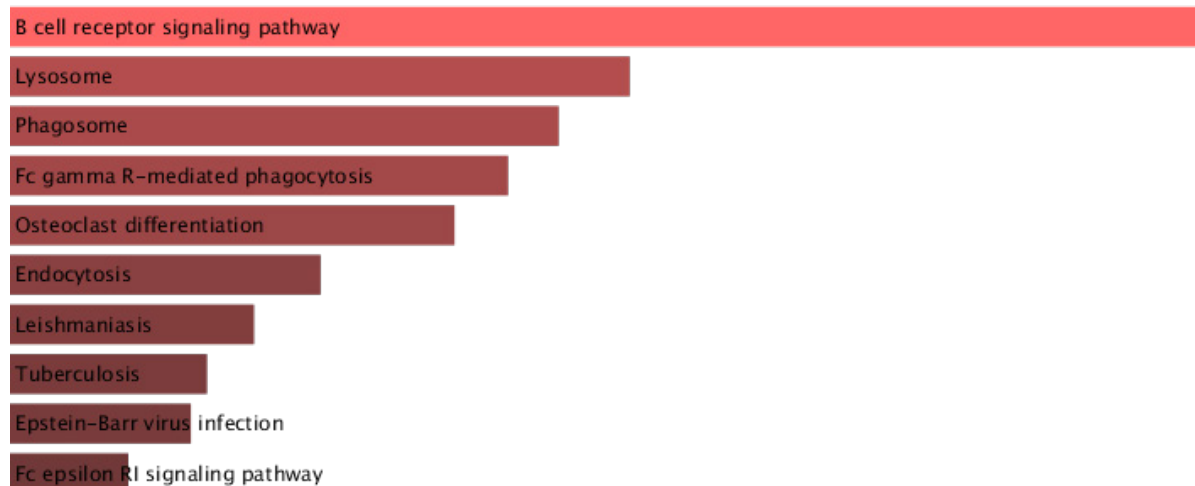
Figure 5E: Scatterplot of all terms in the KEGG_2021_Human gene set library. Each point represents a term in the library.

Table 3: Top ten KEGG 2021 Human significant p-values and q-values, 2021

Terms	p-value	q-value	Overlap genes
B cell receptor signaling pathway	3.67E-12	1.17E-09	*[GSK3B, IFITM1, CD81, INPPL1, PIK3CD, PIK3CB, IKBKB, AKT2, AKT3, BLNK, RAC2, AKT1, RAC3, IKBKG, RAC1, HRAS, VAV3, CR2, MAP2K1, MAP2K2, SYK, PRKCB, VAV1, VAV2, BTK, RAF1, SOS2, CARD11, LILRA6, DAPP1, PIK3R2, LILRA1, LILRA2, LILRA3, LILRA4, RELA, LILRA5, RASGRP3, CD79B, CD79A, PPP3R1, CD19, INPP5D, PLCG2, MAPK1, MAPK3, LYN, CD72, LILRB1, LILRB2, LILRB3, LILRB4, LILRB5, NFKB1, GRB2, KRAS, PTPN6, FCGR2B, NFKBIE, PIK3AP1, CD22, NFKBIB]*
Phagosome	9.23E-12	1.47E-09	*[SCARB1, NCF1, NCF2, NCF4, MPO, ACTG1, TUBB8, TUBB6, LAMP1, LAMP2, ATP6V1E1, HLA-DOA, ATP6V1E2, HLA-DOB, ATP6V0E1, STX7, HLA-B, TAP2, CYBB, HLA-C, TAP1, CYBA, HLA-A, HLA-F, TUBA4A, HLA-E, DYNC1LI1, HGS, ATP6V0D1, ATP6V1A, RAB5B, RAB5C, CORO1A, C3, CLEC7A, ATP6V0A2, CD14, ATP6V1D, HLA-DQA2, ATP6V1C1, HLA-DQA1, ATP6V0A1, ATP6V1F, MSR1, HLA-DRB4, M6PR, MARCO, HLA-DRA, CALR, HLA-DRB3, RAB5A, TUBA8, ITGAM, ITGB5, ITGB2, TCIRG1, CTSS, FCAR, MRC2, SEC61A1, TUBA1C, FCGR3A, TUBA1B, FCGR3B, TUBA1A, CTSL, OLR1, CD36, RAC1, HLA-DPA1, ATP6V0B, ATP6AP1, TUBB, SFTPD, TUBB2B, TUBB2A, CANX, ATP6V1B2, ITGA5, TLR6, SEC22B, ATP6V0C, TLR4, HLA-DQB1, RAB7A, TLR2, VAMP3, DYNC1I2, STX12, C1R, THBS3, HLA-DMB, FCGR1A, RILP, TUBB4B, TUBB4A, FCGR2A, HLA-DPB1, FCGR2B, FCGR2C]*
Lysosome	1.45E-11	1.54E-09	*[SCARB2, HEXB, CLTC, CTSZ, CLTB, CLTA, CTSW, TCIRG1, LITAF, CTSS, AP4M1, LAPTM4B, LAPTM4A, CTSO, LAMP1, NAGLU, GM2A, CTSL, HYAL1, LAMP3, HYAL2, SMPD1, LAMP2, HYAL3, AP1S2, AP1S1, ACP5, CTSH, ACP2, CTSG, CTSF, CTSE, GUSB, CTSD, CTSC, CTSB, ARSA, HGSNAT, ATP6V0B, MANBA, ATP6AP1, SLC11A1, GAA, AP1B1, AP3B2, PLA2G15, NPC2, DNASE2, ATP6V0D1, ATP6V0C, CD63, ASAH1, IDUA, GBA, ABCB9, GNS, GGA2, AP3M1, CLN3, GGA1, GGA3, CLTCL1, NEU1, PSAP, ATP6V0A2, ARSG, AP1M2, ARSB, ATP6V0A1, CTSA, ABCA2, NAPSA, SORT1, FUCA1, M6PR, AP3D1, LAPTM5, CTNS, NAGA, MCOLN1, IGF2R, GALNS, GNPTG, PPT1, MAN2B1, CD68, GLA]*
Endocytosis	2.66E-11	2.12E-09	*[WIPF1, WIPF2, ARPC5L, WASHC2C, WASHC2A, ZFYVE27, VPS35, WASHC1, AP2M1, SH3GL1, SH3GLB1, SH3GLB2, USP8, HLA-B, HLA-C, HLA-A, HLA-F, HLA-E, RNF41, RUFY1, ACAP3, ZFYVE16, ACAP1, RAB31, HGS, RAB35, CHMP4B, CHMP4A, VPS25, STAM2, VPS28, RAB5B, RAB5C, TSG101, AGAP2, AGAP1, NEDD4L, AGAP4, AGAP3, VPS26B, PLD1, PRKCZ, PLD2, SNX3, SNX1, GRK3, RAB11FIP1, GRK2, GRK5, CLTCL1, GRK6, PIP5K1B, PIP5K1C, RAB11FIP3, RAB11FIP4, RAB11FIP5, RAB4A, SMAD3, IQSEC2, SMURF2, IQSEC1, SMURF1, CAV1, IQSEC3, HSPA6, ARPC4, SNF8, ARPC5, IGF2R, EHD1, EHD4, ARPC2, ARPC3, CHMP2A, RAB5A, RNF103-CHMP3, ARF3, SH3KBP1, ARPC1B, ARPC1A, CLTC, CLTB, SNX12, CLTA, AP2A1, ARRB1, ARRB2, AP2A2, RAB22A, CAPZB, PSD4, CHMP1A, CCR5, LDLRAP1, HRAS, GIT1, RAB8A, ACTR2, VPS37C, ARAP3, ...]*

Osteoclast differentiation	2.77E-10	1.77E-08	[SPI1, CSF1, NCF1, NCF2, NCF4, FHL2, PIK3CD, PIK3CB, SIRPB1, IKBKB, FCGR3A, FCGR3B, AKT2, AKT3, BLNK, AKT1, SIRPA, ACP5, IKBKG, RAC1, JUNB, IFNAR2, MAP2K1, SYK, IL1R1, IFNGR1, IFNGR2, CYBA, TRAF2, GAB2, TYK2, TGFBR1, OSCAR, TGFBR2, TNFRSF1A, TYROBP, BTK, LCP2, PPARG, SQSTM1, IRF9, IFNAR1, LILRA6, CSF1R, PIK3R2, LILRA1, LILRA2, LILRA3, LILRA4, RELA, LILRA5, RELB, SOCS3, PPP3R1, SOCS1, PLCG2, MAPK1, FYN, MAP2K7, FCGR1A, MAP2K6, MAPK3, JUND, TGFB1, STAT1, STAT2, LILRB1, LILRB2, LILRB3, MAPK14, LILRB4, NFKB1, LILRB5, MAPK12, FOSL2, NFKB2, MAPK13, MAPK11, FCGR2A, GRB2, TAB1, FCGR2B, MAP3K14, FCGR2C]
Fc gamma R-mediated phagocytosis	1.09E-09	5.78E-08	[NCF1, ARPC1B, ARPC1A, ARPC5L, INPPL1, PIK3CD, PIK3CB, CRKL, FCGR3A, FCGR3B, AKT2, AKT3, CFL1, RAC2, AKT1, RAC1, VAV3, ACTR2, MAP2K1, SYK, PRKCB, SPHK2, SPHK1, PRKCD, PLA2G4C, PLA2G4A, GAB2, VAV1, DNM2, VAV2, HCK, RAF1, CRK, ARF6, ASAP3, WAS, PIK3R2, ASAP1, PLA2G6, PLD1, PLD2, PAK1, INPP5D, PLCG2, MAPK1, PIP5K1B, PIP5K1C, PLCG1, FCGR1A, WASF2, MAPK3, LYN, VASP, MARCKSL1, GSN, MYO10, LIMK2, LIMK1, ARPC4, ARPC5, MARCKS, PTPRC, FCGR2A, ARPC2, ARPC3, RPS6KB2, FCGR2B]
Epstein-Barr virus infection	3.48E-09	1.59E-07	[RBPJ, ICAM1, PSMD8, CCND3, PSMD4, CCND1, AKT2, PSMD2, AKT3, PSMD3, AKT1, CIR1, HLA-DOA, B2M, HLA-DOB, IFNAR2, MAP2K3, MAP2K4, ENTPD1, USP7, HLA-B, TAP2, HLA-C, TAP1, HLA-A, HLA-F, RUNX3, HLA-E, SAP30, BTK, TP53, IFNAR1, PIK3R2, IRAK1, CD19, PLCG2, RIPK1, FADD, MAP2K7, HLA-DQA2, HLA-DQA1, MAP2K6, LYN, HLA-DRB4, GADD45B, GADD45A, EIF2AK2, ISG15, NFKB1, GADD45G, NFKB2, CXCL10, MAVS, HLA-DRA, CALR, TAB1, HLA-DRB3, NFKBIE, MAP3K14, MYD88, NFKBIB, CD40, CDKN1B, TRADD, PIK3CD, PIK3CB, CD3E, ITGAL, IKBKB, CASP9, CASP8, CASP3, BLNK, IKBKG, RAC1, JAK3, HLA-DPA1, CR2, SYK, APAF1, DDX58, TRAF2, TYK2, IRAK4, TAPBP, NCOR2, FCER2, CCNA1, IRF3, OAS1, OAS2, OAS3, IRF7, IRF9, HLA-DQB1, TLR2, RELA, RELB, HLA-DMB, SAP30L, ...]
Tuberculosis	4.42E-09	1.76E-07	[CALML4, LAMP1, AKT2, AKT3, LAMP2, AKT1, HLA-DOA, HLA-DOB, RFX5, ATP6V0D1, CLEC4E, RAF1, RAB5B, RAB5C, CORO1A, C3, PPP3R1, IRAK1, CLEC7A, IRAK2, ATP6V0A2, CD14, FADD, HLA-DQA2, HLA-DQA1, ATP6V0A1, CREBBP, HLA-DRB4, TGFB1, VDR, RFXANK, NFKB1, RFXAP, HLA-DRA, CALM3, HLA-DRB3, RAB5A, MYD88, CIITA, ITGAM, TRADD, ITGB2, LSP1, TCIRG1, CTSS, CASP9, MRC2, FCGR3A, CASP8, FCGR3B, CASP10, CASP3, ITGAX, JAK2, CTSD, CAMP, HLA-DPA1, ATP6V0B, ARHGEF12, CR1, FCER1G, APAF1, ATP6AP1, KSR1, SYK, IFNGR1, SPHK2, IFNGR2, SPHK1, IL18, IRAK4, RHOA, TIRAP, TNFRSF1A, TLR1, TLR9, TLR6, ATP6V0C, TLR4, HLA-DQB1, RAB7A, TLR2, CEBPB, SRC, NOD2, RELA, HLA-DMB, MAPK1, BID, FCGR1A, CAMK2G, MAPK3, CD74, BAD, IL10RB, STAT1, NFYC, CARD9, MAPK14, MAPK12, ...]
Salmonella infection	2.28E-08	8.07E-07	[CYFIP1, FHOD1, ARPC5L, ACTG1, TXN2, PYCARD, TUBB8, TUBB6, AKT2, AKT3, TNFSF10, AKT1, KPNA4, VPS39, KPNA1, MAP2K3, VPS18, MAP2K4, MAP2K1, MAP2K2, TUBA4A, DYNC1LI1, MYL5, PFN1, RAF1, MYL9, NAIP, ARL8A, RAB5B, RAB5C, LY96, KLC1, HSP90B1, KLC4, KLC3, KLC2, RRAS, IRAK1, VPS11, NLRP3, NCKAP1L, RIPK1, CD14, FADD, MAP2K7, VPS16, MAP2K6, GSDMD, MLKL, M6PR, TNFRSF10B, ARPC4, ARPC5, NFKB1, PTPRC, ARPC2, ARPC3, PLEKHM1, PLEKHM2, DYNLRB1, TAB1, ACBD3, RAB5A, MYD88, TUBA8, ARPC1B, TRADD, ARPC1A, PIK3CD, PIK3CB, PIK3CG, IKBKB, TUBA1C, ACTR1A, TUBA1B, TUBA1A, CASP8, CASP5, CASP3, CASP4, CASP1, IKBKG, RAC1, HRAS, ACTR2, RIPK3, EXOC7, TUBB, IL18, RHOG, TRAF2, IRAK4, DYNLL1, DYNLL2, RHOA, TIRAP, RHOB, DNM2, TNFRSF1A, TUBB2B, ...]
Yersinia infection	1.45E-07	4.61E-06	[GSK3B, WIPF1, WIPF2, ARPC1B, ARPC1A, ARPC5L, PIK3CD, PIK3CB, ACTG1, CRKL, PYCARD, IKBKB, RPS6KA3, AKT2, AKT3, RPS6KA1, CASP1, RAC2, AKT1, RAC3, IKBKG, RAC1, MAP2K3, VAV3, MAP2K4, ACTR2, MAP2K1, MAP2K2, ARHGEF12, IL18, RHOG, TRAF2, IRAK4, TICAM1, RHOA, VAV1, VAV2, CD8B, IRF3, CD8A, ARHGEF1, LCP2, ITGA5, CRK, TLR4, SKAP2, ARF6, SRC, PXN, WAS, PIK3R2, NLRC4, MEFV, RELA, IRAK1, NLRP3, CCL2, MAPK1, PTK2B, PIP5K1B, PIP5K1C, PLCG1, MAP2K7, WASF2, MAP2K6, MAPK3, GIT2, LIMK1, ARPC4, ARPC5, BAIAP2, MAPK14, NFKB1, MAPK12, MAPK13, MAPK11, FCGR2A, ARPC2, ARPC3, GNAQ, TAB1, PKN1, MYD88]

Functional analysis of significantly downregulated genes

To validate the molecular functions, biological processes, cellular components, and pathways linked with the differentially expressed genes, enrichment analysis was done on significantly under-expressed genes. According to Gene Ontology (GO) biological process, Gene Ontology (GO) molecular function, Gene Ontology (GO) cellular component, and KEGG pathway analysis based on p value, the fifteen most significant molecular functions, biological processes, cellular components, and ten most significant pathways

associated with the differentially expressed genes are represented. (Figures 6A- 6D).

Significantly enriched KEGG pathways

Nicotine addiction, caffeine metabolism, prion disease, thermogenesis, Huntington disease, drug metabolism, T cell receptor signaling among other pathways were significantly enriched in the downregulated genes (Figure 6E).
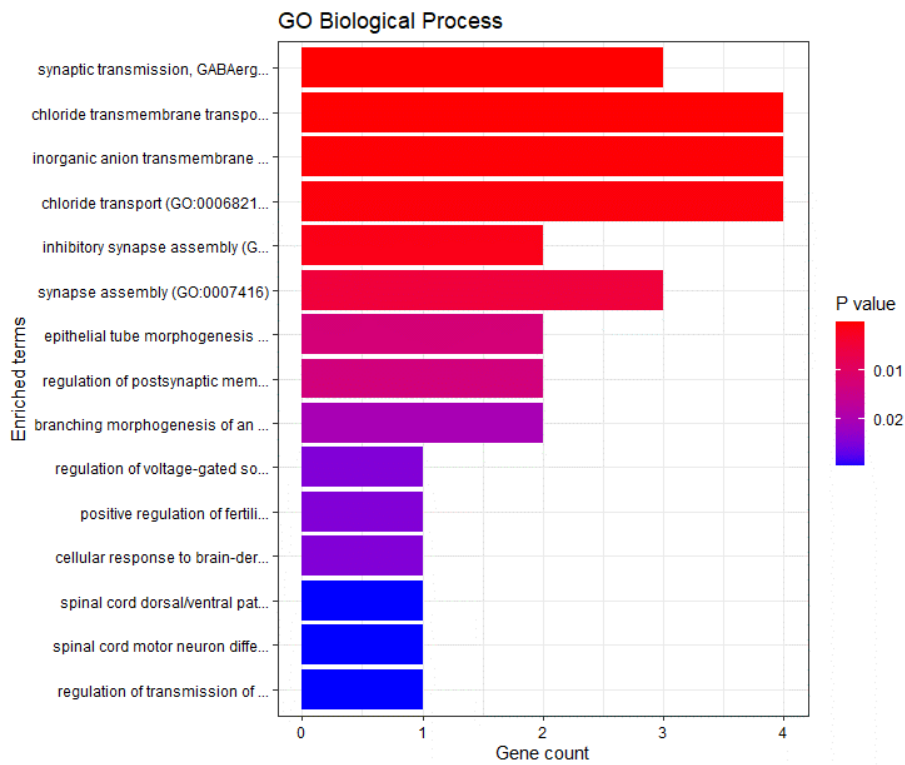
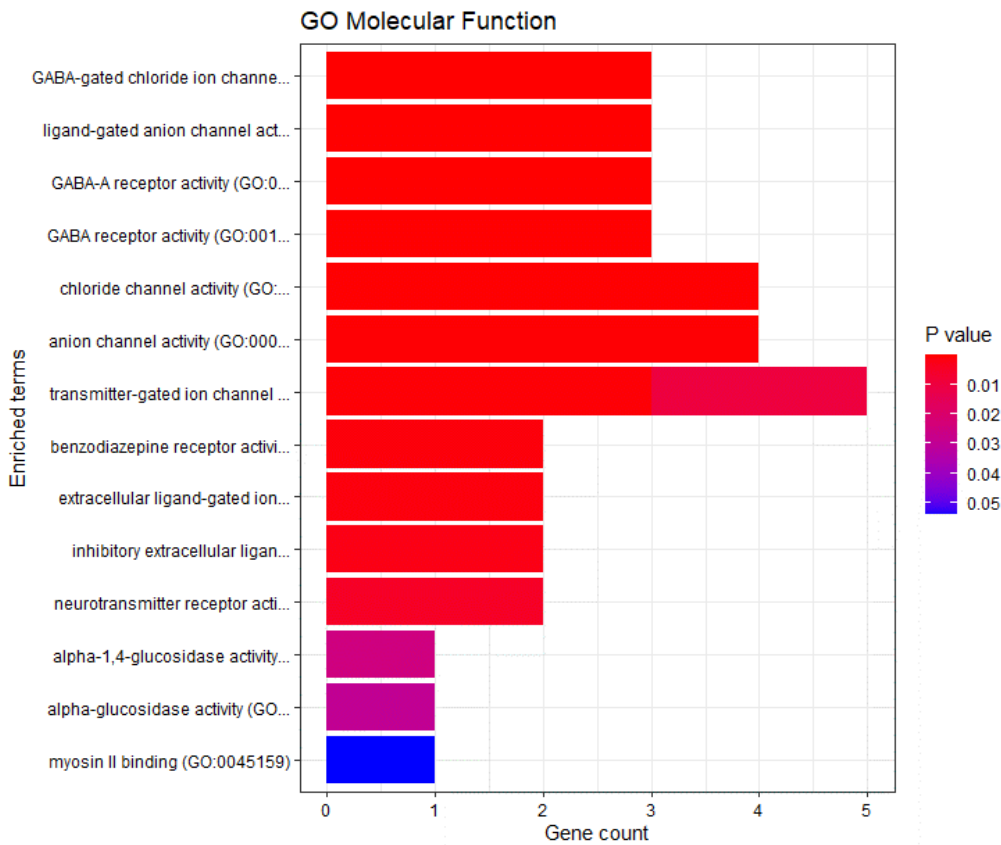**Figure 6A:** GO biological process. **Note:** P value (■): 0.01, (■): 0.02

Figure 6B: GO molecular function. **Note:** P value (■): 0.01, (■): 0.02, (■): 0.03, (■): 0.04, (■):0.05
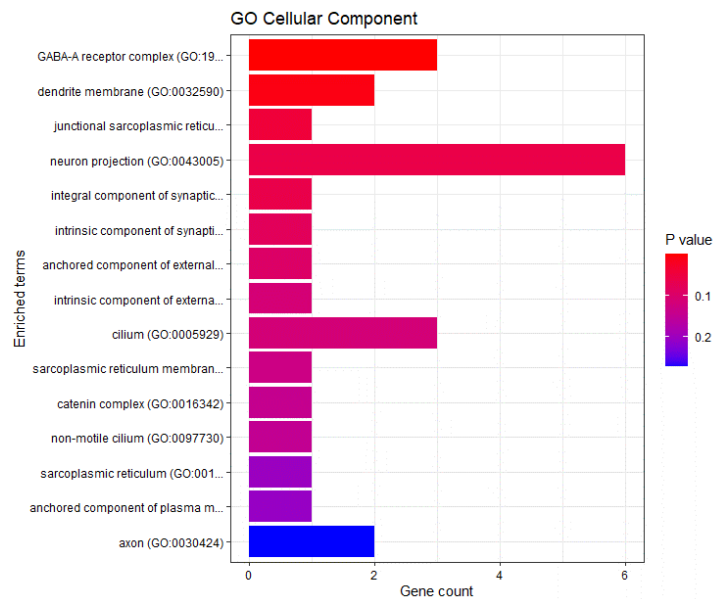
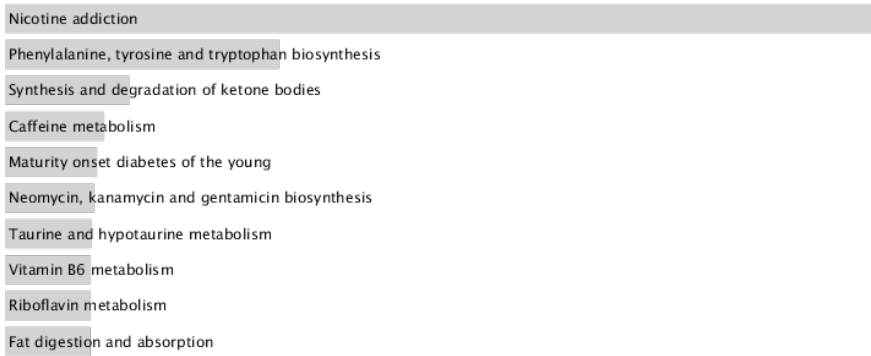**Figure 6C:** GO cellular component. **Note:** P value (🟥): 0.1; (🟦): 0.2

Figure 6D: KEGG pathways. **Note:** *ENRICHR Gene Ontology and KEGG pathways analysis of significantly under-expressed genes of the non-African population.

Figure 6E: Scatterplot of all terms in the KEGG_2021_Human gene set library. Each point represents a term in the library.

DISCUSSION

The study set out to assess the difference in *ACE2* expression in COVID-19 infected patients of African-American and non-African-American descent. Preliminary analysis between healthy African-American and COVID-19 infected African-American individuals did not show any difference in *ACE2* expression. Likewise, a similar comparison between healthy and COVID-19 infected non-African-American individuals did not show any difference in *ACE2* expression level. Our primary finding showed that the *ACE2* gene was significantly downregulated in the African- American cohort compared to non-African-American cohort. The findings from this study might explain Yang and Cadegian's observation of reduced COVID-19 severity with reduced *ACE2* expression [34,35].

There are reports that COVID-19 has a higher infection rate and severity among the African-American population [47,48]. Asides the probable genetic cause of the disproportionate COVID-19 rates and deaths in African-Americans, other social factors such as overcrowding, working conditions, access to healthcare and background chronic health conditions may have played a greater role.

We analyzed gene expression in the two populations and discovered that African-American patients had 7,718 differentially upregulated genes and 16,374 differentially downregulated genes when compared to non-African-American patients. Many of these genes may be important genes for COVID-19 research. Our study confirms that there are significant disparities in Differentially Expressed Genes (DEG) expression levels between African-American and non-African-American COVID-19 patients.

Residue function and enrichment analysis of downregulated genes suggests that nicotine addiction, caffeine metabolism, thermogenesis, drug metabolism, T cell receptor signaling, prion and Huntington disease pathways are actively involved in viral disease manifestation of African-American COVID-19 population as compared to a non-African-American population. Normally residues of the former two pathways prevent disease severity by blocking *ACE2* from binding to COVID-19 spike protein, decreasing viral virulence or replication and inflammation [49,50]. Therefore, the suppression of their corresponding genes in the diseased population clearly supports their significance in close protection against COVID-19 infection. The abundant presence of xanthine and caffeine/nicotine which are directly involved in the caffeine/nicotine metabolism pathway also directly reduce viral propagation in African-Americans by inhibiting red blood cells-*ACE2* complex that allows viral entry [26,51]. Autoimmune genes associated with prion and Huntington disease pathways regulate subtle peripheral and Central Nervous System (CNS) inflammation caused by pro-inflammatory cytokines such as Interleukins (IL) and Tumor Necrosis Factor (TNF) – α hence slowing down neural degeneration [52]. Hypothetically, the viral attack of the patient's nervous system by COVID-19 might have significantly impacted these pathways resulting in downregulation of respective genes [53].

Thermogenic regulation is highly dependent on expression of *ACE2* whereby its downregulation enhances microcirculation, thus subsequent downregulation of the thermogenic pathway in absence of any impairment [54]. Cytochrome enzymes are active agents in toxin and drug degradation and their suppression by infection leads to compromised drug metabolism giving rise to treatment complications especially in COVID-19 patients [34,55]. Even though genes for these enzymes are usually upregulated in African-

Americans as compared to non-African-Americans discrepancies observed here might be attributed to socio-environmental factors beyond our control during sampling [56]. Negative regulation of other pathways such as T cell receptor signaling reduces viral cell entry as well as T cell production controlling immunopathology [57]. Evidently, the respiratory, inflammatory and infectious disease pathways play a role in COVID-19 progression.

Moreover, the expression levels of *TGFB1*, *VASP* and *CYBA* in African-American COVID-19 patients were all elevated in our study among other genes. *TGFB1* and *CYBA* are associated with the osteoclast differentiation pathway; *TGFB1* is involved in regulatory systems that support the balance of osteoblasts and osteoclasts, while over- expression of *CYBA* is linked to decreased blood stem cells [58]. Moreover, the expression of *TGFB1* in Tuberculosis disease is known to be elevated as it impacts the patients' immunological response to the disease [59].

Meanwhile, the expression of *VASP* and *MAPK1* have been reported to be associated with phagocytosis and Fc Gamma R-mediated phagocytosis [60,61]. However, more study should be performed to confirm the function and character of *VASP* in with Fc Gamma R-mediated phagocytosis. Moreover, an upregulated gene linked to *APOL1*-associated kidney disease among African-Americans, NLRP3 was found to be associated with salmonella infection in our study and its expression levels enhanced in our COVID-19 African-American patient transcriptomes [62,63]. Our *TGFB1*, *CYBA*, and *VASP* results are consistent with those previously reported, so we speculate that these may be potential COVID-19 - related genes and more research is required.

CONCLUSION

The expression of *ACE2* gene is downregulated in the cohort of African- American COVID-19 patients in this study. This may explain the low severity of COVID-19 in this population compared to non-African-American populations. Future exploration of the *ACE2* gene and the pathway residues of respiratory, inflammatory and infectious disease pathways might offer new chemical therapeutics for this virus.

DECLARATIONS

Availability and requirements

Project name: Expression Level Analysis of *ACE2* Receptor Gene in African-American and Non-African-American COVID-19 Patients

Project home page: https://github.com/omicscodeathon/*ace2_*covid

Availability of data and materials

The dataset analyzed during the current study as a case study is publicly available at https://github.com/omicscodeathon/*ace2_*covid/blob/main/accessions. The data supporting the results reported in this manuscript is included within the article. The Project repository which also includes the entire code and other requirements can be downloaded from https://github.com/omicscodeathon/*ace2_*covid. The guidelines for this project and related updates, are available at: https://github.com/omicscodeathon/*ace2_*covid/blob/main/README.md

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CONTRIBUTIONS

MN conceived the original idea and developed the pipeline, performed the bioinformatic analysis of the data, and wrote the original draft of the manuscript. AF supervised, wrote, reviewed and edited intensively the manuscript and performed administrative roles. KO performed the bioinformatic analysis of the data, reviewed the manuscript and provided critical feedback and helped update the github repo. BK performed the bioinformatic analysis of the data, wrote, reviewed and edited the manuscript. OD performed the bioinformatic analysis of the data. OIA drafted the original abstract and provided the needed resources and guidance for the successful completion of the project. OIA reviewed the manuscript and provided critical feedback. All authors read and approved the final manuscript.

ETHICS DECLARATION

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

No competing interests were disclose.

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