Differential expression of COVID-19-related genes in European Americans and African Americans

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ABSTRACT

The Coronavirus disease 2019 (COVID-19) pandemic has affected African American populations disproportionately in regards to both morbidity and mortality. A multitude of factors likely account for this discrepancy. Gene expression represents the interaction of genetics and environment. To elucidate whether levels of expression of genes implicated in COVID-19 vary in African Americans as compared to European Americans, we re-mine The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) RNA-Seq data. Multiple genes integral to infection, inflammation and immunity are differentially regulated across the two populations. Most notably, F8A2 and F8A3, which encode the HAP40 protein that mediates early endosome movement in Huntington's Disease, are more highly expressed by up to 24-fold in African Americans. Such differences in gene expression can establish prognostic signatures and have critical implications for precision treatment of diseases such as COVID-19. We advocate routine inclusion of information such as postal code, education level, and profession (as a proxies for socioeconomic condition) and race in the metadata about each individual sampled for sequencing studies. This relatively simple change would enable large-scale data-driven approaches to dissect relationships among race, socio-economic factors, and disease.

Introduction

As of June 1, 2020, the COVID-19 pandemic has infected over 6.3 million people and killed over 370,000 worldwide (<https://coronavirus.jhu.edu/map.html>). Its causative agent, the novel SARS-CoV-2, is an enveloped single stranded RNA virus that infects tissues including lung alveoli^{1,2}, renal tubules^{3,4}, the central nervous system⁵, ileum, colon and tonsils⁶⁻⁸, and myocardium^{9,10}.

The complex combinations of symptoms and disease caused by SARS-CoV-2 include fever, cough, fatigue, dyspnea, diarrhea, stroke, acute respiratory failure, renal failure, cardiac failure, potentially leading to death^{9,11-14}. Symptoms are induced by direct cellular infection and proinflammatory repercussions from infection in other regions of the body¹³⁻¹⁵. A body of evidence indicates that long-term health implications can follow SARS-CoV-2 infection^{16,17}. The attributes of the human host that impact COVID-19 morbidity and mortality are not well understood^{6,13,14,18-23}.

Risk factors for complications of COVID-19 include 65+ years, obesity, and comorbidities such as diabetes, hypertension and heart disease²⁴. Heritable factors in the human host influence COVID-19 symptoms²⁵. However, to date, only a few of the genetic determinants of COVID-19 severity have been even partially elucidated. Genetic variants of Angiotensin-Converting Enzyme2 (ACE2), the major human host receptor for the SARS-CoV-2 spike protein, may be linked to increased infection by COVID-19²⁶⁻²⁹. Human Leukocyte Antigen (HLA) gene alleles have been associated with susceptibility to diabetes and SARS-CoV-2³⁰. The genetic propensity in southern European populations for mutations in the pyrin-encoding Mediterranean Fever gene (MEFV) may elevate levels of pro-inflammatory molecules, leading to a cytokine storm³¹ and greater severity of COVID-19³²⁻³⁵. Identifying those individuals most at-risk for severe COVID-19 infection, and determining the molecular and physiological basis for this risk, would enable more informed public health decisions and interventions.

COVID-19 cases and deaths are disproportionately higher among African Americans. One cause of this disparity are complex socio-economic factors^{15,36,37}. A number of studies have shown differences in genes expression among races³⁸⁻⁴¹. Gene expression reflects the interaction between environmental, physiological, and genetic influences. This study specifically investigates whether the expression of genes that are implicated in the severity of COVID-19 infection varies with race. Understanding differences in expression at the population level could help predict risk factors and identify more personalized, treatments for COVID-19.

Here, we re-mine existing RNA-Seq data and reveal significant differences in expression between European Americans and African American of multiple genes potentially involved in COVID-19-associated inflammation and immunomodulatory response.

Results

We evaluated differential expression by re-mining an aggregated dataset of 7,142 RNA-Seq samples⁴² modified from the normalized and batch-corrected data from the GTEx and TCGA projects⁴³. The Genotype-Tissue Expression (GTEx) project provides data representing “non-diseased” conditions from diverse tissues. The well-curated TCGA project is the largest project available with easily accessible metadata on the races of the individuals who contributed samples representing diseased tissues (tumors) of multiple origins. These large data provide a unique opportunity to evaluate differences in gene expression across populations in multiple tissues in diseased and normal states.

Re-mining existing RNA-Seq data and metadata has several caveats. Because race assignments are self-reported, many of the individuals sampled will be from admixed populations^{41,44}. We are terming those self-reporting as “Black or African American” as “African Americans” and “White” as “European Americans”. The ancestry of the preponderance of African Americans is Western Africa⁴⁴, thus our results for African Americans would mostly reflect more specifically Western Africans. Those self-reporting as White are presumed to be predominantly European Americans, but this group also would include individuals of other populations, including Indians and admixed Hispanic individuals, depending on how these individuals chose to self-report.

Also, we were limited to comparison of differences between gene expression in African American and European American populations, because even in these large studies, the sample numbers for the other three major population groups (Asian, Native American, and Pacific Islanders)⁴⁵ were too low for robust statistical assessment. Even between these two populations, not all conditions (cancers or “non-diseased” tissues) had sufficient samplings of African Americans for robust statistical assessment (Table 1; Supplementary Table S1).

Finally, although the GTEx project analyzes non-diseased tissues, many of the individuals who donated tissues were severely ill or postmortem from varied causes, which would likely effect gene expression in these tissues.

We analyzed the data and metadata with MetaOmGraph (MOG), software that supports interactive exploratory analysis of large, aggregated data⁴². Exploratory data analysis uses statistical and graphical methods to gain insight into data by revealing complex associations, patterns or anomalies within that data at different resolutions⁴⁶.

Differentially expressed (DE) genes among samples from European Americans and African Americans were identified in a tumor-specific or tissue-specific manner using MOG (Mann-Whitney U test). Of the tumor types in the TCGA data, BRCA, COAD, KIRC, KIRP, LUAD, LUSC, THCA, and UCEC had sufficient numbers of samples for DE analysis (Table 1). GTEx normal tissues analyzed were: breast, colon, esophagus, lung, liver, prostate, stomach, thyroid, and uterus (Table 1). We define a gene as DE between two groups if it meets each of the following criteria:

1. Estimated fold-change in expression of 2-fold or more (log fold change, $|\log FC| \geq 1$ where $\log FC$ is calculated as in limma⁴⁷.)
2. Mann–Whitney U test is significant between the two groups (BH corrected p-value < 0.05)

Supplementary Tables S2-S25 contains the full list of DE genes between African American and European American populations for each condition. The numbers of DE genes vary depending on the condition the samples were obtained from. Many genes follow a similar DE trend in diseased as in non-diseased tissues, however, in each case, the fold-change difference of expression among the DE genes was larger in the cancers than in the corresponding non-diseased tissues.

We investigate the distribution of the gene expression values with two additional statistical analyses. We performed the two-sample Kolmogorov–Smirnov test (KS test) to assess whether there is a significant difference in distribution of gene expression between African Americans and European Americans. Hartigan’s dip test was used to test whether a given distribution shows bi or multi-modality. Bi- or multi-modal distributions indicate there may be hidden or unknown covariates affecting the gene expression. Within a race, this could imply presence of sub-population structure. These analyses indicate the not only are genes differentially expressed between the two populations, but the overall distribution in expression values often differed between the populations, for an obvious example, GSTM1 expression in KIRC (Figure 4). Also, in many cases, one or both populations have a bimodal distribution, for example, GSTM1 expression in BRCA has a bimodal distribution in European Americans but not in African Americans (Figure 4).

GO terms related to the biological processes of infection, inflammation and immunity are overrepresented among the genes differentially expressed between African Americans and European Americans. Table 2 lists these GO terms. Supplementary Table S29 provides the overrepresented Gene Ontology (GO) terms among DE genes for each diseased or non-diseased tissue.

We drew from *in silico* studies⁴⁸ and experimental analyses especially human responses to infection by SARS-CoV-2 and other coronaviruses^{49–52} to identify ten genes implicated in cellular responses to SARS-CoV-2 infection that are among those differentially expressed in African American and European American populations. Molecular functions of these genes include receptor kinases, cytokines, other signal transduction molecules, and antioxidants. These genes are integral to central cellular processes that affect pathogenesis by SARS-CoV-2, including endosomal development, autophagy, immunity and

Project	Condition	#AA	#EA	#Upregulated	#Downregulated
TCGA	Breast invasive carcinoma (BRCA)	142	674	83	164
TCGA	Colon adenocarcinoma (COAD)	54	188	30	21
TCGA	Kidney renal clear cell carcinoma (KIRC)	46	410	68	94
TCGA	Kidney renal papillary cell carcinoma (KIRP)	49	166	19	13
TCGA	Lung adenocarcinoma (LUAD)	48	368	16	5
TCGA	Lung squamous cell carcinoma (LUSC)	28	337	2	0
TCGA	Thyroid carcinoma (THCA)	25	292	3	3
TCGA	Uterine Corpus Endometrial Carcinoma (UCEC)	54	70	28	5
GTEX	Breast	12	75	0	0
GTEX	Colon	41	292	13	9
GTEX	Esophagus	80	564	19	11
GTEX	Liver	15	97	0	0
GTEX	Lung	39	269	45	20
GTEX	Prostate	13	89	0	0
GTEX	Stomach	29	159	4	6
GTEX	Thyroid	43	267	25	30
GTEX	Uterus	13	68	0	0

Table 1. Number of DE genes in African Americans compared to European Americans in eight cancer types and nine non-diseased tissue types. Criteria for DE, >2-fold difference in expression, Mann–Whitney U test is significant with BH-corrected p-value < 0.05.

Tissue	GO Term	Fold Enrichment	FDR
COAD	humoral immune response	17	0.003
KIRC	positive regulation of fibrinolysis	76	0.001
LUSC	immune response mediated by microbial peptide	11	0.04
THCA	regulation of T-Cell migration	23	0.03
THCA	killing of cells of other organisms	14	0.03
THCA	antimicrobial humoral response	10	0.03
THCA	receptor-mediated endocytosis	8	0.06
Lung	glutathione derivative biosynthesis)	71	0.001
Esophagus	cellular detoxification of nitrogen)	>100	0.04

Table 2. GO terms related to infection, inflammation and immunity that are most enriched among genes that are DE in African Americans compared to European Americans in cancers and non-diseased tissue types.

Figure 1. Expression of the **IL6ST** interleukin signal transducer gene in African Americans and European Americans across eight cancer conditions. Violin plots summarizing the expression over each tumor sample in the two populations. AA, African American; EA, European American. Horizontal lines represent mean log expression. *, Hartigan's dip test significant (p-value < 0.05); *, KS test significant (p-value < 0.05); *, Mann–Whitney U test significant (BH corrected p-value < 0.05).

inflammation^{6,51,53}. Molecular functions of these genes include receptor kinases, cytokines, other signal transduction molecules, and antioxidants.

Cytokines and the storm

A number of genes of the immune response are differentially expressed between African American and European American populations. Expression of **IL6ST**, a component of the cytokine receptor complex that acts as signal transducer for cytokine interleukins **IL6** and **IL7**, is 2-fold lower in African Americans than European Americans in **BRCA** (Figure 1).

Circulating chemokines **CXCL9** and **CXCL10** are also differentially expressed in African Americans as compared to European Americans in several cancers. **CXCL9** expression is 2-fold greater in **KIRP** and over 2-fold lower in **COAD** and **KIRC**; **CXCL10** expression is 1.5-fold higher in **BRCA**, and over 2-fold lower in **COAD**, **KIRC** and **TCHA** (Figure 2).

The small inducible chemokine **CCL3L3** is upregulated in African Americans by 2- to 3-fold in **BRCA**, **COAD**, and **KIRP** (Figure 3) and in several non-diseased tissues (Supplementary Table S16-S25).

Carcinoembryonic Antigen-related Cell Adhesion Molecules **CEACAM5** and **CEACAM6** are both downregulated 2 to 3-fold in **BRCA** in African Americans. (Supplementary Table 2).

Reactive Oxygen Species

Expression of **GSTM1**, a key enzyme involved in oxidative stress, differs between African Americans and European Americans. Expression is up to 6-fold higher in African Americans in the cancers we evaluated (Figure 4). Distribution of expression in European Americans, but not African Americans is bimodal. **GSTM1** expression is also DE in non-diseased esophagus and thyroid gland (Supplementary Table S18, S24).

F8As, endocytosis, and autophagy

Endocytosis and autophagy are intimately interrelated with Covid-19⁵⁴. One little-studied player implicated in early endosome motility⁵⁵ and hence the endocytotic pathway and autophagy, is the seven tetratricopeptide-like repeat **F8A/HAP40** (HAP40) protein⁵⁶. In human genomes, three genes, **F8A1**, **F8A2**, and **F8A3**, encode the HAP40 protein⁵⁶. The **F8A** genes are located on the X chromosome. **F8A1** is within intron 22 of the coagulation factor **VIII** gene, which has a high frequency of mutations⁵⁷; **F8A2**, and **F8A3** are located further upstream.

F8A1, **F8A2** and **F8A3** are each differentially expressed in African Americans versus European Americans. **F8A1** is more highly expressed by about 2-fold in European Americans in every cancer analyzed (Figure 5) and by 2-fold in non-tumor colon (Supplementary Table S17). Conversely, **F8A2** and **F8A3** are more highly expressed in African Americans in all cancer types. Expression of **F8A2** in African Americans is up to 24-fold greater; expression of **F8A3** is up to 6.6-fold greater. In **LUSC**, **F8A2** and **F8A3** are the only DE genes (Supplementary Table S7). **F8A2** and **F8A3** follow a similar trend in non-diseased

Figure 2. Expression of the **CXCL9** and **CXCL10** circulating chemokine genes in African Americans and European Americans across eight cancer conditions. Violin plots summarize the expression over each tumor sample in the two populations. AA, African American; EA, European American. Horizontal lines represent mean log expression. *, Hartigan's dip test significant (p-value < 0.05); *, KS test significant (p-value < 0.05); *, Mann–Whitney U test significant (BH corrected p-value < 0.05).

Figure 3. Expression of the **CCL3L3** chemokine gene in African Americans and European Americans across eight cancer conditions. AA, African American; EA, European American. Horizontal lines represent mean log expression. *, Hartigan's dip test significant (p-value < 0.05); *, KS test significant (p-value < 0.05); *, Mann–Whitney U test significant (BH corrected p-value < 0.05).

Figure 4. Expression of the mitochondrial glutathione-S-transferase gene, **GSTM1** in African Americans and European Americans across eight cancer conditions. GSTM1 is a key player in metabolism of ROS. Violin plots summarize GSTM1 expression over each tumor sample in the two populations. AA, African American; EA, European American. Horizontal lines represent mean log expression. *, Hartigan's dip test significant (p-value < 0.05); *, KS test significant (p-value < 0.05); *, Mann–Whitney U test significant (BH corrected p-value < 0.05).

tissues, being more highly expressed in African Americans by up to 4-fold in colon, esophagus, and thyroid (Supplementary Table S17-S18). Distribution of F8A2 and F8A3 expression is bimodal in European Americans for most cancers. Thus, part of the difference in levels of F8A2 and F8A3 expression between the two populations is due to their distribution, with low levels of expression in a subset of the European American population.

Because of the paucity of literature on HAP40⁵⁸ and because, to our knowledge, the relationships among F8A1, F8A2, and F8A3 genes have not been described, we investigated further the sequences, sequence variants, and the expression patterns of these genes.

The sequences of the HAP40 proteins of F8A1, F8A2, and F8A3 are identical to each other in human reference genome GRCh38.p13 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.39). Allele variants of HAP40 proteins encoded by F8A1, F8A2, and F8A3 were mined from The Genome Aggregation Database (gnomAD)⁵⁹, a open database, which contains sequences of over 140,000 exomes and genomes from individuals of diverse populations as categorized by clustering of genetic features (rather than being self-reported). Individuals are assigned to one of the five major populations and to sub-populations within these. The search of gnomAD identified no variants in the HAP40s encoded by F8A2 or F8A3, and a single very rare variant (<1/1000) of F8A1 found only in European (non-Finnish) populations. The variant encodes a missense mutation (https://gnomad.broadinstitute.org/gene/ENSG00000197932?dataset=gnomad_r2_1). No structural variants were identified for HAP40 of F8A1 or F8A3; F8A2 has a rare duplication of 54 aa.

To our knowledge, F8A2 and F8A3 gene expression has not been described. We analyzed coexpression of the three F8A genes in the context of the other 18,212 genes represented in the full TCGA-GTEX dataset, using the “Pearson correlation” function in MOG. Although the F8A genes are proximately located on the X chromosome, the genes are *not* highly coexpressed with each other ($|\text{Pearson Corr.}| < 0.46$). Furthermore, no F8A gene is coexpressed with *any* other of the 18,212 genes represented in the dataset ($|\text{Pearson Corr.}| < 0.46$) (Supplementary Table S30). Of the 18,212 genes, the expression of F8A1 is most *negatively* (anti-) correlated with those of F8A2 and F8A3, with Pearson Correlations of -0.45 and -0.24 , respectively (Supplementary Table S30). Mutual information (MI) can detect linear as well as complex non-linear associations, whereas Pearson’s correlation measure quantifies linear dependencies. Using the MI function in MOG, we found that F8A2 and F8A3 genes are most associated with F8A1 (Supplementary Table S30), indicating that there may be a negative interaction among these genes.

Discussion

Genetics of human populations contribute to the propensity and severity of diseases^{37,39,41,41,45,60–65}. Sometimes the contribution is straightforward; a single allele variation found in Ashkenazi Jews, causes the vast majority of Tay-Sachs disease⁶⁶. Sometimes it is more complex; for example, hypertension, which more prevalent in African American than European American populations⁶⁰ in part due to detrimental APOL1 mutations that are more frequent in West African populations⁶². Despite the paucity of studies focused on Western African populations, the propensity and severity of several other diseases among this population have been attributed to genetics^{41,62,67}.

The individual’s immune system is key to fighting viral infections. However, conversely, many COVID-19 deaths have been attributed to a cyclic over-excitement of the innate immune system. This latter process, often termed a cytokine storm, results in a massive production of cytokines and the body attacking itself, rather than specifically destroying the pathogen-containing cells¹⁴. Thus, people with comorbidities, the elderly, and immunosuppressed individuals, may be at a greater risk for COVID-19 morbidity and mortality because they may not respond to infection with sufficient immune response⁵⁰ and/or because they may be more likely to develop a cytokine storm¹⁴. We focused on ten genes that are differentially expressed between African Americans and European Americans are implicated in these biological processes.

The most dramatic differences in gene expression were in expression of the F8A genes, F8A1 being upregulated in European Americans, and F8A2 and F8A3 being upregulated in African Americans. Each F8A gene encodes an identical HAP40 protein. F8A1, F8A2 and F8A3 genes each have a very distinct pattern of expression across the thousands of samples of tissues and cancers in the TCGA/GTEX dataset.

HAP40 function has been researched only in the context of its critical role in early endosome maturation in Huntington’s disease⁵⁶. In Huntington’s, HAP40 forms a bridge between the huntingtin protein and the regulatory small guanosine triphosphatase, RAB5; formation of this complex reduces endosomal motility by shifting endosomal trafficking from the microtubule to the actin cytoskeleton⁵⁵. High F8A1 expression has been reported in several conditions: Huntington’s⁶⁸, a SNP variant for type 1 diabetes risk⁶⁹, cytotrophoblast-enriched placental tissues from women with severe preeclampsia⁷⁰; and mesenchymal bone marrow cells as women age⁷¹. Although its non-disease biology has been little explored, because of its role in early endosome motility in Huntington’s, HAP40 is considered a potential molecular target in therapy of autophagy-related disorders⁷².

Endosome motility and development play an important but complex role in the innate immune response, which can either promote or hinder the battle between SARS-CoV-2 and its human host^{51,54,73,74}. Coronaviruses including SARS-CoV-2

Figure 5. Expression of the HAP40 putative early endosome trafficking genes: **F8A1**, **F8A2** and **F8A3** in African Americans and European Americans across eight cancer conditions. Although the function of HAP40 has not been investigated in normal individuals, this protein is a key component of Huntington's Disease; in Huntington's, HAP40 shifts endosomal trafficking from the microtubules to actin⁵⁵. Violin plots summarize the expression over each tumor sample in the two populations. AA, African American; EA, European American. Horizontal lines represent mean log expression. *, Hartigan's dip test significant (p-value < 0.05); *, KS test significant (p-value < 0.05); *, Mann-Whitney U test significant (BH corrected p-value < 0.05).

mainly enter host cells via binding to the ACE2 receptor followed by endocytosis^{20,29,51}. Nascent early endosomes are moved along the microtubule cytoskeleton, fusing with other vesicles; varied molecules can be incorporated into the membrane or the interior^{51,54,73,74}. This regulated development enables diverse fates. For example, in the context of SARS-CoV-2, endosomes might release viral RNA or particles; they might merge with lysosomes and digest their viral cargo; or might fuse with autophagosomes (autophagy) and subsequently with lysosomes that digest the cargo^{51,54,73,74}. SARS-CoV-2 might reprogram cellular metabolism, suppressing autophagy and promoting viral replication⁷⁵. The cell might modify autophagy machinery to decorate viral invaders with ubiquitin for eventual destruction, activate the immune system by displaying parts of the virus, or catabolize excess pro-cytokines. Autophagy might induce cytokine signaling, which could promote protective immune response or engender a destructive storm of cytokines, inflammation and tissue damage⁵⁴.

Cytokines and other immunomodulatory molecules, including CCL3L3, CXCL9, CXCL10, CEACAM5 and CEACAM6, were differentially expressed between African Americans and European Americans. CCL3L3 is a member of the functionally-diverse C-C motif chemokine family. It encodes CCL3, which acts as ligand for CCR1, CCR3 and CCR5 recruits and activates granulocytes; it also inhibits HIV-1-infection⁷⁶. CCL3L3 is upregulated in younger and impoverished white males⁷⁷. Circulating chemokines CXCL9 and CXCL10 initiate human defenses, and potentially instigate autoimmune and inflammatory diseases, by activating G protein-coupled receptor CXCR3⁷⁸⁻⁸⁰. CEACAM5 and CEACAM6 are members of the C-Type Lectin Domain Family. This gene family encodes a diverse group of calcium (Ca²⁺)-dependent carbohydrate binding proteins, several of which, including CEACAM5 and CEACAM6 have been implicated as having specific cell adhesion, pathogen-binding and immunomodulatory functions⁸¹. CEACAM5, a driver of breast cancer⁸² and modulator of inflammation in Crohns Disease⁸³, and CEACAM6, an inhibitor of breast cancer when coexpressed with CEACAM8⁸⁴, are both downregulated 2 to 3-fold in BRCA in African Americans.

Reactive Oxygen Species (ROS) generated in the mitochondria promote the expression of proinflammatory cytokines and chemokines, thus playing a key role in modulating innate immune responses against RNA viruses⁸⁵⁻⁸⁹. Mitochondrially-targeted glutathione S-transferase, GSTM1, which was more highly expressed in African Americans than European Americans, is a key enzyme in the metabolism of ROS, as well as xenobiotics including pharmaceuticals⁸⁸. GSTM1 is induced by nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that integrates cellular stress signals⁹⁰⁻⁹³. Low expression of GSTM1 can lead to increased mitochondrial ROS, which may ultimately result in a cytokine storm that triggers inflammation and/or autoimmune disease. Conversely, if GSTM1 is too highly expressed, pharmaceuticals may be metabolized and thus rendered inactive, and ROS may be metabolized too rapidly to maintain a sufficient signaling role in the immune system. Allele frequencies of GSTM1 vary among Asian, African and European populations⁹⁴; the biological significance of these alleles is being investigated^{95,96}.

CEACAM5 and CEACAM6, both downregulated 2 to 3-fold in BRCA in African Americans, are members of the C-Type Lectin Domain Family. This gene family encodes a diverse group of calcium (Ca²⁺)-dependent carbohydrate binding proteins; CEACAM5 and CEACAM6 have been implicated as having specific cell adhesion, pathogen-binding and immunomodulatory functions⁸¹. CEACAM5 is a driver of breast cancer⁸² and modulator of inflammation in Crohns Disease⁸³, and CEACAM6 is an inhibitor of breast cancer when coexpressed with CEACAM8⁸⁴.

By revealing differential expression of genes implicated in COVID-19 morbidity and mortality between African Americans and European Americans, we emphasize the importance of integrating gene expression data into the mix of factors considered in studying this pandemic. Our study indicates that, under both diseased and non-diseased conditions, many genes involved in infection, inflammation, or immunity are differentially expressed between African Americans and European Americans. One contributing explanation of the finding that disease-related genes are overrepresented among DE genes is that the selection pressure due to disease is very strong on both (ancestral) regions, but these regions have very different complements of pathogens. Humans living in Europe and those living in Western Africa would have had to evolve the ability to resist the prevalent local pathogens.

Archived expression data has tremendous value. However, studies such as this one are hampered by several factors. For example, obtaining adequate sample sizes for statistical analysis of populations is very important but difficult or expensive to address. Ethnic bias and practical factors (such as subject availability) often result in insufficient numbers of subjects from many populations to be represented in medical studies; this lack of representation prevents the development of precise prognosis or therapy based on genetics^{39,97}. Similarly, diverse socioeconomic contexts may not be well represented among the individuals sampled. Yet these are clearly a factor in disease⁹⁸. The statistical predictor provided for the U.S. by the Robert Wood Johnson Foundation (<https://www.rwjf.org/en/library/interactives/whereyouliveaffectshowlongyoulive.html>) reflects the concept that "your zip code can be greater than your genetic code" (although unfortunately there is no accompanying genetic information).

Another factor that would greatly increase the utility of archived expression data is improving and extending the metadata for all (future) studies; this would be relatively simple to implement. Well-constructed metadata is key to the usefulness of data. Among the vast body of human RNA-Seq data being deposited, fields for age and gender are typically represented and available

in the metadata. However, except in specialized studies, metadata on the race and ethnic heritage of the sampled individuals are often not included, or are very difficult to access. The same is even more true of fields that would provide socio-economic information, such as postal code or risk factors such as occupation. Because of the absence of socioeconomic metadata, even in GTEx and TCGA, the arguably most comprehensive RNA-Seq datasets to date, we were unable to distinguish genetic effects from environmental causes of the differences in gene expression. Without routine inclusion of diverse metadata for human 'omics samples, data re-mining is hampered, and important information is lost.

Conclusion

Multiple genes implicated in COVID-19 are differentially expressed in African American and European American populations. The differential expression is evident despite the fact that race is self-reported in and metadata, and that many Americans are racially admixed⁴¹.

Gene expression represents the interaction of genetic and environmental factors. Routine inclusion of information on ethnicity, race, postal code (as a proxy for socioeconomic condition), and profession in the metadata for each individual sampled would empower large-scale data-driven approaches to dissect the relationships between race, socio-economic factors, and disease.

By highlighting the wide-ranging differences in expression of several disease-related genes across populations, we emphasize the importance of harvesting this information for medicine. Such research will establish prognostic signatures with vast implications for precision treatment of diseases such as COVID-19.

Methods

The MOG tool was used to interactively explore, visualize and perform differential expression and correlation analysis of genes. We downloaded the precompiled MOG project http://metnetweb.gdcb.iastate.edu/MetNet_MetaOmGraph.htm⁴², created using the data processed by Wang et. al., in which expression values have been normalized and batch corrected to enable comparison across samples⁴³. This *MOG_HumanCancerRNASeqProject* contains expression values for 18,212 genes, 30 fields of metadata detailing each gene, across 7,142 samples representing 14 different cancer types and associated non-tumor tissues (TCGA and GTEx samples) integrated with 23 fields of metadata describing each study and sample.

Since the data was normalized and batch corrected, we used Mann-Whitney U test, a non-parametric test, to identify differentially expressed genes between two groups. An R script was written to perform KS and dip tests, and create the violin plots and executed via MOG interactively.

Pearson correlation values were computed, after data was $\log_2$ transformed within MOG, in MOG's statistical analysis module.

Information on how to reproduce the results are available at <https://github.com/urmi-21/COVID-DEA>.

Data availability

We subscribe to FAIR data and software practices⁹⁹. MOG is free and open source software published under the MIT License. MOG software, user guide, and the *MOG_HumanCancerRNASeqProject* project datasets and metadata described in this article are freely downloadable from http://metnetweb.gdcb.iastate.edu/MetNet_MetaOmGraph.htm. MOG's source code is available at <https://github.com/urmi-21/MetaOmGraph/>. Additional files are available at <https://github.com/urmi-21/COVID-DEA>.

Supplementary data

Supplementary data are available at bioRxiv.

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Conflict of interest statement.

None declared.

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