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MAYO CLINIC

COVID-19: Understanding Inter-Individual Variability and Implications for Precision Medicine

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Abstract

Coronavirus disease 2019 (COVID-19) is characterized by heterogeneity in susceptibility to the disease and severity of illness. Understanding inter-individual variation has important implications for not only allocation of resources but also targeting patients for escalation of care, inclusion in clinical trials, and individualized medical therapy including vaccination. In addition to geographic location and social vulnerability, there are clear biological differences such as age, sex, race, presence of comorbidities, underlying genetic variation, and differential immune response that contribute to variability in disease manifestation. These differences may have implications for precision medicine. Specific examples include the observation that androgens regulate the expression of the enzyme transmembrane protease, serine 2 which facilitates severe acute respiratory syndrome coronavirus 2 viral entry into the cell; therefore, androgen deprivation therapy is being explored as a treatment option in males infected with COVID-19. An immunophenotyping study of COVID-19 patients has shown that a subset develop T cytopenia which has prompted a clinical trial that is testing the efficacy of interleukin-7 in these patients. Predicting which COVID-19 patients will develop progressive disease that will require hospitalization has important implications for clinical trials that target outpatients. Enrollment of patients at low risk for progression of disease and hospitalization would likely not result in such therapy demonstrating efficacy. There are efforts to use artificial intelligence to integrate digital data from smartwatch applications or digital monitoring systems and biological data to enable identification of the high risk COVID-19 patient. The ultimate goal of precision medicine using such modern technology is to recognize individual differences to improve health for all.

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CURRENT STATUS OF THE COVID-19 PANDEMIC: A DISEASE DEFINED BY INDIVIDUAL DIFFERENCES AND HETEROGENEITY IN SUSCEPTIBILITY AND OUTCOMES

As of December 1, 2020, there were 13.72 million confirmed cases and 270,642 deaths due to coronavirus disease 2019 (COVID-19) in the United States (Figure 1).[1] Unfortunately, after "flattening the curve," the United States has experienced a surge in new cases since early June 2020 (Figure 2) starting in in states such as Florida, Arizona, Nevada, and Texas, subsequently spreading to Midwestern United States.[2] As of the week ending November 21, 2020, there were 79,501 COVID-19 laboratory-confirmed hospitalizations in the United States, among which 55,416 occurred in those older than the age of 50 years; hence, predominantly affecting older patients.[2] There are also clear race and ethnicity differences in age-adjusted COVID-19–associated hospitalization rates, being highest in the American Indian/Alaska native, Black, and Hispanic populations (Figure 3).[2] Age-adjusted hospitalization and mortality rates from COVID-19 have also been reported to be higher in males than females, highlighting the role of

biological sex in disease outcomes.[3,4] Patients who test positive for COVID-19 disease can be asymptomatic or present with multiorgan failure. Recent studies have suggested the role of genetics in being protective or conferring susceptibility to COVID-19 infection. Understanding the differences observed in biological factors such as age, sex, race, presence of comorbid conditions, as well as the host and viral genome and the roles they play in the variability in COVID-19 presentation and susceptibility may provide clues into disease pathophysiology, therapeutic targets, and enable identification of the high-risk patient for therapeutic intervention and vaccination.

ARTICLE HIGHLIGHTS

- The hallmark feature of COVID-19 that has confounded public perception and delivery of medical care for the disease is the wide variation observed in disease susceptibility and progression.
- Innate biological differences in age, sex, race, the genome and differential immune responses of the person exposed to severe acute respiratory syndrome coronavirus 2 in addition to differences in the vral genome itself plays an important role in the inter-individual variability observed.
- Predicting which patients are at risk for severe COVID-19 disease would enable delivery of the right care to the right patient.
- Advances in data analytics, multi-omic and digital technologies, and the use of artificial intelligence may help the development of a precision medicine approach to the COVID-19 pandemic.

SEX DIFFERENCES AND COVID-19 DISEASE

Males are at a greater risk of COVID-19–related morbidity and mortality as compared with females across various age groups (Figure 4).[3-8] Studies describing sex differences have been descriptive and did not adjust for confounding concomitant morbidities. Men are more likely to be smokers and have a higher risk of hypertension, cardiovascular disease, and diabetes mellitus, all of which are risk factors for adverse outcomes in COVID-19 disease.[9,10] However, the prevalence of these comorbidities is low in the younger age groups in which sex-related differences in outcomes were observed. The susceptibility to adverse outcomes may be related to an increased inflammatory response (Figure 5) in males and a lack of the protective effect of estrogen receptor signaling present in females.[11,12] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus binds to the angiotensin-converting enzyme (ACE) 2 receptor; and a recent study in heart failure patients who did not have COVID-19 disease showed that circulating ACE2 levels are higher in men as compared with women independent of other factors.[13] The difference in ACE2 levels may be related to sex-related differential expression of the *ACE2* gene because it is located on the X chromosome or sex-related differential regulation of interferon production that affects *ACE2* gene expression.[14] There is also increased bi-allelic X chromosome–linked *TLR7* gene expression observed in females as these genes escape inactivation as opposed to males possessing only a single copy of these genes.[15] Toll-like receptor (TLR) 7 is abundantly present in lung tissue and upon recognition of viral RNA produces type 1 interferon, thus launching an early antiviral response.[16-18] Females have higher B cell counts and also tend to have a greater antibody response than males to vaccination that may be related to the effect of testosterone on antibody production.[19,20]

CHARACTERISTICS OF PATIENTS WITH SEVERE VERSUS NO OR MILD DISEASE

Coronavirus disease 2019 has a wide spectrum of severity, from the asymptomatic spreader to patients with extreme cardiorespiratory failure requiring maximal mechanical cardiac and respiratory support.[21]

Early reports from China have shown that male sex, diabetes, and low albumin level are associated with worse COVID-19 disease severity and increased mortality.[22] More recent data from the United States and Europe have confirmed that male sex and diabetes are associated with worse outcomes in hospitalized COVID-19 patients. Additionally, studies suggest that smoking, hypertension, and obesity are also associated

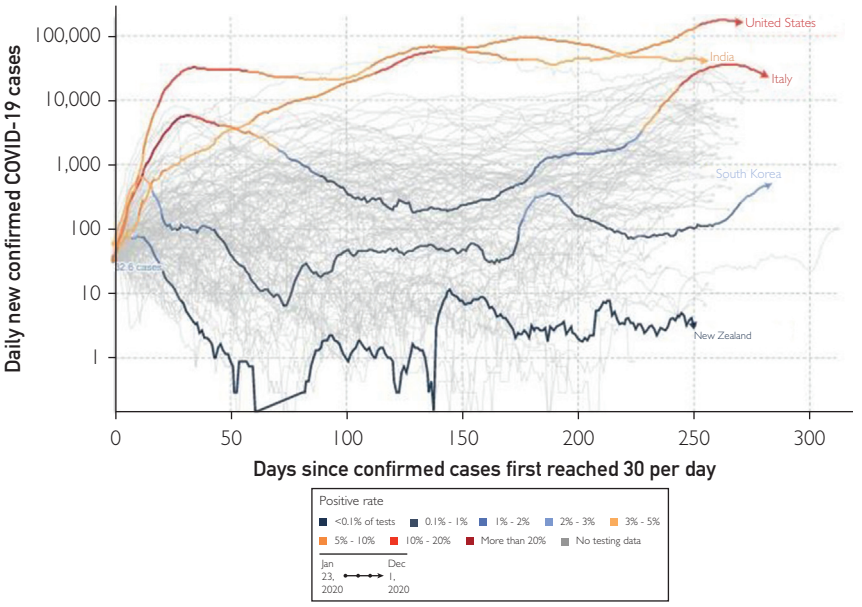

FIGURE 1. Daily new confirmed coronavirus disease 2019 (COVID-19) cases by country shown in a rolling 7-day average up to December 1, 2020 (Source: Johns Hopkins University CSSE COVID-19 Data, https://ourworldindata.org).

with more severe illness and worse prognosis.[3,23] The odds ratio (OR) for progression to severe illness was higher in diabetic patients (64-fold) presenting with fever (>37.5°C) and chills (six-fold), and infiltrate on x-ray (13-fold), suggesting that these patients should be closely observed even if initial symptoms are mild.[24] Recently, it has been observed that COVID-19 patients with pre-existing cardiovascular disease have more severe disease and an increased risk of adverse events, including death.[25-32]

In contrast, we now know that a good majority of affected patients remain asymptomatic or have very mild symptoms. In one population, the majority of such patients were 20- to 40-year-old men, reporting cough, sputum production, and hyposmia — commonly associated with hypogeusia and nasal congestion.[22] However, with increased surveillance testing, a high rate of asymptomatic and presymptomatic infection (up to 50%) in older patients who are considered higher-risk for severe COVID-19 illness is being reported, such as nursing home residents. These asymptomatic/presymptomatic patients seem to have high levels of viral RNA in their upper airway secretions suggesting significant potential for transmission regardless of symptoms.[33,34] Even more concerning is that asymptomatic patients may have: 1) a longer median duration of viral shedding than symptomatic ones; and 2) weaker immune responses, with significantly lower virus-specific immunoglobulin G antibody titers and cytokine levels.[35] Data on patients younger than 18 years old is sparse, but overall it seems to suggest that COVID-19 disease is asymptomatic or mildly symptomatic in the pediatric population. The reported rate of hospitalization in this population is low (5.7% to 20%), with infants younger than 1 year old and children with chronic underlying conditions being at higher risk for more severe illness. Severe pediatric infections (requiring hospitalization) are more prevalent in males (57%) than females, which is consistent with findings in adults.[36] More

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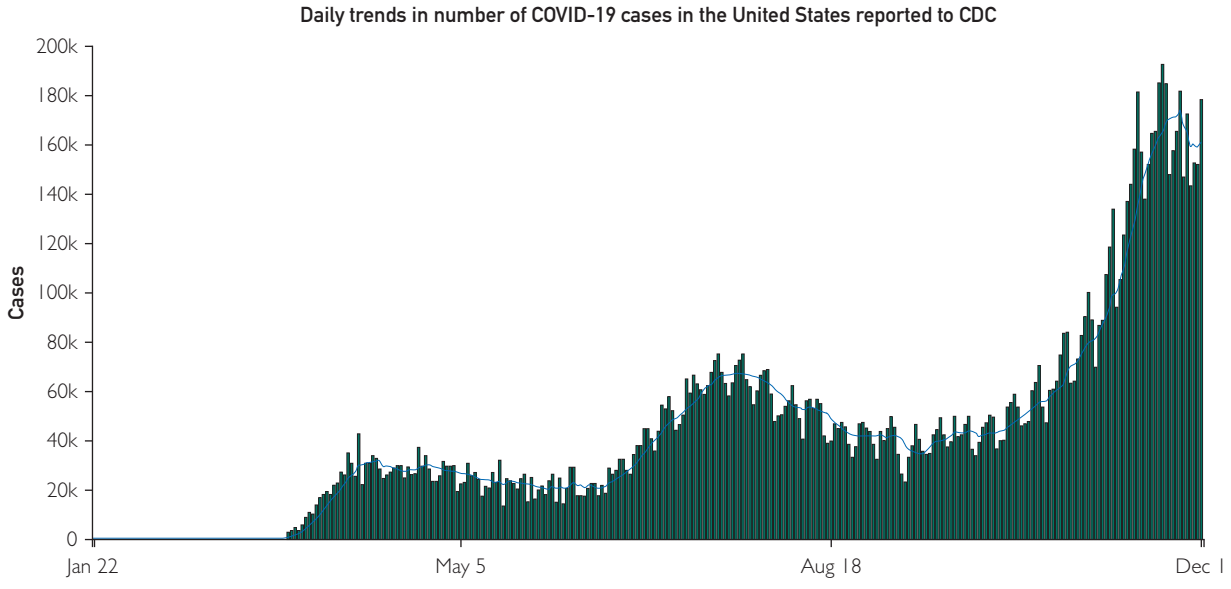

FIGURE 2. Number of new coronavirus disease 2019 (COVID-19) cases reported on a daily basis in the United States since the beginning of the outbreak up to December 1, 2020. Bars denote new cases by day and graph line shows 7-day average. (Source: Centers for Disease Control and Prevention, https://www.cdc.gov).

recently, there have been descriptions of clusters of children with COVID-19 infection who have developed multisystem inflammatory syndrome with predominant gastrointestinal and cardiovascular organ involvement including occurrence of myocarditis and Kawasaki disease (KD)—like features.[37,38] The relationship between the genetic architecture of KD and this manifestation of COVID-19 remains to be defined; however, there is growing

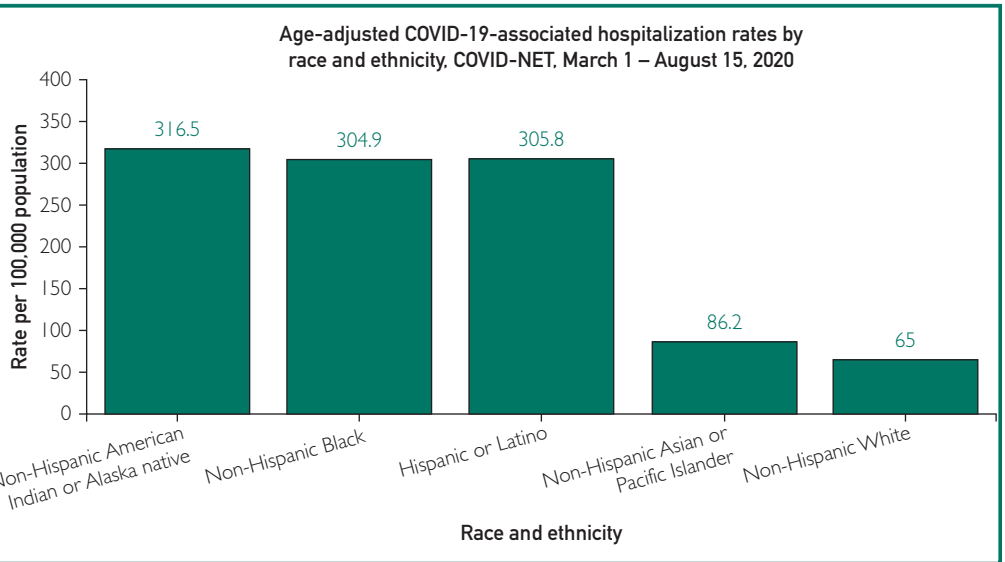

FIGURE 3. Age-adjusted hospitalization rates by race and ethnicity for coronavirus disease 2019 (COVID-19) disease from March 1, 2020, to August 15, 2020 (Source: Centers for Disease Control and Prevention, https://www.cdc.gov). COVID-NET, Coronavirus Disease 2019 Associated Hospitalization Surveillance Network.

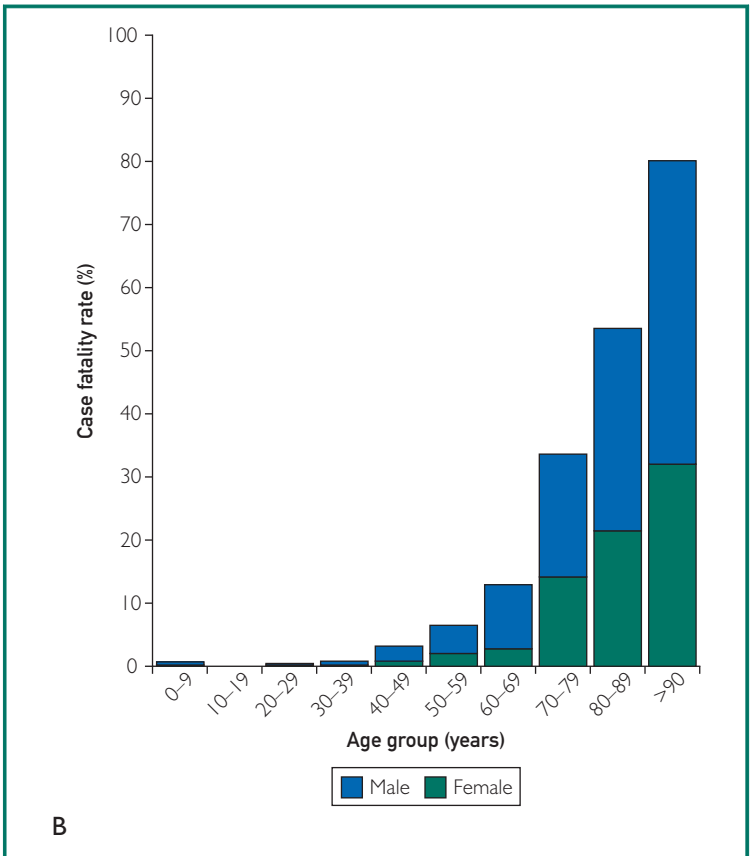

FIGURE 4. Coronavirus disease 2019 case fatality rate expressed as a percentage in different age groups separated for males (blue) and females (green) (Adapted from Scully et al[4]).

concern that the ensuing SARS-CoV-2 infection—related inflammatory response and cytokine storm may result in this more severe form of disease,[39] especially among Blacks, Hispanics, or South Asians[37,38,40,41] and those with increased body mass index.[42]

Recent data also suggest that younger people can present with severe infections more often than previously reported,[43] which suggests that other than age alone, innate differences play a role. The discovery of the mechanism for SARS-CoV-2 infection as the requisite binding of the virus to the membrane-bound form of ACE2 for internalization of the complex by the host cell may also provide insight into who may be at risk for severe disease. Wrapp et al[44] suggest that the greater virulence of SARS-CoV-2 compared with SARS-CoV may be explained by the significantly higher affinity that the COVID-19 viral S1 protein exhibits for ACE2. There is a clear spectrum of ACE2 expression in both humans and other mammals.[45] Therefore, the human ACE2 expression level and pattern in various tissues may be important when considering variation in susceptibility and disease severity across infected patients. For example, children younger than 10 years old, most of whom are asymptomatic or have mild disease, have the lowest expression of ACE2 receptors in their nasal epithelium.[46] However, some develop a severe KD-like syndrome characterized by profound inflammation of multiple organs, including the cardiovascular system, termed multisystem inflammatory syndrome in children. Clinical features that predict which children develop multisystem inflammatory syndrome in children are not well understood, but some have proposed that sex (males > females), obesity, and genetic factors are important.

DISSECTING REASONS FOR VARIABLE MORTALITY RATES ACROSS COUNTIES AND COUNTRIES

The global COVID-19 pandemic has revealed substantial variability in incidence and mortality according to factors such as geographical location and social vulnerability. This variability has largely been shaped by variable policies related to travel restrictions, voluntary versus mandatory social distancing, and availability of testing and contact tracing, as well as differences in health care systems and management.[47,48]

In addition to public policy efforts, there may be biologic determinants that contribute to variability in COVID-19 severity across countries as described in this paper. For example, East Asian populations have higher allele frequencies in the expression quantitative trait loci (eQTL) variants leading to higher ACE2 tissue expression compared with European populations, which would suggest that Asian populations may be more susceptible to more severe disease due to increased viral uptake via the ACE2 route.[45] Some data suggest that countries with universal policies using Mycobacterium bovis Bacillus Calmette-Guérin (BCG) vaccination have fewer COVID-19 cases and a lower case fatality rate than countries that lack universal

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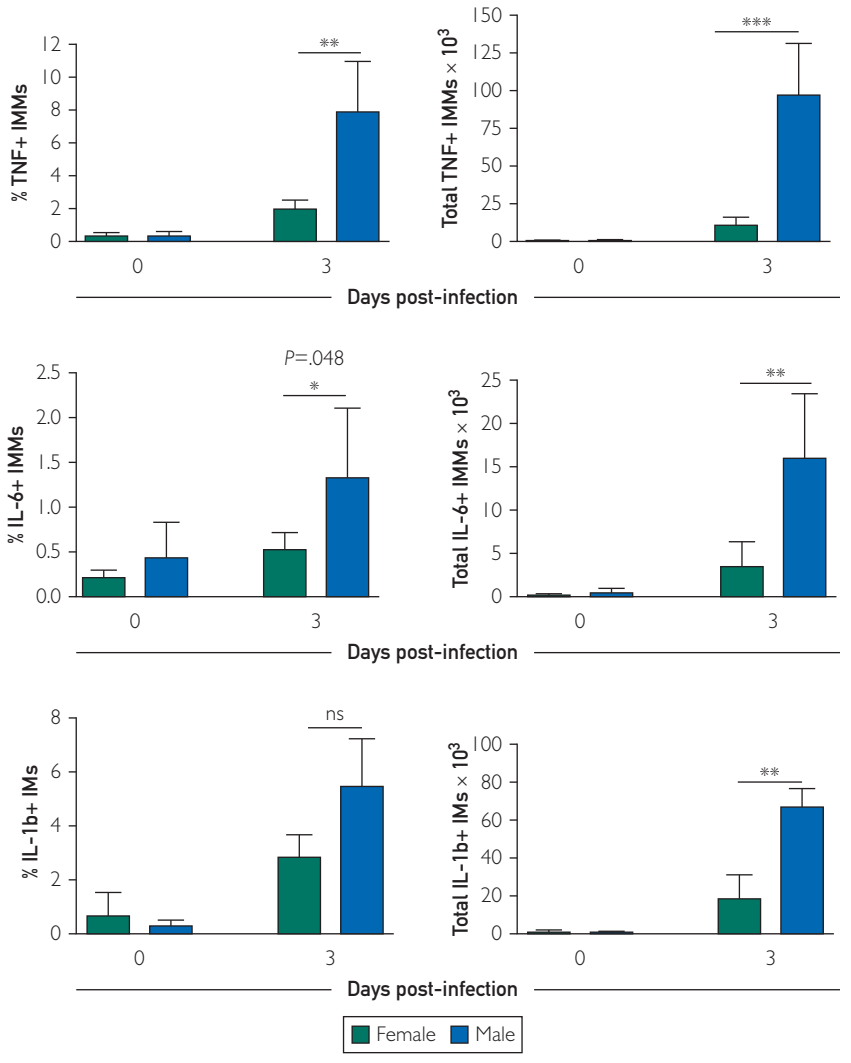

FIGURE 5. The percentage and number of cytokine/chemokine producing inflammatory monocyte macrophages (IMMs) expressed in male and female mice lung tissue after infection with mouse-adapted severe acute respiratory syndrome coronavirus (SARS-CoV) (Adapted from Channappanavar et al[11]). IL, interleukin; IM, inflammatory monocyte macrophages; TNF, tumor necrosis factor.

BCG vaccination.[49] The BCG vaccine is thought to induce "trained immunity," a concept that refers to a long-term epigenetic and metabolic reprogramming of innate immune cells that results in heightened proinflammatory activity.[50,51] Mycobacterium bovis Bacillus Calmette-Guérin vaccinations' protective-effect hypothesis was questioned in an Israeli population-based cohort that showed no difference in the rate of COVID-19 test positivity between those in a BCG-vaccinated versus unvaccinated cohort.

In addition to variability in incidence, mortality from COVID-19 varies widely by county across the United States (Table). For example, during the height of the outbreak in March and April 2020, the number of patients who were hospitalized and had COVID-19–related deaths were highest in the Bronx and lowest in Manhattan in New York City.[52] Even more striking is the clear disparity in case incidence and case fatality by race-ethnicity as described above.[53-55] The disproportionate rates of COVID-19

infections in people of color can be attributed to the insidious and widespread effects of systemic health and social inequities throughout the United States. For example, people of color are more likely to have cardiovascular comorbidities that increase susceptibility to severe disease. In addition, they are more likely to be front-line workers and live in neighborhoods with a higher degree of social vulnerability. Despite these hypotheses, there is controversy as to whether racial differences in outcomes persist after adjusting for differences in medical and socioeconomic comorbidities.[56-58] In an adjusted analysis, the odds of hospitalization was higher for Blacks as compared with Whites. However, after accounting for differences in medical comorbidities and neighborhood characteristics, the risk of in-hospital mortality was similar between Blacks and Whites. Given the striking disparities noted early in the pandemic, ongoing efforts aim to characterize COVID-19 disease with social determinants of health, including poverty, residential segregation, and availability of medical services, and to examine its effects on outcomes.[59]

GENETIC DETERMINANTS OF SUSCEPTIBILITY TO SEVERITY OF COVID-19 INFECTION

Genetic variation likely contributes to individual differences in susceptibility and severity of COVID-19 infection following exposure to SARS-CoV-2. Our understanding of the role of genetic variants in COVID-19 infection is evolving. Although few large-scale rigorous genetic epidemiological studies, including genome-wide association studies (GWAS), have been published in the peer-reviewed literature, early evidence implicates variation in several genes.

ACE, ACE2, and *TMPRSS2*

The transmembrane protease serine 2 (TMPRSS2) and the endosomal cysteine proteases cathepsin B and L activate the spike protein of SARS-CoV-2 allowing the virus to bind to the ACE2 receptor on cell surfaces and subsequently to enter the cell through endocytosis.[60,61] Furthermore, the *ACE* gene encoding the enzyme ACE1 is characterized by a deletion/insertion polymorphism in intron 16, and the D allele is associated with decreased expression of the receptor ACE2. Therefore, studies have focused on determining whether genetic variants in *ACE*, *ACE2*, and *TMPRSS2* impact COVID-19 infection.

Population studies in European, North African, and Middle Eastern countries have found a negative correlation between the D allele in *ACE* and COVID-19 prevalence and mortality.[62,63] However, this correlation appears to be inconsistent with the apparent lower prevalence and mortality of COVID-19 in East Asian populations, in which the D allele is less frequent.[64] Furthermore, these studies did not account for socioeconomic differences among countries that were likely to impact the course of COVID-19.

A comparison of whole-exome sequence data from 131 COVID-19 cases and 258 controls in Italy showed a higher prevalence of *ACE2* variants that impair expression or function of the ACE2 receptor in the control cohort, suggesting that these variants are protective against SARS-CoV-2.[65] In silico analyses suggest that some variants in *ACE2* may either weaken or strengthen binding of the SARS-CoV-2 spike protein to the ACE2 protein, respectively, decreasing or increasing susceptibility to infection.[66,67] Differences in epigenetic regulation of *ACE2* have also been observed, with decreased DNA methylation of the gene in women relative to men, and decreasing methylation with age[68] likely leading to increased gene transcription. Cao et al[45] analyzed coding variants in *ACE2* and eQTL variants that affect ACE2 expression in various East Asian, South Asian, African, and European populations. Although they did not detect differences among populations in variants that impair SARS-CoV-2 spike protein binding, they determined that East Asian populations have greater allele frequencies of eQTL variants associated with higher ACE2 expression, possibly leading to increased susceptibility to infection. A GWAS was conducted in 676 cases with SARS-CoV-2 positive tests and 1334 controls from the UK Biobank.[69] Although there was an overall negative association between *ACE2* expression and test positivity for

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TABLE. United States Counties With the Most Confirmed Cases and Deaths[a]

Total number of cases		New cases per 100,000 population		Total number of deaths		Case fatality rate, %	
Los Angeles (CA)	310,595	Los Angeles (CA)	13.9	Los Angeles (CA)	7076	Los Angeles (CA)	2.29
Cook (IL)	195,740	Cook (IL)	50.92	Queens (NY)	6063	Queens (NY)	7.95
Miami-Dade (FL)	187,757	Miami-Dade (FL)	34.33	Kings (NY)	5759	Kings (NY)	7.79
Harris (TX)	163,287	Harris (TX)	20.88	Cook (IL)	5510	Cook (IL)	2.81
Maricopa (AZ)	160,184	Maricopa (AZ)	9.14	Bronx (NY)	4064	Bronx (NY)	7.30
Dallas (TX)	97,875	Dallas (TX)	18.08	Miami-Dade (FL)	3669	Miami-Dade (FL)	1.95
Broward (FL)	87,456	Broward (FL)	25.37	Maricopa (AZ)	3609	Maricopa (AZ)	2.25
Clark (NV)	83,419	Clark (NV)	20.30	Wayne (MI)	3060	Wayne (MI)	7.13
Queens (NY)	76,276	Queens (NY)	6.27	Harris (TX)	2814	Harris (TX)	1.72
Kings (NY)	73,892	Kings (NY)	4.65	New York (NY)	2552	New York (NY)	7.53

[a]Data presented are the top 10 US counties with the most confirmed total number of cases, followed by the current number of new cases per 100,000 in the county population. Similarly, the top 10 US counties with the most confirmed total number of deaths, followed by the case-fatality rate for those counties is presented.

SARS-CoV-2, some eQTLs associated with lung tissue expression appeared to be associated with test positivity. In a GWAS of 835 SARS-CoV-2–infected cases and 1255 population-derived controls from Italy, and 775 cases and 950 controls from Spain, there was an association with rs11385942 at chromosome 3p21.31.[70] Among the six genes associated with this locus, *SLC6A20* encodes the sodium/amino-acid (proline) transporter 1 (SIT1) that functionally interacts with ACE2.

In silico analyses have identified 21 single nucleotide polymorphisms that may impact splicing, microRNA regulation, post-translational modifications, and protein folding of TMPRSS2.[71] Other investigators have identified coding single nucleotide polymorphisms that are predicted to cause a loss of function of TMPRSS2 and eQTL variants that impact *TMPRSS2* expression.[72] In both studies, differences in allele frequencies in different populations were hypothesized to underlie differences in the prevalence of COVID-19. However, no direct clinical correlations have been made in COVID-19 patients.

ABO Blood Group

In other infections, the ABO protein may serve as a receptor or a co-receptor for bacteria, parasites, and viruses. Recent studies have suggested that ABO antigens modify the cellular distribution of receptors and, depending on which blood group antigens are expressed, differentially modulate spike protein binding to the host cell.[73] Several studies have associated the A and the O blood group with higher and a lower susceptibility and severity of COVID-19 infection, respectively.[70,74-77]

Human Leukocyte Antigen

Human leukocyte antigen molecules form a complex with small pathogen-derived peptides at the surface of infected cells, which is recognized by CD8+ or CD4+ T lymphocytes to trigger an immune response. Human leukocyte antigen molecules are encoded by several genes that together are characterized by several thousand alleles with significant heterogeneity. Using a bioinformatics strategy, Barquera et al[78] determined the binding affinities between 438 HLA proteins and peptides from the SARS-CoV-2 proteome. They found that the frequencies of strongest and weakest HLA binders differed among populations from different geographic regions, suggesting differences among populations in protection against SARS-CoV-2. Among 669 cases from the UK Biobank who tested positive for SARS-CoV-2, a single HLA variant (DQA1_509, $P=1.0\times10^{-5}$) was enriched in positive cases.[76] However, in a GWAS of 835 SARS-CoV-2–infected cases and 1255 population-derived controls from Italy, and

775 cases and 950 controls from Spain, no associations were found with HLA loci.[70]

Toll-Like Receptors

The TLR pathway activates the immune system and inflammation. Genetically engineered mice that are null for various members of the TLR pathway exhibit greater susceptibility to infection by SARS-CoV.[79,80] Therefore, it is speculated that variants in *TLR* genes in humans may also modulate susceptibility to infection by SARS-CoV-2.[81] Indeed, in a small case series of four male patients belonging to two families requiring intensive care unit admission, loss-of-function variants were identified in *TLR7* (c.2129_2132del, p.[Gln 710Argfs*18] and c.2383G>T, p.[Val795-Phe]) and these variants were found to be associated with impaired type I and II interferon responses in vitro.[82]

Other Genes

There are emerging reports implicating variation in other genes in the susceptibility to COVID-19 infection. In the UK Biobank SARS-CoV-2–positive case-control GWAS cited above,[69] a significant association with having a positive SARS-CoV-2 test was found for rs286914 (OR, 1.52) in erythroblast transformation specific homologous factor (*EHF*), a transcriptional repressor involved in lung inflammation and response to injury and a modifier of disease severity in cystic fibrosis. In a comparison of whole-exome sequencing data from 35 COVID-19 cases and 150 controls in Italy, a gene burden test of loss-of-function variants identified olfactory receptor family 4 subfamily C member 5 (*OR4C5*) and Kruppel-associated box zinc-finger protein 717 (*ZNF717*) as protective.[83] *OR4C5* may participate in natural immunity leading to virus and cell death. *ZNF717* is a transcriptional regulator involved in a range of cellular processes, including cell proliferation, differentiation and apoptosis, and in the regulation of viral replication and transcription. Using a candidate gene approach, Zhang et al[84] investigated a synonymous variant (rs12252) in the interferon-induced transmembrane protein 3 gene (*IFITM3*) in 80 Chinese cases hospitalized with COVID-19. *IFITM3* encodes an immune effector protein critical to viral restriction and acts to restrict membrane fusion. There was an association between homozygosity for the C allele (CC vs CT/TT) and disease severity (OR, 6.37). Finally, an uncontrolled observation was made that 71% of male patients hospitalized with COVID-19 had clinically significant male androgenetic alopecia, whereas the expected prevalence of a similar age-matched White population is approximately 31% to 53%.[85] Interestingly, *TMPRSS2* has an androgen response element, and androgens are known to increase *TMPRSS2* transcription.

A recently published GWAS identified variants in or near genes that are linked to host antiviral defense mechanisms (oligoadenylate synthetase — *OAS1*, *OAS2*, *OAS3*, interferon receptor — *IFNAR2* genes) and the inflammatory response (tyrosine kinase 2 — *TYK2*, and dipeptidyl peptidase 9 — *DPP9* genes) that were significantly associated with critical illness in COVID-19 patients. These genes could potentially be targeted by precision medicine approaches in genetically susceptible individuals to prevent progression to critical COVID-19 disease.[86]

Ancestral Haplotypes

A haplotype on chromosome 3 inherited from *Vindija 33.19* Neanderthals, who lived approximately 50,000 years ago in southern Europe, confers an OR for requiring hospitalization from COVID-19 of 1.6 (95% CI, 1.42 to 1.79).[87] The Neanderthal haplotype appears in South Asia at a frequency of 30%, in Europe at 8%, among admixed Americans at 4%, in East Asia at very low frequencies, and in Africa at almost zero frequency. Thus, differences in the frequency of the Neanderthal haplotype may underlie differences in susceptibility to severe COVID-19 among populations.

VIROLOGIC DETERMINANTS OF COVID-19 TRANSMISSION AND SEVERITY

Heterogeneity in infection risk or disease severity may not just to be due to differences in the host but may also partially derive from virologic diversity. Mutations in viral genomes have long been recognized to

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contribute to transmission and escape from pre-existing immune responses (eg, influenza virus), or to antiviral treatment response (eg, HIV). Likewise, evidence suggests that SARS-CoV-2 is continually evolving genetically. Despite an apparently slow mutation rate,[88] the sheer scope of the pandemic has caused widespread evolution globally[89-91] and the emergence of seven viral clades.[92,93] Consequently, data are emerging that viral mutations may affect disease severity or transmission. For instance, a 382-nucleotide deletion (Δ382) in the open reading frame 8 (ORF8) region of the SARS-CoV-2 genome has been associated with milder disease in Singapore.[94] On the other hand, the D614G variant in the SARS-CoV-2 spike protein may increase transmissibility through improved viral fitness without affecting disease severity.[95] It is likely that additional viral mutations will be described that affect response to antiviral therapies. Together, these emerging data suggest the importance of obtaining viral genotypes to risk stratify patients or personalize therapeutic options.

PHARMACOLOGICAL THERAPIES FOR THE TREATMENT OF COVID-19: NOT ALL PATIENTS RESPOND THE SAME

There are currently 4397 clincial trials registered in Clinicaltrials.gov as of January 7th, 2021, with 2482 being treatment or interventional trials. Although specific patient characteristics predicting response to COVID-19 therapies have not yet been described, patient characteristics that predict hospitalization and worse outcomes have. When thinking about precision medicine approaches to COVID-19 treatment, it is important to recognize that many published studies of COVID-19 therapies have focused on hospitalized patients or those who require intensive care unit admission and/or ventilator support. With this framework in mind, results from select randomized clinical trials for COVID-19 therapies are discussed below to highlight the variability in response and its implications for developing precision medicine approaches.

Outpatients

The high prevalence of COVID-19–positive patients who are not hospitalized makes it an important group to study, but the low event rate in these patients and the uncertainty in predicting those who will develop complications poses significant challenges to demonstrate efficacy of a therapeutic intervention. An example of this challenge is the recently completed placebo-versus-hydroxychloroquine clinical trial in which 821 asymptomatic subjects were enrolled, most of whom had a high-risk exposure to a COVID-19 patient.[96] Hydroxychloroquine blocks viral entry by inhibiting glycosylation of host receptors and endosomal acidification and shows in vitro activity against SARS-CoV-2.[97] The incidence of new COVID-19 disease in this trial was low at 13% and just two hospitalizations occurred in the overall study population during follow-up. Study participants were predictably low risk, with the median age being 40 years; the majority of the participants were women and more than 70% did not have comorbidities. This study, which was powered to show a 50% relative reduction in new symptomatic infections, had low-risk participants enrolled and a low event rate resulting in no benefit being shown for the use of hydroxychloroquine as compared with placebo. Therefore, development of a predictive tool using demographic, clinical, biochemical, and genetic parameters to identify high-risk patients may be especially important to deliver individualized care rather than therapy for all. A randomized controlled trial (NCT04332991) being funded by the National Heart Lung Blood Institute to evaluate the safety and effectiveness of hydroxychloroquine was stopped on June 20, 2020, after enrolling 479 of its intended 500 participants, stating that the drug was unlikely to be of benefit to hospitalized patients with COVID-19.[98] Also, in June 2020, the US Food and Drug Administration revoked the emergency use authorization for hydroxychloroquine, citing emerging scientific data that the drug is unlikely to be effective in treating COVID-19.[99]

Hospitalized Patients

Hospitalized COVID-19 patients (more than 88% were critically ill) had a shorter time to recovery when treated with remdesivir as compared with placebo.[100] Remdesivir is an investigational inhibitor of viral RNA-dependent RNA polymerase and was shown to be active against SARS and Middle East respiratory syndrome.[101] There were statistically significant differences in the prespecified subgroups indicating heterogeneity in response. For example, White patients seem to derive benefit with remdesivir as opposed to Black or Asian patients, and a similar trend was observed in non-Hispanic patients as compared with Hispanic patients. Although such findings do not lead to the conclusion that the drug should or should not be used in these subgroups, they do emphasize the importance of representing various racial and ethnic groups in COVID-19 clinical trials to assess for biological differences in disease presentation and response to interventions.

The role of corticosteroids in the treatment of acute lung injury and acute respiratory distress syndrome is controversial.[102,103] The Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial showed that dexamethasone 6 mg daily for a median of 7 days (interquartile range, 3 to 10 days) reduced the 28-day mortality rate by 17% as compared with usual care.[104] There appeared to be variable response with a greater reduction in mortality in the subgroup of patients on oxygen (rate ratio 0.82; 95% CI, 0.72 to 0.94) and invasive mechanical ventilation (rate ratio 0.64; 95% CI, 0.51 to 0.81) at the time of randomization, but no benefit in those not receiving respiratory support (rate ratio 1.19; 95% CI, 0.91 to 1.55). The patients who were on respiratory support at randomization were younger, a greater proportion was male, and they had less comorbidity such as heart disease as compared with those not receiving respiratory support, which may account for the favorable response to treatment.

Using Biomarkers to Guide Individualized COVID-19 Therapy

Sophisticated immunophenotyping has shown the distinct heterogeneity in response to COVID-19 infection that may have implications for individualized treatment.[105] For example, the occurrence of subset-selective T cytopenia in COVID-19 disease that is similar to H1N1 influenza has prompted a clinical trial that is testing the efficacy of interleukin-7 (IL-7) in COVID-19 patients with lymphopenia.[106] Early measurement of IL-6, IL-10, and interferon-γ inducible protein (IP-10) elevated levels of which could help identify patients who are at risk for a poor prognosis and increased length of hospitalization[105] potentially could be used to enable early and swift intervention. Highly elevated IP-10 in this study appeared to segregate patients who developed hyperinflammation and such immune signatures perhaps could be used to identify patients who may benefit from monoclonal antibodies targeting pro-inflammatory cytokines.

IMPLICATIONS OF COVID-19 ANTIBODY TESTING FOR PRECISION MEDICINE

Severe acute respiratory syndrome coronavirus 2 antibody testing has been reported to have clear utility for determining the number of individuals with a positive antibody test within a population at a single time point, or at repeated time points, to obtain information about the true prevalence of disease and determine the proportion of asymptomatic to symptomatic cases, the infection fatality ratio (proportion of deaths to total number of infections), and for modeling purposes (with the caveat that the study sample must be representative of the population of interest).[107,108] Recently SARS-CoV-2 antibody testing was used to retrospectively test blood donations from sera collected from December 13, 2019, to January 17, 2020, and demonstrate that virus was present in the United States at that time, underscoring how antibody testing can provide invaluable information at the population level.[109]

Coronavirus Structure and Viral Antigens Used for Antibody Tests

Coronavirus[110,111] genome encodes four structural proteins, envelope (E), membrane (M), nucleocapsid (N) and spike (S)

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proteins, as well as 16 nonstructural proteins. The spike protein contains the S1 domain responsible for receptor binding (also known as the receptor binding domain [RBD]), and the S2 domain, responsible for facilitating fusion and entry into host cells. The S2 domain is highly conserved among coronaviruses,[112] whereas the S1 domain is the most unique.[113] The S1 RBD of SARS-CoV-2 is also responsible for inducing the host immune response.[114,115] In fact, the SARS-CoV-2 RBD is the primary inducer of neutralizing antibodies, antibodies that can bind to the virus and prevent infection by blocking the viral replication process.[113-115] Currently available antibody tests predominantly target the spike protein and/or the nucleocapsid protein.[116] Antibody tests that target the nucleocapsid protein are more sensitive, whereas those targeting the S1 protein have been touted as more specific.[113,116]

Indications for Testing

The benefit of SARS-CoV-2 antibody testing at the individual level is currently being debated. Although some have proposed that individuals who have SARS-CoV-2 antibodies will be immune to recurrent disease, there are currently no data to support this assumption. There is also no consensus on what level of antibodies (and which antibodies) would confer individual immunity. Furthermore, there have been concerns about false-positive results (and resulting false reassurance) that may occur due to cross-reactivity with the presence of antibodies to other coronaviruses, such as the alpha and beta coronaviruses that cause the common cold, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus; recently machine learning approaches have been used to address this concern.[107,117] Unknown sensitivity, specificity, and positive and negative predictive values have contributed to reluctance to recommend use of these tests for individual patient-care decisions.[118-120]

One instance where SARS-CoV-2 antibody tests may have utility in individual cases is in helping with the diagnosis of COVID-19 infection if the patient presents late in the course of their disease when polymerase chain reaction testing for viral antigens may be negative.[121,122]

Variability in Patient Characteristics and Seroconversion

Data on whether seroconversion is different based on different patient characteristics are just beginning to emerge. Several investigators have observed that individuals with more severe disease and worse outcomes have higher titers of SARS-CoV-2 antibodies compared with those with less severe disease.[35,123-126] Individuals with mild disease also appear to be much more likely than those with more severe disease to revert to seronegativity in the convalescent period or to never seroconvert at all.[125] This difference in response is even more apparent in asymptomatic individuals with reports of ~80% of asymptomatic individuals never generating an antibody response at all[125] and ~40% of asymptomatic individuals reverting to seronegativity in the convalescence period.[35]

Some investigators have focused specifically on patient characteristics that determine production of neutralizing antibodies in response to SARS-CoV-2. In one study, patients who were 31 years and older developed higher neutralizing antibody level than those who were 16 to 30 years old.[126] This was confirmed in another study using slightly different age cut-off values (40 to 85 years old vs 15 to 39 years old).[28] Others have observed that asymptomatic individuals had lower neutralizing antibody levels compared with symptomatic individuals.[35] Importantly, it has been reported that up to 30% of individuals infected with SARS-CoV-2 developed very low or undetectable levels of neutralizing antibodies.[28]

A recent study used a deep immune profiling approach and integrated it with clinical data to define distinct immunotypes (characterized by variable activation of, and proliferation of, different subsets of B and T cells) that predicted response to COVID-19.[127,128] Data from these types of approaches have important implications for development of therapeutics and vaccines for COVID-19. For instance, determining which subsets of

B or T cells may be protective in the setting of natural infection may provide a target for vaccine development and a correlate measure of induced immunity in the absence of large-scale efficacy trials.

VACCINATION FOR COVID-19: VARIABLE RESPONSE AND TARGETING THE HIGH-RISK PATIENT

The race to develop a safe and efficacious vaccine against SARS-CoV-2 has been unprecedented, with more than 150 potential vaccines in various stages of clinical and pre-clinical development worldwide in less than 6 months since the start of the pandemic.[129]

There are several challenges in vaccine development for COVID-19 that has specifically related to heterogeneity in response.[130] An important concern is the risk of disease enhancement that was addressed in a recent consensus report.[131] Another is the concern that the efficacy of candidate vaccines may be diminished in elderly individuals, who are more susceptible to serious infection and death from COVID-19. In addition, both the innate and adaptive immune responses are altered and less effective in many people as they age.[132] Genetic and environmental factors that account for a wide range of variability in response to vaccines have not yet been well elucidated in COVID-19. Deep immune profiling has defined distinct immune responses, characterized by differences in activation of subsets of B and T cells, to infection with COVID-19.[127,128] Understanding the characteristics that lead to these distinct immunotypes has important implications for vaccine development. If indeed an immune response profile to COVID-19 disease exposure is determined to be protective as in convalescent plasma, those individuals who comprised one of the immunotype groups that were unable to mount any immune response to COVID-19 infection may translate to them having a similar lack of response to a vaccine.

Results of the first phase 1 COVID-19 vaccine trial were recently reported.[133] Although the number of participants was low (N=45; with 15 in each dosing regimen) and relatively homogenous (age range, 18 to 55 years; 89% white), there was significant variability in immune response within each dosing regimen, as measured by anti-spike antibody titer and serum-neutralizing activity. The amount of variability between vaccine recipients may be dramatic. In preliminary reports of the Oxford vaccine,[134] anti-spike immunoglobulin G antibody response units varied between vaccine recipients by up to 3 orders of magnitude. Similar variability in antibody production titer has been described in early phase studies of different vaccine formulations.[135,136] It will be important to identify patient characteristics that lead to this variability in future trials. It would also be important to identify vaccine formulations that lead to less variability in response. However, in the current state, it is difficult to make these comparative analyses between vaccine trials due to nonstandardized assays measuring immunogenicity correlates. In addition to variability in response to vaccination, it will be important to consider targeting patients at high risk for COVID-19 outcomes given the initial logistic challenges of vaccinating the entire population.

FUTURE DIRECTIONS — RECOGNIZING AND ADDRESSING HETEROGENEITY IN COVID-19 DISEASE USING ARTIFICIAL INTELLIGENCE, DIGITAL PLATFORMS, SENSOR TECHNOLOGY, AND PERFORMING LARGE-SCALE GENETIC STUDIES

Predicting the heterogeneity in presentation and outcomes becomes especially important in a pandemic when the availability of resources such as intensive care unit beds and ventilators may be limited. Once a COVID-19 test is positive, determining the at-risk patient (Figure 6) becomes important not only from a surveillance perspective to be able deliver effective care expeditiously but also enables individualized targeting of those patients for medical intervention that may attenuate disease course. The rapidity of disease progression almost makes it imperative to be able to use electronic health records to develop predictive algorithms to identify high-risk patients. In one example, a clinical decision support tool was developed to assess COVID-19 disease severity using a multiplex

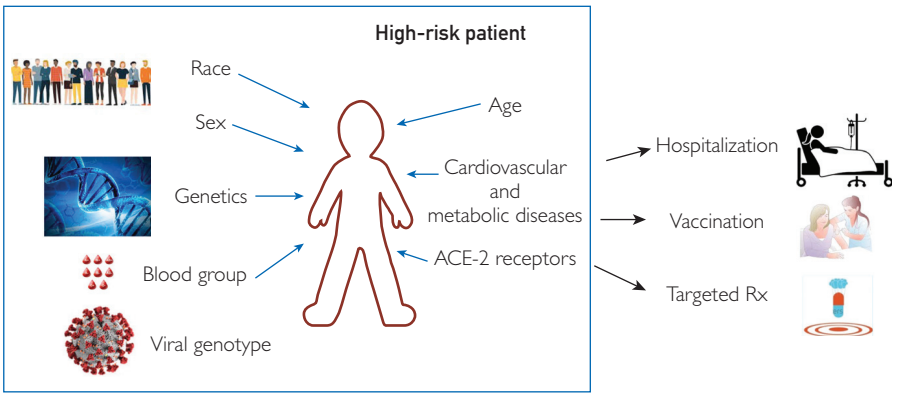

FIGURE 6. Proposed schematic depicting the demographic and biological variables that may characterize the high-risk patient. ACE-2, angiotensin-converting enzyme 2; Rx, drug therapy.

and multiclass platform with demographic data such as age and sex and biological data such as cardiac troponin, C-reactive protein, procalcitonin, and myoglobin levels of 160 hospitalized COVID-19 patients.[137]

An increasingly important component of precision medicine is the ability to obtain individualized data using digital technology such as smartwatch applications or digital monitoring systems that collect heart rate, temperature, pulse oximetry, and sleep patterns that may efficiently and quickly identify infection and clinical decline using artificial intelligence algorithms (WSJ Pro Artificial Intelligence Hospitals) allowing monitoring of some coronavirus patients at home[138]; Thomas Reuters Germany launched a new smartwatch application to monitor coronavirus spread.[139,140] Predictive and pre-emptive platforms using artificial intelligence to analyze electronic health records on a large scale coordinated with information obtained from individual patients using digital and sensor technology need to be undertaken. Identifying the high-risk patient is important in the context of the high-risk patient being exposed to and subsequently developing COVID-19 disease. The risk of infection can be pre-emptively identified for geographical locations in which high-risk patients reside, by using mobile phone data to track population outflows from hot spots.[141] A high-risk patient could also be identified by mobile phone application technology that stores contacts of COVID-19 patients using Bluetooth signals when they may be in the asymptomatic infectious phase of the illness.[142] The clinical use of digital technology that collects individual patient information remains challenging due to patient confidentiality issues; therefore, appropriate electronic informed consent should be obtained and data should be collected using a Health Insurance Portability and Accountability Act–compliant digital platform.[143] In addition, separate passwords for clinical applications, encryption of devices used for clinical work, secure data storage, and protected patient Web portal for communication should be used.[144]

As discussed above, an important host factor that determines susceptibility and severity of COVID-19 illness is the host genome. Although our understanding of the genetic determinants of COVID-19 has improved, its role in susceptibility to the disease remains limited. Identifying such genetic loci in an agnostic manner may not only assist in determining the risk profile of the patient but also could provide insight into the pathophysiology of disease and identify potential therapeutic targets. The findings of the genetic studies summarized above will require validation in additional cohorts with long-term follow-up. To successfully perform such studies, large consortia are required and one such collaboration is the COVID-19 host genetics initiative that comprises of array-based genotyping in 69% and exome/

whole-genome sequencing in 29% of the participants studies. The ultimate goal of such digital and genomic tools in the modern era is to be able to recognize individual differences to improve health for all using precision medicine.

CONCLUSION

Coronavirus disease 2019 is making a rapid resurgence around the world. There are demographic characteristics such as age, race, ethnicity, sex, and biological differences in factors such as ACE2 expression, immune regulation, and genetics that define the well-known variability observed in COVID-19 disease manifestation, susceptibility, and progression. Identifying and validating these individual differences and leveraging digital platforms including the use of artificial intelligence in developing predictive algorithms may help in individualizing targeted therapy including hospitalization and assist in the logistics of vaccine administration.

Abbreviations and Acronyms: **ACE** = angiotensin-converting enzyme; **eQTL** = quantitative trait loci; **GWAS** = genome-wide association studies; **TMPRSS2** = transmembrane protease serine 2; **TLRs** = Toll-like receptors

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