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Role of Genetic Variants and Gene Expression in the Susceptibility and Severity of COVID-19

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Since its first report in December 2019, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly emerged as a pandemic affecting nearly all countries worldwide. As the COVID-19 pandemic progresses, the need to identify genetic risk factors for susceptibility to this serious illness has emerged. Host genetic factors, along with other risk factors may help determine susceptibility to respiratory tract infections. It is hypothesized that the *ACE2* gene, encoding angiotensin-converting enzyme 2 (ACE2), is a genetic risk factor for SARS-CoV-2 infection and is required by the virus to enter cells. Together with ACE2, transmembrane protease serine 2 (TMPRSS2) and dipeptidyl peptidase-4 (DPP4) also play an important role in disease severity. Evaluating the role of genetic variants in determining the direction of respiratory infections will help identify potential drug target candidates for further study in COVID-19 patients. We have summarized the latest reports demonstrating that *ACE2* variants, their expression, and epigenetic factors may influence an individual's susceptibility to SARS-CoV-2 infection and disease outcome.

Key Words: Angiotensin-converting enzyme 2 (*ACE2*) variants, Transmembrane protease, Serine 2 (*TMPRSS2*), Epigenetics, COVID-19, SARS-CoV-2 infection

INTRODUCTION

Coronavirus disease 2019 (COVID-19) emerged in Wuhan city, China, in December 2019. On January 12, 2020, the World Health Organization (WHO) named the causative virus as novel coronavirus (nCov); the virus was renamed on February 11, 2020, as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the international virus classification commission [1]. During the initial days of the epidemic, various countries followed and implemented different testing strategies, based on the accessibility of diagnostic methods [2, 3]. COVID-19 causes respiratory illness that may range from a mild viral pneumonia to acute respiratory distress syndrome (ARDS) leading to multi-organ failure. The elderly population and individuals suffering from chronic illness have to be extra cautious, as the mortality rate is considerably high among these groups [4]. The massive spread of the virus across countries within a few months of onset led the WHO to declare COVID-19 a pandemic. As of July 19, 2020, the total number of cases and deaths worldwide stood at 14,043,176 and 597,583, respectively [5].

SARS-CoV-2 is transmitted to lung epithelial cells through aerosols. Various immune cells in the body may also augment the infection; peripheral neutrophilia and lymphocytopenia significantly induce the immune response and cause deleterious ARDS, multi-organ dysfunction, and finally the death in some patients. Additionally, a sudden surge of pro-inflammatory cyto-

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kines may lead to fatal outcomes, indicating that cytokines are crucial in the pathophysiology of COVID-19 [6].

These findings have raised the question of whether genetic or epigenetic variation can be used to identify symptom susceptibility or severity in this disease. A review of a number of studies has suggested that genetic factors along with other risk factors can determine an individual's susceptibility to respiratory tract infections [7]. As angiotensin-converting enzyme 2 (ACE2) receptor on host cells acts as an entry point for SARS-CoV-2, it is hypothesized that the *ACE2* gene constitutes a genetic risk factor for SARS-CoV-2 infection [8]. In this regard, *ACE2* variants previously reported to be associated with other disorders, such as hypertension and other cardiovascular diseases, pose promising genetic factor candidates [9, 10]. Moreover, variation in the expression of *ACE2* and associated epigenetic factors may play a significant role in determining an individual's susceptibility to COVID-19. This review provides an update on the possible role of genetic variants, gene expression, and epigenetic factors in the pathophysiology of COVID-19.

Coronaviruses

Coronaviruses are members of the enveloped single-stranded RNA viruses that cause respiratory, enteric, and cardiovascular diseases in humans and animals. Various coronaviruses, such as NL63, 229E, and OC43, have been found to be altered for human spread [11]. Many different types of coronaviruses have arisen from various bat species in China; these are mostly direct SARS-CoV ancestors [12]. Initially, the SARS-CoV that triggered an epidemic in humans originated from Chinese horseshoe bats, *Rhinolophus*, which originally adapted to the wild Himalayan palm civet [13]. The novel betacoronavirus (SARS-CoV-2) that causes COVID-19 shares 79.5% nucleotide identity with SARS-CoV [14].

During earlier SARS outbreaks, studies demonstrated, in an autopsy series using culture techniques, viral isolation, and *in situ* hybridization, that SARS-CoV exists in pneumocytes [15, 16]. The existence of coronavirus-like particles and viral particles in pneumocytes has also been revealed by transmission electron microscopy [17]. Further evidence indicates that ACE2 serves as the functional receptor for SARS-CoV, suggesting that alveolar pneumocytes are a potential entry point for SARS-CoV in the lung [18]. ACE2 staining was strongly positive for type I and II pneumocytes, whereas weak staining was observed in bronchial epithelial cells.

ACE2 structure and function

ACE2 is an ACE homologue discovered in 2000. The *ACE2* gene is found on chromosome Xp22 and comprises 18 exons [19]. ACE2 has only one catalytic domain, acts as a zinc metallopeptidase, and its distinct and counterbalancing functions in the renin-angiotensin system are characterized by different substrate specificities. ACE2 functions as a simple carboxy peptidase; it hydrolyzes angiotensin I (Ang I) to Ang 1-9 and Ang II to Ang 1-7 [20]. ACE2 has higher affinity to Ang II than to Ang I; its catalytic activity with Ang II is 400-fold higher than with Ang I [21].

ACE2 has multiple roles including catalytic activities with specific substrates; it acts as a negative regulator of the renin-aldosterone system, B0 AT1 amino acid transporter, and receptor for coronaviruses (SARS-CoV) [22]. ACE2 restricts the adverse effects of Ang II on profibrotic and vasoconstrictors. The hydrolysis of Ang II into Ang 1-7 decreases oxidative stress, and Ang 1-7 has counter regulatory mechanisms including antifibrotic and vasodilatory actions. Consequently, ACE2 disturbance leads to elevated Ang II levels and reduced heart function [22]. ACE2 is active in several tissues and is widespread in the lungs, kidneys, heart, and testis. Low levels of *ACE2* mRNA expression have been associated with hypertension and heart failure and reduced levels of cardiac ACE2 have been reported in hypertension and diabetic heart disease. These multiple physiological roles of ACE2 have been hijacked by SARS-CoV-2 to serve as a receptor, resulting in COVID-19 [23].

ACE2 as SARS-CoV-2 primary receptor

Previously, Li, *et al*. [24] reported that ACE2 is a key receptor for the HCoV-NL63 coronavirus, which caused the SARS outbreak. In addition to SARS-CoV-2, the ACE2 receptor has been linked with MERS and Hartnup disorder [25]. Cellular ACE2 expression levels differ among individuals. A recent study reported that the distribution of ACE2 expression is organ-specific [26].

ACE2 variation and SARS-CoV-2 receptor binding domain

ACE2 utilization by SARS-CoV-2 can be explored to gain insights into the mechanism of restricting SARS-CoV-2 entry into host cells. Although ACE2 is expressed in most vertebrates, SARS-CoV-2 cannot always utilize it as a receptor. The ability of SARS-CoV-2 to utilize ACE2 was predicted by observing few essential single amino acid (AA) variant sites, which were found to be important for SARS-CoV-2 [27]. Recent reports showed that SARS-CoV-2 can utilize ACE2 expressed in humans, Chinese horseshoe bats, swine, and civets, but not that expressed in mouse [28]. In another study, Qiu, *et al*. [29] evaluated ACE2 homology

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among five different species and observed 11 discrete mouse AA residues (T20, Y83, S218, A246, K353, P426, T593, N636, A714, R716, and A774), which are conserved in other ACEs. Although the binding of human ACE2 and the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein is quite complex, out of the 11 conserved AA residues, only three (T20, Y83, and K353) are located near the interface and thereby may facilitate binding [29]. Consequently, AA residues considered important for SARS-CoV receptor binding may also be critical for SARS-CoV-2 as their RBD AA sequences are 74.6% identical. Of the previously reported important sites (K31, Y41, K68, Y83, K353, D355, R357, and M383) [30], substitutions in K68 and M383 slightly inhibit SARS-CoV binding, while those in K31D, K353 H/A/D, D355A, Y41A, Y83F, and R357A strongly inhibit the SARS-CoV binding and could also abolish the interaction [31]. Using these substitution methods, Qiu, et al. [29] identified nine critical AA sites and evaluated various types of ACE2 utilization by SARS-CoV-2.

Structural basis for SARS–CoV–2 recognition

In SARS-CoV, receptor recognition and membrane fusion occur through the viral S glycoprotein, which is present on the surface. As soon as the virus enters the host, the trimeric S protein is split into two subunits, S1 and S2, followed by viral release of the S1 subunit for post-fusion confirmation. Subsequently, the S1 subunit binds with the peptidase domain of ACE2 via its RBD, whereas membrane fusion takes place via the S2 subunit. Once the S1 subunit binds to the ACE2 peptidase domain, a single cleavage site is exposed on S2, which is subsequently sliced by various host proteases. This mechanism is crucial for viral infection. The SARS-CoV-2 S protein can also take advantage of ACE2 for host infection in a similar manner.

ACE2 also serves as a chaperone for membrane trafficking of the AA transporter B0 AT1(SLC6A19). B0 AT1 is involved in sodium-dependent uptake of neutral AA in intestinal cells. With the stabilization of B0 AT1, the comprehensive structure of ACE2 in a dimeric assembly has been reported by high-resolution studies [23]. Docking of the S protein trimer onto the structure of the ACE2 dimer together with the RBD of the S protein revealed that two S protein trimers bind to an ACE2 dimer simultaneously. Using a structure-based rational strategy, neutralizing antibodies or decoy ligands with greater affinities for either coronavirus S protein or ACE2 could be produced for viral infection treatment modalities [23].

ACE2 variants and their association with SARS–CoV–2

Recently, it was suggested that ACE2 variants affect ACE2 levels in humans. To validate this, the Leeds Family Study estimated ACE, ACE2, and neutral endopeptidase plasma levels in 534 individuals. It was predicted that various genetic profiles could account for up to 67% of the phenotypic variation in circulating ACE2 [32]. Of the various ACE2 variants, rs2106809 was hypothesized to primarily affect the circulating ACE2 levels that are comparatively higher in the CC or CT genotype than in the TT genotype. Moreover, microRNAs (miRNAs) can act as intermediaries in potential pathways that can regulate endothelial activities through posttranscriptional degradation and/or translational repression [33]. Important variations in the intermolecular interaction between the SARS-CoV-2 S protein and ACE2 alleles have been elucidated by molecular superimposition and comparative modeling. Of note, the ACE2 alleles, rs73635825 (S19P) and rs143936283 (E329G), produce proteins demonstrating weaker binding affinity and missing key residues for SARS-CoV-2 S protein complex formation. These findings might indicate an intrinsic resistance mechanism to SARS-CoV-2 infection [34]. Thus, these alleles might undergo positive selection under new selection pressure provided by SARS-CoV-2. However, as protein-protein and ligand-protein interactions are vital, molecular dynamic simulation could be used to prove an important interlink between various ACE2 alleles and the SARS-CoV-2 S protein. Thus, analytical means for predicting the binding affinity of intermolecular complexes may offer valuable insights. Furthermore, the correlation between viral binding and ACE2 variants in patients could help identify various mechanisms underlying the pathophysiology and severity of COVID-19.

ACE2 expression in individuals across different ethnicities

ACE2 expression at cellular levels differs among individuals. Both ACE2 variants and their expression might affect individual susceptibility to SARS-CoV-2 infection and disease severity [14]. This information might provide a means for controlling viral infection by suppressing ACE2 expression levels in cells. COVID-19 results in progressive respiratory failure and sometimes even death owing to alveolar lung damage. The binding affinity of SARS-CoV-2 to ACE2 is 10–20-fold higher than that of SARS-CoV [35]. Recent epidemiological studies on COVID-19 positive cases from China have reported that the overall incidence of COVID-19 is marginally higher in males; however, the proportion of males far exceeds that of females among chronically ill and fatal cases. Individuals over 60 years of age or those with chronic illnesses, such as hypertension and type 2 diabetes mellitus,

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are often associated with systemic inflammation or cytokine storm. Variations in immunity and ACE2 expression may lead to specific vulnerability and intensity of SARS-CoV-2 infection [36, 37].

In East Asia, the viral infection has spread exponentially; older males, in particular, are more affected by SARS-CoV and the recent SARS-CoV-2 infection. *ACE2* expression, which varies between males and females, also differs between the world's three main racial groups: African, Asian, and Caucasian. Recently, Chen, *et al.* [38] reported higher expression of *ACE2* in some organs, such as the adrenal gland, adipose tissue, esophagus, and heart, of Asian females than that in males. Expression levels also vary among other ethnic groups. Expression levels are moderately increased in the lungs, blood vessels, muscles, and ovaries. Typically, *ACE2* expression decreases with age in both sexes and all ethnic groups. However, significant findings were only detected in Caucasian group, as the sample size was comparatively larger. Another important finding was that males had a more significant age-dependent decrease compared with females. For example, in the Caucasian group, few females demonstrated decreased *ACE2* expression with increasing age in the blood, colon, and adrenal gland. However, in males, the age dependent decrease in *ACE2* expression was observed in the blood, colon, adrenal gland, nerves, adipose tissue, and salivary glands [38]. The patterns of *ACE2* expression found in this study were entirely different from those revealed by other studies projecting the inductive effect of increased *ACE2* expression in the population on SARS-CoV-2 virus infection susceptibility and associated fatality.

Consistently, the prevalence of expression quantitative trait loci (eQTL) that show higher *ACE2* expression is nearly 100% in East Asians and >30% in other racial groups. *ACE2* expression at the molecular level depends on many factors such as the positive impact of estrogen and androgen levels (both decrease with age) and the binding of virus infection-related transcription factors at *ACE2* regulatory regions [39]. In addition, type 2 diabetes mellitus and inflammatory cytokines are involved in the repression of *ACE2* expression.

ACE2 expression analyses of the lung tissues of Asian and Caucasian group remain controversial. The ACE2 expressing cell ratio, detected using single-cell RNA-seq analysis, indicated that Asian donors had significantly increased *ACE2* expression compared with African-American and Caucasian donors [40]. However, no differential expression of *ACE2* has been observed between males and females or between younger and older individuals in normal human tissues [41]. As *ACE2*-expressing cells comprise a minor proportion of lung tissue cells [40], these conclusions may have been influenced by the purity of the *ACE2* positive cells and sample size. Cao, *et al*. [39] reported that the heterogeneity in *ACE2* expression among different ethnic groups can be explained by variations in the distribution and allele frequencies of *ACE2* eQTLs in divergent populations. In addition, the average difference in allele frequencies of eQTLs between South Asian and East Asians has suggested a potential deviation in *ACE2* expression in various populations and ethnicities in Asia. Table 1 summarizes the studies that have assessed *ACE2* variants and expression in association with susceptibility and disease severity.

ACE2 expression associated with age and SARS–CoV infection

A different trend of *ACE2* expression has been observed in children. In general, children with confirmed COVID-19 were mildly symptomatic or mostly asymptomatic. Children usually have higher plasma ACE2 levels than adults [42]. For example, ACE2 plasma levels in children (six months to 17 years of age) were 13–100 U/L compared with 9–67 U/L in the adult population (using a furanacryloyl-L-phenylalanylglycylglycine-based enzymatic activity assay) [33]. Fewer COVID-19 cases have been reported in children than in higher age groups. In a study including 1,099 patients in China, only 0.9% of the confirmed cases were under nine yrs of age and only 1.2% were 10–19 years old [37]. This phenomenon agrees with a previously reported mouse study, in which younger animals were found to be resistant to SARS-CoV infection [43]. The correlation between COVID-19 severity and age can be explained based on immune decline (immune-senescence), as well as an unusual child-specific plasma ACE2 profile. However, ACE can traverse the placenta; it helps the mother pass on her immunity and other serum antibodies to the baby. During the second and third trimester of pregnancy, ACE2 plasma and urine levels were found to be elevated along with an increase in local placental/uterine output and ACE2 functions. This explains the systemic hemodynamic role in placental-fetal blood flow increase and rapid growth of the fetus [44].

Transmembrane protease serine 2

In humans, the *TMPRSS2* gene encodes transmembrane protease, serine 2 (TMPRSS2), an enzyme belonging to the serine protease family. This protein contains a type 2 transmembrane domain along with a receptor class A domain. In addition, a scavenger receptor cysteine-rich domain and a protease do-

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Table 1. ACE2 variants and expression cause inter-individual variability and susceptibility to COVID-19 in different populations

Population	Reference	Method	Variants	Interpretation	Effect on ACE2
Italian population	[6]	Computational study using Network of Italian Genomes database	rs775181355 207 G>T or p.Val506Ala	p.Val506Ala is indeed the only AA change reported in the European non-Finnish population	Predicted as probably damaging for ACE2 protein structure by PolyPhen and deleterious by SIFT
Italian population	[6]	Computational study using Network of Italian Genomes database	p.Asn720Asp, p.Lys26Arg, and p.Gly211Arg	These variants are moderately expressed in Italian and European populations; they do not occur in the Eastern Asia population	Predicted to interfere with SARS-CoV-2 spike protein, thus destabilizing the protein structure
	[27]	Homology modelling predicted ACE2 allelic variants	rs73635825 (S19P) and rs143936283 (E329G)	Intrinsic resistance with these alleles owing to low binding affinity	Normal ACE2 expression but lack of some key residues in complex formation with SARS-CoV-2 spike protein
Population allele frequencies from 1,000 Genomes Phase 3	[31]	eQTLs in many tissues, from GTEx database	38 most significant eQTLs in ACE2	High (close to 100%) allele frequency in East Asians	All are positively correlated with higher ACE2 expression, indicative of their contributions to higher ACE2 expression in East Asians
East Asian, European, African, South Asian, mixed American	[32]	eQTL variants using GTEx database	rs4646127, rs2158082, rs5936011, rs6629110, rs4830983, and rs5936029 Lys26Arg, Ile486Val, Ala627Val, Ser692Pro, Asn720Asp, Asn638Ser, and Leu731Ile/P	Major allele frequency (most common variant) is high (>95%) in East Asian population compared with the European population (<50–60%)	Higher major allele frequency is associated with greater susceptibility and higher ACE2 expression.
European and East Asian	[45]	eQTLs variants, from GTEx database	rs2285666 (also called G8790A)	The A allele is more frequent in the Italian population	The AA genotype confers higher ACE2 expression level
European and East Asian populations	[45]	eQTLs variants, from GTEx database	rs463727, rs34624090, rs55964536, rs734056, rs4290734, rs34783969, rs11702475, rs35899679, and rs35041537	SNVs frequent in European population, whereas totally absent in Asian population	Two haplotypes were supposed to increase TMPRSS2 expression in an androgen-specific way

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; SNV, single nucleotide variant; eQTL, expression quantitative trait loci; GTEx, genotype-tissue expression; PolyPhen, Variant phenotyping; SIFT, sorting intolerant from tolerant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; AA, amino acid.

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main have also been identified [45]. These proteases are involved in several pathological and physiological processes that take place in the human body. *TMPRSS2* has been shown to be upregulated by androgenic hormones in prostate cancer cells; however, in androgen-independent prostate cancer, it was found to be downregulated [46].

TMPRSS2 can also cleave and activate the SARS-CoV-2 S protein during membrane fusion. This protease cleaves the SARS-CoV-2 receptor, the ACE2 carboxypeptidase, and ACE2 cleavage has been shown to augment viral entry into the various host cells [47]. TMPRSS2 also serves as an activator of SARS-CoV entry into the host cells via the same mechanism. It is mostly expressed in the epithelial cells of human lungs, absorptive enterocytes, and upper endothelial cells of the esophagus, and it activates influenza A virus and meta-pneumo virus in culture cells [48]. The virus replication stage at which proteases cleave the viral glycoproteins in the plasma membrane varies between SARS-CoV and other viruses such as meta-pneumo virus and influenza A virus [48]. In the influenza A virus and meta-pneumo virus, entry is enabled by TMPRSS2 making a simple cut in the cell membrane glycoprotein, whereas in SARS-CoV-2, the S protein is cleaved after the receptor induces a few conformational changes in protease structure [49]. Initially, the protease cleavage site in the S protein was predicted to be exposed after receptor binding; however, it was found to be located very near the C-terminal region of S protein [50]. Further studies have reported that the S protein of mouse hepatitis virus type 2 (MHV-2) usually undergoes conformational changes in two phases, which are facilitated by progressive receptor binding and proteolysis, to be activated for fusion [51]. The MHV-2 S protein was found to be similar to the SARS-CoV S protein. Fusion and effective cleavage, while the receptor binds to the target cells, is very tightly controlled. Premature proteolysis of the activating cleavage site is also prevented efficiently in this way [52].

Cellular factors required for SARS-CoV-2 entry might provide information regarding viral transmission. Thus, protease dependence of SARS-CoV-2 entry has been studied [47].

Endosome cysteine proteases cathepsin B/L (CatB/L) are employed for SARS-CoV-2 S protein priming in cells along with TMPRSS2 protease activity. Priming by TMPRSS2 is essential for viral entry and for further spread in the affected host, whereas CatB/L activity is non-essential. Various studies have indicated that SARS-CoV-2 infection rate is also affected by TMPRSS2 proteolytic activity, which raises questions regarding the residual S protein priming conducted by CatB/L. It is hypothe-

sized that furin-mediated pre-cleavage at the S1/S2 site in virally infected cells allows protease dependent entry into these cells, similar to MERS-CoV [47]. In addition, TMPRSS2 is required for homeostasis maintenance and various developmental changes and thus constitutes a drug target for future use. Importantly, TMPRSS2 protease activity is blocked by the serine protease inhibitor camostat mesylate.

Human trials have been recently approved in Japan for testing potential anti-pancreatitis agents; however, the condition is not associated with viral infection. These compounds and various others have been found to significantly increase antiviral activity and thus might be considered as an effective off-label treatment option for SARS-CoV-2-induced respiratory distress [47]. A recent study has reported that using a purified soluble form of TMPRSS11a, receptor binding to the pseudo-typed SARS-CoV S protein significantly enhanced virus multiplication rate. In addition, proteolytic cleavage was shown to be similar to trypsin [53]. A specific spatial orientation of the TMPRSS2 protease with respect to the SARS-CoV-2 S protein is a major factor underlying receptor binding. Additionally, receptor-bound S protein interaction with TMPRSS2 occurs in a specific spatial orientation at the cell surface resulting in inefficient cleavage of SARS-CoV-2 S protein and subsequent membrane fusion. Arginine and lysine residues within ACE2 AAs 697–716 have been predicted to be of prime importance for TMPRSS2-mediated cleavage and histone acetyltransferase activity. In addition, ACE2 needs to be processed for SARS-S-driven entry mediated by these proteases. TMPRSS2 increases SARS-S-entry, because it competes with metalloprotease A disintegrin and metalloprotease 17 for ACE2 processing [54].

TMPRSS2 variants

TMPRSS2 variants have been postulated to account for SARS-CoV-2 epidemiological data. Recently, allele frequency differences of rs12329760 (a known *TMPRSS2* variant) and eQTL haplotypes (genetic loci that explain genetic variations in mRNA expression levels) were reviewed in Italian and East Asian populations [55]. In the Italian population, death rates and variation in COVID-19 severity in both sexes were significantly higher than those in the East Asian population. Furthermore, the Italian population demonstrated a remarkable decrease in the load of detrimental TMPRSS2 protease variants compared with other Europeans, suggesting augmented TMPRSS2 protein/activity. Thus, TMPRSS2 plays an important role in disease severity. The catalytic triad of TMPRSS2 comprises a set of three coordinated AA, H296, D345, and S441, found at the active site of prote-

ases; this special catalytic pocket structure helps in the hydrolysis of the highly specific substrate protein. Comparison between the Italian and East Asian (also Europeans) populations showed that four variants exhibited significantly distinct frequencies. Three of these single nucleotide variants (SNVs) are synonymous, and one is a missense substitution, p.Val160Met. Because of this substitution, the serine protease catalytic triad is spared, and the effect takes place on a residue away from catalytic triad. Initially, this TMPRSS2 protease variant was shown to have functional links with the risk of prostate cancer [56]. In terms of eQTLs, several variants demonstrating a significant effect on *TMPRSS2* expression in the lung epithelial tissue [genotype-tissue expression (GTEx) data] have been reported in the 3' region of the gene sequence [55].

In particular, two different haplotypes can be inferred from the GTEx frequency data [55]:

the haplotype that is more regularly seen is the "European" haplotype that comprises SNVs rs463727, rs34624090, rs55964536, rs734056, rs4290734, rs34783969, rs11702475, rs35899679, and rs35041537. This haplotype was found to be co-expressed and co-regulated with an eQTL (rs8134378). The eQTL (rs8134378) for TMPRSS2 is located 13 kb upstream of an androgen responsive enhancer, possibly upregulating the gene in an androgen-specific manner [57]. However, this haplotype was missing in the Asian population.

Three SNVs, rs2070788, rs9974589, and rs7364083, comprise another haplotype, which was found to have increased *TMPRSS2* expression. Compared with East Asians, the Italian population had a 9% increase in this minor allele frequency [55].

Dipeptidyl peptidase–4 (DPP4)

DPP4 is a transmembrane glycoprotein. It is an ecto-peptidase that cleaves amino-terminal dipeptides causing T cell activation and hence functions in host cell immune-regulation against viral infections. It also functions as a binding protein and ligand of extracellular factors like collagen and fibronectin. DPP4 is expressed by the epithelial and endothelial cells of vessels, as well as by the kidneys, intestines, lungs, and smooth muscle cells of the vasculature. DPP4 accelerates lung inflammation and causes fatal respiratory distress in MERS-CoV infection. Both SARS-CoV-2 and MERS-CoV affect the lower respiratory tract and cause ARDS, thus suggesting a link between SARS-CoV-2 and DPP4.

Molecular interactions between SARS-CoV-2 and DPP4 have been studied via computational model-based docking and 3D

structures of the SARS-CoV-2 S glycoprotein and human DPP4 [58]. This model suggests a tight interaction between the S glycoprotein domains and DPP4 receptor. DPP4 binding residues K267, T288, A289, A291, L294, I295, R317, Y322, and D542 interact with the MERS-CoV S protein. The SARS-CoV-2 S1 domain has shown a similar -interaction with DPP4 suggesting that MERS-CoV and SARS-CoV-2 share similar pathways while interacting with host cells. Thus, DPP4 inhibitors, such as gliptins, could be used in COVID-19 patients to significantly reduce viral infection and multiplication rate, as well as reduce cytokine storm and inflammation in the lower airways [59].

Epigenetic aspects of immune response in SARS–CoV–2 infection

Macrophages and dendritic cells constitute key "hazard" signals among various cells of the innate immune system. These signals are stimulus-specific and cell-specific to ensure the onset of spatial and temporal responses. Such cell-specific signals are facilitated by cytokine, interleukin, and chemokine secretion or direct cell-cell communication. Consequently, their epigenetic ability to alter within minutes of a stimulus is not only important for rapid antiviral host response activation, but also necessary to maintain a sustained and specific defense response. It has been suggested that epigenetic regulation is accountable for virus entry priming and retention of this highly regulated host immune response over the initial activation wave [60].

Previous studies have focused on epigenetic aspects of the activation and formation of the innate and adaptive immune responses. The primary responsibility of the innate system is manifested via interferon and tumor necrosis factor genes. Interferons are effective mediators in preventing viruses from entering and activating the pathogenic immune response [61, 62]. Consequently, many viruses have possibly developed virulent mechanisms to counteract specific interferon-stimulated gene effectors [63]. Interferons and immune responses are sensitive to epigenetic modifications through different epigenetic pathways [64]. In contrast, interferon-stimulated genes generally show lower amounts of inducing histones, such as H3K4me3 and H4Ac, and lower RNA polymerase II occupancy [65]. Further chromatin remodelers and transcription factors are needed for the initiation of transcription at these genes including ATP-dependent chromatin remodeling complex SWItch / sucrose non-fermentable (SWI / SNF) [66, 67].

Viruses have also developed various pathways to antagonize and disrupt epigenetic regulatory mechanisms, such as viral protein interference with host histone modification enzymes

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[68], chromatin remodeling machinery, and modified histones [61]. Current epigenetic studies have focused on the effects of genetic processes on DNA, rather than emphasizing the role of the genetic code itself. Major areas of focus include chromatin remodeling, histone methylation, and many other processes that affect how transcription is initiated, as well as how DNA is packaged in cells. Recently, Pinto *et al.* have reported that histone modification (H3K4me1, H3K4me3, H3K27Ac) in *ACE2* gene is associated with increased *ACE2* expression and they have also mentioned upregulation of NAD-dependent histone deacetylase Sirtuin 1 (SIRT1) is associated with increased *ACE2* expression [69]. Similarly, Sawalha *et al.* have observed that decreased DNA methylation of *ACE2* promoter (hypomethylation) was associated with increased *ACE2* expression [70].

Corley, *et al.* [69] analyzed public datasets and revealed that DNA methylation levels associated with the *ACE2* gene varied by tissue type, sex, and lung disease, as well as biological age in airway epithelial cells. In particular, the significant relationship between *ACE2* DNA methylation and biological age indicates that age-dependent differences in host response may be mediated by aging-associated *ACE2* dysregulation and increased *ACE2* expression. These *ACE2* gene-associated DNA methylation findings convincingly indicate that *ACE2* is regulated transcriptionally, post-transcriptionally, and post-translationally within the body [69].

Approximately 60% of mammalian genes are thought to be influenced by miRNAs, especially cancer, metabolism, development, and apoptosis regulatory pathways. Since the first viral miRNAs were discovered in the human Epstein-Barr virus (EBV) [71], nearly 320 viral miRNA precursors have been identified. Most of the characteristics of miRNA-regulated gene expression appear to be particularly beneficial for viruses. For example, viruses targeting specific human genes via viral miRNAs create a suitable environment for virus replication and survival. In addition, viral miRNAs constitute a potential escape from the host immune system, because the host itself produces the miRNAs in the same way. Recently, Saçar Demirci, *et al.* [72] identified miRNA-mediated interactions in SARS-CoV-2 infection by computational analysis. Interestingly, the viral miRNAs appear to target a total of 1,367 human genes, while 479 human miRNAs might target SARS-CoV-2 genes, which are mainly responsible for viral entrance, replication, biogenesis, and infection.

CONCLUSIONS AND FUTURE PERSPECTIVES

The rapid surge in COVID-19 cases and the lack of specific management guidelines to date highlight the urgency and importance of identifying genetic factors that may influence disease susceptibility and severity. At present, information regarding genetic variants of *ACE2* and *TMPRSS2* among various populations in association with SARS-CoV-2 infection rate and disease severity is limited. In addition, studies based on genomic data analysis from data banks need to be validated by experimental analysis. It has been reported that *ACE2* expression varies in different ethnic populations with respect to the allele frequency of its variants, although different confounding factors, such as genetic variability, age-related variation, and other co-morbid factors, might be involved in disease progression. Thus, additional studies are imperative to gain a better understanding of the pathophysiology and the involvement of *ACE2* variants, expression, and epigenetic aspects in COVID-19 patients.

AUTHOR CONTRIBUTIONS

SC and KS: Literature review and manuscript drafting and revision; PM: conceptualization and design, proofreading and editing, and critical revision; SM and PS: proofreading and supervision. All authors have read the manuscript and provided final approval.

CONFLICTS OF INTEREST

None declared.

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