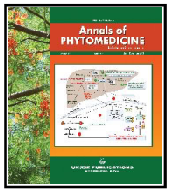
Annals of Phytomedicine 12(2): 12-20, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.3

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Review Article : Open Access

Discovering the epigenetic pathways underlying SARS-CoV-2 infections and exploring recent epigenetic-associated clinical trials

M. Masi Malaiyan, P. Mohan Kumar*, P. Logesh, K. Sibi**, S. Tamizharasi*, J. Nandhakumaran**, S. Janani and T. Prabha◆**

Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-638052, Tamil Nadu, India
** Department of Pharmaceutics, Nandha College of Pharmacy, Erode-638052, Tamil Nadu, India*
*** Department of Pharmacology, Nandha College of Pharmacy, Erode-638052, Tamil Nadu, India*

Article Info

Article history
Received 17 October 2023
Revised 4 December 2023
Accepted 5 December 2023
Published Online 30 December 2023

Keywords
Epigenetics
DNA methylation
Histone modification
COVID-19
SARS-CoV-2
Clinical trials

Abstract

One of the worst risks to public health in recent years, the COVID-19 epidemic has affected nearly everyone on the planet. Many aspects of the replication of SARS-CoV-2 cycle are regulated by epigenetic mechanisms, including the expression of the viral receptor ACE2, the expression of genes encoding cytokines as part of the host immune response, and the role of various histone modifications in multiple COVID-19 characteristics. The proteins from SARS-CoV-2 directly bind to several proteins in the host over the period of disease. Numerous relationships involving viral protein sequences and epigenetic proteins, such as histone deacetylases and proteins with bromodomains, have been found through correlation-based studies. Owing to the diverse ways that epigenetic processes influence the life cycle of viruses and the host's immune reaction to infection, epigenetic modulators have been proposed as potential targets for COVID-19 treatments, and epigenetic indicators have been identified as emerging COVID-19 indicators. A summary of current therapy on clinical trials that should help to prevent the globalization of SARS-CoV-2 is discussed in this review article.

1. Introduction

1.1 Epigenetics

The study of the entire set of epigenetic alterations on a cell's genetic material, known as the epigenome, is known as epigenomics (Fuso and Raia, 2020). Reversible changes to a cell's histones or DNA that impact gene expression without changing the DNA sequence are known as epigenetic alterations. Epigenomic maintenance is an ongoing process that participates in vital biological systems such as DNA repair, which significantly contributes to the integrity of eukaryotic genomes (Schafer and Baric, 2017).

1.2 History and epidemiology of coronavirus

SARS-CoV-2 belongs to the beta-coronavirus family, together with SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV). Compared with other RNAs, it is characterized by the production of transcripts encoding unknown ORFs with fusion, deletion, and/or frameshift. Corona has had a particularly negative impact on India. On January 30, 2020, the first case of COVID-19 in India was confirmed. Globally, one in every three new cases was reported from India (World Health Organization Information Bulletin). It is the new epicenter of the global pandemic (Sahithya *et al*., 2021). The Spanish flu (H1N1 influenza virus) in 1918-1919 caused about 20-50 million deaths. The Asian flu (H2N2-influenza virus) in 1957-1958 caused about 1-4 million deaths. The Hong Kong flu (H3 N2-influenza virus) in 1968 caused about 1-4 million deaths. HIV/AIDS, as described by some as a pandemic and by WHO as a global epidemic, started with the first case in the Democratic Republic of Congo in 1976 and since then has affected more than 37.9 million people, with about 770,000 deaths in 2018 from HIV-related illnesses (Khan *et al*., 2020). Cases in India are progressively increasing with each passing day. As of August 10th, 2020, there were over 2.3 million infections in India (the second million in exactly three weeks since the nation hit a million infections on July 16), with Andhra Pradesh, Karnataka, Uttar Pradesh, West Bengal, and Bihar accounting for 42% of new cases. The most severely impacted states in India include Uttar Pradesh, Delhi, Karnataka, Maharashtra, Tamil Nadu, and Andhra Pradesh.

1.3 Structure of SARS-CoV-2

Coronaviruses belong to the family Coronaviridae and are divided into alpha (α-CoV), beta (β-CoV), gamma (γ-CoV), and delta (δ-CoV) coronaviruses. The alpha and beta coronaviruses can infect mammals, and the viruses infecting humans are genetically similar to the β-CoV genus (Bellik *et al*., 2020). The 27-32 kb-long SARS-CoV-2 genome is made up of a single-stranded positive-sense RNA with 9860 amino acids that codes for a wide range of structural and non-structural proteins. The enclosed particle, known as a virion, is covered in proteins. Within the capsid lies the single-stranded RNA genome. It is composed of the proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) (Behura *et al*., 2022; Senthilkumar *et al*., 2021).

1.4 Current treatment for COVID-19

The treatment for COVID-19 appeared to be quite promising in the beginning. Several antiviral medications, including remdesevir,

Corresponding author: Dr. T. Prabha
Professor, Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Erode-638052, India
E-mail: drtpappa@yahoo.com
Tel.: +91-9965557346

favipiravir, lopinavir/ritonavir, and umifenovir, have been prescribed alone or in conjunction with the antimalarial drugs chloroquine or hydroxychloroquine compounds (Atlante *et al*., 2020).

Paxlovid is another medication authorized for COVID-19 in adults. It consists of nirmatrelvir, which blocks the function of an enzyme required for the virus to replicate, and ritonavir, an antiviral treatment that helps reduce the degradation of nirmatrelvir.

In certain instances, rheumatoid arthritis medications such as baricitinib and tocilizumab have been advised by the FDA to treat COVID-19. Among these, one that appears to be effective against COVID-19 is baricitinib, which has antiviral and anti-inflammatory properties. As an injectable, tocilizumab is used. It appears to function by lowering inflammation in response to COVID-19.

Molnupiravir is a different medication approved by the FDA to treat mild-to-moderate patients who are at risk of developing a serious illness and who are unable to take other types of treatment.

Research evidence suggests that nutritional interventions play an important role in COVID-19 management and treatment. WHO (World Health Organization) reports that a balanced diet contributes to the efficient functioning of the immune system. This diet comprises a good load of vitamins, minerals, carbohydrates, polysaccharides, lipids, fibers, and other essential macro and micronutrients (Indhuleka *et al*., 2021).

1.5 Mechanistic links between epigenetics and COVID-19

Healthy cellular processes like growth and development depend on epigenetic mechanisms. However, alterations in the genes that should ideally guard against specific diseases could increase a person's susceptibility to them. Certain foods and environmental pollutants are among the attributes that might cause epigenetic modifications (Yahaya *et al*., 2021). It is believed that coronaviruses modify the human epigenome, which enables them to evade the host's defenses and effectively initiate and disseminate infection. Numerous elements of the SARS-CoV-2 replication process, such as the production of cytokines and the viral receptor ACE2, are influenced by epigenetic processes.

2. Epigenetic approach to COVID-19 management

Histone modification and DNA methylation control ACE2 and the epigenetic enzymes that control these processes may be targeted for affecting the host immune system. Among these enzymes are lysine demethylase 5B (KDM5B), histone acetyltransferase 1 (HAT1), histone deacetylase 2 (HDAC2), and DNA methyltransferase 1 (Al Aboud *et al*., 2018).

2.1 Histone acetyltransferase inhibitor (HATI)

Important functions for histone acetyltransferases in the NF-κB (nuclear factor kappa) cascade and the IFN-I (interferon) responses are played by p300 and CREB-binding proteins. Anacardic acid, MG149, and C646 are examples of HATIs that are being shown to decrease NF-κB signaling and the synthesis of IL-6 (interleukin), which decreases excessive inflammation. Research has revealed that the SARS-associated CoV-2 helicase, Nsp13, interacts with p300; however, the exact chemical interaction is not established.

2.2 Histone lysine methyltransferase inhibitor (HKMTI)

Histone lysine methyltransferases G9a, which catalyzes H3K9me2, SUV39H1 (an inhibitor of variation 3-9 homolog 1 that catalyzes H3K9me3), and Ezh2 (enhancers of zeste homolog 2 that catalyzes H3K27me3) can manage essential immune cell processes including differentiation, development, elasticity, cytokine description, and other processes. HKMTIs are expected to induce hyperinflammation, which will efficiently correct the dysregulated immunological homeostasis caused by COVID-19. Moreover, certain HMTIs, such as the G9a inhibitor DZNep and the Ezh2 inhibitor GSK126, exhibit antiviral qualities.

2.3 DNA methyltransferase (DNMTI)

Decitabine is a nucleoside-based DNMTI that has been shown to reduce inflammation and the IFN reaction by suppressing the methylation of DNA in macrophages. As such, it may be useful in reducing the effects of cytokine storms. In particular, decitabine is currently being investigated in clinical settings for the treatment of patients with COVID-19 pneumonia-ARDS.

2.4 Bromodomain and extra-terminal protein inhibitor (BETI)

Which include JQ-1 and dBET6, have been shown to work by interfering with the association between viral transmembrane "E" proteins and BRD4 (Bromodomain-containing protein 4). A variety of novel bromodione and extra-terminal domain inhibitors have emerged in recent decades that have a higher affinity with either bromodomain protein, *viz*., BD1 or BD2, including OTX015, apabetalone (formerly referred to as RVX-208), and ABBV-774. JQ-1 and OTX015 have been shown to decrease SARS-CoV-2 infection and ACE2 concentrations in H1437 adenocarcinoma of the lung cells.

2.5 HDAC activator (histone deacetylases)

Significant epigenetic regulators, histone deacetylases (HDACs), control processes such as transcription, replication, and maturation of viruses, as well as the inflammatory immune response. A significant class of HDACs that regulate cellular energy stress and pathogen defense are called sirtuins (SIRT1-7). Potential SIRT1 activator resveratrol has been demonstrated to block NF-κB activity, which promotes an anti-inflammatory impact and may play a role in combating hyper-inflammation in COVID-19.

2.6 Vitamins and natural products

Vitamins such as vitamin D and certain naturally occurring bioactive compounds, such as flavonoids (quercetin and hesperetin), polyphenols (resveratrol and curcumin), and their derivatives (epigallocatechin-3-gallate and or EGCG), have the potential to modify epigenetic modifications and can significantly restore immunological dysregulation. Exogenous vitamin D injections have been found to either lower the severity of autoimmune illnesses or postpone their course. Vitamin D has also been described as having the ability to induce immunological tolerance. This suggests a potential function for it in reducing the COVID-19 cytokine storm.

3. Diverse epigenetic role in COVID-19 pathogenesis

3.1 The role of DNA methylation in COVID-19 pathogenesis

DNA methylation is an epigenetic method that involves attaching the methyl group to a specific cytosine protein in a cytosine-guanine sequence (CpG). Genes can be silenced by methylating a gene promoter in a collection of CpG sites (also known as CpG islands) that are present in cells (Bhat *et al*., 2022). To increase their infectivity,

certain microorganisms can be epigenetically modified by the host cell. Specifically, SARS-CoV-2 infiltrates host cells by binding to a receptor encoded by the angiotensin-converting enzyme2 (ACE2) gene. Yet, the methylation and expression of ACE2 determine the virus's binding affinity, meaning that it is impacted by the immune system's level of function. Thus, some of the individual differences in COVID-19 vulnerability may be explained by the varied methylation and expression of ACE2 in different people. In contrast to hypermethylation, hypomethylation boosts ACE2's expression and capacity to bind viruses. SARS-CoV-2 affinity and overexpression of ACE2 can be caused by hypomethylation of ACE2, which is a consequence of immune-mediated illnesses in particular. Disease-mediated hypomethylation of ACE2 is more likely to affect women. It is due to the possibility of faulty X chromosome inactivation brought on by the decreased DNA methylation. This could further increase the expression of ACE2 and other X-linked genes. The X chromosome cannot be inactivated without DNA methylation, and females' expression of one copy of the X chromosome is suppressed. It is required to keep female cells' expression levels appropriate and on par with those of male cells (Corley *et al*., 2020). In a study on COVID-19 patients, DNA methylation analysis at two CpG sites important to the ACE2 gene showed that female individuals were significantly hypomethylated compared to male subjects. This could be because the X chromosome's inactivation is disrupted, up-regulating its genes, such as the ACE2 gene, making them more susceptible to COVID-19.

3.2 The role of histone modifications in COVID-19 pathogenesis

Acetylation, methylation, phosphorylation, and ubiquitylation are some of the mechanisms that can change histones. When it comes to histone acetylation and methylation, histone deacetylases (HDACs) and histone demethylases (HDMs) catalyze the former and the latter, respectively. Histone acetyltransferases (HATs) and histone methyltransferases (HMTs) catalyze the former. In particular, the results indicated that histone modification up-regulated genes linked to ACE2, including lysine demethylase 5B (KDM5B), histone acetyltransferase 1 (HAT1), and histone deacetylase 2(HDAC2). This finding signifies that there may be a significant likelihood of severe COVID-19 expression in people with such conditions (Jirtle and Skinner, 2007).

3.3 The role of non-coding RNAs in COVID-19 pathogenesis

Deacetylation and demethylation are catalyzed by histone deacetylases (HDACs) and histone demethylases (HDMs), respectively, whereas histone acetyltransferases (HATs) and histone methyltransferases (HMTs) catalyze histone acetylation and methylation, respectively. For instance, the results indicated that histone modification up-regulated genes linked to ACE2, including lysine demethylase 5B (KDM5B), histone acetyltransferase 1 (HAT1), and histone deacetylase 2 (HDAC2). This finding suggests that there may be a significant likelihood of severe COVID-19 expression in people with such conditions. Figure 1 represents the epigenetic pathway underlying SARS-CoV-2 infections.

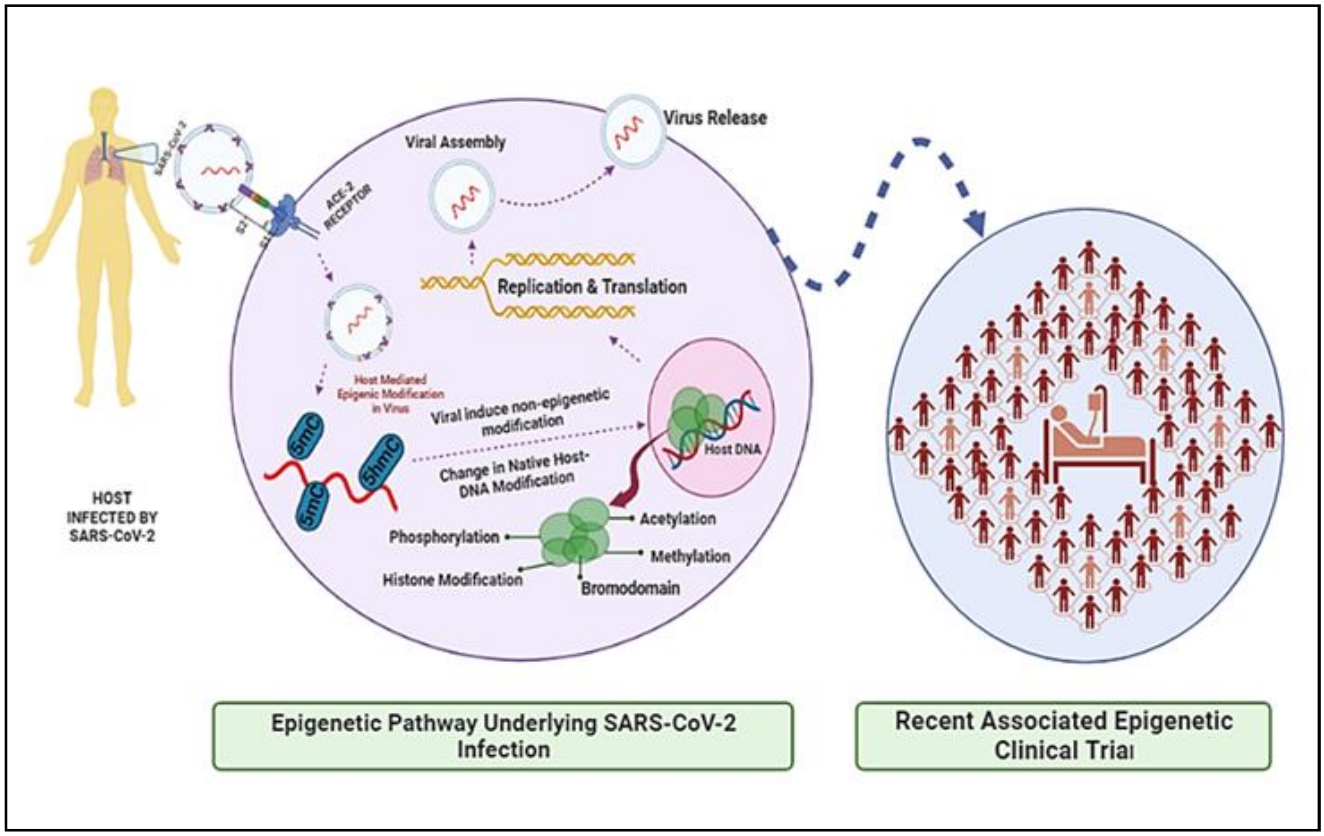


Figure 1: Epigenetic pathway underlying SARS-CoV-2 infections.

4. Clinical studies on epigenetic modification during COVID-19 disease condition

DNA methylation has been related to viral infection and immune system control. To determine potential loci modified by methylation of DNA (an epigenetic marker) that might be associated with the development of COVID-19 in patients without additional medical disorders, the author, de Moura *et al*. (2021), carried out an epigenome-wide association study (EWAS). The blood samples from 407 COVID-19 patients aged 61 who did not have any comorbidities were taken from their circulation in the peripheral regions. Among them, 194 (47.7%) had moderate manifestations that did not necessitate hospitalization, while 213 (52.3%) experienced a serious clinical manifestation necessitating a breathing aid. They also investigated the methylation status of 850,000 sites for CpG in the patient's DNA. The methylation state of 44 sites of CpG was found to be linked to the clinical manifestations of COVID-19, where the 20 recognized coding genes contain 23 (52.3%) of these loci. Researchers examined those EWAS-identified sites to develop a DNA epigenetic profile associated with the severity of the disease. The authors discovered that in COVID-19 patients, DNA methylation regions are epigenetic vulnerability sites for breathing difficulties. These possible indicators could aid in the medical identification and management of patients with SARS-CoV-2 when combined with additional medical, cellular, and genetic characteristics.

Tunaligil *et al*. (2021), explored the epigenetic modifications in COVID-19 patients to investigate the numerous mechanisms that are involved in the degree of severity of the disease and the rise in risk due to infection. Based on the best available data, the ratios of hospitalization with and without intensive care units in the presence of disorders with raised ACE2 expression were compared. Additionally, two distinct age groups (19-64) and $\geq$65 have been compared. The age group 65 and older with previous diseases is shown to have the most severe COVID-19 disease. This investigation explores the effects of COVID-19 on various patient groups using an empirical, non-experimental methodology. Their findings held promise for use in clinical trials.

Reverse transcription-polymerase chain reaction (RT-PCR) is an important test for the diagnosis of COVID-19. However, PCR-based testing has certain factors that limit results and may not produce a positive early in the course of the infection before symptoms take place. DNA methylation patterns can differentiate between an asymptomatic infection and one that is symptomatic. Coronaviruses have an impact on methylation in peripheral blood. Using Illumina's Infinium methylation EPIC BeadChip array, Arnold *et al*. (2022), analyzed peripheral blood samples from 164 patients who tested positive for SARS-CoV-2 by RT-PCR, 8 of whom did not demonstrate any symptoms. An epigenome-wide association study discovered ten methylation sites linked to infection, and a quantile-quantile plot revealed little inflation. Thus, these findings imply that changes in methylation patterns may distinguish between asymptomatic and symptomatic infections.

Illumina Infinium methylation EPIC BeadChip850K was chosen by Calzari *et al*., 2023. They looked at genome-wide variations in DNA methylation in an Italian population of comorbid patients and compared the severe (n = 64) and mild (123) prognoses. Studies have revealed that an epigenetic signature present at the time of hospitalization can considerably predict the probability of severe outcomes. After more investigation, a negative prognosis after contracting COVID-19 was linked to an acceleration of age. Patients who have a poor prognosis have a much higher prevalence of stochastic epigenetic mutations. The findings were repeated *in silico* using COVID-19-negative patients and previously published datasets. Using novel methylation data as well as previously disclosed datasets, scientists confirmed in the blood that epigenetics plays a role in the immune response after COVID-19 infection, allowing the discovery of a particular signature capable of differentiating disease evolution. In addition, the study also found that epigenetic drift and age acceleration are linked to a poor prognosis. All of these data demonstrate that host epigenetics undergo significant and unique changes in response to COVID-19 infection, which can be used for individualized, prompt, and targeted therapy of COVID-19 patients during the earliest phases of hospitalization.

It has been demonstrated that ACE2 and NRP1 receptor activation or protease activity inhibition are useful tactics for preventing viral replication and infectivity. An epigenetic medication called valproic acid has been given clinical approval. As a histone deacetylase inhibitor, it has strong antiviral and anti-inflammatory properties. Here, Saiz *et al*. (2021), suggested valproic acid as a viable treatment option for COVID-19, a virus where hyperinflammation and quick viral replication are key components. The impact of valproic acid and additional HDAC blockers on the activity of the ACE2 and NRP1 receptors, in addition to their capacity to decrease infection, viral generation, and inflammation, is studied by using a variety of cell strains, such as HK-2, Huh-7, HUVEC, Caco-2, and BEAS-2B. Through its HDAC suppressive effect, valproic acid treatment dramatically reduced the expression of host-derived proteins ACE2 and NRP1 in each of the cell lines. Hence, preinfection therapy of cells using valproic acid reduces the synthesis of contagious SARS-CoV-2 infections but not of other ACE2- and NRP1-independent pathogenic viruses like HCoV-229E and VSV. Moreover, after an hour of post-SARS-CoV-2 infection, valproic acid administration lowers the amount of viral infection in a dose-dependent way despite substantially modifying N protein concentrations or the amount of genome and subgenome messenger RNAs. The pro-inflammatory cytokines like IL-6 and TNF-α released by SARS-CoV-2 are inhibited by the existence of valproic acid. According to the investigation's outcomes, valproic acid blocks three important routes, which impact how severe is the COVID-19 infection. This diminishes the infectivity of SARS-CoV-2 by downregulating the expression of ACE2 and NRP1, lowers viral production, most likely due to its impact on virus development or virion stability, and attenuates the inflammatory response that is triggered. Therefore, until vaccination campaigns are implemented globally, giving valproic acid to COVID-19 patients may be deemed a safe course of treatment.

The epigenome-wide pattern of DNA methylation of COVID-19 convalescents was compared to uninfected subjects from before and following the outbreak of the pandemic by Huoman *et al*. (2021). DNA was extracted from systemic blood mononuclear cells treated *in vitro* with the SARS-CoV-2 virus and from healthy controls, COVID-19 convalescents, and individuals without symptoms who had SARS-CoV-2-specific T cell responses. The illumina methylation EPIC 850K array was then used, and statistical and bioinformatic studies were carried out, including evaluations of module identification, route over representation, and differential DNA methylation. Differential DNA methylation patterns were identified to distinguish COVID-19

convalescents from uninfected controls in an experimental SARS-CoV-2 infection scenario. Corresponding *in vitro* analyses identified six of the 66 genes comprising the SARS-CoV-2-induced module: TP53, INS, HSPA4, SP1, ESR1, and FAS. Gonadotropin-releasing hormone receptor pathways, and muscarinic cholinergic receptor signalling, were found to be involved, according to over-representation analysis. Furthermore, the SARS-CoV-2 interactome was influenced by a substantial number of network and differentially methylated genes from both contexts. Permanent epigenomic information is preserved by modified patterns of DNA methylation in COVID-19 convalescents, suggesting their recovery after the infection with SARS-CoV-2. Investigations exploring the relationship between *in vitro* infections and real-world exposure demonstrate the critical role DNA methylation plays in immune system reactions to SARS-CoV-2 infections.

Mongelli *et al*. (2021), studied pyrosequencing of particular CpG islands already identified as appropriate for biological age estimation in a group of 117 COVID-19 survivors and 144 non-COVID-19 individuals. The data show that the post-COVID-19 cohort has a continuous age rise, with a delta age acceleration of 10.45-7.29 years (+5.25 years over the normal range) compared to 3.68-8.17 years in the COVID-19-free sample ($p \leq 0.0001$). This finding is confirmed by the noticeable shortening of telomeres in the post-COVID-19 cohort compared to COVID-19-free patients ($p \leq 0.0001$). Furthermore, when compared to the COVID-19-free population, ACE2 expression was lower in post-COVID-19 patients, although DPP-4 expression remained unchanged. Based on these findings, we propose that some epigenetic modifications are associated with the post-COVID-19 syndrome, particularly in younger individuals (60 years).

Pang *et al*. (2021), conducted a thorough assessment of DNA methylation states and an epigenetic timeframe in blood from normal individuals before and after non-hospitalized COVID-19 test confirmed patients. Initially, at a median of 8.35 weeks, they examined the DNA methylation levels in blood samples of 21 individuals both before and after they received a COVID-19 diagnosis. Following the diagnosis of COVID-19 in the blood, 261 CpGs were revealed to be differentially methylated at an FDR-adjusted P value of 0.05. The gene body and the northern and southern shelf sections of genes linked to metabolic pathways included these CpGs. The study revealed the commonality of genes found in transcriptional SARS-CoV-2 infection datasets. Estimates of the phenomenon age and the grim age based on principal component analysis were raised by an average of 2.1 and 0.84 years in adults over 50 following infection. It was found that changes in immune cell type components in CD4+T cells, B cells, granulocytes, plasma blasts, depleted T cells, and immature T cells, along with age, were associated with variations in epigenetic clocks post-COVID-19. In humans older than 50 who got Moderna, complementary longitudinal research on epigenetic clock evaluations was conducted in 36 individuals before and following both Moderna and Pfizer's mRNA-based COVID-19 vaccination. The results showed a mean difference of 3.91 years was removed from basic component-based Horvath epigenetic clock estimates. The drop-in estimates of the epigenetic clock were discovered to be significantly connected with modifications to immune cell composition in plasmablasts and B cells, as well as chronological age, both before and following vaccination. These results imply that epigenetic clocks may be useful as a biomarker of COVID-19 vaccination responses.

According to host epigenome manipulation following COVID-19 infection, DNA methylation profiles can be used to distinguish COVID-19 infection patients from those unaffected and aid in analyzing the seriousness of COVID-19 disease, even in its early stages of manifestation. Konigsberg *et al*. (2021), analyzed blood samples from 164 COVID-19 patients with longitudinal measures of disease severity and 296 patient controls to improve immune response identification using illumina's infinium methylation EPIC array. 13,033 genome-wide significant methylation sites for case vs. control status were found using epigenome-wide association analysis. Among differentially methylated locations, genes and pathways associated with interferon signalling and viral response were considerably improved. Researchers find highly substantial connections between earlier published genes in genetic association studies (for example, IRF7 and OAS1). Models created with sparse regression employing machine learning approaches produced highly predictive results: cross-validated optimal fit AUCs were 79.1%, 80.8%, and 84.4% for hospitalization, ICU admission, and progression to death, respectively, and 93.6% for case-versus-control status. To sum up, the researchers found a potent epigenetic signature unique to COVID-19 in peripheral blood. This signature was fuelled by important immune-related pathways associated with infection status, disease severity, and clinical deterioration, offering insights into the diagnosis and prognosis of patients with viral infections.

Rathod *et al*. (2021), examined the possibility of sex-specific associations between DNAm at CpGs of coronavirus-related loci and asthmatic allergy. There were n = 242 individuals in the Isle of Wight born group of people, and they were all over twenty six years old. The non-specific and sex-specific connections involving the presence of asthmatic or rhinitis allergy and DNAm at CpGs on coronavirus-related loci have been examined using regression models. Recently discovered CpGs were evaluated for their operational importance by examining DNA associations with gene activity in blood. For asthma and allergy, there were substantial effects of interaction with sex at 27 and 40 CpGs, respectively. Despite gender, DNAm was associated with allergy at 45 CpGs and asthma at 21 CpGs. 14 CpGs for asthmatics and 17 CpGs for allergies showed the possibility of having epigenome regulatory function on gene expression, while the observed CpGs were evaluated for their practical significance; of those, 6 CpGs for asthmatics and 7 CpGs for allergies were predicted to be sex-specific. People who suffer from allergies and asthma might be particularly vulnerable to COVID-19 since these conditions are associated with mutations in the genome. The sex-specificity of the relationship between asthma/allergy and DNAm at particular CpGs, as well as the relationship between the expression of genes and DNAm at CpGs associated with asthma/allergies, might be responsible for the reported sex-specificity in COVID-19 morbidity and mortality conditions.

There is a strong correlation between a mother's mental health during pregnancy and the growth of her unborn child. The consequences of stress exposure may be mitigated during pregnancy and may include modified patterns of DNA methylation in specific genes linked to stress (*e.g*., the serotonin transporter gene, SLC6A4, and the glucocorticoid receptor gene, NR3C1). Nazzari *et al*. (2022), reported on the methylation status of NR3C1 and SLC6A4 in Italian mothers and infants exposed to the COVID-19 pandemic lockdown throughout various trimesters of pregnancy. Between the periods of May 2020 and February 2021, 283 mother-child pairs were enrolled for

deliveries. To evaluate the methylation status of NR3C1 (44 CpG sites) and SLC6A4 (13 CpG sites), buccal cells were taken within 24 h of administration. Using principle component (PC) analyses, the methylation data dimension was reduced to one PC per mother and baby gene methylation. Even after correcting for confounders, the effect remained substantial. During the pandemic, pregnant women and children may have changed epigenetic indicators of stress-related genes. Women and newborns should be rigorously checked for mental wellness during and after the pandemic since these epigenetic marks have been previously linked to an elevated risk of maternal psychiatric disorders and poor child development.

Viruses, like SARS-CoV-2, can manipulate the epigenetic landscape of host immune cells to avoid detection. In a study, Corley *et al*. (2020), compared the DNA methylation (DNAm) profiles of nine terminally ill individuals with unaffected hospitalized influenza patients, untreated HIV infection, and mild-to-moderate COVID-19 HIV co-infected individuals with critically ill COVID-19 patients who had proven SARS-CoV-2 plasma viremia. Cell-type deconvolution analysis showed a high proportion of estimated neutrophils, suggesting the presence of DNA changes linked to granulopoiesis, and identified lymphopenia in severe COVID-19 individuals. They found a distinct DNA pattern for severe COVID-19, which correlated with results from single-cell transcriptional investigations of severe COVID-19 and infection models. This signature was defined by hypomethylation of inflammatory genes and hypermethylation of IFN-related genes. GrimAge reports that epigenetic clock analysis linked severe COVID-19 to higher DNA damage and a higher chance of death, hence endorsing the epigenetic clock as a disease and mortality risk predictor. These results point out the presence of a severe COVID-19 DNA pattern in blood, which may help explain pathogenic mechanisms, correlate clinical assessments, and discover novel targets for SARS-CoV-2 therapy.

Franzen *et al*. (2021), performed targeted bisulfite amplicon sequencing on diverse DNA methylation datasets of blood samples with epigenetic ageing characteristics. As a whole, epigenetic clocks are closely related to patients' chronological age, whether they have acute respiratory distress syndrome or not. Additionally, lymphocytes did not show significantly increased telomere degradation. Therefore, in older adults, these indicators are not able to reliably predict a higher risk of severe COVID-19 infection.

A significant factor in coronavirus diseases is cells. Determining the reason behind the 'cis' and 'trans' regulating regions' epigenetic chromatin access and assembling transcriptome immune profiles may help understand COVID-19. Li *et al*. (2021), conducted a single-cell assay for transposase-accessible chromatin and single-cell RNA sequencing *via* peripheral blood single-nuclear cells of moderate individuals, healthy volunteers, and seriously ill individuals who were diagnosed with COVID-19. Using scATAC-seq (9 cases) and scRNA-seq (15 cases), respectively, 76,570 and 107,862 single cells were used to assess chromatin access and transcriptome immune profiles. In the three groups, the scATAC-seq identified 28,535 unique peaks, of which 41.6 and 10.7% were identified in the enhancer and promoter areas, respectively. In comparison to HCs, CD4+T and CD8+T cells from SCPs and MPs showed enrichment of inflammatory pathways, including the tumor necrosis factor pathway and the mitogen-activated protein kinase (MAPK) system, between the genes that are peak-located in all T cells and their subsets. The TBX21 motifs were less accessible in SCP CD4+ T cells as opposed to MPs. Moreover, scRNA-seq showed that SCPs and MPs had a smaller percentage of T cells overall-in particular, CD4+T cells-than HCs. In both the SCP and MP cases, transcriptome data showed significantly greater levels of inflammatory and histone-related genes (NFKBIA, S100A9, and PIK3R1) in total T cells, CD4+T cells, and CD8+T cells. Among the CD4+T cells, SCPs and MPs showed a decrease in T helper-1 (Th1) cells. In SCPs in CD8+T cells, activation markers such as CD69 and HLA class II genes (HLA-DRA, HLA-DRB1, and HLA-DRB5) were shown to be enhanced. An analysis of the scRNA-seq and scATAC-seq data together showed some degree of consistency among the two approaches. In COVID-19 patients, they have generated a transcriptome immunological profile, chromatin epigenetic state, and landscape of T cells. Compared to what was previously achievable with merely scRNA-seq data, this has made it possible to dissect the characteristics of the T cells involved in more detail and at a higher resolution. Based on this discovery, they hypothesize that defective activity in SCP CD4+ T cells in conjunction with T-cell inflammatory states may play an important role in the development and resolution of COVID-19.

Godoy *et al*. (2022), investigated both the transcriptome and DNA methylome of blood vessel cells from COVID-19 individuals. The DNA samples from 48 severe COVID-19 patient's CD14+, CD15- monocytes and 11 normal subjects were hybridized on methylation EPIC bead chip arrays. Besides, ten individuals with severe COVID-19 had single-cell transcriptomics generated sequentially. Improvements in interaction between monocytes and various immune cell types have been determined using CellPhoneDB. They discovered that variations in gene expression were correlated with alterations in DNA methylation at CpG sites linked to antigen-presentation and interferon-related genes. Although separate DNA methylation anomalies in viral infection genes were discovered, these modifications heavily overlapped with those seen in bacterial sepsis. They also discovered that these modifications included a few of the DNA methylation changes caused by inflammatory cytokines and myeloid differentiation. The production of T-helper 1 cell cytokines and genes related to interferons has been correlated to a series of DNA methylation changes associated with the repetitive organ failure assessment (SOFA) score. Studies conducted by CellPhoneDB on single-cell transcriptomes of different immune cell types suggest that monocytes, along with different cell types, including regulatory T cells and NK cells, may interact in a variety of ways.

Nussle *et al*. (2023), the study aims to examine the epigenetic signs of lifestyle changes associated with COVID-19 degree of severity and whether COVID-19 severity prediction was enhanced by adding these marks in addition to the EPICOVID signature. This study makes use of data from two publicly available studies, which may be obtained *via* the Gene Expression Omnibus (GEO) platform (accession numbers: GSE168739 and GSE174818). The GSE168739 sample is a retrospective, cross-sectional examination of 407 people with confirmed COVID-19 across 14 Spanish hospitals, whereas the GSE174818 sample is a single-center observational study of patients admitted to the hospital for COVID-19 symptoms (n = 102). YC was calculated using the epigenetic age estimations from Gonseth-Nussle (a), Horvath (b), Hannum (c), and PhenoAge (d). COVID-19 severity was determined using study-specific criteria such as hospitalization status (yes/no) (GSE168739) or vital status (alive/dead) at the end of follow-up (GSE174818). To evaluate the relationship between YC, lifestyle exposures, and COVID-19 severity, logistic regression techniques were employed. In primary prevention,

epigenetic age holds promise, especially as a motivator for lifestyle modifications aimed at lowering the likelihood of severe COVID-19 symptoms.

Salgado *et al*. (2021), investigated a large number of transcriptomes from COVID-19 patient-derived tissues and cells affected by MERS-CoV, SARS-CoV, and SARS-CoV-2. Using integrative investigations of gene co-expression networks and de-novo pathway enrichment, several gene groups and protein channels that are increased by transcription reasons or epi-factors associated with infection by SARS-CoV-2 are reported. They identified TRIM25, MOV10, RELA, and EP300 as intriguing candidates among over 60 more proteins that are involved in the epigenetic response during infection with viruses and may have medical significance. These findings suggest that focusing on the epigenetic machinery may be a workable COVID-19 treatment option.

Muhammad *et al*. (2021), discovered possible candidate genes in COVID-19 that may be controlled by COV-2-induced DNA methylation alterations. Using *in silico* transcriptome data from the COVID-19 lung autopsy, the differentially expressed genes with CpG islands in their promoter regions were initially identified. An *in vitro* model of SARS-CoV-2-infected lung epithelial cells (NHBE and A549) showed similar gene regulation. Following the infection with SARS-CoV-2, pulmonary epithelium cell levels of DNA methyltransferases such as DNMT1, DNMT3A, and DNMT3B were markedly reduced. Just two of the genes in COVID-19 displayed decreased levels, which is indicative of regulatory hypermethylation, while twelve genes displayed elevated expression, which is indicative of regulatory hypomethylation. Only HSPA1L and ULBP2 were observed to be increased in AZA-treated bronchial epithelial and immune function cells, indicating epigenetic modification. The promoter methylation and mRNA expression levels of these two genes were assessed in genomic DNA and RNA obtained from whole blood samples of asymptomatic, severe COVID-19 patients and similarly matched healthy controls to confirm the hypomethylation of these two genes during SARS-CoV-2 infection. In both asymptomatic and severe COVID-19 blood samples, HSPA1L methylation was significantly lower, and mRNA expression was increased, suggesting epigenetic regulation by SARS-CoV-2 infection. Given that HSPA1L facilitates host-virus replication, it has been suggested that HSPA1L be a possible target for antiviral prophylaxis and therapy.

Epigenome-wide association studies look at the relationship between specific CpG site methylation and EWAS. In a single cohort of nearly 18,000 Scottish people, Hillary *et al*. (2023), investigated the association between blood DNA methylation and the incidence of 19 illness states and the prevalence of 14 diseases. Methods and results: DNA methylation at 752,722 CpG sites was measured in whole-blood samples from 18,413 participants (aged 18 to 99) in the family-structured, population-based cohort study Generation Scotland. The EWAS study discovered both longitudinal correlations and cross-sectional links among baseline CpG methylation and 19 event conditions, as well as 14 prevalent sickness states. Prevalent instances were self-reported on health questionnaires at the beginning of the experiment. Incident instances were found by linking to Scottish primary and secondary (ICD-10) care data; this allowed the censoring date to be set for October 2020. The mean interval between symptoms and diagnosis ranged from 5 years for chronic pain to 11.7 years for hospitalization due to COVID-19. The 19 disease states were chosen based on their appearance on the World Health Organization's list of the ten main mortality and sickness burdens or their involvement in baseline self-report questionnaires. Age at methylation type, gender demographics, estimated white blood cell composition, and five prevalent lifestyle risk factors were all considered while adjusting the EWAS models. They discovered more than 100 links between blood methylation sites and prevalent diseases that were independent of important confounding risk factors, as well as a need for more uniformity among EWAS on human disease.

Pregnancy stress may leave epigenetic fingerprints of stress-related genes (such as the serotonin transporter gene SLC6A4), which may alter how preborn infants behave. Provenzi *et al*. (2021), began a longitudinal cohort study in April 2020 to investigate the behavioural and epigenetic effects of prenatal stress exposure connected to COVID-19 in mothers and infants. The stress related to COVID-19 throughout pregnancy was assessed after the baby was born. SLC6A4 methylation was evaluated at 13 CpG sites in the buccal cells of mothers and neonates. The temperaments of infants were assessed when they were three months old. The data for 108 mother-infant couples was fully accessible. SLC6A4 methylation in infants at seven CpG sites was significantly connected to higher prenatal stress related to COVID-19. SLC6A4 methylation at these locations predicted infant temperament at three months.

5. Conclusion

SARS-CoV-2 infection can be detected by numerous levels of epigenetic regulation, such as DNA methylation and histone changes. The host cell tries to mount a strong immune response to viruses, which is regulated in part by epigenetic systems, while the virus attempts to bypass the reaction and modify the cell to create an environment that promotes viral replication, virus particle assembly, and virus particle spread to infect new cells.

COVID-19 is a tough disease to combat due to its severe contagiousness and ability to spread swiftly before symptoms appears, making it difficult to slow the virus down by quarantining affected patients. Advanced methods and techniques are required to fight the COVID-19 disease. Therapies targeting epigenetic proteins can tip the scales in favour of the sick patient by preventing the virus from multiplying and spreading. To eradicate the global epidemic of COVID-19, effective treatments must be available to the population worldwide in a timely and cost-effective manner.

The studies mentioned in this review are a good starting point for understanding SARS-CoV-2, but there is still a lot more to learn. More research into the underlying biology of SARS-CoV-2 and kindred viruses is needed to bring this pandemic to a close and help ensure that another viral epidemic is not as disruptive in the future.

In all, an emerging involvement of epigenetic enzymes and alterations, as well as their prospective activities in COVID-19 treatment, is shown. In the future, research should focus on elucidating the functional significance of these chemical changes in virus survival, infection efficiency, and disease severity, as well as the ability of the virus and host cells to evade attack.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Al Aboud, N. M.; Tupper, C. and Jialal, I. (2022). Genetics, epigenetic mechanism. StatPearls Publishing, Treasure Island (FL).

Arnold, C. G; Konigsberg, I.; Adams, J. Y.; Sharma, S.; Aggarwal, N.; Hopkinson, A. and Monte, A. A. (2022). Epigenetics may characterize asymptomatic COVID-19 infection. Human genomics, **16**(1):27.

Atlante, S.; Mongelli, A.; Barbi, V.; Martelli, F.; Farsetti, A. and Gaetano, C. (2020). The epigenetic implication in coronavirus infection and therapy. Clinical Epigenetics, **12**:1-12.

Behura, A.; Naik, L.; Patel, S.; Das, M.; Kumar, A.; Mishra, A. and Dhiman, R. (2022). Involvement of epigenetics in affecting host immunity during SARS-CoV-2 infection. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 166634.

Bellik, Y.; Bey, M. B.; Fatmi, W.; Kouidri, M.; Souagui, Y. and Ammar Selles, S. M. (2020). Micronutrients and phytochemicals against COVID-19: mechanism and molecular targets. Ann. Phytomed., **9**(2):15-29.

Bhat, S.; Rishi, P. and Chadha, V. D. (2022). Understanding the epigenetic mechanisms in SARS-CoV-2 infection and potential therapeutic approaches. Virus Research, **318**:198853.

Calzari, L.; Zanotti, L.; Inglese, E.; Scaglione, F.; Cavagnola, R.; Ranucci, F. and Gentilini, D. (2023). Role of epigenetics in the clinical evolution of COVID-19 disease. Epigenome-wide association study identifies markers of severe outcome. European Journal of Medical Research, **28**(1):1-15.

Corley, M. J. and Ndhlovu, L. C. (2020). DNA methylation analysis of the COVID-19 host cell receptor, angiotensin I converting enzyme 2 gene (ACE2) in the respiratory system reveal age and gender differences. Preprints, 2020030295.

Corley, M. J.; Pang, A. P.; Dody, K.; Mudd, P. A.; Patterson, B. K.; Seethamraju, H. and Ndhlovu, L. C. (2021). Genome-wide DNA methylation profiling of peripheral blood reveals an epigenetic signature associated with severe COVID-19. Journal of Leukocyte Biology, **110**(1):21-26.

De Moura, M. C.; Davalos, V.; Planas-Serra, L.; Alvarez-Errico, D.; Arribas, C. and Ruiz, M. (2021). Epigenome-wide association study of COVID-19 severity with respiratory failure. EBio. Medicine, **66**:103339.

Franzen, J.; Nuchtern, S.; Tharmapalan, V.; Vieri, M.; Nikolic, M.; Han, Y. and Wagner, W. (2021). Epigenetic clocks are not accelerated in COVID-19 patients. International Journal of Molecular Sciences, **22**(17):9306.

Fuso, A. and Raia, T. (2020). The relevance of epigenetics in the SARS-CoV-2 infection and COVID-19 disease organisms. Journal of Biological Sciences, **4**(1):11-14.

Godoy-Tena, G; Barmada, A.; Morante-Palacios, O.; de la Calle-Fabregat, C.; Martins-Ferreira, R.; Ferreté-Bonastre, A. G. and Ballestar, E. (2022). Epigenetic and transcriptomic reprogramming in monocytes of severe COVID-19 patients reflects alterations in myeloid differentiation and the influence of inflammatory cytokines. Genome Medicine, **14**(1):134.

Hillary, R. F.; McCartney, D. L.; Smith, H. M.; Bernabeu, E.; Gadd, D. A.; Chybowska, A. D. and Marioni, R. E. (2023). Blood-based epigenome-wide analyses of 19 common disease states: A longitudinal, population-based linked cohort study of 18,413 Scottish individuals. PLoS Medicine, **20**(7): e1004247.

Huoman, J.; Sayyab, S.; Apostolou, E.; Karlsson, L.; Porcile, L.; Rizwan, M. and Lerm, M. (2021). Epigenome-wide DNA methylation profiling of healthy COVID-19 recoverees reveals a unique signature in circulating immune cells. MedRxiv, 260014.

Indhuleka, R.; Sanjana, J.; Janet. and Ragavi, V. (2021). Significance of nuts in strengthening immune system during COVID-19. Ann. Phytomed., **10**(2):119-121.

Jirtle, R. L. and Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. Nature Reviews Genetics, **8**(4):253-262.

Khan, A. A.; Khan, S.; Khan, U. and Das, K. (2020). The COVID-19 pandemic: A scoping review. Ann. Phytomed., **9**(1):18-26.

Konigsberg, I. R.; Barnes, B.; Campbell, M.; Davidson, E.; Zhen, Y.; Pallisard, O. and Barnes, K. C. (2021). Host methylation predicts SARS-CoV-2 infection and clinical outcome. Communications Medicine, **1**(1): 42.

Li, S.; Wu, B.; Ling, Y.; Guo, M.; Qin, B.; Ren, X. and Lu, H. (2021). Epigenetic landscapes of single-cell chromatin accessibility and transcriptomic immune profiles of T cells in COVID-19 patients. Frontiers in Immunology, **12**, 625881.

Mongelli, A.; Barbi, V.; Gottardi Zamperla, M.; Atlante, S.; Forleo, L.; Nesta, M. and Gaetano, C. (2021). Evidence for biological age acceleration and telomere shortening in COVID-19 survivors. International Journal of Molecular Sciences, **22**(11):6151.

Muhammad, J. S.; Saheb Sharif-Askari, N.; Cui, Z. G; Hamad, M. and Halwani, R. (2021). SARS-CoV-2 infection-induced promoter hypomethylation as an epigenetic modulator of heat shock protein A1L (HSPA1L) gene. Frontiers in Genetics, **12**:622271.

Nazzari, S.; Grumi, S. and Mambretti, F. (2022). Maternal and infant NR3C1 and SLC6A4 epigenetic signatures of the COVID-19 pandemic lockdown: When timing matters. Translational Psychiatry, **12**(1): 386.

Nussle, S.; Bochud, M. and Gonseth-Nussle, S. (2023). Investigating the association of measures of epigenetic age with COVID-19 severity: evidence from secondary analyses of open access data. Swiss Medical Weekly, **153**(4):40076-40076.

Pang, A. P.; Higgins-Chen, A. T.; Comite, F.; Raica, I.; Arboleda, C.; Went, H. and Corley, M. J. (2022). Longitudinal study of DNA methylation and epigenetic clocks prior to and following test-confirmed COVID-19 and mRNA vaccination. Frontiers in Genetics, **13**:819749.

Provenzi, L.; Mambretti, F.; Villa, M.; Grumi, S.; Citterio, A.; Bertazzoli, E. and Borgatti, R. (2021). Hidden pandemic: COVID-19-related stress, SLC6A4 methylation, and infants' temperament at 3 months. Scientific Reports, **11**(1):15658.

Rathod, A.; Rathod, R.; Zhang, H.; Rahimabad, P. K.; Karmaus, W. and Arshad, H. (2021). Association of asthma and rhinitis with epigenetics of coronavirus related genes. Epigenetics Insights, **14**:251686572110 39224.

Sahithya, M. D.; Srinath, M.; Kumar, B. C and Giri, C. C. (2021). Molecular *in silico* docking interventions in various SARS-CoV-2 receptor targets involving bioactive herbal compounds of *Andrographis paniculata* (Burm. f.) Nees. Ann. Phytomed., S22-S39.

Saiz, M. L.; De Diego, M. L.; López-García, D.; Corte-Iglesias, V.; Baragano Raneros, A.; Astola, I. and Suarez-Alvarez, B. (2021). Epigenetic targeting of the ACE2 and NRP1 viral receptors limits SARS-CoV-2 infectivity. Clinical Epigenetics, **13**:1-18.

Salgado-Albarrán, M.; Navarro-Delgado, E. I.; Del Moral-Morales, A.; Alcaraz, N.; Baumbach, J.; González-Barrios, R. and Soto-Reyes, E. (2021). Comparative transcriptome analysis reveals key epigenetic targets

in SARS-CoV-2 infection. NPJ Systems Biology and Applications, 7(1):21.

Schafer, A. and Baric, R. S. (2017). Epigenetic landscape during coronavirus infection. Pathogens, 6(1):8.

Senthilkumar, N.; Sumathi, R.; and Babu, D. S. (2021). Prospection of antiviral compounds from forest plants under ongoing SARS-COV-2 pandemic. Ann. Phytomed., S195-S208.

Tunaligil, V.; Meral, G; Kati, A.; Chattopadhyay, D. and Mandal, A. K. (2021). COVID-19 Epigenetics and implications for public health. Coronaviruses, 2(2):235-240.

Yahaya, T.; Oladele, E.; Muhammed, A.; Haruna, A. and Liman, U. (2021). Involvement of epigenetics in the pathogenesis, testing, and management of coronavirus disease 2019 (COVID-19) Pandemic: A narrative review. Pharmaceutical and Biomedical Research, 120:103-114.

Citation **M. Masi Malaiyan, P. Mohan Kumar, P. Logesh, K. Sibi, S. Tamizharasi, J. Nandhakumaran, S. Janani and T. Prabha (2023). Discovering the epigenetic pathways underlying SARS-COV-2 infections and exploring recent epigenetic-associated clinical trials. Ann. Phytomed., 12(2):12-20. http://dx.doi.org/10.54085/ap.2023.12.2.3.**