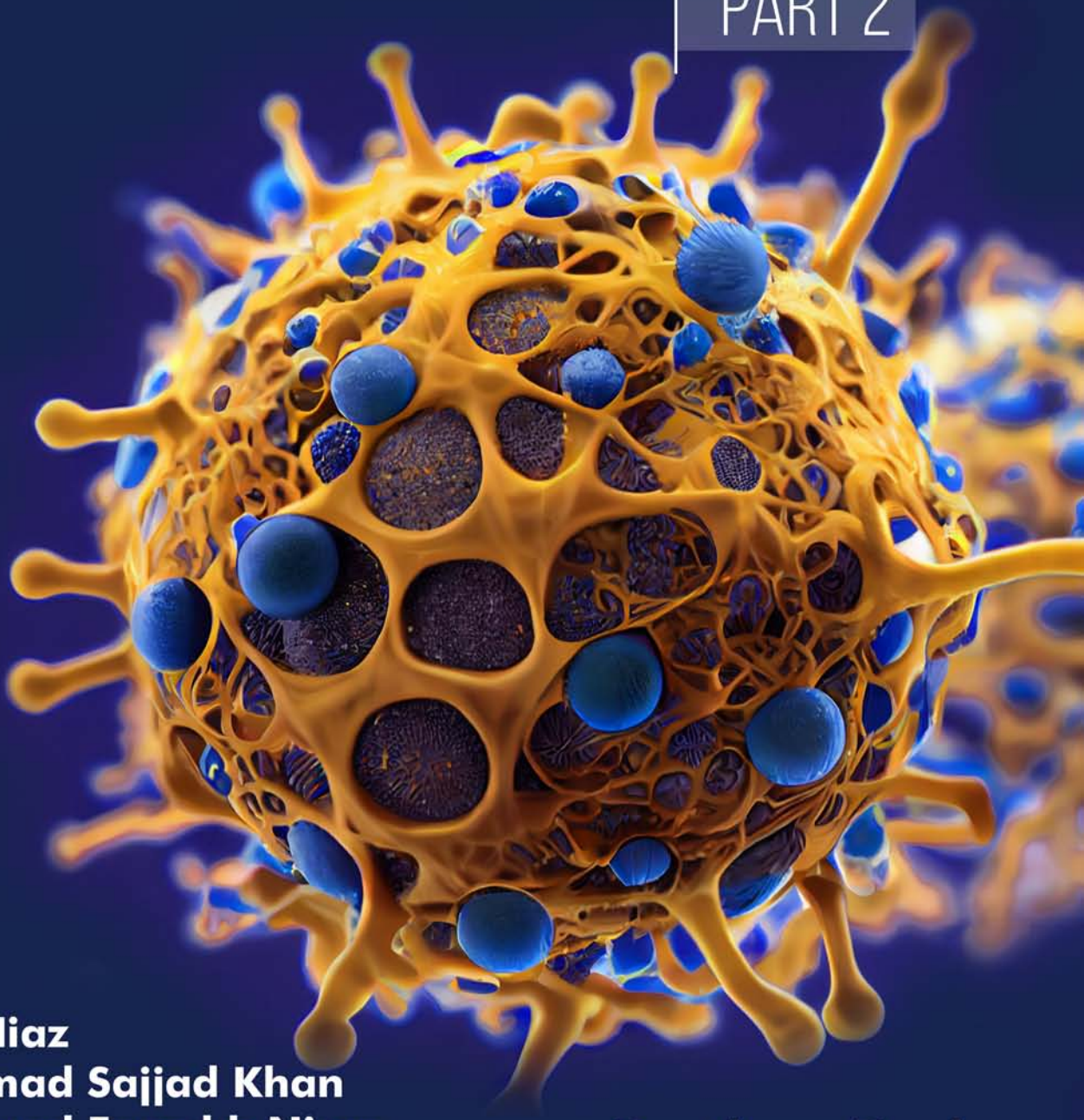
GENETIC DIVERSITY OF CORONAVIRUSES

FROM SARS-CoV TO SARS-CoV-2

PART 2



Editors:
Kamal Niaz
Muhammad Sajjad Khan
Muhammad Farrukh Nisar

Bentham Books

Genetic Diversity of Coronaviruses: From SARS-CoV to SARS-CoV-2

(Part 2)

Edited by

Kamal Niaz
Department of Pharmacology and Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

Muhammad Sajjad Khan
Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

&

Muhammad Farrukh Nisar
Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

Genetic Diversity of Coronaviruses: From SARSCoV to SARS-CoV-2 – *(Part 2)*

Editors: Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar

ISBN (Online): 978-981-5322-19-4

ISBN (Print): 978-981-5322-20-0

ISBN (Paperback): 978-981-5322-21-7

First published in 2025.

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
No. 9 Raffles Place
Office No. 26-01
Singapore 048619
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

FOREWORD

The connection between human health, animals, and the environment has been widely recognized as important for the ecosystem we inherit and intend to improve for the generations to come. This, of course, is not a responsibility of a region or a country; rather is the responsibility of all of us. Efforts have to be collaborative and transboundary in approach. Of the many types of challenges, respiratory diseases have emerged as a real threat in the recent past. Scientists have been working to reduce their load and easily spread it from animals to human beings. Newly emerging respiratory diseases such as severe acute respiratory syndrome-coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pose a serious threat to the human population and are reported in the year 2003, 2012, and 2019, respectively.

This book is very relevant in this connection. This book consists of four key modules. The first module provides a clearly defined genetic mutation and progression of SARS-CoV in the human population. The second elaborates on the genetic mutational changes of MERS, and the third one summarizes the genetic mutation of SARS-CoV-2. The last one elaborated on the correlation of coronaviruses with various disorders, especially epigenetic, neurological disorders, and artificial intelligence. The learning outcomes of this book are developing knowledge, skills, and competencies in scientists, students, employers, and human resource specialists. This book focuses on developing a detailed set of guidelines regarding epidemiology, genetic alteration, a structural protein, quantitative analysis, and diagnostic approaches of SARS-CoV, MERS-CoV, and SARS-CoV-2, and will give step-by-step awareness to the researchers about these outbreaks. This book will be adopted to give reliable knowledge to all scientists globally. I hope that this book will be distributed widely in Pakistani higher institutions soon for thorough implementation at all levels of postgraduate studies.

Rakesh Srivastava
GLAX Health (Improve Health Through Innovation)
LLC, 8000 Innovation Park Drive, Baton Rouge
LA 70820, USA

PREFACE

Coronaviruses, such as severe acute respiratory syndrome-coronavirus (SARS-CoV), Middle East respiratory syndrome-coronavirus (MERS-CoV), and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) have posed significant public health threats in the last two decades. It has been revealed that bats act as natural reservoirs for these viruses, and periodic monitoring of coronaviruses in bats, dogs, civets, and other wild animals may thus provide important clues about emergent infectious viruses that transfer to humans. The Eastern bent-wing bat *Miniopterus fuliginosus* (*M. fuliginosus*) and genus *Rhinolophus* are distributed extensively throughout China and other countries. Therefore, there is a need to analyze the genetic diversity of coronaviruses transmitted to humans. The only coronavirus genus found was *alphacoronavirus*. The established *alphacoronavirus* genome sequences showed high similarity to other *alphacoronaviruses* found in other *Miniopterus* species and other animals. It suggests that their transmission in different *Miniopterus* species may provide opportunities for recombination with different *alphacoronaviruses*. The genetic information for these novel *alphacoronaviruses* will improve our understanding of the evolution and genetic diversity of coronaviruses, with potentially important implications for the transmission of human diseases. This virus is different from the previously isolated MERS-CoV and SARS-CoV and is the seventh that infects humans. SARS-CoV-2 spreads rapidly and infects a large number of the population globally. Besides, a new variant of coronavirus disease-19 (COVID-19) known as B.1.1.7 is spreading globally, especially in the United Kingdom (UK), with an unusually large number of mutations in the proteins. This variant spreads more easily and quickly than other variants. The new variant is defined by 14 mutations resulting in amino acid changes and three deletions, some of which are believed to influence the virus's transmissibility in humans. The World Health Organization (WHO) has reported that one of the mutations identified (N501Y) is altering an amino acid within the six key residues in the receptor-binding domain. It is indicated that the rate of transmission of the variant, known as B.1.1.7 or VUI 202012/01 (variant under investigation, the year 2020, month 12, variant 01), was 71% (95% confidence interval 67% to 75%), which is higher than for other variants. It may also have a higher viral load.

In this book proposal, we consolidated the genetic diversity/mutation that occurred in 2002-21. Since SARS-CoV-2 is the closest to SARS-CoV and MERS-CoV, the approaches discussed here will be similar and/or varying by a slight degree. In the last 18-19 years, this is the third outbreak of the same coronavirus with a slight mutation that shocked the whole world. This book should be prioritized as up-to-date literature on genetic mutations that have occurred in the form of SARS-CoV, MERS-CoV, and SARS-CoV-2. It will act as a suitable reference if any such virusappears in the future. This book proposal has been classified into four parts: Part I: Genetic Mutation of SARS-CoV, Part II: Genetic Mutation of MERS-CoV, Part III: Genetic Mutation of SARS-CoV-2, and Part IV: Correlation of Coronaviruses with Various Disorders.

With the emergence of new coronavirus variants, different host tropisms permit a thorough analysis of their genomic diversity/mutations that acquire adaptability to their host. Thus, in Part I, we start the book with chapters dealing with a mutation in SARS-CoV, the host genetic diversity of SARS-CoV, newly emerging variants of SARS-CoV, the genetic architecture of host proteins involved in SARS-CoV, and the landscape of host genetic factors correlating with SARS-CoV. In Part II, a critical analysis of the MERS-CoV involves the potential to mutate its genome by opposite genetics and to get better recombinant viruses with described mutations. Such processes will assist in studying the capabilities of particular genes and their effects on virus survival and pathogenesis. These strategies can even help in determining host

factors correlating with MERS-CoV genome growth and proliferation. In Part III, we discuss mutation in SARS-CoV-2, the host genetic diversity of SARS-CoV-2, newly emerging variants of SARS-CoV-2, the genetic architecture of host proteins involved in SARS-CoV-2, and the landscape of host genetic factors correlating with SARS-CoV-2. Part IV includes the correlation of coronaviruses with various disorders, especially with epigenetic alteration, neurological disorders, and artificial intelligence.

This book will appear as a baseline for scientists and health professionals to better understand the genetic diversity of SARS-CoV, MERS-CoV, and SARS-CoV-2. However, this single book would not have succeeded without the enthusiasm and determination of publishers and investigators to take time from their hectic schedules and endow on time. We thank the scrutineers who contributed, directly and indirectly, to bring it to reality.

Kamal Niaz
Department of Pharmacology and Toxicology
Faculty of Bio-Sciences
Cholistan University of Veterinary and Animal Sciences (CUVAS)
Bahawalpur 63100, Pakistan

Muhammad Sajjad Khan
Department of Breeding and Genetics
Faculty of Animal Production and Technology
Cholistan University of Veterinary and Animal Sciences (CUVAS)
Bahawalpur 63100, Pakistan

&

Muhammad Farrukh Nisar
Department of Physiology and Biochemistry
Faculty of Bio-Sciences
Cholistan University of Veterinary and Animal Sciences (CUVAS)
Bahawalpur 63100, Pakistan

List of Contributors

Abdul Basit	Department of Microbiology, University of Jhang, Jhang 38000, Pakistan
Abdullah	Department of Pharmacy, University of Malakand, Chakdara Dir Lower, KP, Pakistan
Ahmad Ali	Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Alam Zeb	Directorate of Livestock and Dairy Development Department, Khyber Pakhtunkhwa, Peshawar, Pakistan
Amjid Islam Aqib	Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Arooj Fatima	Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Ayesha Saeed	Department of Biochemistry, Govt. College University, Faisalabad, Pakistan
Aziz ur Rahman	Department of Pharmacy, University of Malakand, Chakdara Dir Lower, KP, Pakistan
Chen Shanyuan	School of Life Sciences, Yunnan University, Kunming 650091, China
C-Neen Fatima Zaheer	Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
Fazlullah Khan	Department of Pharmacy, Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad, Pakistan
Firasat Hussain	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Furqan Shafqat	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Hina Afzal Sajid	Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
Ihtisham Ulhaq	Department of Biosciences, COMSATS University, Islamabad, Pakistan
Ijaz Ahmad	Department of Human, Legal and Economic Sciences, Telematic University "Leonardo da Vinci", Chieti, Italy
Imtiaz Ali Khan	Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan
Jannat Bibi	Institute for Chinese Olympic Advanced Study, Beijing Sport University, Beijing 100084, China
Kamal Niaz	Department of Toxicology and Pharmacology, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Kashif Prince	Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Kashif Rahim	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

Maryam Bashir	Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Mehmet Ozaslan	Division of Molecular Biology and Genetics, Department of Biology, Gaziantep University, Gaziantep 27000, Turkey
Momin Khan	Directorate of Livestock and Dairy Development Department, Khyber Pakhtunkhwa, Peshawar, Pakistan
Muhammad Ashir Nabeel	Department of Theriogenology, University of Agriculture, Faisalabad, Pakistan
Muhammad Dilawar	Department of Zoology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Farrukh Nisar	Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Farrukh Tahir	Department of Biochemistry, University of Jhang, Jhang 38000, Pakistan
Muhammad Humayun	Department of Biosciences, COMSATS University, Islamabad, Pakistan
Muhammad Ibrar	Department of Allied and Health Sciences, Iqra National University Swat Campus, Swat, Pakistan
Muhammad Kalim	Department of Microbiology and Immunology, Wake Forest University, Winston-Salem 27101, USA Houston Methodist Hospital Research Institute, Houston Methodist Hospital, Houston, Texas 77030, USA
Muhammad Luqman Sohail	Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Naveed	Department of Clinical Pharmacology, School of Pharmacy, Nanjing Medical University, Nanjing 211166, China
Muhammad Rafiq	Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Saeed	Department of Poultry Sciences, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Safdar	Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Sajjad Khan	Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Muhammad Shuaib	School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China
Muhammad Yasir Waqas	Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

Muhammad Zia Ahmad	Faculty of Social Sciences, University of Sargodha, Sargodha 40100, Punjab, Pakistan
Qurat ul Ain Babar	Department of Biochemistry, Govt. College University, Faisalabad, Pakistan
Rabia Liaqat	Department of Pathology, University of Agriculture, Faisalabad, Pakistan
Saadia Muneer	Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
Sana Tehseen	Department of Botany, Faculty of Science and Technology, Government College Women University, Faisalabad, Pakistan
Sartaj Ali	Department of Biosciences, COMSATS University, Islamabad, Pakistan
Shafeeq Ur Rehman	Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Sobia Noreen	Department of Pharmaceutical Technology, University of Innsbruck, Innsbruck 6020, Austria
Tean Zaheer	Department of Parasitology, University of Agriculture, Faisalabad, Pakistan
Umair Younas	Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Vishal Kiran	Government College University Lahore, Lahore, Pakistan
Yasir Razzaq Khan	Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan
Yasmeen Junejo	Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan

CHAPTER 1

Genetic Architecture of Host Proteins Involved in MERS-CoV

Firasat Hussain[1,*], Muhammad Kalim[2,3], Kashif Rahim[1], Shafeeq Ur Rehman[1], Furqan Shafqat[1], Muhammad Shuaib[4], Umair Younas[5], Amjid Islam Aqib[6], Imtiaz Ali Khan[7] and **Chen Shanyuan[8]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Department of Microbiology and Immunology, Wake Forest University, Winston-Salem, NC 27101, USA*

[3] *Houston Methodist Hospital Research Institute, Houston, Texas 77030, USA*

[4] *School of Ecology and Environmental Science, Yunnan University, Kunming 650091, China*

[5] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan*

[6] *Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[7] *Department of Entomology, The University of Agriculture, Peshawar 25000, Pakistan*

[8] *School of Life Sciences, Yunnan University, Kunming 650091, China*

Abstract: Middle East respiratory syndrome coronavirus (MERS-CoV), a novel coronavirus linked to severe respiratory tract illness, was initially identified in 2012. Since then, 1401 individuals have been infected with this virus in 26 countries, with 543 people (39%) dying. Severe respiratory infection, sometimes accompanying shock, acute renal damage, and coagulopathy are all symptoms of these disorders. This pandemic has sparked worldwide worry because of its human-to-human transmission *via* intimate contact. The Eastern Province, Riyadh, and Makkah were severely hit. In 2014, the pandemic progressed fastest in Makkah, Riyadh, and Eastern Province in 2013. Effective therapeutic and immunological solutions based on solid molecular research were critical, with the threat of an epidemic looming. The MERS-CoV intrinsic genetic heterogeneity across different clades may have set the way for cross-species transmission and alterations in inter-species and intra-species tropism. Host protease blockers include transmembrane serine protease 2 (TMPRSS2), cathepsin L, and furin. According to sequence comparison and modeling research, the viral spike features a putative receptor-binding domain (RBD) that enables this interaction.

* **Corresponding author Firasat Hussain:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan;
E-mail: firasathussain@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

The dipeptidyl-peptidase 4 (DPP4)-propeller engages with the receptor-binding subdomain but not the intrinsic hydrolase domain. The receptor binding subdomain of MERS CoV and severe acute respiratory syndrome coronavirus (SARS CoV) is drastically different. This chapter aims to explain the genetic architecture of host proteins involved in MERS-CoV and compare it with other coronaviruses.

Keywords: MERS-CoV, Protein S, Adaptive immune response, LY6E protein, DPP4, Dipeptidyl peptidase 4, CD26 protein.

INTRODUCTION

Given the present epidemic, the severe respiratory syndrome coronavirus-2 (SARS-CoV-2) has received a lot of study interest. Nonetheless, the Middle East respiratory syndrome-coronavirus (MERS-CoV), a formerly highly virulent coronavirus, remains a source of worry, particularly in Saudi Arabia and neighboring nations. Patients with mild to deadly MERS-CoV have a greater risk of spreading the virus because they shed more viral offspring than those with moderate symptoms [1, 2]. Reduced propagation and outbreak containment have been achieved by recognizing and isolating these individuals in healthcare institutions and instituting adequate infection control [3, 4]. Innovative MERS-CoV cases continue to be recorded, particularly in the Arabian Peninsula [4]. In the Arabian Peninsula, clade B viruses became common in dromedaries, producing zoonotic disease and, in some cases, clusters of human-to-human transmission. Camels in East Africa, Egypt, Ethiopia, Sudan, Djibouti, Kenya, North Africa, Morocco, West Africa, Nigeria, and Burkina Faso had viruses that belonged to different clade C sub-lineages [5, 6].

MERS-CoV is a zoonotic disease that causes asymptomatic to severe pneumonia in humans. The virus only produces a slight illness in dromedary camels but travels quickly amongst them. MERS-CoV, an innovative coronavirus, was discovered in people in the Middle East in 2012, followed by many European countries [7, 8]. A significant proportion of infected individuals (> 50%) experienced severe respiratory disease and clinical signs that were comparable to those reported during the 2003 SARS epidemic caused by the SARS-CoV [9]. Preliminary epidemiological studies show that this fatal virus may be spread from human to human, raising worldwide concerns about the opportunity of a MERS pandemic [10, 11]. The MERS-CoV virus requires a significant surface S glycoprotein to interact with and infiltrate the focus cell [12, 13].

According to recent studies, coronaviruses are exceptionally compatible with evading immune detection and decreasing immunological response [14]. This explains why they have such a lengthy incubation period, which may last 2 to 11 days. In a prior study, pegylated interferon (IFN) was more operative, contrary to

MERS-CoV, than SARS-CoV in cell culture in a macaque model [15]. MERS-CoV clearance from the respiratory system needs an IFN-mediated innate immune response. Overexpression of the LY6E (Lymphocyte Antigen 6 Family Member E) gene did not influence S1/S2 cleavage or the mutant MERS-CoV S pseudo-particle cell entrance level.

To summarize, LY6E is a CoV-restricting factor that prevents CoV invasion and defends the host from a severe viral illness. According to recent findings, coronaviruses appear unusually compatible with avoiding immune detection and lowering immunological reactivity. This helps to explain why they have such a long incubation period, ranging from 2 to 11 days [16]. Most processes, specifically the detection and signaling of IFN-I, depend on suppressing innate immune responses. Small and inexpensive measures are needed again for control, and comprehensive research is required to understand the pathogenesis and develop a vaccine against MERS-COV. Mice seem to be the most favorable small-animal species for this objective due to their abundance and the occurrence of a comprehensive level of knowledge, especially in heritability and serology. Unsurprisingly, rodents, gerbils, and badgers are immune to MERS-CoV because they lack the MERS-CoV binding site, sentient CD26 (hCD26), and synthetase activities occurred (DPP4) [17 - 19]. Receptor-binding domain (RBD)-protective trimer's immunity could be improved even more by stabilizing its trimeric configuration with disulfide bridges, trying to add other multimeric patterns, including GCN4, adapting spacer sequence data in between RBD and Fd, or incorporating such strategies, as has been completed for other virus particles [20]. Even though RBD-Fd influences the minimum concentration activation of cell-mediated immunity (data not shown), its robust control is primarily due to neutralizing antibodies. This supported the fact that almost all mice that withstood MERS-CoV infestation had reasonably extreme serum-thwarting levels of antibodies, implying that neutralizing antibodies, instead of cell-mediated immunity, could provide a significant function in attempting to avoid MERS-CoV infestation, which is based on RBD-trimeric form vaccine candidates.

The amplicon shares well with RBD–CD26 binding site interaction, and antibody adhesion prevents the viral RBD from conversing, including its cell surface receptor. As a result, one primary method of LCA60 suppression is the potent inhibition of virus–receptor interrelations. Algorithmic docking has proven experimental studies, and the creation of varieties enabled the identification of RBD toxins crucial for adhesion and neutralization. LCA60 has an innovative specific antigen that is unchanged by 3B11 and other phage-derived neutralizing antibodies. Proteins 7 and 8 are cofactors for polymerase activation, and protein 10 is a 20 O-methyltransferase. Three transmembrane proteins, 3, 4, and 6, function as membrane anchors for the replicative transcription complex. Protein

CHAPTER 2

Landscape of Host Genetic Factors Correlating with MERS-CoV

Abdullah[1], Aziz ur Rahman[1], Muhammad Ibrar[2] and **Fazlullah Khan[3,*]**

[1] *Department of Pharmacy, University of Malakand, Chakdara Dir Lower, KP, Pakistan*

[2] *Department of Allied and Health Sciences, Iqra National University Swat Campus, Swat, Pakistan*

[3] *Department of Pharmacy, Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad, Pakistan*

Abstract: The current outbreak of SARS-CoV-2 has raised various clinical and scientific questions, including the effect of host genetic factors on pathogenesis and disease susceptibility. MERS-CoV is a highly pathogenic virus in humans, causing high mortality (30-40%) and morbidity. CoVs are found to be widespread in man, poultry, and mammals. MERS-CoV enters the host cells by attachment with DPP4 receptors; it hijacks the host cell cycle, which helps in its survival and proliferation. Understanding the innate immune response against MERS-CoV is essential in the treatment development and precautionary measures. Nonstructural protein 1 (nsp1) has attracted greater attention as a potential virulence factor and a possible target for vaccine development. Downregulation of Th2, inadequate Th1 immune response, and overexpression of inflammatory cytokines IL-1α IL-1β, and IL-8 occur in the lower respiratory tract of patients infected with MERS-CoV. Research has shown that high viral load, high expression of inflammatory cytokines, and the downregulation of Th1 and Th2 response result in severe infection, contribute to lung inflammation, develop acute respiratory distress syndrome (ARDS) and pneumonia, and cause high fatality.

Keywords: Cell cycle, Genetic, Interleukin, MERS-CoV, Nonstructural protein.

INTRODUCTION

Several unique features characterize infectious diseases, such as a single agent causes them, they can cause an epidemic due to person-to-person transmission, and they can strongly impact human evolution [1, 2]. Moreover, infectious diseases may be eradicated, but new ones may emerge, thus forming a dynamic stage for human-infection interplay [3]. Innovative research on infectious diseases

* **Corresponding author Fazlullah Khan:** Department of Pharmacy, Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad, Pakistan; Tel: +92-3469433155; E-mail: fazlullahdr@gmail.com

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

has shown strong inter-individual differences attributed to host genetic makeup. Heritability studies have provided the primary evidence to corroborate this [4, 5], consequently increasing interest in understanding the genetic background of infectious diseases in the last decade of the 20th century [4, 5]. Consequently, more than 4000 gene studies focusing on respiratory infections were published between 2001 and 2010 [6]. As respiratory infectious diseases have the potential to cause epidemics and pandemics, extensive interest in the host factors has developed. In this connection, the Spanish influenza of 1918 has been recorded as the largest recorded pandemic, causing approximately 25-100 million deaths [7, 8].

Similarly, the severe acute respiratory syndrome-coronavirus (SARS-CoV) outbreak in 2003 showed how quickly a novel respiratory pathogen could spread on a global scale [9]. In September 2012, a beta coronavirus was found in the Middle East that causes severe respiratory infection in humans [10]. The International Committee on Taxonomy of Viruses named the virus Middle East Respiratory Syndrome-coronavirus (MERS-CoV) in 2013 [11]. Camel is the vector for MERS-CoV [12]. CoVs have a positive-sense, large, single-stranded RNA genome with a length of 27-32 kb, the largest among other viruses [13, 14]. Gene 1, which occupies two-thirds of the genome, consists of 2 large overlapping open reading frames (ORFs), ORF1a and ORF1b, having a ribosomal frameshifting signal at the junction of the 2 ORFs [15]. As the virus enters the host cell, its genome translation generates 2 large precursor polyproteins 1a (pp1a) and 1ab (pp1ab), which are processed by ORF1a-encoded viral proteinases 3C-like proteinase (3CL^{pro}) and papain-like proteinase (PL^{pro}) into 16 nonstructural proteins (nsp1-16). These are numbered according to their order from the N-terminus to the C-terminus of the ORF [15]. The nsps play a key role during viral RNA transcription and replication [16 - 18]. In addition, protease, helicase, RNA-dependent RNA polymerase, and some nsps are RNA processing enzymes [15, 19]. MERS-CoV infection is characterized by elevated systemic inflammatory chemokines/cytokines and immunopathology [20 - 22]. The high level of inflammatory chemokines and cytokines correlates with poor disease outcomes, increased infiltration of inflammatory cells into the lungs, and immunopathology [23, 24]. In addition, cytokine storms may occur due to stimulating chemokines, cytokines, and innate immune cells [25, 26]. Moreover, the high interleukin 8 (IL-8) level plays a vital role in acute SARS infection, severe immunopathology, and viral bronchiolitis pathogenesis [27, 28]. The high level of inflammatory IL-1α and IL-1β cytokines is linked with acute inflammatory response and tissue damage, consequently causing severe pathogenesis, mortality, and inflammatory loop induction [29, 30]. MERS-CoV evades the interferon (IFN) signaling cascade and nuclear factor-κB (NF-B) signaling pathway and antagonizes the antiviral immune response [31, 32]. Pathogenesis of MERS-CoV infection is

complex, involving the dissemination of the virus to other organs and severe damage to the lungs [33, 34]. As susceptibility to infectious agents lies at least partly hidden or masked in immune response or inborn errors, an insight into host genetic factors may prove invaluable [35]. This makes infectious disease a high research priority, keeping in view the mobility of modern humans and the changing nature of the pathogen.

Species Susceptibility

MERS-CoV can infect humans, bats, and camels. While guinea pigs, mice, hamsters, and ferrets are unaffected. SARS-CoV-2 better replicates in cats and ferrets than in pigs, dogs, ducks, and chickens [36]. Bats have been extensively investigated as they serve as natural reservoirs of many coronaviruses. Studies have focused on co-evolutionary aspects between CoVs and the bat genome and other studies related to host genetic factors [37]. These include well-known genes such as ACE2 receptor genes for SARS-CoV-1 [38] and the DPP4 receptor genes for MERS-CoV [39].

Host Receptors Involved in MERS-CoV Entry

MERS-CoV enters its host near or at the plasma membrane or endosomes. The spike protein of MERS-CoV binds with dipeptidyl peptidase 4 (DDP4). Because of this interaction, the protein cleavage sites of S protein are exposed. In the presence of cell surface proteases (hTMPRSS2), cleavage of S protein occurs, and thus, the fusion of the virus occurs at or near the plasma membrane. In the absence of cell surface proteases, endocytosis of MERS-CoV occurs, facilitated by endosomal proteases (cathepsin L) [40]. It has been found that MERS-CoV prefers plasma membrane entry instead of endosomal entry [41, 42].

Translation and the Unfolded Protein Response in MERS-CoV-Infected Cells

To facilitate virus assembly and replication of the viral genome, virus infection alters cellular gene expression. CoVs utilize the translational machinery of the host cell for viral protein synthesis. The host mRNA translation is stopped, the host antiviral response is inhibited, and the translational machinery is used for the non-canonical mode of protein synthesis of the virus [43]. During replication of CoV, the increased modification and production of viral proteins and virion budding-related endoplasmic reticulum (ER) membrane depletion cause overloading of the folding capacity of the ER, which results in ER stress [44]. This causes the activation of the unfolded protein response (UPR), causing the cell to reverse to homeostasis and reducing the risk posed by protein misfolding for the correct functioning of the cell [45]. In humans, three ER-resident transmembrane sensors control the UPR that are activating transcription factor-6

CHAPTER 3

History of SARS-CoV-2

Qurat ul Ain Babar[1], Ayesha Saeed[1], Maryam Bashir[2], Kamal Niaz[3], Muhammad Rafiq[2], Muhammad Yasir Waqas[2], Muhammad Farrukh Tahir[4], Amjad Islam Aqib[5], Muhammad Dilawar[6] and **Muhammad Farrukh Nisar[2,*]**

[1] *Department of Biochemistry, Govt. College University, Faisalabad, Pakistan*

[2] *Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[3] *Department of Toxicology and Pharmacology, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[4] *Department of Biochemistry, University of Jhang, Jhang 38000, Pakistan*

[5] *Department of Medicine, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[6] *Department of Zoology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

Abstract: Human CoVs (hCoVs) were discovered in people suffering from the common cold during the early 1960s. This family is comprised of four well-known genera, viz. α-CoV, β-CoV, γ-CoV, and Δ-CoV. Mammals, including humans, pigs, cats, and bats, may be infected by α-CoV and or β-CoV. γ-CoV mainly affects avifauna, whereas Δ-CoVs affect both birds and mammals. The coronavirus (CoV) outbreak has caused great devastation globally. CoVs are positive-sense, non-segmented single-stranded RNA viruses of the order nidovirales and the family Coronaviridae. Deep sequencing examination of lower respiratory tract pathological studies on affected people revealed the presence of a new coronavirus strain, which was termed SARS-CoV-2. Four structural proteins, viz. envelope protein (E), membrane protein (M), nucleocapsid protein (N), and spike protein (S) have also been determined. Following the very initial reports of the novel severe acute respiratory syndrome (SARS) coronavirus back in late 2019 from Wuhan, China, a plethora of research attempts arose on how SARS-CoV-2 made its entry into humans. There is still a difference in ideology for its laboratory escape or the zoonotic spread, but the exact phenomenon is not known yet. Completing a thorough review, the studies suggest that the virus's origin is more complicated than previously known.

Keywords: HCoVs, α-coronavirus, Single-stranded RNA, SARS, Wuhan.

[*] **Corresponding author Muhammad Farrukh Nisar:** Department of Physiology and Biochemistry, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan; Tel: +92-345-7100555; E-mail: mfarrukhnisar@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

INTRODUCTION

The coronavirus (CoV) outbreak has caused devastation across the continents. Coronaviruses (CoVs) are positive-sense, non-segmented, single-stranded RNA viruses of the order Nidovirales and the family Coronaviridae [1]. Human CoVs (hCoVs) were first discovered in people suffering from the common cold during the 1960s. This family comprises four well-known genera viz. alpha-coronavirus (α-CoV), beta-coronavirus (β-CoV), gamma-coronavirus (γ-CoV) and delta-coronavirus (Δ-CoV). Mammals, including humans, pigs, cats, and bats, may be infected by α-CoV or β-CoV. γ-CoV mainly affects avifauna, whereas Δ-CoVs affect both birds and mammals. The hCoV-NL63 and hCoV-229E of the genus α-CoV, hCoV HKU1, and hCoV-OC43 of the β-CoV lineage A are commonly implicated in the common cold in immunologically strong adults [2]. Because they spread across species and may infect a variety of animals, these viruses are referred to as zoonotic viruses. CoVs involved in the pathogenesis, such as the Middle East Respiratory Syndrome (MERS) viruses, Severe acute respiratory syndrome (SARS) viruses, and other gastrointestinal or respiratory infections, have been reported in animals and birds [3]. SARS-CoV was first discovered during SARS epidemics in Guangdong province, China, back in early this century [4, 5], while MERS-CoV caused major respiratory illness epidemics in the Middle East during 2012 [6]. These viruses have become pandemic, with SARS causing over 8096 illnesses in 26 countries and over 800 fatalities, while MERS has been found in 2500 documented human infections in 27 countries with 860 deaths (Table **1**) [7, 8]. The outbreak level of CoVs is normally linked with recombination and mutation allowing the generation of new viral strains having greater sensitivity to their newer hosts [9 - 15]. The 2019 novel coronavirus disease (CoVID-19) caused by SARS-CoV-2 was first reported in early December 2019 in Wuhan, Hubei province, China.

Table 1. Cumulative number of cases (Based on WHO 31st December 2003 data).

Areas	Female	Male	Total	Number of Deaths	Date Onset First Probable Case	Date Onset Last Probable Case
Australia	4	2	6	0	26-Feb-03	1-Apr-03
Canada	151	100	251	43	23-Feb-03	12-Jun-03
China	2674	2607	5327	349	16-Nov-02	3-Jun-03
China, Hong Kong Special Administrative Region	977	778	1755	299	15-Feb-03	31-May-03
China, Macao Special Administrative Region	0	1	1	0	5-May-03	5-May-03
China, Taiwan	218	128	346	37	25-Feb-03	15-Jun-03

(Table 1) cont.....

Areas	Female	Male	Total	Number of Deaths	Date Onset First Probable Case	Date Onset Last Probable Case
France	1	6	7	1	21-Mar-03	3-May-03
Germany	4	5	9	0	9-Mar-03	6-May-03
India	0	3	3	0	25-Apr-03	6-May-03
Indonesia	0	2	2	0	6-Apr-03	17-Apr-03
Italy	1	3	4	0	12-Mar-03	20-Apr-03
Kuwait	1	0	1	0	9-Apr-03	9-Apr-03
Malaysia	1	4	5	2	14-Mar-03	22-Apr-03
Mongolia	8	1	9	0	31-Mar-03	6-May-03
New Zealand	1	0	1	0	20-Apr-03	20-Apr-03
Philippines	8	6	14	2	25-Feb-03	5-May-03
Republic of Ireland	0	1	1	0	27-Feb-03	27-Feb-03
Republic of Korea	0	3	3	0	25-Apr-03	10-May-03
Romania	0	1	1	0	19-Mar-03	19-Mar-03
Russian Federation	0	1	1	0	5-May-03	5-May-03
Singapore	161	77	238	33	25-Feb-03	5-May-03
South Africa	0	1	1	1	3-Apr-03	3-Apr-03
Spain	0	1	1	0	26-Mar-03	26-Mar-03
Sweden	3	2	5	0	28-Mar-03	23-Apr-03
Switzerland	0	1	1	0	9-Mar-03	9-Mar-03
Thailand	5	4	9	2	11-Mar-03	27-May-03
United Kingdom	2	2	4	0	1-Mar-03	1-Apr-03
United States	13	14	27	0	24-Feb-03	13-Jul-03
Viet Nam	39	24	63	5	23-Feb-03	14-Apr-03
Total	-	-	**8096**	**774**	-	-

COVID-19 is responsible for serious respiratory infections particularly pneumonia, initially caused by an unknown source depicted by pneumonia-like symptoms and was eventually linked to a seafood market in Wuhan [16]. Deep sequencing examination of lower respiratory tract pathological studies on affected people revealed the presence of a new coronavirus strain, which was termed SARS-CoV-2. In China, the cumulative death toll surpassed 4645 after 84778 diagnoses and hundreds of suspects to date, representing a significant decrease in recent days. Later on, SARS-CoV-2 spread in over 137 countries worldwide and almost in every continent, resulting in about 7,823,289 cases and 431,541 fatalities. According to WHO, initially, the death rate of COVID-19 in China was

CHAPTER 4

Hosts Genetic Diversity of SARS-CoV-2

Kashif Prince[1], Arooj Fatima[2], Sana Tehseen[3], Muhammad Sajjad Khan[4], Muhammad Saeed[5], Firasat Hussain[2], Muhammad Naveed[6], Kashif Rahim[2] and **Umair Younas[7,*]**

[1] *Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Department of Microbiology, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[3] *Department of Botany, Faculty of Science and Technology, Government College Women University, Faisalabad, Pakistan*

[4] *Department of Breeding and Genetics, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[5] *Department of Poultry Sciences, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[6] *Department of Clinical Pharmacology, School of Pharmacy, Nanjing Medical University, Nanjing 211166, China*

[7] *Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

Abstract: The coronavirus disease-19 (COVID-19) spread worldwide in no time. Finally, the World Health Organization declared it a pandemic in March 2020. The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) can mutate, and many mutations have been observed worldwide. The severity of symptoms varies from mild to critical cases, and the incubation period ranges from 5-14 days. Various studies have shown that the diversity of SARS-CoV-2 within the hosts is prevalent, and some genomes are more susceptible to the alterations due to mutation. Some of the tissues that exhibited the highest ACE2 expression in different host tissues (humans) were kidneys, thyroid, heart, adipose tissue, small intestine, and testicles. Endothelial cells have also been the site for SARS-CoV-2. Chinese people were the first to be reported with the polymorphism detection for the ACE2 gene. Different variants of the ACE2 gene that are closely linked with hypertension were rs464155, rs4240157, and rs4830542. There has been a close association between ACE2 and TMRSS2 and SARS-COV-2, SARS-CoV-1, and influenza virus. The inducibility of heme oxygenase-1 (HO-1) enzyme to reactive oxygen species is regulated by the GT dinucleotide repeat mutation and polymorphism of the HO-1 gene.

[*] **Corresponding author Umair Younas:** Department of Livestock Management, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan, E-mail: umairyounas@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Keywords: SARS, Coronavirus, ACE2, Mutation, Virus, Genome.

INTRODUCTION

After the first coronavirus case reported in 2019, it has repeatedly modified itself. The new form of coronavirus, *i.e*., severe acute respiratory syndrome-coronaviru--2 (SARS-CoV-2), has a high capability to mutate itself and has done this to a great extent worldwide [1]. Not only do viruses vary from host to host, but within the host, different types of variants of viruses can be found with various mutations [2]. In 2019, Wuhan, China, reported the first case of coronavirus disease-19 (COVID-19), and it spread all over the world in no time. Finally, the World Health Organization declared the COVID-19 pandemic in March 2020. COVID-19 is a disease affecting the respiratory system, and the symptoms observed in this disease are persistent cough and fatigue [3]. However, a subpopulation of people infected with the virus remains asymptomatic [4]. The severity of symptoms varies from mild to critical cases, and the incubation period also varies from 5-14 days [5, 6]. The ability of coronavirus to proofread (due to nsp-14, *i.e* ., non-structural proteins) is attributed to a decrease in the rate of mutation as compared to other positive-sense ssRNA viruses, but studies have shown the diversity and prevalence of SARS-CoV-2 within the hosts, and some genomes are more susceptible to the alterations due to mutation [7, 8]. Virus isolation and sequencing efforts have led to the cross-examination of the SARS-CoV-2 genome, exhibiting better or more in-depth consideration of the vertical progress of the viral evolution along with its origin and the worldwide pattern of global spread [9, 10]. Researchers have conducted studies to address the ACE2 expression in human tissue samples and analyze the results without considering gender and age factors [11]. Some tissues that exhibited the highest ACE2 expression in different host tissues (humans) were kidneys, thyroid, heart, adipose tissue, small intestine, and testicles. Endothelial cells have also been the site for SARS-CoV-2; therefore, ACE2 expression was noted, thus explaining the possible explanation for the disease affecting various patients' organs at one time [11, 12].

The intermediate level of ACE2 expression was noticed in tissue samples of the liver, esophagus, adrenal gland, lungs, colon, and bladder. In contrast, blood vessels, spleen, bone marrow, muscle, uterus, and brain exhibited the lowest level of ACE2 expression. Given the immune patterns of women and men, the expression of ACE2 was shown to be downregulated and upregulated in the lungs [13]. It was reported that there are vasodilation and antifibrotic effects of angiotensin [14, 15]. In various conditions like heart failure, diabetes, and hypertension, there has been less cardiac expression of ACE2 [16, 17]. Chinese people were the first to be reported with the polymorphism detection for the

ACE2 gene. Different variants of the ACE2 gene that are closely linked with hypertension were rs464155, rs4240157, and rs4830542 [18 - 21]. It was noted that eleven common and rare variants were found to be closely linked with the increased expression of ACE2. These expressions were irregularly distributed for the various population groups [22]. It was noted that in the East-Asian population group, the increased expression of the ACE2 gene was closely related to the ACE2 gene polymorphism (variant 4,646,127), and this finding allows us to understand this vital issue more appropriately [22]. There has been a close association of ACE2 and TMRSS2 with SARS COV-2, SARS CoV-1, and influenza virus to facilitate the virus while entering the infected host cell. The spike protein of SARS CoV-2 is cleaved by TMPRSS2, which is supposed to be an androgen-reactive serine protease enzyme, leading to the activation and entry of the virus [23], as shown in Fig. (**1**). There have been many studies conducted on the polymorphism of single nucleotide in TMPRSS2, as in the case of breast cancer, the high patient endurance is correlated with the rs2276205 (A>G) having a low-frequency allele [24]. Similarly, in the case of prostate cancer, there is a high frequency of TMPRSS2 rs12329760 (C>T) mutation in men with a family history of disease. Additionally, the ERG gene fusion, often associated with prostate cancer, is observed in these cases, suggesting a genetic predisposition to the condition [25, 26].

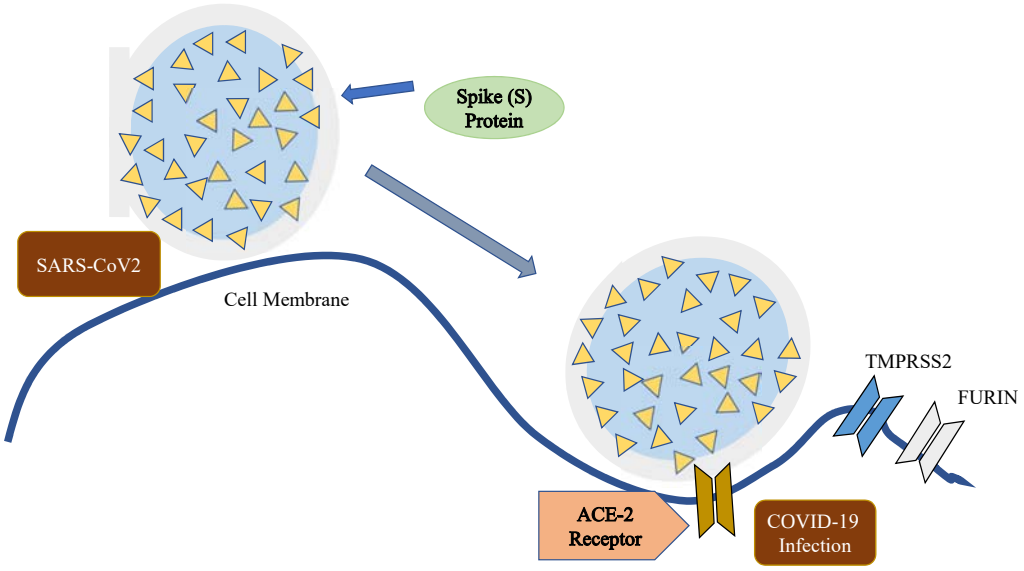


Fig. (1). Demonstration of COVID-19 infection activation: the viral spikes interact with ACE-2 receptors while invading the cell. Spikes are cleaved by TMPRSS2 (membrane protease 2), host cell protease, and furin, activating COVID-19 infection [83].

CHAPTER 5

Newly Emerging Variants of SARS-CoV-2

Muhammad Safdar[1, *], Mehmet Ozaslan[2], Yasmeen Junejo[3], Umair Younas[4], Muhammad Zia Ahmad[5], Jannat Bibi[6] and **Sobia Noreen[7]**

[1] *Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Division of Molecular Biology and Genetics, Department of Biology, Gaziantep University, Gaziantep 27000, Turkey*

[3] *Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[4] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[5] *Faculty of Social Sciences, University of Sargodha, Sargodha 40100, Punjab, Pakistan*

[6] *Institute for Chinese Olympic Advanced Study, Beijing Sport University, Beijing 100084, China*

[7] *Department of Pharmaceutical Technology, University of Innsbruck, Innsbruck 6020, Austria*

Abstract: Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus appeared at the end of 2019 and was subsequently named coronavirus disease-19 (COVID-19). Its worldwide emergence resulted in a large number of infections. Many studies depicted that the information about genomic variations in viruses has important effects on the prognosis and treatment of transmissible diseases. In this chapter, we collected various genomic variants, performed a phylogenetic analysis of recently registered genomes at various databases, and characterized the SARS-CoV-2 operating on silicon tools. Many complete sets of SARS-COV-2 are available in different databases such as GenBank and verified by National Genomics Data Center (NGDC) and National Microbiology Data Center (NMDC) databases. We found various variants, and the most common variants were 3037C>T (ORF1ab), 14408C>T (ORF1ab), 23403A>G (S), 25563G>T (ORF3a), 1059C>T (ORF1ab) and 241C>T (5' UTR) in online data samples. In addition, the complete genome sequence identity of the SARS-COV-2 results was 96.2% similar to that of a bat. These identified variations have increased the frequency of the spread of SARS-CoV-2. This information assists a comprehensive collection that combines genomic characterization, epidemiological and graphical records.

* **Corresponding author Muhammad Safdar:** Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences (CUVAS), Punjab, Bahawalpur 63100, Pakistan; Tel: +92-341-6946240, E-mail: msafdar@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Keywords: Genomic variants, Phylogenetic analysis, SARS-COV-2, ORF3a , epidemiological records .

INTRODUCTION

The World Health Organization (WHO) declared COVID-19 a global pandemic, and this crisis continues to result in millions of deaths worldwide. Furthermore, it has a significant impact on the world economy. Despite the efforts of healthcare workers and scientists worldwide, the number of confirmed cases and deaths continues to rise. As mentioned in the literature, there are currently a limited number of clinically approved vaccines and antiviral medications available to control the spread of the virus [1 - 3]. However, efforts to develop new treatments and vaccines continue, and ongoing vaccination campaigns are underway in many countries to mitigate the impact of the pandemic [1]. This chapter demonstrates the current genome present in the online databases to better understand the variation and genomic characteristics of SARS-CoV-2. It is important to note that the genome is 30 kb, and its structure corresponds to particular features of SARS-CoV and MERS-CoV [2 - 4].

High-quality, complete genome sequences of viral isolates, regardless of their virulence or other unique characteristics, are essential for accurate COVID-19 sequencing and analysis [5]. Therefore, the spread of COVID-19 is expected to persist, with increasing genetic diversity due to seasonal outbreaks. The evolution of its subtype can be revealed through ongoing genome data analysis, and the subtype evolution dynamics will reveal the genome data analysis [6]. Different databases make genomic information of SARS-COV-2 available to the public for research and discovery of new drugs [7]. For data sharing, users must agree to cooperate with all data participants and give appropriate credit. Some countries like China, the Philippines, and Japan have contributed to the success of this initiative, even though they did not share it. WHO supports the rapid release of available coronavirus sequences that provide comprehensive information to the public and researchers through databases. COVID-19 variants and microRNAs are involved in their pathogenesis. However, the role of microRNAs in viral infections is not clear. MicroRNA-mediated gene regulation plays a role in the host's response to different COVID-19 variants. It is necessary to determine the specific microRNAs involved and their functional significance in COVID-19 pathogenesis and its variants.

Therefore, this chapter helps provide comprehensive and instant data that incorporates genomic variants, epidemiology, and clinical features of COVID-19 patients simultaneously [8]. By mining and utilizing the databases and building a statistical framework based on genetic, antigenic, and epidemiological

information, more insights are gained into new variants, thereby advancing the potential for preventing and controlling COVID-19 [3, 9, 10]. In this chapter, we focused on discussing the variants between the old and recently sequenced genomes of SARS-CoV-2 and phylogenetic analysis for the origin of this virus.

NGDC and NMDC Databases

We downloaded different available genomes from GenBank and verified them from NGDC and NMDC databases. Previously, some genomes were excluded from the analysis due to excessive variations with gaps. A study utilized the NC_045512 genome sequence as a reference for genomic coordinates to analyze real samples [11]. As a result, the coordinates of the genome should be adjusted for comparison with previous studies [12]. Before conducting the analysis, the genomes were compared and matched to the reference genome. The alignment process used default clearance penalties of 10 and extension penalties of 0.5. The differences were removed compared to NC_045512 to create variants [13]. In this study, the researchers used protein annotations to convert nucleotide-level variants to amino acid codon variants, which were then aligned with the genes [14]. The study also identified nucleotide mutations in the genomes of the coronaviruses. Additionally, a whole-genome-based phylogenetic tree of the coronaviruses was constructed using the maximum-likelihood method with BEAST, and the GTR+I+G nucleotide model of substitution was used. This type of analysis can help researchers understand the genetic relationships between different strains of the virus and how it has evolved. In the phylogenetic trees, it is used to identify COVID-19 variants and understand their origin and relationships with others. By comparing the genetic sequences of different variants, some scientists have determined that the mutations track the spread of the variants worldwide.

Identification of Variants

Many variants were found in different online databases, and some variants were found to be unique (Tables **1** and **2**). Some distinct variants of the genomes, such as missense, synonymous, and non-coding alleles, are shown in Tables **1** and **2**. The variants such as 3037C>T(ORF1ab), 14408C>T (ORF1ab), 23403A>G (S), 25563G>T (ORF3a), 1059C>T (ORF1ab) and 241C>T (5' UTR) [7] were present. Both 3037C>T and 23403A>G (S) are synonymous. Furthermore, 14408C>T causes amino acid modifications such as P4803L in ORF1ab, and 25563G>T causes amino acids to change Q8521H [7]. It is worth noting that the majority of the sub-strains carrying the 14408C>T and 25563G>T variants are found outside of Wuhan, and the most commonly observed base change is C>T. After that, the frequency of the SARS-CoV-2 ORF1ab gene showed that this gene was the most

CHAPTER 6

Genetic Architecture of Host Proteins Involved in SARS-CoV-2

Abdullah[1], Muhammad Ibrar[2] and **Fazlullah Khan[3,*]**

[1] *Department of Pharmacy, University of Malakand, Chakdara Dir Lower, KP, Pakistan*

[2] *Department of Allied and Health Sciences, Iqra National University Swat Campus, Swat, Pakistan*

[3] *Department of Pharmacy, Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad, Pakistan*

Abstract: Proteins are the functional units of the cell that allow viruses to reproduce inside host cells. Proteins are essential for a cell's proper operation. Gene variations can reveal potential new therapeutic targets. Examining the innate immune system, coagulation, and other host proteins about the severity or mortality of COVID-19 reveals potentially changeable maladaptive host responses. Proteins are considered to be the high prevalent biological group of pharmacological factors, and high-throughput proteomics methods are quickly being employed to find prospective target molecules for innovative development of new drugs and repurposing studies. Researching the naturally occurring variations in the human gene sequence that code for therapeutic targets can also show how treatments work and ensure that people are safe. Researchers can create novel or repurposed therapeutics by examining the host protein's genetic makeup that interacts with SARS-CoV-2 or supports host responses to COVID-19.

Keywords: COVID-19, RNA viruses, Ritonavir, Nucleocapsid, Spike proteins.

INTRODUCTION

During December 2019, many cases of respiratory infections due to unknown reasons were reported in Wuhan city, spreading rapidly to other countries. The infectious virus was isolated, identified, and named 2019 novel coronavirus, and the condition was diagnosed as coronavirus disease-2019 [1, 2]. The virus has a more substantial transmission capacity, and the rapid increase in confirmed COVID-19 cases makes control and prevention extremely challenging. Novel pathological conditions caused by RNA viruses with genetic recombination, mutation, and cross-species transmission are considered a critical health chall-

* **Corresponding author Fazlullah Khan:** Department of Pharmacy, Faculty of Pharmacy, Capital University of Science and Technology (CUST), Islamabad, Pakistan; Tel: +92-3469433155, E-mail: fazlullahdr@gmail.com

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

enge worldwide, exemplified by COVID-19 [3]. Researchers struggle to search for possible strategies to control disease and develop therapies for prevention in the future. Currently, there is an intense need to find specific targets for treating the disease and develop new treatment strategies or repurpose existing drugs. Finding agents might prevent vulnerable persons from contracting COVID-19 and successfully treat people with severe COVID-19 symptoms [4 - 6].

It is critical to comprehend the genetic makeup of pharmacological targets to determine the genetic connections between molecular targets and illnesses [7, 8]. Human drug targets' human gene sequence variations can be employed to directly assist therapeutic mechanisms and guarantee human safety. Major pharmaceutical companies have implemented this strategy to find and validate therapeutic targets for a range of non-communicable disorders as well as for drug repurposing. Recently, extensive research has been done on the genomes and plasma based on aptamer-proteome data of over ten thousand people who had not been exposed to COVID-19. Understanding the genetic structure of 179 host proteins that are important to SARS-CoV-2 was the goal of these investigations. These results emphasise the possibility of using genetic variations as tools to confirm therapeutic targets in newly emerging genome-wide association studies [1] on COVID-19 [9, 10].

SARS-CoV-2 Relevant Proteins

A single-stranded RNA virus (SSRV) called SARS-CoV-2 has a genome that is about 30 kb in size. It has fourteen open reading frames (ORFs), which can be used to encode several different proteins. The nucleocapsid (N) protein, the spike (S) protein, the membrane (M), and the envelope (E) protein are the four primary structural proteins of the virus [11].

Spike Proteins

Many experiments have been performed to target the S protein of SARS-CoV-2, which is found on the virus's outer coat and is necessary for viral entry into host cells. The ability of the S protein's spike protein to trigger antiviral antibodies has made it a key target for the development of a SARS-CoV-2 vaccine. S protein-targeting antibodies can prevent the virus from infecting and replicating in the host cell [12]. The spike protein is the most antigenic part of any viral protein and is responsible for inducing humoral immunological reactions [13] and the production of neutralizing antibodies, both of which result in protective immunity against viral infections. As a result, the spike protein has been chosen as a crucial target for SARS-CoV-2 immunization [14, 15]. Around 35 of the 47 vaccine candidates currently undergoing clinical testing use spike proteins and different technological platforms. The full-length S protein (FLSP), the receptor-binding

protein (RBD) domain, the S1 subunit, the S2 subunit, the N-terminal domain (NTD), and the membrane fusion peptide (FP) are a few examples of the S protein's possible antigens [16].

The Nucleocapsid Protein

The N protein, which is found in the viral envelope, is essential for forming the helical nucleocapsid during virion assembly by wrapping the RNA. In comparison to the other four human coronaviruses, a recent study suggests that the SARS-CoV-2 N protein (S2N protein) demonstrates a higher level of conservation with SAS-CoV and MERS-CoV. It is interesting to note that compared to the S protein, the S2N protein appears to trigger a higher humoral and cellular immune response against SARS-CoV-2 [17].

Membrane Protein (M Protein)

The coronavirus M protein, which is principally in charge of starting the virus budding process, is situated in the virus envelope between S proteins and minor amounts of the E protein and is essential for virus assembly [18]. The M protein interacts with several additional SPs during viral assembly, including the N protein, E protein, and S protein [19]. Significant CD4+ and CD8+ T cell-mediated immune responses against the S2M protein have been found in virus-infected and recovered patients in recent investigations. Notably, S2M was highly recognized, and significant reactivity was seen [20]. Previous research has demonstrated the immunogenicity of the SARS-CoV M protein by demonstrating that synthetic peptides derived from immunodominant epitopes triggered potent antibody-induced immune responses in immunized rabbits [21]. The envelope protein and Nsp3 protein, which include numerous functional domains and play significant roles in helping viral pathogenesis, are two other proteins that require attention in addition to the M protein.

Local Genetic Architecture of Protein Targets

About 220 DNA sequence variations that affect 97 proteins in trans have been found using a total of 106 aptamers. Seven of these proteins were recognized and demonstrated by multiple aptamer pairs or triplets [22]. Thirty six of the 96 proteins were identified, and 15 of these previously identified proteins interact with proteins that are encoded in the genome of SARS-CoV-2 (such as COMT, PLOD2, DCTPP1, SDF2, ERO1LB, GLA, ERLEC1, MFGE8, EIF4E2, IL17RA, MARK3, FKBP7, PTGES2, PLAT, and COL6A1) and are targets for currently available drugs [23]. In addition, 16 proteins, including PLG, IL2RA, F2, F5, F8, F9, F10, CD14, FGB, IL6ST, IL1R1, IL2RB, VWF IL6R, SERPINE1, and

CHAPTER 7

Landscape of Host Genetic Factors Correlating with SARS-CoV-2

Ihtisham Ulhaq[1], Abdul Basit[2], Firasat Hussain[3], Muhammad Humayun[1], Umair Younas[4], Sartaj Ali[1], Amjad Islam Aqib[5] and **Kashif Rahim[3,*]**

[1] *Department of Biosciences, COMSATS University, Islamabad, Pakistan*

[2] *Department of Microbiology, University of Jhang, Jhang 38000, Pakistan*

[3] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[4] *Department of Livestock Management, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[5] *Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

Abstract: The researchers revealed a novel coronavirus in the Chinese population on 7th January 2020, named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The previous coronaviruses proved merely the tip of the iceberg after the emergence of the recently identified SARS-CoV-2. The potential of pandemic status significantly revealed the concealed capabilities of virulence and contagiousness of the betacoronaviruses group. This book chapter discusses the landscape of host genetic factors correlating with SARS-CoV-2. All SARS-CoV-2 genes code for the structural and non-structural proteins that have distinct interactions with host proteins. NSP13 is associated with centrosome and insulin signals in humans, NSP5 is associated with the ATPases of host cells, and NSP9 is associated with the nuclear pore's host proteins. The ORF8ab and ORF8b avoid the host immune responses and inhibit the signaling cascade of INF-β. Cytokine storm is associated with TLR2, FOXO1, and MYC genes of SARS-CoV-2 that further cause host cell death during infection. STAT1, IFIH1, IRF9, OAS1-3, and PML are associated with the immune response to SARS-CoV-2 infection, particularly the production of type I interferon. The SARS-CoV-2 entry is affected by the TMEM106B gene, and this gene can prevent virus-induced cell death. Replication of SARS-CoV-2 reduces due to deletions in TMEM106B and VAC14 genes. Genetic variants also influence the host susceptibility in the major histocompatibility complex antigen loci (HLA). The susceptibility of COVID-19 is considerably associated with the genetic variation in HLA and plays a significant role in identifying populations at higher risk.

[*] **Corresponding author Kashif Rahim:** Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan, E-mail: kashifrahim@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

Keywords: SARS-CoV-2, COVID-19, Host, Genetic factors, GWAS, TWAS, HLA, Virus-host interactions.

INTRODUCTION

At the end of December 2019, Chinese people were diagnosed with a respiratory tract infection of an unidentified etiology in Wuhan, Hubei Province [1]. Initially, physicians considered it pneumonia due to the clinical conditions of the patients. During laboratory investigations, a novel coronavirus was identified from throat samples of hospital-admitted patients through Real-time PCR (Polymerase Chain Reaction) and next-generation sequencing [2]. Therefore, a new coronavirus was ascertained on 7th January 2020 and given the name novel coronavirus 2019 by WHO (World Health Organization) [3]. ICTV (International Committee on Taxonomy of Viruses) later named it SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) as it was genetically parallel to the previous SARS coronavirus that emerged in 2002 [4]. The person infected with SARS-CoV-2 had the symptoms of coughing, sneezing, fatigue, difficulty breathing, and sometimes diarrhea, while the disease was named COVID-19 (Coronavirus Disease 2019) officially by WHO [5]. The acknowledgment of the *Coronaviridae* family comes about by ICTV **(**The International Committee on Taxonomy of Viruses), which works on viral nomenclature and classification [6]. Coronaviruses are respiratory pathogens belonging to the family of *Coronaviridae*. Coronaviruses have the largest genome size among RNA viruses with a positive sense [7]. Coronaviruses have a non-segmented genome covered in an envelope. The order Nidovirales includes the largest family, *Coronaviridae,* which is divided into two subfamilies: *Orthocoronavirinae and Torobirinae* [8]. *Orthocoronavirinae* is classified into four genera: alpha, beta, gamma, and delta. Each of these genera targets different animal species for infections. Alphacoronavirus and betacoronavirus have broad host tropism as they infect birds, animals, and humans, while deltacoronavirus and gammacoronavirus infect only birds [9]. Betacoronaviruses infect various animals. Host tropism is broad and has zoonotic potential but is frequently reported in humans, bats, and camels [10 - 13]. The size of the betacoronavirus genome is 26-32kb, while the overall virus size is about 60-140nm [14, 15]. The recently identified SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) genome contains 29844 to 29891 coding nucleotides, lacking the hemagglutinin-esterase gene [16]. The virus's genome is covered in a capsid whose symmetry is helical and constructed from structural proteins. The genome and capsid have collectively been termed the nucleocapsid. Further, the nucleocapsid is wrapped by a membrane, an envelope assembled by lipids and proteins [17]. These membranous structures prevent the environmental factor's influence on the virus and ensure its safety in unfavorable conditions [18]. Moreover, spikes protrude from the envelope made of glycoproteins, making the

virus appear like a crown. Based on this morphology, it has been named coronavirus by ICTV [19]. Furthermore, the glycoproteins in spikes are essential for viral structural proteins (VSP). Additionally, they play a role in the virus's binding to the susceptible host cell and entering genetic material [20]. There are two domains in spike glycoproteins with different roles; one is associated with the viral envelope, and the other is part of the receptor-binding portion [21]. SARS-CoV-2 is phylogenetically similar to SARS-CoV, and there is a higher similarity in the receptor-binding domain and S gene of both SARS coronaviruses, indicating their capability for human-to-human transmission [22]. Genetic analysis of RBD in the S gene revealed that new mutations would deviate from the host range and cellular tropism of SARS-CoV-2 [23 - 26]. The enzyme RNA-dependent RNA polymerases in RNA viruses triggered the mutations and frequently made genetic recombinations [27 - 30] that ultimately caused an evolution in SARS-CoV-2. Single nucleotide polymorphisms (SNPs) identified two major subtypes of SARS-CoV-2 as L and S; the subtype L is more destructive and contagious [31]. The mutations and genetic recombinations make the virus more lethal and are strongly associated with disease severity and mortality rate [31]. All the functional characteristics of SARS-CoV-2, such as transmission, pathogenesis, response to host defenses, and receptor affinity, are altered due to the high mutation rate that leads to genetic diversity [31]. Full-length genome analysis of SARS-CoV-2 showed that the protein profile of the SARS-CoV-2 has a strong association with the human host proteins [32]. This book chapter aims to analyze the SARS-CoV-2 –host interactions on genetic level genome-wide associated studies (GWAS), transcriptome-wide associated studies (TWAS), regional human leukocyte antigen (HLA) associations, the role of pro and anti-viral genes, and cross trait associations, as shown in Fig. (**1**).

Genome-Wide Associated Studies (GWAS)

After a few weeks of COVID-19 emergence in December 2019, on January 24, 2020, the first genome of SARS-CoV-2 was sequenced [33]. Moreover, it had 75–80% genetic similarity to SARS-CoV [34, 35]. Besides, SARS-CoV and SARS-CoV-2 have genetic similarities with bat-derived SARS-related coronavirus and RaTG13 [36]. The size of the betacoronavirus genome is 26-32kb, while the overall virus size is about 60-140nm [37, 38]. The recently identified SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) genome contains 29844 to 29891 coding nucleotides, lacking the hemagglutinin-esterase gene [39]. The virus's genome is roofed in a capsid whose symmetry is helical and constructed from structural proteins. The genome and capsid have collectively been termed the nucleocapsid.

CHAPTER 8

Epigenetic Mutations and Coronaviruses

Amjad Islam Aqib[1,*], Yasir Razzaq Khan[1], Tean Zaheer[2], Rabia Liaqat[3], Muhammad Luqman Sohail[1], Ahmad Ali[1], Hina Afzal Sajid[4], Firasat Hussain[5] and **Saadia Muneer[6]**

[1] *Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Department of Parasitology, University of Agriculture, Faisalabad, Pakistan*

[3] *Department of Pathology, University of Agriculture, Faisalabad, Pakistan*

[4] *Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan*

[5] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[6] *Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan*

Abstract: All genetic variations are the outcome of mutations in the genetic material. The greater the mutation ratio, the greater will be the genomic diversity. Currently, epigenomics enables us to locate, read, and translate the epigenetic mechanism that monitors and reins the whole genome of coronaviruses at different stages. Many researchers reported the role of epigenetic mutations in the development and progression of several common viral infections, especially age-related diseases. Many families of viruses can counter the immune response by utilizing a cascade of epigenetic events and taking over the regulatory capacity for their benefit. Coronaviruses possess the same mechanism to affect epigenetic machinery, *i.e.*, by improving mutations in the epigenetic code, DNA methylation, post-translational alterations of histone proteins and other proteins linked with epigenome, or direct dysregulation of enzymes.

Keywords: Epigenetic mutations, Enzyme dysregulation, DNA methylation, Post-translational alterations.

INTRODUCTION

Coronaviruses are enveloped viruses; they have a crown-like appearance under an electron microscope with an RNA genome that is single-stranded and a positive sense strand. Their genome size ranges from 26-32 kilobases (kb) in length. It is

* **Corresponding author Amjad Islam Aqib:** Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan; Tel: +92 343 7474583; E-mail: amjadwaseer@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

known that coronaviruses have giant RNA genomes. These have been structured into three classes, namely γ- coronaviruses, β- coronaviruses, and α-coronaviruses, based on their genetic portfolio and antigenicity. Coronaviruses primarily infect birds and mammals by crossing species barriers and causing various infections ranging from general cold to further serious illnesses, such as the Middle East respiratory syndrome (MERS) and SAR, which particularly impact the farming industry. Recently, in 2019, they destroyed the world, causing severe respiratory diseases that eventually led to global lockdowns that stalled life on Earth with millions of deaths. The disease symptoms range from upper respiratory tract infection (URTI), like the common cold and flu, to infection of the lower respiratory tract (LRTI), for instance, bronchitis, pneumonia, and even severe acute respiratory syndrome (SARS). Transmission of coronaviruses results from close contact and *via* respiratory droplets generated by sneezing and coughing [1, 2]. Coronaviruses have been known to circulate among other animals and have been identified to infect human beings, as in SARS and MERS. Four other coronaviruses cause respiratory indications resembling the cold [3, 4].

A novel coronavirus, designated coronavirus disease-2019 (COVID-19), was identified when a number of pneumonia cases were discovered in Wuhan, China, and its province Hubei by the end of 2019 [5]. In late January 2020, thousands of coronavirus cases were confirmed by laboratories in China, with a constant rise in cases in adjacent areas eventually spreading throughout China first and then the rest of the world. Epidemiologic investigation in Wuhan associated the coronavirus with a seafood bazaar where the most patients had visited or worked [6, 7]. This market was eventually closed for sterilization. The seafood marketplace also sold living bats, snakes, rabbits, and different kinds of animals [8]. However, as the virus spread, many laboratories claimed that it had no contact with this market. There was a surge in cases recognized among healthcare labors and other interactions of coronavirus-infected patients.

In China, transmission through human-to-human contact was confirmed, including in the United States [9, 10]. A report of a trivial cluster using five cases recommended that there has been a spread from an asymptomatic patient that occurred all through the incubation period; eventually, all sufferers that fall in this group are known to have mild infection [11].

There is a small E protein in the coronavirus, and 76 to 109 amino acids are present in its integral membrane protein. Further studies on prime and secondary configuration suggested that E has an amino terminus that is hydrophilic, containing 7 to 12 amino acids. It is small and tracked by a bulky transmembrane domain (TMD) that is hydrophobic, consisting of 25 amino acids, as it finishes with a hydrophilic carboxyl terminus that is long and consists of the bulk of the

protein. A pore in membranes is ion-conductive, formed by the amphipathic α-helix TMD [12].

Why are Viral Mutations Important?

All genetic variations are the outcome of mutations in the genetic material. Mutations result from hereditary material or nucleic acid damage and are not restricted to replication only [13]. The mutation is the first step of evolution as it yields new strands of DNA/RNA for a particular gene, ending up creating a new allele. New DNA/RNA strand sequences (a new allele) can also be produced by recombination technology that creates a specific gene. The mutation rate is a variation in genetic material when passed to the subsequent generation of an organism, and it is described as a probability. Usually, in the case of viruses, a generation is determined by a cell infection phase; this comprises adherence to the cell exterior, fusion of the cell membrane, uncoating, replication, gene expression, encapsulation, and finally, discharge of the infective particles. The frequency at which mutations occur in the virus should not be confused with the virus's mutation rate, as both go different ways. The former is a measure of genomic disparity, which is the combination of several factors, such as recombination, random genetic drift, and natural selection. The greater the mutation ratio, the larger will be the genomic diversity. Even though genetic diversity is always dependent on several factors, the mutation level is of prime concern as it is the crucial basis of genomic variation [14]. Likewise, alteration rates must not be tangled through molecular evolutionary ratios. According to the neutral theory of molecular evolution, these two have a linear association. However, the mutation is an inherited/biochemical procedure. Molecular evolution is linked to the infatuation of novel alleles in populations. Understanding pharmacogenomics and viral modification rates is crucial for better consideration and managing the pathogenesis, medication resistance, vaccination, immune avoidance, and the emergence of new strains causing new illnesses [15].

The Baltimore taxonomy of viruses is built conforming to the genomic material of the virion: positive-strand RNA viruses such as tobacco mosaic virus, rhinoviruses, noroviruses, and hepatitis C virus, and negative-strand RNA viruses such as rabies virus, influenza virus, and Ebola virus; likewise there are RNA viruses that are double-stranded for instance rotaviruses and bursal disease virus; also there are retroviruses, for example, human T cell leukemia virus, HIV, *etc*., and para-retroviruses, *i.e*., hepatitis B viruses [13]. After the classification of RNA viruses, there are single-stranded DNA viruses (including bacteriophage and parvoviruses) and double-stranded (including herpes viruses, papillomaviruses, adenoviruses, and poxviruses). Viruses are non-living infectious particles that entirely rely on human cell machinery to replicate, and in biological systems, they

CHAPTER 9

Neurological Complications of SARS-CoV, MERS-CoV, and SARS-CoV-2

Amjad Islam Aqib[1,*], Tean Zaheer[2], Rabia Liaqat Khan[3], Yasir Razzaq Khan[1], Ahmad Ali[1], Hina Afzal Sajid[4], Vishal Kiran[5], C-Neen Fatima Zaheer[6], Firasat Hussain[7] and **Muhammad Ashir Nabeel[8]**

[1] *Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Department of Parasitology, University of Agriculture, Faisalabad, Pakistan*

[3] *Department of Pathology, University of Agriculture, Faisalabad, Pakistan*

[4] *Center of Excellence Molecular Biology, University of the Punjab, Lahore, Pakistan*

[5] *Government College University Lahore, Lahore, Pakistan*

[6] *Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan*

[7] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[8] *Department of Theriogenology, University of Agriculture, Faisalabad, Pakistan*

Abstract: This chapter comprises the neurological pathogenesis of Coronaviridae in the central nervous system (CNS). These viruses manifest their virulence factors involving multiple organs of the body, initiating from febrile conditions, respiratory distress, and hypoproteinemia leading to edematous fluid accumulation. They pave their path to CNS by directly affecting the cranial plus vagus nerve fibers and synapses or through systematic circulation. The viruses can have an affinity with various receptor sites present on organs that help in hematogenous and retrograde mobility towards CNS. Comorbidities occur excessively due to these viruses in the living system involving vital organs such as the liver, heart, and lungs. Neurological dissemination of these viruses is characterized by a permanent loss of nerves or part of the CNS, either entirely or partially. Prevention is suggested, accompanied by adequate treatment and care management to avoid extensive spreading of the virus throughout CNS.

Keywords: Comorbidities, MERS-COV, Neurological complications, SARS COV-2, SARS-COV.

[*] **Corresponding author Amjad Islam Aqib:** Department of Medicine, Faculty of Veterinary Science, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan; E-mail: amjadislamaqib@cuvas.edu.pk

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

INTRODUCTION

Coronaviruses are classified as enveloped viruses and belong to the Nedovirals and Coronaviridae families. There are four genera: *Alpha*, *Beta*, *Gamma*, and *Delta*. Their genome is positive-sense, single-stranded RNA [1]. Among them, all the genera except gamma coronaviruses are responsible for the infection in a variety of mammals. Despite the ability of all four genera to transmit between species, they all harm humans by causing severe respiratory syndromes like the Middle East respiratory syndrome, which is brought on by MERS-CoV. Additionally, SARS-CoV is involved in respiratory syndrome with severe acuteness (SARS), and SARS-CoV-2 has recently been linked to the COVID-19 virus [2]. Scientists first described the CoV disease in 1931, and in 1965, they isolated the first humanoid virus (HCoV-229E). Only two types were available in late 2002. Among coronaviruses, HCoV-OC43 and HCoV-229E were known to exist when the prevalence of SARS was first observed. Since then, six more coronaviruses have been discovered that affect humans. Wuhan, China, saw the first SARS-CoV-2 detection in December 2019, which quickly became a global pandemic within months [3]. The pandemic has had an important impact on the world economy, social interactions, and public health, resulting in a rise in mortality rates worldwide [4]. COVID-19, a disease caused by SARS-CoV-2, is a recognized medical condition. Symptoms of COVID-19 are similar to those of the common flu, including fever (90%) and cough (70%), as well as myalgia and lethargy (50%). Headache (8%), diarrhea (5%), ageusia, and anosmia can also occur as the first signs [4]. In contrast, the lesser fraction of severe cases showed some severe lower respiratory tract infections that frequently needed respiratory aid. 5% of asymptomatic cases of this virus have also been detected [5].

The worst condition of the pandemic has led the health systems to prevent and combat COVID-19. Evidence shows that patients and clinical settings face more problems in gaining access to health benefactors despite a considerable upsurge in telemedicine. For example, it has been reported that deaths due to cardiac arrest at home increased by about 800% due to the COVID-19 pandemic in New York. On the other hand, it was predicted that individuals with neurological disorders faced problems in receiving help from their neurologists. The neurological community is offering the utmost caution to their patients with all the suffering faced due to the COVID-19 virus.

Transmission to CNS

Coronaviruses' Potential Mechanism of Nervous System Infection

Aside from the respiratory system, coronaviruses can evade immune responses and infect other organs, like the central nervous system. Since coronaviruses can be detected in brain tissue using *in situ* hybridization, which demonstrates there are viral remnants in the brain of infection, this neuroinvasive nature of coronaviruses has been demonstrated. Autopsy studies have provided proof of the opportunistic capacity of these pathogens to infect the brain [6]. Additionally, research has shown that coronaviruses can enter the nasal cavity and infect the brain. The infection can enter the central nervous system to peripheral nerve terminals and then travel through synapses in the CNS either retrogradely or trans-synaptically from infected tissues. Coronaviruses can result in cognitive and behavioral impairments as a result of this invasion [7]. The SARS-CoV's capacity to spread throughout the body *via* systemic circulation enables the virus to enter infected individuals' cerebral circulation. This slow flow increases the chance for the virus spike protein to interact with the ACE2 receptor and can explain why the virus is more successful in infecting the endothelial cells of the microcirculation. This interaction is the first step in the virus' ability to spread throughout the body. The cribriform plate is connected to the nasal cavity and has a porous structure, allowing for easy passage of the virus. The olfactory bulb is responsible for sensing odors, providing another potential entry point for the virus. The virus might be able to enter the brain as a result of this movement [8]. Another possible mechanism for the invasion of the brain by coronaviruses is through the lymphatic or hematogenous route. These mechanisms allow coronaviruses to possess neurotropic and neuroinvasive characteristics. The virus may initially infect neurons, glial cells, white blood cells, or even the blood-brain barrier's endothelial cells (BBB), which can result in cell death and neurodegeneration [9]. According to the scant research on COVID-19's neurological effects, it appears that SARS-CoV-2 can harm the nervous system in two different ways:

- Direct invasion of neural tissue
- Maladaptive inflammatory responses

The central nervous system (CNS) can become infected by SARS-CoV-2 directly through hematogenous and neuronal regurgitation pathways. The virus can attack by infecting CNS endothelial cells, which are prevalent in ACE2. The virus then uses the infected cells to enter the CSF and gain access to the nervous system. From there, it can spread to other areas of the brain and spinal cord, leading to inflammation and tissue damage [10]. The investigation produced data that supported this hypothesis. SARS-CoV-2 is believed to be capable of attaching to

CHAPTER 10

Artificial Intelligence and Coronaviruses

Shafeeq Ur Rehman[1,#], Furqan Shafqat[1,#], Momin Khan[2], Alam Zeb[2], Ijaz Ahmad[3] and **Kamal Niaz[4,*]**

[1] *Department of Microbiology, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

[2] *Directorate of Livestock and Dairy Development Department, Khyber Pakhtunkhwa, Peshawar, Pakistan*

[3] *Department of Human, Legal and Economic Sciences, Telematic University "Leonardo da Vinci", Chieti, Italy*

[4] *Department of Pharmacology and Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan*

Abstract: For the third time in the last few decades, novel coronavirus-19 (2019-nCoV or COVID-19) has been described as the most fatal coronavirus ever, capable of infecting not just animals but even humans all over the world. Healthcare policy makes use of advanced technologies such as artificial intelligence (AI), big data, the internet of things (IoT), and deep machine learning to tackle and forecast emerging diseases. AI is increasingly being used to help in disease identification, prevention, reaction, rehabilitation, and clinical analysis. Since these developments are currently in their initial phases of development, slow improvement in their application for significant deliberation at local and foreign strategy levels is being made. Nevertheless, a current case shows that AI-driven technologies are improving in reliability. Companies like BlueDot and Metabiota used AI technology to predict the coronavirus disease-19 (COVID-19) in China before it surprised the world in late 2019 by spying on its effects and propagation. One approach is to use computational techniques to discover new target drugs and vaccines in silico. Machine learning-based algorithms trained on particular biomolecules have provided affordable and quick-to-implement tools for the development of successful viral treatments during the last decade. Drug repurposing is a technique for finding new uses for accepted or experimental drugs. For novel diseases like COVID-19, a drug repurposing approach is a viable approach. Future directions of AI are drug discovery and vaccination, biological research, remote video diagnosis, tracking patient contacts, COVID-19 recognition and therapy *via* smart robots, and identification of non-contact infection. This chapter aims to explore AI-based techno-

* **Corresponding author Kamal Niaz:** Department of Pharmacology and Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur 63100, Pakistan; Tel: +923129360054; E-mails: kamalniaz@cuvas.edu.pk, kamalniaz1989@gmail.com

# Shafeeq Ur Rehman and Furqan Shafqat have equal contribution

Kamal Niaz, Muhammad Sajjad Khan & Muhammad Farrukh Nisar (Eds.)

logy for diagnosis, management, drug repurposing medications, novel drug discovery, and vaccines for coronaviruses (SARS-CoV and MERS), including during the COVID-19 pandemic.

Keywords: Artificial intelligence, Drug, MERS, SARS-CoV, SARS-CoV-2.

INTRODUCTION

Coronaviruses are a group of viruses that can cause a variety of infections, from mild flu to severe acute syndrome. Coronaviruses are members of the Coronaviridae group. Its order is divided into two subfamilies: (1) *Coronavirinae*, which includes the genera *Alphacoronavirus*, *Betacoronavirus, Gammacoronavirus*, and *Deltacoronavirus*, and (2) *Torovirinae*, which includes the genera *Torovirus* and *Bafinivirus*, as well as an unidentified genus. The Middle East respiratory syndrome coronavirus (MERS-CoV) virus has been found in camels [1]. MERS-CoV is thought to be transferred to humans from camels *via* pulmonary particle spit or the consumption of raw camel flesh or dairy. The virus's pathogenic status was verified utilizing viral bioinformatics tools [2]. One way to learn more about the virus and develop detection and management methods is through artificial intelligence (AI).

The introduction of modern computer simulation techniques and their widespread acceptance in many industries worldwide have resulted in better risk analysis for local and global markets. The healthcare sector, in particular, has been developed with a rise in health knowledge assisted by the existence of different techniques such as machine learning, the IoT, Big Data, AI, and many others. Prognostic computing tools' accessibility and corresponding use in the medical industry globally have resulted in noteworthy changes in surgical procedures, personalized healthcare, and epidemiology. These are supposed to improve accuracy in this domain, particularly regarding diagnostic precision [3]. Forecasting technologies are still used in the healthcare workforce's recruiting and evaluation, and they are being bolstered to incorporate questions about inclusivity and meritocracy [4]. AI is one of the tools for understanding the virus and developing prevention and management strategies. From symptomatic monitoring to early diagnostic tests and quicker drug production, AI can assist at any level of the healthcare process. In China, AI-based technologies are being used to detect coronavirus outbreaks [5]. Computer vision, speech detection, natural linguistic processing, and digital anatomy knowledge processing are only a few AI technologies. Similarly, AI has transformed drug development by uncovering secret trends and facts in biomedical data. AI has been used by pharmaceutical firms and start-ups for drug research and production [6].

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

T

V

Kamal Niaz

Prof. Kamal Niaz (KN) obtained Ph.D. in the field of toxicology and pharmacology at Tehran University of Medical Sciences (TUMS), Tehran-Iran in 2017. He is working as assistant professor in the Department of Pharmacology & Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary & Animal Sciences (CUVAS), Bahawalpur. He has developed excellent research and communication skills and has expertise in the domain of environmental toxicants, diabetes, endocrine disruptor, and genomic disruption. His area of expertise include phytomedicine and ecotoxicity, epigenetic alteration due to toxicants and the role of these chemicals in diabetes. He has published more than 50 articles in national and international journals with h-index-17, and 107+ impact factors along with 40 book chapters in various publishers such as Elsevier, Springer, and Bentham Science. He is working as a volunteer reviewer in different prestigious journals Frontier of Veterinary Sciences, Food and Chemical Toxicology, Scientific Report, Science of the Total Environment, and Current Protein & Peptide Science, etc.

Muhammad Sajjad Khan

Prof. Dr. Muhammad Sajjad Khan did Ph.D. in dairy science (genetics and statistics) University of Wisconsin, Madison, USA. He has over 35 years of research and teaching experience. He published over 650 publications in international journals and 5 books. His main focus is on the development of animal recording and genetic evaluation procedures for improved utilization and conservation of animal genetic resources as well as development of chicken breeds for backyard poultry industry. MS Khan has over 5600 citations with 33 h-index.

Muhammad Farrukh Nisar

Prof. Muhammad Farrukh Nisar completed doctorate from College of Bioengineering, Chongqing University, Chongqing, China in December 2016, and working as an assistant professor in the Department of Physiology & Biochemistry, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, since, December 2018. He is mainly involved in research areas including photo biochemistry, biochemistry, biochemical pathways, oxidative stress, and cellular defense mechanisms, cell biology, cell signaling and pathways biology, screening of natural drugs as potential anti-cancer(skin) and skincare drugs, phytomedicine, photo- and chemo-therapies to treat skin cancer/conditions, natural drugs, and cell signaling, photomedicine and free radicals biology (ROS/RNS). He has published more than 70 research articles so far on multifarious aspects of biomedical/life sciences with a cumulative impact factor of over 120. He is actively involved in exploring novel biomedical materials for drug delivery, photosensitive drug release, and plant isolations to find novel anticancer, antioxidant, and anti-aging drugs.