NEAR EAST UNIVERSITY

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DEPARTMENT OF MOLECULAR MEDICINE

Association between PAI-1 4G/5G polymorphism and COVID-19 patients who infected with different SARS-CoV-2 variants

M.Sc. THESIS IN MOLECULAR MEDICINE

Evren MOĞOL

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study

Evren Moğol

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ABSTRACT

Association between PAI-1 4G/5G polymorphism and

COVID-19 patients who infected with different SARS-CoV-2 variants

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May,2020

AIM:

This study aims to investigate the association between *PAI-1* 4G/5G polymorphism and COVID-19 patients who infected with Delta or Omicron (B.1) SARS-CoV-2 variants.

BACKGROUND:

The COVID-19 disease is a potentially fatal infection that caused the loss of millions all over the world and has left some survivors with ongoing health issues. An infection of the respiratory system can be caused by the SARS-CoV-2 virus; this illness can affect either the upper or lower respiratory tracts. This coronavirus, like most other coronaviruses, is mostly transmitted from person to person by close contact. The severity of the illness might fluctuate from mild to severe at any given time.

Variable COVID-19 symptoms include fever, cough, headache, exhaustion, and loss of smell and taste. The majority of infected persons may experience mild to moderate symptoms, whereas only 14 percent may develop severe symptoms and 5 percent may suffer life-threatening symptoms such as respiratory failure or multi-organ dysfunction. The symptoms that are most common are mild to moderate. Individuals above the age of 65 have a significantly increased risk of developing severe symptoms. In certain patients, there have been reports of organ damage as well as a range of effects following recovery. Ongoing studies spanning many years are being conducted in order to investigate the COVID-19's potential long-term effects.

New, more infectious variants such as Omicron and Delta have emerged over the course of the pandemic as the SARS-CoV-2 genome mutated. The pathophysiology of COVID-

19 involves a number of different signaling pathways and biological components, one of which is PAI-1. PAI-1 is responsible for regulating fibrinolysis, and it has been discovered that severe COVID-19 patients have a significantly increased risk of thrombosis as a result of PAI-1 overexpression. Numerous research are undertaken to analyze the impact of PAI-1 within the context of the COVID19 pandemic.

METHODS:

A total number of 408 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test were used in this study. The control group consisted of individuals who were SARS-CoV-2 RT-qPCR negative. On the other hand, the case group consisted of patients who were SARS-CoV-2 RT-qPCR positive for the Delta or Omicron variants of SARS-CoV-2.

The SARS-CoV-2 positive patients were included to variant identifying analysis by using TaqMan allele specific primers for mutations of SARS-CoV-2 Delta or Omicron variant. The variant identifying analysis was done by use of Multiplex SARS-CoV-2 VOC RT-qPCR identifying kit (Near East University, Nicosia, Cyprus) according to manufactory instructions.

RESULTS:

A total number of 408 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine SARS-CoV-2 RT-PCR test was used in this study to investigate the allelic frequencies of SERPINE1 -675 4G/5G polymorphism (rs1799889) in COVID-19 patients who are infected by SARS-CoV-2 Delta or Omicron variants and compared them with those who were tested negative for SARS-CoV-2 as a control group. The results indicated that there is a strong association between The PAI-1 -675 4G/5G polymorphism and increased risk of SARS-CoV-2 Delta variant compared to Omicron BA.1 variant infection.

CONCLUSION:

In the current study, our main objective was to identify if there is an association between PAI-1 -675 4G/5G polymorphism and COVID-19 causing the SARS-CoV-2 Delta and Omicron (B.1) variant.

To sum up, the results of this study displayed higher risk of infection from the Delta variant compared to the Omicron variant for the individuals who have the PAI-1 -675 4G/5G polymorphism.

Key Words: thrombosis, omicron, PAI-1, SARS-CoV-2, polymorphism, delta

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List of Abbreviations

ACE2: Angiotensin-Converting Enzyme 2

bp: Base pair

cDNA: Complementary deoxyribonucleic acid

HWE: Hardy-Weinberg equilibrium

MERS-CoV: Middle East Respiratory Syndrome coronavirus

nM: Nanomolar

PAI-1: Plasminogen activator inhibitor-1

PCR: Polymerase chain reaction

qRT- PCR: Quantitative reverse transcriptase – polymerase chain reaction

RBD: receptor binding domain

RNA: Ribonucleic acid

SARS-CoV: Severe acute respiratory syndrome coronavirus

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

SNP: Single nucleotide polymorphism

WHO: World Health Organization

µl: Microliter

µM: Micromolar

CHAPTER 1

INTRODUCTION

1.1 Introduction

Since late 2019, a newly discovered coronavirus strain named SARS-Cov-2 has spread from Wuhan, China. Soon after, the new Coronavirus disease (COVID-19) classified as a pandemic under the name of COVID-19 pandemic by the WHO (Habas et al., 2020).

Millions of patients have suffered poor end results from Covid-19 and a portion of patients who have survived the disease are suffering from permanent health complications (Rauf et al., 2020). As of May 2022, there have been a total of 520 million cases worldwide with 6.2 million cumulative deaths from COVID-19. The United States of America has suffered the greatest causalities with 985.000 total deaths (WHO, 2022).

1.2 A New Coronavirus Disease (COVID-19)

The most common method of transmission for SARS-CoV-2 is via respiratory droplets from the infected individual in forms of breath, cough or close contact conversation. Mentioned droplets could be inhaled, or settle in the individuals' eyes, nose or mouth. Another possibility of transmission is when the droplets contaminate a surface and an uninfected individual interacts with the said surface (Cevik et al., 2020).

Survival of the coronavirus on surfaces depends on the material of the surface it contaminates. While the coronavirus could only survive for hours on surfaces such as cardboard or copper, it can survive up to several days on surfaces like plastic or steel. Fortunately, if an individual does not come in contact with the infected surface in a short duration, the sum of virus will rapidly decline and the remaining amount won't be enough to infect an individual (Rauf et al., 2020).

During the initial months of the pandemic, number of cases doubled at the rate of seven and a half days. In the first and second week of January 2020, the virus expanded to other Chinese provinces mostly due to Chinese New Year migration and Wuhan's status

as a transportation center and major railway hub. China recorded roughly 140 new cases every day in January 2020, including two instances in Beijing and one in Shenzhen. Later, official statistics revealed that 6,174 persons had manifested symptoms, and it is conceivable that even more had contracted the disease (CDC Weekly, 2020).

A paper published in The Lancet on January 24, 2020, indicated human transmission, highly suggested personal protective equipment for health care workers, and noted that testing for the virus was necessary due to its "pandemic potential." The WHO designated the coronavirus a worldwide health threat early 2020 and in the March 11th of the same year, pandemic (WHO, 2020).

1.2.1 Etiology

The coronavidae virus family divides into four subtypes of Alpha, Beta, Gamma and Delta, of these mentioned subtypes; the main concerning coronavirus subtype for the human health is Beta Coronaviruses, such as Mers-CoV or the one responsible for the current pandemic, SARS-CoV-2 The remaining three infect mammalians, birds and both of them respectively (Weiss & Leibowitz, 2011).

Coronaviruses envelope their genome; the biggest among the RNA viruses; twice. The first envelopment is achieved by the protein nucleocapsid then three other structural proteins come into plays which are membrane, envelope and the spike protein. The first two is tasked with the construction of the virus; the latter however, is tasked with entering the cell. In achieving this purpose, it is located on the outer membrane of the virus in the form of spikes where the name corona is originated from. Moreover, it is the main indicator of which type of cell it can infect and one of the main proteins to initiate a response from the host (Perlman & Netland, 2009; Weiss & Navas-Martin, 2005).

There have been found seven human CoVs (HCoVs) that are capable of infecting humans (Chan et al., 2013). Common ones such as HCoV-OC43 and HCoV-HKU1 can induce the common cold. Additional ones are SARS-CoV and MERS-CoV (Chen et al., 2020). On average, one out of ten patients experience poor end results from the former, while it is 35 out of 100 for the latter (Chan et al., 2020).

Lu et al., (2020) has found that the genome of the current coronavirus is almost 80% identical to one of the coronaviruses sampled from a bat, while it is about three fourths identical to the initial SARS-COV. Sars-Cov-2 contains a 29.9 kb RNA genome which is single-stranded and positive-sense with more than %66 of them being open reading frames (Kim et al., 2020). These open reading frames are responsible for the translation of 2 proteins which are modified to become 16 different proteins that aren't involved in the structure of the virus but; are involved in the modification of the endoplasmic reticulum of the host cell to turn it into a site for replication of the newly generated virus (Knoops et al., 2008; Masters, 2006). The other %33 of the genome is tasked with encoding the proteins spike, envelope, nucleocapsid and membrane which will form the structure of the virus (Li et al., 2020).

1.2.2 Pathogenesis

The main target of the coronavirus is the cells that are located in the human respiratory system, with the most notably the alveolar endothelial cells that are tasked with the production of the surface of the lungs. Even if its main target of infection is AECs, it can infect any cell that express ACE2 receptor such as macrophages (Chu et al., 2020). Early in the infection, it can replicate at high rates when the innate immune system is compromised (Wölfel et al., 2020). If the virus replicates at high rates, it can cause pathogenesis in the infected tissue and in turn, these cells induce the increase of proinflammatory cytokines and chemokines after these occurrences (Ackermann et al., 2020; Channappanavar et al., 2016).

With the increase in the present inflammatory cells, the production of additional cytokines and chemokines are elevated to combat the viral infection. This reaction can be detected with the assessment of cytokine levels in the patients' serum. Plus, the severity of the disease is proportional to these cytokine levels (Huang et al., 2020).

The infected cells can induce apoptosis if two conditions are met. The first one is if there is a soaring amount of viral replication in the beginning of the infection and the second is if there is an immense amount of response from the cytokines that will initiate

Fas/FasL and TRAIL-DR-5 complexes (Rodrigue-Gervais et al., 2014; Varga et al., 2020). An additional factor in these cells' death comes from monocyte macrophages that are capable of the generation of TNF- α. The result of apoptosis is the disruption of the lung structural integrity, mainly the barrier of alveolar cell and the blood vessels where the exchange of oxygen happen and this disruption will lead to vascular leakage and alveolar edema (Channappanavar et al., 2016).

Pericytes are responsible from ensuring the endothelial cell activity remains consistent in blood microvessels (Chen et al., 2020). However, they express one of the highest amounts of ACE2 and if they are infected, it can cause microcirculation abnormalities due to the malfunction of the endothelial cells.

T-cells that are specific for the coronavirus are a must if the host is to be free from the virus and prevent additional damage to the tissues. When SARS-CoV-2 causes an overwhelming inflammatory response, however, TNF- α-mediated cell death lowers the T-cell response, resulting in uncontrolled inflammatory reactions (Channappanavar et al., 2016; Zhao et al., 2010). Although SARS-CoV-2 may infect T cells in a non-productive manner, it is unknown if this infection causes T-cell death (Wang et al., 2020). Regardless of the cause, T-cell depletion in the later stages of an infection may increase viral survival and prolong the sickness (Fink & Cookson, 2006).

1.3 SARS-CoV-2 Genomic Evolution and Survival

Throughout human history, viruses have undergone fast evolution, with each new virus there is a new potential for it to be problematic for the health of the mankind. In the last 20 years, the mankind experienced several epidemics from multiple kinds of them, with SARS-CoV-2 being the last (Majumder & Minko, 2021).

RNA viruses usually mutate very fast because they are unable to perform proofreading (Sanjuán, 2016). However, the exoribonuclease (ExoN) domain of nsp14 has been linked to a proofreading process in the coronavirus family (Robson et al., 2020). It is known that the nsp14-ExoN is substantially conserved among CoVs (Gribble et al.,

2020). In spite of the expectation that this would slow or even prevent mutations to occur, there have been multiple variants of SARS-CoV-2 (WHO, 2022).

The main determinant how a coronavirus selects its host and its pathogenicity is its spike glycoprotein, thus it is the primary diagnostic and therapeutic target. It is composed of S1 and S2 subunits and both of them have domains for different purposes. S1 is responsible from the host-cell attachment and this is achieved by the cooperation between RBD and the N-terminal domain. S2, on the other hand, is responsible from the entrance of the virus into the host cell and it has 6 domains compared to S1 subunit's 2. These are: transmembrane, internal fusion peptide, C-terminal, fusion peptide and two heptad-repeat domains (Xia, 2021).

The most notable change in SARS-CoV-2 from its predecessor is an additional site of a furin cleavage in the interception of S1 and S2 subunits and this cleavage is known to make it more infectious (Peacock et al., 2021). Therefore, any additional changes occurring in the S protein could potentially affect SARS-CoV-2's infectivity, and because it is the main target of the both immune cells and possible treatments, it is one of the central places for a mutation to occur (Gupta et al., 2021).

1.3.1 Viral Classification and Nomenclature Tools

It is essential to accurately identify each distinct strain of SARS-CoV-2 to monitor newly developing variations and to provide a prompt reaction in the event that it becomes necessary to do so. As an example, many nations in Europe shut their borders in December 2021 owing to the potential for higher infection rates brought on by the Omicron variant, which was a continuation of measures that had been done previously. It is possible that a bigger number of casualties would have occurred as a consequence of a lengthier reaction time if this variation had not been recognized as promptly as possible. The WHO collects the genetic data of SARS-CoV-2 lineages and stores it in three distinct databases so that it can respond as quickly as possible. Pango, GISIAD, and Nexstream are their respective names. Each database gives researchers from all around the world the opportunity to upload the genomic sequences of their SARS-CoV-

2 strains and provides them with an open-access library of all SARS-CoV-2 strains that have ever been discovered. Because of this mutually beneficial relationship, new approaches to battling the pandemic may be found and implemented much more quickly. It is reasonable to assume that not everyone will be familiar with lineages and sublineages, yet this is still an unreasonable expectation. The WHO established the Technical Advisory Group on Virus Evolution in order to improve the general public's level of knowledge and make the discussion of novel variations more accessible. Prior to this name change, this group was known as the WHO Virus Evolution Working Group. In addition to Pango, GISIAD, and Nexstream officials, this group is comprised of experts in virology and microbiological nomenclature. Because of its ease of use and widespread familiarity, the current approach for recognizing important variations makes use of the letters of the Greek alphabet, such as Omicron or Mu. This choice was made because of the alphabet's simplicity (DW, 2021; WHO, 2021).

1.3.2 Variants of Concern (VOC)

If a variant of SARS-CoV-2 is more infectious or less susceptible to vaccines or possible methods of treatment, it is classified as variant of concern. As of May 2022, five variants of concern have been identified (WHO, 2020).

Alpha (B.1.1.7)

The UK initially detect this variant and it was named Alpha on December 14, 2020. It has 23 unique mutations with 8 of them being in the spike protein. Fortunately vaccines are fully effective against this variant even though it has an additional 50% infectivity (Gupta et al., 2021; WHO, 2020).

Beta (B.1.351)

The second variant of concern is found in South Africa on December 18 2020 and in a short amount of time it was detected in other countries as well. The most notable feature of this variant is that it is observed relatively more in healthy young individuals, possibly because of the additional mutations it harbors (Khan et al., 2021; Tegally et al., 2021).

Gamma (P.1)

Gamma variant was detected in the tourists returning to their country from Brazil on January 2 2021. This variant is responsible from producing ten times greater viral loads compared to previous ones possibly because of the 10 mutations found in its S protein (Faria et al., 2021; Fujino et al., 2021).

Delta (B.1.617.2)

A new variant harboring two unique mutations, which are dubbed E484Q and L452R, started infecting more and more people in India. Because there weren't any known variants containing the mentioned two mutations, the name Delta was given on May 11 2021. The Delta variant expanded rapidly around the globe and became the most infectious variant until November 2021. As of May 2022, it is still in circulation (del Rio et al., 2021; WHO, 2021).

Omicron (B.1.1.529)

The dominance of the Delta variant started to fade with the detection of a new variant that is almost 3 times more infectious. Being the most mutated variant to date, this variant was named Omicron on November 2021. The spike protein itself has more than 30 mutations; in comparison the Delta variant harbors half of that amount (Gu et al., 2022; Tian et al., 2021; WHO, 2021).

1.3.3 Variants of Interest (VOI)

If a unique SARS-CoV-2 variant were to be detected in groups of people or countries but it is found to not lessen the effectiveness of the available treatments or vaccines then it is monitored under the name of variant of interest and as of May 2022 there are no VOIs in circulation (WHO, 2020; 2022).

Epsilon (B.1.427)

COVID-19 cases in California rose between November 2020 and March 2021. During this rise, Epsilon variant steadily rose (Carroll et al., 2021). The L452R spike mutation

identified in the Epsilon variation boosts infectivity and diminishes sensitivity to antibody neutralization, according to in vitro experiments (Yang et al., 2021). The WHO categorized Epsilon as a VOI, however as of May 2022, the Epsilon variant was no longer designated a VOI (WHO, 2022).

Zeta (P.2)

Zeta variant was discovered in Brazil in April 2020 with some of the mutations present from the Gamma variant (Voloch et al., 2020). The WHO no longer considers Zeta to be a VOI as of May 2022 (WHO, 2022).

Eta (B.1.525)

Similar to Zeta, the Eta variant harbors some of the mutations from the gamma variant but it differs from zeta in that it does not have most of the notable mutations such as of N501Y (Chakraborty et al., 2021). As of May 2022 it is not regarded as a VOI (WHO, 2022).

Theta (P.3)

The Theta variant was found for the first time in the Philippines on February 18, 2021, when two concerning mutations were discovered in Central Visayas. 14 amino acid substitutions were detected in all samples, including seven alterations in spike protein. As of May 2022, the theta variant was no longer regarded as a VOI (Ferraz et al., 2021; WHO, 2022)

Iota (B.1.526)

The spike protein mutation E484K has been observed in this variant (Annavajhala et al., 2021). By February 2021, it had swiftly spread throughout New York and accounted for around one in four viral sequences. By 11 April 2021, 18 countries have found the variant. By the end of July 2021, the proportion of the Iota variant compared to the total sum of infections in the United States of America has decreased rapidly because of the Delta variant (Annavajhala et al., 2021). The Iota variant was no longer regarded a VOI as of May 2022 (WHO, 2022)

Kappa (B.1.617.1)

The concentration of patients who are infected with the Kappa variant climbed to 50% between December 2020 and March 2021 in India until the Delta variant became dominant (Newey, 2021). As of May 2020, the Kappa variant is not considered as a VOI (WHO, 2022).

Lambda (C.37)

In August of 2020, the Lambda variant was first spotted in Peru. On 14 June 2021, the WHO classified it as a variant of interest and named it Lambda (Robertson, 2021). The Lambda genome has 7 alterations positioned in its protein of entrance and as of May 2022,it is no longer regarded as a variant of interest (Robertson, 2021; WHO, 2022).

Mu (B.1.621)

The Mu variant was first found in Colombia in January 2021, and on August 30, 2021, the WHO recognized it as a variant of interest. The Mu genome has a total of 21 alterations; nine of them are found in spike protein (WHO, 2021). As of May 2020, it is not considered as a VOI (WHO, 2022).

1.4 SARS-CoV-2 Variant Pathogenesis and Host Response

As the COVID-19 pandemic progresses, new mutations are observed with each emerging variant (WHO, 2022). Some mutations happen in the virus' spike protein, thus each variant could show new pathogenic effects. In a study done by Vincent et.al on the pathogenic effects of Alpha and Beta variants in rhesus macaques have found no significant increase in pathogenicity of the mentioned variants in fact, the Beta variant have shown decreased pathogenicity despite the reports of increased disease severity. However, they have found that the Beta variant better in the upper respiratory track, which the opposite can be said for the Alpha variant and the initial form of the SARS-CoV-2 virus when it first emerged (Munster et al., 2021).

Mohandas et al., (2021) has tested the pathogenicity of variant Delta on the Syrian mouse models and informed that the Delta variant has increased viral replication

especially in the first week of the infection and higher transmissibility than the Alpha variant. The main area of infection remained identical to the previous ones.

In case of the Omicron variant, McMahan et al., (2022) has conducted an experiment to assess the pathogenicity of the Omicron variant in hamsters. Their results showed that hamsters did not lose weight even after being infected with higher dosages of Omicron variant compared to the previous variants which resulted in significant weight loss with lesser dosage. Moreover, the mice had lower viral loads in their lower respiratory track with decreased pathology in their lungs although the viral load in their Nasal turbinates had increased significantly.

1.5 Human Genetic Variations

The term "human genetic variation" refers to the genetic differences that may be found within populations as well as between them. In the human population, there can be many different variants of a single gene, which is an example of polymorphism. Due to the fact that mutations can occur at any stage of human development and that the number of copies of each gene can vary, it is impossible for two individuals to be genetically identical. Even monozygotic twins have some genetic differences (Bruder et al., 2008). As of 2017, there were 324 million differences found in sequenced human genomes (NCBI, 2017), while in 2015, it was estimated that the average difference between an individual's genome and the reference genome was 20 million base pairs (Auton et al., 2015). There are numerous scales of genetic diversity among people, from major abnormalities in the human karyotype to single nucleotide modifications (Kidd et al., 2008).

Single-nucleotide polymorphisms (SNPs) make up the majority of polymorphisms in the genome with an occurrence rate of one in every 1,000 nucleotides on average. The occurrence of other types of polymorphisms, including as alterations in copy number, insertions, deletions, duplications, and rearrangements, is far less frequent. There is no requirement that every SNP will result in detrimental impacts to the individual. The vast

majority of SNPs are located in non-coding sections of the genome; however, some SNPs can be found in both coding and non-coding regions and are the cause of certain inherited genetic diseases. Some of the most well-known instances of pathogenic SNPs include thalassemia, Duchenne muscular dystrophy, and cystic fibrosis (NIH, 2007).

1.5.1 Genetic polymorphism and COVID-19

It is possible that changes in the activity of particular proteins, caused by the existence of frequent polymorphic genetic variants in a population, might result in increased susceptibility to infection, enhanced viral replication, or heightened inflammatory response (Sabater Molina et al., 2022).

ACE2, being the main receptor of entrance for SARS-CoV-2, is the most logical starting point of looking into the association of genetic polymorphisms and SARS-CoV-2. Sabater Molina et al., (2022) have researched the Ace2 SNPs (rs2074192, rs1978124) and have associated these SNPs with the severity of the disease, especially in older adult males due to ACE2 being located in the X chromosome and therefore leaving no chance of heterozygosity in males to alleviate the severity of the disease. On the other hand, in Italy, no link between illness severity and gender susceptibility to ACE2 was discovered. Nonetheless, in the same study, TMPRSS2 levels and genetic variations were identified as potential disease modulators, which contributed to the reported epidemiological data among Italian patients. It has been shown that not only the ACE2 gene, but also the ACE1 gene, may influence the clinical course of COVID-19 (Asselta et al., 2020).

Abbas et al., (2021) have conducted a research on the association of COVID-19 susceptibility and polymorphisms in Glutathione S-transferase (GST) gene, mainly GSTM1 and GSTT1 homozygous deletion polymorphisms due to these polymorphisms cause loss of enzyme activity and are associated with increased risk of oxidative stress associated respiratory diseases. Because the present oxidants in the air we breathe is already putting a strain onto the immune system already and when the additional damage is inflicted by SARS-CoV-2, the innate immunity becomes incapable to defend the disease . Their findings have shown that individual who have GSTT1 homozygous

deletion have higher risk of mortality and lower overall survival but this deletion has no significant effect on COVID-19 susceptibility. In case of GSTM1, individuals with GSTM1 homozygous deletions had neither higher rate of mortality, nor higher rate of infection to COVID-19, thus they ruled out GSTM1 as a factor.

1.5.2 COVID-19 and Thrombosis

COVID-19 generates a prothrombotic condition, and the high frequency of documented significant thrombotic events raises concerns about the pathophysiology of these events (Connors & Levy, 2020). COVID-19 has been associated with micro and macrovascular thrombotic problems in the veins of the lungs, brain, and gut (Levi et al., 2020). There have been reports of strokes as the presenting symptom in previously healthy young patients, and venous thromboembolism formation despite the use of anticoagulants (Oxley et al., 2020). The occurrence of thrombotic events in asymptomatic individuals has been reported. In addition, thromboses have been observed both in the acute context and in the weeks after critical illness, indicating that the pro-thrombotic condition may persist for several weeks or even longer after hospital discharge (Wong, 2003).

The cumulative incidence of thrombotic events was 49% in a Dutch group of 184 COVID-19 patients in the ICU, the most of which were pulmonary embolisms despite the use of antithrombotic medications. In this group, patients having a thrombotic episode had a fivefold higher risk of death (Klok et al., 2020). In another Italian group of 388, the percentage of thrombotic events was 21 (Lodigiani et al., 2020).

The reason why coagulation, thus thrombosis is a common occurrence in severe COVID-19 is because of endothelial cells' role in fibrinolysis regulation and vessel wall integrity. When SARS-CoV-2 damages alveolar endothelial cells, it stimulates pro-inflammatory cytokines (Levi & van der Poll, 2017). This increase in inflammatory activity contributes to the development of microvascular thrombosis, such as pulmonary microvasculature obstruction (Kasinathan & Sathar, 2020). Additionally, the development of the TF-Factor VIIa complex is involved with thrombin production and

fibrin deposition in multiple organs, such as lungs (GLAS et al., 2013). With an increase in fibrin, fibrinolysis process also piques and in turn raises plasminogen levels (Loskutoff & Quigley, 2000).

1.5.3 The SERPINE1 (PAI-1) Gene and Diseases

SERPINE1 gene is among the serine protease inhibitors superfamily (*SERPIN*) and it's located on chromosome 7 (7q21.3–q22). *SERPINE1* encodes the PAI-1 protein which suppresses the activity of plasminogen activator proteins such as urokinase plasminogen activator (u-PA) and tissue type plasminogen activator (t-PA) (Klinger et al., 1987). The mentioned proteins convert plasminogen to plasmin, which is a contributor to fibrinolysis (Loskutoff et al., 1983). The PAI-1 protein dampens this conversion to prevent premature clot breakage (LIJNEN, 2005).

Complete PAI-1 deficiency is associated with significant bleeding, which can be life-threatening in rare situations. So far, fewer than 10 families with PAI-1 deficiency have been recorded and it is autosomal recessive in disease (Heiman, 1993).

Overexpression of PAI-1 can also lead to a set of diseases such as insulin resistance and cause obesity which is a factor in metabolic syndrome that can increase the risk of developing cardiovascular diseases (Alessi & Juhan-Vague, 2006). Additionally, depending on the factor that induces the expression of PAI-1, its overexpression could also cause various diseases such as atherothrombosis, diabetes or myocardial infarction (Hamsten et al., 1987; Kohler & Grant, 2000; Lyon & Hsueh, 2003).

A number of SNPs in PAI-1 have been linked to different disorders. For instance, the rs2227631 polymorphism was strongly connected with coronary artery disease risk in dominant and allele models, while the homozygous genotype of rs6092 had a deleterious influence on men's triglyceride levels (Henry et al., 1997; Su et al., 2006). However, PAI-1 4G/5G polymorphism has received the most extensive investigation than any other PAI-1 SNP.

An insertion/deletion polymorphism, PAI 4G/5G polymorphism happens whether a guanosine nucleotide is present or not at the -675 bp location in the SERPINE1 gene promoter region (Dawson et al., 1993). 4G allele transcripts greater amounts of protein than the 5G allele because of the site for binding is absent which was reserved to an inhibitor. This absence is observed as higher PAI-1 levels in people who are 4G homozygous compared to the ones with the homozygous 5G allele. In case of the heterozygous individuals, their levels are in between the homozygous ones (Eriksson et al., 1995).

Currently this polymorphism is being tested if it can be a predictor of various diseases. Yıldırım et al., (2017) has found out the frequency of PAI-1 4G/5G polymorphism observed to be greater in people suffering from endometrial cancer compared to the group of control. Katko et al., (2021) has found out that the 4G homozygous patients with Graves' disease are more susceptible to developing Thyroid eye disease. Madách et al., (2010) demonstrated that severe sepsis patients are more likely to suffer from septic shock if they have the 4G allele. Divella et al., (2015) informed that this polymorphism, especially the 4G allele, can be used as a prognostic marker for patients with Hepatocellular carcinoma (HCC).

1.5.4 PAI-1 4G/5G variation and COVID-19

A recent study showed a connection of COVID-19 and PAI-1 4G/5G polymorphism in which the 4G homozygote patients have the highest plasma PAI-1 concentrations followed by 4G/5G heterozygotes and the least amount of plasma concentration was found in patients with 5G homozygote also the polymorphism associated with severity of the disease (Vatseba & Virstyuk., 2021). Abdullaev et al., (2021) also support previous findings, and they have observed that of all COVID-19 patients identified with PAI-1 4G/5G polymorphism have showed thrombotic events. The postmortem examinations have also done in the same study and they revealed that the thrombotic events were mainly raised from pulmonary artery thrombosis or pulmonary embolism with deep vein thrombosis. In addition, the ratio of women who have suffered from pulmonary embolism was twice as much compared to men.

Furthermore, JAK et al., (2021) have reported a four-day-old fetal autopsy that has shown signs of placental injury caused by coagulation alteration due to fetal SARS-CoV-2 infection, which the mother who had infected in the last trimester of pregnancy and she had homozygote in the 4G allele. They conclude that SARS-CoV-2 passed the placental barrier from the mother who is heterozygous to this polymorphism to infect the fetus and the polymorphism could increase the damage of the SARS-CoV-2 infection.

1.6 Work in thesis

This study investigates the allelic frequencies and the distributions of the genotypes of PAI-1 4G/5G polymorphism in patients who are infected by the SARS-COV-2 Delta and Omicron (BA.1) variants.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Suppliers

HiMedia Insta Q96™ Real Time (HiMedia, Mumbai, India), Tianlong GeneRotex96 Rotary Nucleic Acid Extraction System (TIANLONG, Shaanxi, China), Bio-Rad MyCycler™ Thermal Cycler System (Bio-Rad, California, USA), Appleid Biosystems Veriti Thermal Cycler (Applied Bıosystems, Waltham, Masachusets, USA), DNR Bio Imaging Systems MiniBIS Pro (DNR Bio Imaging Systems, Neve Yamın, Israel), Cleaver Scientific gel electrophoresis instrument and power supply (Cleaver Scientific, Rugby, UK).

2.1.2 Sample Collection

A total number of 408 individuals who admitted to Near East University Hospital COVID-19 PCR Diagnosis Laboratory for routine test was used in this study. The control group consisted of 204 individuals who were SARS-CoV-2 RT-qPCR negative. On the other hand, the case group consisted of 204 patients whom 100 of them were SARS-CoV-2 RT-qPCR positive, infected with SARS-CoV-2 Delta variant and 104 of them were SARS-CoV-2 RT-qPCR positive, infected with SARS-CoV-2 Omicron (BA.1) variant

2.1.3 Chemical Reagents

2.1.3.1 Molecular Weight Markers

GelPilot 50 bp DNA ladder (QIAGEN, Hilden, Germany) catalogue no. 239025) and GeneRuler 50 bp DNA ladder (Thermo Scientific™, Pittsburg, USA, catalogue no. SM0371) were utilized as a molecular weight marker.

2.1.3.2 Oligonucleotide Primers

The primers pairs which were designed for *PAI-14G/5G* polymorphism (SNP: rs1799889) (Table 1).

Primers	**Sequence**
PAI-1 (rs1799889)	
Forward	5'-CACAGAGAGAGTCTGGCCACGT-3'
Reverse	5'-CCAACAGAGGACTCTTGGTCT-3'

Table 1: The sequence of *PAI-1* forward and reverse primers for multiplex PCR

2.1.3.3 Enzymes

FastDigest BSlI (Thermo Fisher Scientific, Waltham, Massachusetts, USA, catalogue no. K1991) was used to digest of PCR products following DNA amplification.

2.1.3.4 Standard Solutions

A 10x stock of Tris-Borate/ EDTA (TBE) which is an electrophoresis buffer (108 gr Tris and 55 gr Boric acid and 40 ml 0.5 M EDTA pH 8.0) was dissolved in 1 ml water. 10X TBE buffer was diluted to 1X (100 ml from 10X TBE + 900 ml Dıstilled water).

2X PCR Master Mix (Thermo Fisher Scientific, catalogue no. K0172), which is the solution includes *PAI-1* DNA Polymerase, dNTPs, and all the components required for the PCR, was used for amplification of DNA.

2.1.3.5 Other Chemical Agents

Agarose powder (Sigma-Aldrich, catalogue no. 11388983001) was used to gel electrophoresis showing PCR-RFLP products of samples. Ethidium bromide (EtBr) (Sigma-Aldrich, catalogue no. E1385) which is a fluorescent dye was used for making agarose gel visible.

2.1.3.6 Computers

GelCapture Software was used to viev, analyze the gel images and store the imaging data. Statistical Package for the Social Sciences (SPSS) was used for the data statistics.

2.2 Methods

2.2.1 The Detection of SARS-CoV-2 from VNAT Solution by Real-Time Polymerase Chain Reaction

SARS-CoV-2 detection was obtained with UNIPLEX SARS-CoV-2 RT-qPCR diagnosis kit (Near East University, Nicosia, Cyprus) from VNAT Solution by 2X PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA, catalogue no. K0172), Real-Time Polymerase Chain Reaction according to manufactory manuals.

2.2.2 SARS-CoV-2 Mutation Typing and variant identifying using TaqMan Allele Specific Primers (ASPs)

The SARS-CoV-2 positive patients were included to variant identifying analysis by using TaqMan allele specific primers for SARS-CoV-2 mutations. The variant identifying analysis was done by use of Multiplex SARS-CoV-2 VOC RT-qPCR identifying kit (Near East University, Nicosia, Cyprus) according to manufactory instructions.

2.2.3 Nucleic Acid Extraction

The viral RNA from the VNAT solution was extracted by viral DNA RNA extraction kit (Tianlong, China, catalogue no. T014H).

Human DNA was isolated from VNAT solutions which diagnosis were done for COVID-19 using DNA extraction kit (Tianlong, China, catalogue no. T191H).

2.2.4 Amplifying the *PAI-14G/5G* Polymorphism Target Regions by PCR

PCR was used to amplify of target mutated regions of the *PAI-1* gene from isolated genomic DNA. The PCR step was done for all 408 samples using for *PAI-1*restriction enzymes. PCR reaction achieved in 20 µl volume, 15 µl PCR mixture and 5 µl DNA and the PCR reaction mixture composition was presented in Table 2. Thermal cycling conditions to amplify of target mutated regions of the *PAI-1* gene including the temperature regimes and the durations of each step were presented in Table 3.

Component	**1X**
PCR Master mix	5 µl
*PAI-1*Forward primer (10µM)	1 µl
*PAI-1*Reverse primer (10µM)	1 µl
PCR grade Distilled water	8 µl

Table 2. Master Mixture composition for *PAI-1*PCR

Stage	**Temperature**	**Time**	**Cycles**
Initial denaturation	94 ℃	5 minute	1 cycle
Denaturation	94 ℃	30 seconds	35 cycles
Annealing	63 ℃	30 seconds	
Extension	72 ℃	1 minute	
Termination	72 ℃	7 minutes	1 cycle

Table 3. Thermal cycling conditions for *PAI-1* PCR

Following the PCR, the gel electrophoresis was done for the yield amplified products. A 3.5% concentrated agarose gel was prepared (7 grams agarose / 200 ml TBE buffer). After dissolving agarose, the mixture was cooled down and 10µL EtBr was added. 10 µl PCR product with 2 µl loading dye and 5µl 50bp ladder were loaded into the agarose gel. An average of 100 volts used in the running of the samples by Bio-Rad electrophoresis device. The average time of completion was 90 minutes. An ultraviolent trans-illuminator was used to visualize the bands

2.2.5 Genotyping the *PAI-1 4G/5G* Gene Polymorphism by Restriction Fragment Length Polymorphisms (RFLPs)

After visualization of the bands RFLP analysis was done for *PAI-14G/5G* polymorphism by the use of mutation specific restriction enzymes. RFLP analysis achieved of 20 µl total, 10 µl of RFLP mixture and 10 µl of amplified product from PCR. The exact RFLP mixture composition was presented in Table 4. The samples incubated at 37°C for 60 minutes for *PAI-14G/5G* polymorphism for the RFLP analysis.

Component	
Distilled water	4.5 µl
Digest Green Buffer	5 µl
PAI-1 restriction enzyme	0.5 µl
PCR product	10 µl

Table 4. RFLP mixture for *PAI-14G/5G* polymorphism.

A 3.5% concentrated agarose gel, containing ethidium bromide was prepared (7 grams agarose / 200 ml TBE buffer) to separate the RFLP products. The 10 µl RFLP products and 2 µl loading dye and 5 ml 50 bp ladder were loaded into agarose gel. An average of 100 volts used in the running of the samples by Bio-Rad electrophoresis device. The

average time of completion was 90 minutes. An ultraviolent trans-illuminator was used to visualize the bands.

In the presence of mutation, for *PAI-1* restriction endonuclease recognizes the sequence and cut the PCR product into 72bp (4G allele) and 27bp (5G allele) fragments.

2.2.6 Statistical Analysis

Data statistics was done by utlizing SPSS software (Statistıcal Package for the Social Sciences, SPSS Inc, Chicago, IL, USA, and version 25). Descriptive data and genotype data of the study group were expressed as mean ± standard deviation (SD) or number and frequency, where applicable. Normal and non-normal distributed quantitative variables were differentiated with Student's t-test and Mann–Whitney U test between two groups, respectively. The genotype and allelic frequency distributions of PAI-1 675 4G/5G polymorphisms between the study groups were compared using Chi square (χ^2). Pearson's chi-square test or the Fisher's exact test were used to verify the association of the categorical variables between study groups, when the conditions for using the chi-square test were not verified. Hardy-Weinberg equilibrium (HWE) was assessed by Fischer's exact test. OR and 95 % CI were estimated by binary logistic regression analysis adopting codominant, dominant, recessive and additive inheritance models. Akaike's information criterion (ACI) was utilized in the selection of the most suitable inheritance model for the data available. To assess the differences between groups, the data were log transformed to meet ANOVA criteria and then subjected to one-way ANOVA with Tukey's post-hoc analysis. Relative risks were assessed of PAI-1 675 4G/5G polymorphism in COVID-19 Delta and Omicron (BA.1) variant patients by calculating odds ratios (ORs) and 95% confidence intervals (CIs) that were considered separate outcomes. In all cases differences were considered significant at $p < 0.05$.

CHAPTER 3

RESULTS

3.1 Introduction

A significant characteristic of COVID-19 infection is the appearance of thrombosis in small and big veins, thus SARS-CoV-2 is the reason of endothelial dysfunction (Rapkiewicz et al., 2020). PAI-1, being a key marker of endothelial dysfunction, has been consistently found in high plasma levels in patients with severe COVID-19 (Zuo et al., 2021).

Multiple studies have looked into the function of pai-1 in COVID-19. For example, Khan, (2021) and Zuo et al., (2021) are in agreement from their findings that older patients and those with previous cardiometabolic disorders who are more likely to have greater baseline PAI-1 levels are at an increased risk for severe infection and worse outcomes.

Even though elevated plasma PAI-1 levels increase the risk of COVID-19 severity, a few studies researched the association of this polymorphism so far (Lapić et al., 2022). The 4G allele is known to elevate PAI-1 levels. As of May 2020, there aren't any studies available on its impact on multiple covid-19 variants (Eriksson et al., 1995).

In the current academic work, we investigated the allelic frequencies and genotypic distribution between *PAI-1* 4G/5G gene polymorphism (rs1799889) in COVID-19 patients who are infected by the SARS-CoV-2 Delta and Omicron variants and compared them with SARS-CoV-2 negative individuals as a control group.

3.2 General characteristics of the study group

The study group includes 204 COVID-19 patients (100 SARS-CoV-2 Delta variant patients and 104 SARS-CoV-2 Omicron (BA.1) variant patients) and 204 non-infected patients as a control group. The mean age of COVID-19 patient's ± SD was 48.49±11.54 and control group 47.24±12.34 (p=0.290). The gender distribution of the patients' group is 114 (55.9%) female and 90 (44.1%) male for control group 94 (46.1%) female and 110 (53.9%) (p=0.060).

3.3 Allelic and genotypic distribution frequency of PAI-1 -675 4G/5G polymorphism in study group.

The allelic and genotypic frequency distributions of *PAI-1 6754G/5G* polymorphism in COVID-19 patients who are infected by the Delta and Omicron BA.1variants and the control group are presented in Table 5

Notable differences were observed in genotype frequencies of PAI-1 -675 4G/5G polymorphism between SARS-CoV-2 patients and control group (***p=0.001***).

Furthermore, the risk allele of PAI-1 -675 4G/5G polymorphism was found to be statistically significant (OR=39.05, 95% CI=/18.88-80.78, ***p=0.001***) in SARS-CoV-2 Delta and Omicron BA.1 variants infected patients compared to controls (Table 5).

SNP	**Genotypic Frequencies n (%)**		**P-Value**	**Allelic Frequencies**			
χ^2	**OR/CI(95%)**		**P-Value**				
Genotype	**Cases (n=204)**	**Control (n=204)**		**Allele**	**Cases (n=204)**	**Control (n=204)**	
PAI-1 675-4G/5G							
5G/5G	**72(35.3)**	**192(97)**	**4G/5G**	**82(40.2)**	**4(2)**	***0.001***	**4G/5G**
0.55/0.45	**0.98/0.02**	**201.96**	**39.05/18.88-80.78**	***0.001***			
4G/4G	**50(24.5)**	**2(1)**					

Table 5: The genotypic and allelic frequency distributions of PAI-1 -675 4G/5G SNP in the study group

The study group includes 204 COVID-19 patients who are infected by the SARS-CoV-2 Delta and Omicron BA.1 variants and 204 non-infected patients as a control group. OR: Odds Ratio, CI: Confidence Interval. χ^2 and HWE tests were used to compare the genotypic and allelic frequency distributions of polymorphisms between the groups. In all cases, differences were considered significant at $p< 0.05$.

Mutation analysis of *PAI-1 6754G/5G* polymorphism showed that 64.7% of SARS-CoV-2 positive patients (case group) were carried at least one mutant allele (homozygous or heterozygous), while the control group has consisted of 3% were carried mutation at least one allele. The distribution differences of the *PAI-1 6754G/5G* polymorphism within the two groups were statistically significant (OR=0.17, 95% CI=0.07-0.4, ***p=0.001***) (Figure 1).

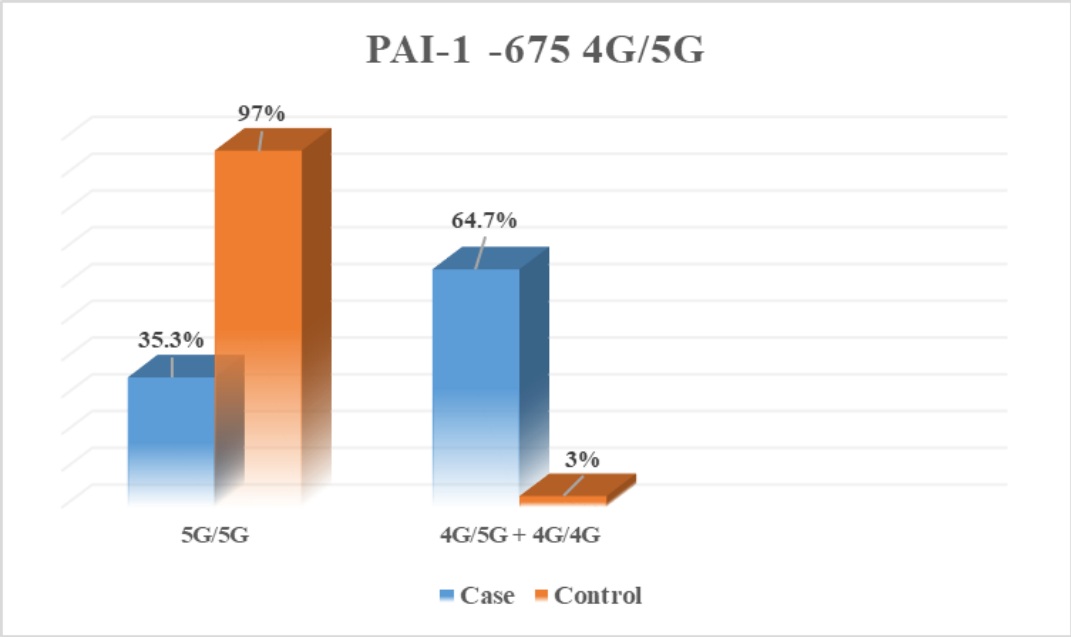

Figure 1: *PAI-1 675 4G/5G* polymorphism genotype distribution. 5G/5G (Wild Type), 4G/5G (Heterozygote), 4G/4G (Homozygote)

3.4 The distribution of PAI-1 6754G/5G polymorphism in the patients with SARS-CoV-2 Delta and Omicron BA.1 variants

Furthermore, we also investigated of the distribution of PAI-1 6754G/5G polymorphism in SARS-CoV-2 patients who were infected with Delta and Omicron BA.1 variants. We observed that 21% of Delta variant infected patients had 5G/5G (wild

type) genotype, while 79% of Delta variant infected patients carried at least one mutant allele (homozygous or heterozygous). However, 5G homozygosity observed more in the SARS-CoV-2 patients who were infected with Omicron BA.1 variant (49%), and the frequency of the mutant genotypes (homozygous or heterozygous) was lower (51%) compared to the patients infected with Delta variant group (OR=3.62, 95% CI=1.95-6.70, ***p=0.001***) (Figure 2).

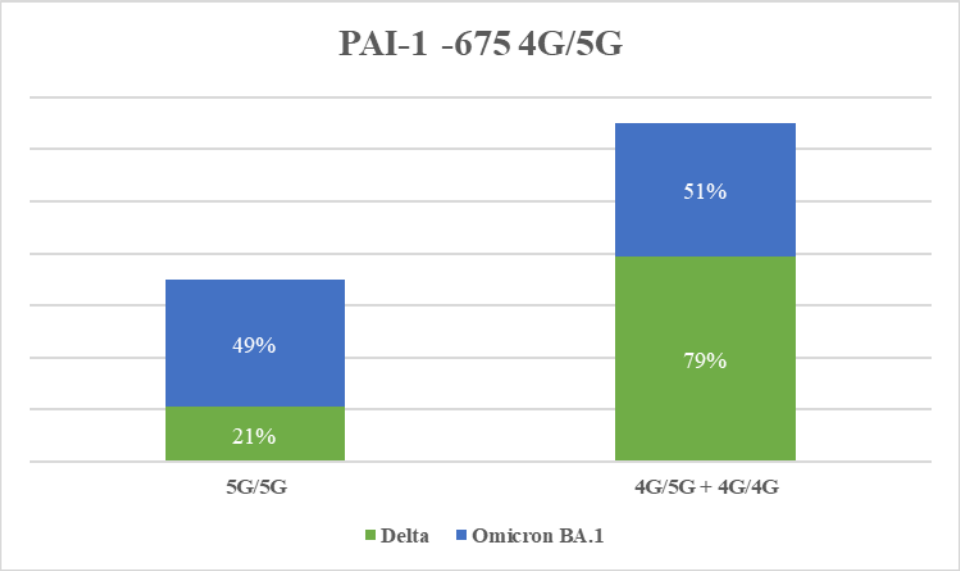

Figure 2: The distribution of PAI-1 6754G/5G polymorphism in the patients with SARS-CoV-2 Delta and Omicron BA.1 variants. *PAI-1* polymorphism genotype distribution 5G/5G (Wild Type), 4G/5G (Heterozygote), 4G/4G (Homozygote)

3.5 Analysis of PAI-1 6754G/5G polymorphism based on the four genetic inheritance models in the patients with SARS-CoV-2 Delta and Omicron BA.1 variants

The genotype frequencies were analyzed by four genetic models: additive, co-dominant, dominant, and recessive models in COVID-19 cases. The PAI-1 -675 4G/5G polymorphism, a significant association between this polymorphism and increased risk of SARS-CoV-2 Delta variant compared to Omicron BA.1 variants cases, and the

analysis showed that in all four models, co-dominant genotype (5G/5G) vs (4G/4G) (OR=2.85, 95% CI.=1.34-6.05, ***p=0.005***); co-dominant genotype (4G/4G) vs (5G/5G) (OR=0.35, 95% CI.=0.16-0.74, p=***0.005***); dominant (OR=3.62, 95% CI.=1.95-6.70, ***p=0.001***); recessive (OR=0.76, 95% CI.=0.40-1.45, p=0.417); additive (OR=0.57, 95% CI=0.11-1.66, ***p=0.002***).

Table 6: Analysis of SNPs based on the four genetic inheritance models.

SNP	**Model of Inheritance**	**OR (95 % CI)**	**p-Value**	**AIC[a]**
PAI-1 -675 4G/5G	**Co-dominant**			
	5G/5G vs 4G/4G	2.85 (1.34-6.05)	***0.005***	-
	4G/4G vs 5G/5G	0.35 (0.16-0.74)	***0.005***	-
	Dominant			
	5G/5G vs 4G/5G+4G/4G	3.62 (1.95-6.70)	***0.001***	13.15
	Recessive			
	4G/4G vs 4G/5G +5G/5G	0.76 (0.40-1.45)	0.417	15.39
	Additive			
	4G/4G vs 4G/5G vs 5G/5G	0.57 (0.11-1.66)	***0.002***	16.32

The AIC: the preferred inheritance model is the one with the minimum AIC value. OR; Odds ratio, CI; Confidence interval, AIC; Akaike's information criterion. p-value≤0.05 considered statistically significant. p-values in bold remained significant after Bonferroni correction.

CHAPTER 4

DISCUSSION

4.1 Introduction

Since 2019, COVID-19 has become the number one priority in general public health worldwide. Its ability to infect immense amount of people made it clear with approximately 525 millions of people have been infected and 6.3 millions of those people could not recover from the virus (WHO, 2022). The reason why it is so infectious is because it is an airborne disease that targets the respiratory system of the host and the initial symptoms appear a few days after the infection. Given the type of substance it infects, it can infect people up to 3 days after the said surface become contaminated. Fortunately, the possibility decreases rapidly in the matter of hours (Rauf et al., 2020). Coronaviruses possess some degrees of proofreading capabilities which made scientist hopeful about avoiding a case of mutation though that was not the case (Sanjuán, 2016). It mutated number of times in the span of two years, even five of them labeled as a variant of concern and many more are put under watch (WHO, 2022). Fortunately, vaccines proved to be the most effective method of combatting this pandemic. It is found that their widespread usage has dramatically reduced hospitalizations and the burden on the health system reduced significantly especially in the cases that required ventilators to be deployed (Knoll & Wonodi, 2021).

The main reason why ventilators are needed in infected patients is due to pathogenic effects of SARS-CoV-2. The virus causes thrombosis in the patients' deep veins especially in the lungs and in severe cases; this thrombosis harms the patient to a degree that they are unable to breathe without assistance. Anticoagulants such as heparin are utilized to combat in this type of instances and to detect the severity of coagulation PAI-1 serum levels is in use as a reliable marker (Khan, 2021).

4.2 The association between PAI-1 -675 4G/5G polymorphism and COVID-19 disease

Individuals who harbor the 4G allele in their *SERPINE-1* gene found to have elevated PAI-1 serum levels and depending on whether they are homozygote or heterozygote differentiates this plasma level. 4G homozygote people naturally have the highest plasma levels followed by heterozygote and 5G homozygote people have the least level of plasma PAI-1 (Hamsten et al., 1987). An assumption can be made that 4G homozygote people are under greater risk if they ever had a severe COVID-19 infection because of the combination of their already present high PAI-1 levels and the additional increase caused by infection could cause poor end results (Khan, 2021).

Vatseba & Virstyuk., (2021) tangled this assumption and provided that the PAI-1 levels with respect to COVID-19 severity and PAI-1 levels indeed lined up as 4G homozygote, 4G/5G heterozygote and 5G homozygote with 4G homozygote individuals having the highest PAI-1 levels and make up the most populated patient group of them all; although this study did not test a specific variant. Even if our study did not measure the plasma levels, it associated how many COVID-19 patients have which type of polymorphism with respect to whether they are Delta or the Omicron variant infected. One observation we made is that 4G/4G and 4G/5G people contracted the Delta variant more and the reverse is applicable for the 5G/5G people and the Omicron variant. Our study with the mentioned one may not seem to have any overlapping connections on the surface, but theirs was done before the emergence of the Omicron and at the time when the Delta variant was the most dominant strain on the planet. From there we can assume the most cases they had were with the Delta variant and in that instance, it is in line with our findings of 4G allele harboring individuals have higher chance of being infected with the Delta variant and with most of Vatseba & Virstyuk., (2021)'s patients being either 4G homozygous or heterozygous.

4.3 The impact of PAI-1 -675 4G/5G polymorphism on COVID-19 patients

Abdullaev et al., (2021) conducted a study where they found that COVID-19 patients in their study group who displayed thrombotic events had at least one 4G allele. Moreover, when they did post mortem examinations, the mentioned thrombotic events

were mainly raised from pulmonary artery thrombosis or pulmonary embolism with deep vein thrombosis. Furthermore, another case reported a 4G homozygous patient suffering from placental injury due to SARS-CoV-2 induced coagulation alterations (JAK et al., 2021).

In addition, another aspect of Vatseba & Virstyuk., (2021)'s experiment was their categorization of patients into three groups depending on their COVID-19 severity. These groups are: mild, moderate and severe. Mild group was entirely composed from 5G homozygous individuals meanwhile, heterozygous patients make up the 83% of the moderate group and the severe group was 62% 4G homozygous and 32% 4G/5G heterozygous patients.

It is worth mentioning that all of these studies were conducted in a time where the Delta variant was the most dominant variant around the globe before the Omicron variant existed. Our findings indicate that a greater proportion of people infected by the Delta variant were either 4G homozygous or 4G/5G heterozygous, whereas the Omicron variant mainly infected 5/G homozygous people . Recent studies have shown that even if the Omicron variant was more infectious than the Delta variant, its COVID-19 symptoms are milder compared to the previous variants (Wang et al., 2022). This result is reinforced by the fact that in animal models, the lung damage caused by the Omicron variant was at least nine times lighter compared to the damage done by the previous variants (Zhao et al., 2022).

In the light of these findings, one of the reasons why the Delta variant cause more severe form of COVID-19 compared to the Omicron variant could be due to the Delta variant is seen more among the people who tend to have greater levels of plasma PAI-1, who have higher chances of suffering from a thrombotic event (Hamsten et al., 1987). This possibility is further supported by the reduced percentage of thromboembolism in the COVID-19 patients who were admitted to the ICU during the Omicron wave compared to the previous ones. It is worth mentioning that the increase in the total vaccination rates could also be a factor in this percentage reduction (Ho et al., 2021).

4.4 Conclusion

The life-threatening condition known as COVID-19 has been responsible for the deaths of millions of individuals. Within the span of two years, a total of five different variants of concern have surfaced. Because of their significantly heightened contagiousness, Delta and Omicron are two of them that stand out above the others. Vaccines continue to be the only way of protection available against COVID-19 as of May 2022, and it is a proven fact that they are successful in the prevention of this disease. Each passing day sees a rise in the proportion of vaccinated people compared to the entire population, which contributes to an overall improvement in the vaccines' efficacy. Because of these immunizations, governments will begin relaxing the requirements they imposed, like as restrictions on who may travel and where they can go, beginning in May of 2022. The vaccination rates in some regions of the world are, regrettably, not yet high enough; as a result, the prospect of a new variant being released into the world is always a possibility.

PAI-1 is responsible for the regulation of the fibrinolytic system due to its capacity to prevent plasmin from transforming into plasminogen, which is the process that dissolves fibrin cloths. However, if there is a higher concentration of PAI-1 in the body than what is considered normal, it can lead to a wide variety of physiological problems. This disruption would be thrombosis in the context of COVID-19; more specifically, it would take the form of pulmonary embolism; however, additional consequences such as hemodynamic disturbances or cardiac problems are also a possibility. In the most severe cases of COVID-19, it has been shown that the infection causes PAI-1 levels to remain in an elevated condition. This keeps the clots from dissolving and can, in the worst case scenarios, lead to consequences such as damage to several organs. A polymorphism known as the PAI-1 4G/5G polymorphism is seen in certain individuals, and this polymorphism causes these individuals' PAI-1 levels to persist at a relatively high level. People who are homozygous for this polymorphism have the greatest PAI-1 levels because the 4G allele of this polymorphism has an increasing influence on plasma PAI-1 levels and other factors. On the other side, those who are heterozygous have lower levels

compared to them, however these levels are not quite as low as those of people who are 5G homozygous. Several researches explored the connection between this polymorphism and COVID-19; however, these investigations did not consider the influence that this polymorphism could have on the various SARS-CoV-2 variants. In our research, we found out that those who have the 4G allele are more likely to be affected by the Delta variant, particularly if they are homozygous for it. This polymorphism can be a factor in addition to all of the others since the severity of the disease induced by this variation is greater than that caused by the other variants. It would suggest that this polymorphism does not contribute to an increased risk for the Omicron form.

4.5 Final remarks and future work

Our main objective was to find out the possible connection between the 4G/5G polymorphism of the PAI-1 gene with the Delta and Omicron variants of the SARS-CoV-2

The result of this study has found a significant association between this polymorphism and the SARS-CoV-2 Delta and Omicron variants. If an individual harbors this polymorphism, they are more vulnerable to contracting the Delta variant and have a higher chance of severe course of infection. Individuals who have this polymorphism do not appear to have increased susceptibility when the variant in question is Omicron.

The experiment was carried out with 408 participants from various ages however, age groups were not considered as a study variable. Therefore, not considering age variables can be a limitation of this study, along with the small sample size. Absence of the previous variant could be considered as a limitation as well. In the future, the same study could be performed with a greater group size and age variables could be included in it

CHAPTER 5

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EDUCATION

Near East University — **Nicosia, TRNC**
Msc. of Molecular Medicine — *3.95GPA*
September 2020 - June 2022

University of Gothenburg — **Gothenburg, Sweden**
Erasmus Programme
January 2017 – July 2017
Plant biology, biotechnology and eukaryotic molecular biology modules

Sabancı University — **Istanbul, Turkey**
Bsc. of Molecular, Biology, Genetics and Bioengineering — *2.65GPA*
September 2013 - December 2019

Final Science High School — **Bursa, Turkey**
2009 - 2013 — *3.7 GPA*

EXPERIENCE

Ergoren Lab. — **Nicosia, TRNC**
Laboratory Assistant, — *August 2020 – June 2022*
Near East University Medical Genetics Lab
Thesis Title: Association between PAI-1 4G/5G polymorphism and COVID-19 patients who infected with different SARS-CoV-2 variants.

- Planned and executed my own thesis project by utilizing PCR, RFLP and gel electrophoresis methods
- Presented my research at the international student congress.

Gozuacık Lab. — **Istanbul, Turkey**
Laboratory Assistant, — *September 2018 –January 2020*
Sabanci University Faculty of Engineering and Natural Sciences

- Gained experience in CRISPR-Cas9, bacteria cloning, transfection, qPCR analysis, western blot analysis and mammalian cell culture.
- Did my own research on cancer metastasis and its relation with the autophagy process.

Sanofi Pharmaceuticals — **Istanbul, Turkey**
Internship, — *June 2018 – August 2018*
Regulatory Affairs Department

- Explored the process of industrial scale pharmaceutical production and the R&D facility
- Translated over 100 documents into English
- Wrote and proofread medication prospectuses of 5 medicines to be approved by the Turkish Ministry of Health

Mine Sibel Gurun Lab. **Bursa, Turkey**
Laboratory Assistant, *June 2016 – September 2016*
Uludag University School of Medicine, Department of Pharmacology

- Learned the luquid chromatography-mass spectrometry technique.
- Gathered leafs from urban/suburban areas to find out the reason of declining endemic silk beetle population

Unal Egeli Lab. **Bursa, Turkey**
Laboratory Assistant, *June 2015 – September 2015*
Uludag University School of Medicine, Department of Pharmacology

- Gained my initial laboratory experience

Civil Involvement Project **Istanbul, Turkey**
Volunteer *September 2013 – June 2014*
Gebze Animal Shelter | Kurtköy Primary School

- Provided additional assistance and raised funds for the local animal shelter
- Taught animal welfare to primary school children.

SKILLS/INTERESTS/VOLUNTEER WORK/ADDITIONAL AFFILIATIONS/ETC

Volunteer neighborhood representative in the Bursa City Council **Bursa, Turkey**
June 2019 – Present

- Bi-weekly meetings to discuss the present problems and how to provide solutions. E.g. Provide food for animal shelters and street animals, organization of soup stands in populated areas.
-

Tennis Instructor **City, ST**
Role *Month 20xx – Month 20xx*

- Role doesn't have to be an official title that was given to you, just think about what you did. Projects are NOT just random school projects, but very elaborate or personal projects you're proud of

Languages

Fluent in Turkish
Fluent in English
Beginner in German