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Original Article

# SARS-CoV-2 receptor a distinct genetic profile specific to the Iraqi Kurdish population

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**Abstract**

SARS-CoV-2, the virus responsible for COVID-19, enters host cells by binding its spike protein's receptor-binding domain (RBD) to the human angiotensin-converting enzyme 2 (ACE2) receptor's peptidase domain (PD). This interaction plays a crucial role in the virus's ability to invade host cells and establish infection. Numerous studies have identified specific residues crucial for their binding interaction. Our objective was to determine whether natural variations in the ACE2 receptor could impact its affinity for the S-protein RBD. To explore this, we focused on investigating the effects of natural variations in the ACE2 PD residues on its binding affinity to the S-protein RBD interface of SARS-CoV-2. We conducted a genotyping study in the Iraqi Kurdish population and identified significant genetic variations in key binding residues of the ACE2 PD residues, including N330K, K353R, R357Q, P389H, and R393H. These variations suggest a distinct genetic profile specific to the Kurdish population regarding their interaction with the SARS-CoV-2 virus. Understanding the implications of these variations is essential for comprehending the mechanisms of viral infection, developing targeted therapeutics, and refining treatment strategies and vaccine design. Additionally, studying these variations can provide insights into population-specific vulnerabilities, help monitor viral evolution and transmission, and guide the development of effective interventions.

**Keywords:** ACE2 receptor, SARS-CoV-2, Iraqi kurdish population, Western Asia, ACE2 polymorphism.

## 1. Introduction

COVID-19 is a respiratory illness caused by a recently discovered coronavirus known as SARS-CoV-2. The virus enters host cells by binding its spike (S) protein to the human angiotensin-converting enzyme 2 (ACE2) receptor [1-3]. This interaction allows for the invasion of host cells, and the SARS-CoV-2 S-protein exhibits a significantly higher affinity to ACE2 compared to the S-protein of the original SARS-CoV, making it highly contagious [4-8]. The severity of disease among patients infected with SARS-CoV-2 varies considerably. Clinical outcomes can be influenced by host genetic factors, similar to what has been observed in other infectious diseases like HIV and malaria [9-13]. The incidence and severity of COVID-19 differ across regions and ethnicities, prompting increased interest in exploring the potential role of human genetic variation in SARS-CoV-2 transmission and pathogenicity [11-12]. In humans, infection by the SARS-CoV-2 virus results in a highly unpredictable disease with varying degrees of severity. While some individuals remain uninfected despite exposure, others experience a complete lack of symptoms, whereas a portion unfortunately succumb to the disease [1]. Host risk factors such as age, sex, and underlying health conditions contribute significantly to this variability. However, genetics may also play a crucial role in determining the incidence and prognosis of the disease [14-15]. Despite the identification of certain prognostic factors, there remains a substantial amount of unexplained variability [2]. For the virus to successfully invade host cells, it must establish a direct binding interaction between the receptor-binding domain (RBD) of the viral S1 protein and the extracellular domain (PD) of the host ACE2 peptidase [16-18]. This binding event is crucial for the virus to gain entry into the host cells and initiate the infection process [19]. In various structural studies focusing on the interaction between the SARS-CoV-2 S-protein RBD and ACE2 PD, several key residues have been identified. These residues play a critical role in facilitating their interaction [20-23]. The ACE2 S-protein-RBD interface involves the following key residues: S19, Q24, T27, F28, D30, K31, H34, E35, E37, D38, Y41, Q42, L45, L79, M82, Y83, T324, Q325, G326, E329, N330, K353, G354, D355, R357, P389, and R393 [24-25]. These specific residues are of particular importance in understanding the molecular mechanisms underlying the interaction between ACE2 and the S-protein RBD of SARS-CoV-2 [26-30]. Our study aimed to investigate the potential impact of natural variations in the ACE2 receptor on its affinity to the S-protein RBD interface, which is crucial for the cellular entry of the SARS-CoV-2 virus. We hypothesized that cer-

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tain variations in ACE2 could either decrease or increase its binding affinity to the S-protein RBD, potentially influencing the susceptibility of individuals to the virus. To explore this possibility, we conducted a genotyping study of the human ACE2 gene, specifically examining Exons 1, 2, 8, and 9, These exons are known to harbor the crucial residue responsible for binding to the virus's S-protein RBD. Our goal was to assess the impact of ACE2 single nucleotide polymorphisms (SNPs) on the genetic variability of ACE2, which may contribute to the diverse clinical outcomes observed in COVID-19. Through our investigation, we aimed to evaluate whether ACE2 polymorphisms have the ability to alter host susceptibility to SARS-CoV-2 by affecting the interaction between ACE2 and the virus's S-protein RBD.

## 2. Materials and Methods

### 2.1. Study participants

In this study, a total of 240 Iraqi Kurdish individuals (120 males and 120 females) were enrolled in Table 1. All participants belonged to the Kurdish ethnicity. The average age of the participants was 64 years, ranging from 32 to 78 years. Among the participants. Nasopharyngeal/oropharyngeal swab samples were collected from Erbil/Rojawa Emergency Hospital, resulting in a total of 240 samples. The presence of COVID-19 infection in individuals was confirmed using the RT-PCR method. Based on the World Health Organization (WHO) definitions for COVID-19 severity classification, the participants were categorized into three groups: control, moderate, and severe. Each group consisted of 40 males and 40 females, resulting in a total of 80 individuals in each group.

### 2.2. ACE2 genotyping

Four exons (exons 1, 2, 8, and 9) of the ACE2 gene, which is the receptor for COVID-19, were genotyped. These specific exons were chosen due to their critical role in the entry of the virus and the binding domain of the receptor-virus interaction. Genomic DNA was extracted from the samples using the DNeasy Blood K kit (Qiagen, Hilden, Germany) following the instructions provided by the manufacturer. The quality and integrity of the extracted DNA were assessed using a NanoDropTM spectrophotometer (Thermo Scientific, UK). PCR reactions were conducted in a 25 µL reaction volume using the GoTaq® Green Master Mix (Promega, USA). The PCR primers were manually designed by the author and the primer sets used are provided in Table 2. The optimized PCR cycling conditions were as follows: an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 45 seconds, and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for 10 minutes. Gel electrophoresis was carried out using a Cleaver gel electrophoresis system (UK) to visualize the PCR products. The DNA bands were visualized using a gel documentation system (Proxima 2500, India). Figure 1 displays the results of the gel electrophoresis, showing the amplified PCR products.

### 2.3. Sample sequencing

To identify genetic variations in the ACE2 gene exons (Exons: 1, 2, 8 and 9) among the study groups, all PCR products were prepared for sequencing. The prepared samples were then sent to Macrogen Labs in Korea for analysis. At Macrogen Labs, the analysis of the samples was conducted using capillary electrophoresis. This technique utilizes thin and long acrylic fiber capillaries to separate

**Table 1**. Study design & participants

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **240 participants (120 Female & 120 male)** | | | | | | | | |
| **DNA Extraction & Quantification** | | | | | | | | **Total n.** |
| | | | **Female** | | | **Male** | | |
| | | | Control | Moderate | Severe | Control | Moderate | Severe | n=240 |
| | | | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | | **PCR** | | | | | | |
| **ACE2 Exons** | | | **PCR reaction= 240 (60 Participants/ Exon)** | | | | | | **Total PCR** |
| | | | **Female** | | | **Male** | | | |
| Exon 1 | Exon 2 | Exon 8 | Exon 9 | Control | Moderate | Severe | Control | Moderate | Severe | **n= 240** |
| | | | | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | | | | n=120 | | | n=120 | | |
| | | | | **DNA sequencing** | | | | | | |
| | | | | **Bioinformatic analysis** | | | | | | |

**Table 2**. Primers used in this study & the expected amplicon size.

| ACE2 gene | Primer & Sequence | $T_m$ C° | $T_a$ C° | Expected amplicon size |
|---|---|---|---|---|
| Exon 1 | F: 5'-GTTATCTGGGACTCCAAAATCAGGGATA-3'<br>R: 5'-GTCTAGGGAAAGTCATTCAGTGGATGTG-3' | 62.8<br>63.6 | 59 | 384 bp |
| Exon 2 | F: 5'-GGACACCTTACCTAGGCATAGAGAGAGA-3'<br>R: 5'-CTTCAGCGGAGTAGAGGACTAAATCACT-3' | 65.3<br>63.3 | 59 | 443bp |
| Exon 8 | F: 5'-AGTATCAGTTGTGTAAGTATCAGCCCCAC-3'<br>R: 5'-AGTGAGAACATGTGGTATTTGTTTTTCG-3' | 63.2<br>59.1 | 59 | 300bp |
| Exon 9 | F: 5'-GCCATGAGAAAATGTCCATACCATTGTC-3'<br>R: 5'-GAGGTGGGTACTCAAGATTCACTGGTGA-3' | 62<br>66.8 | 59 | 384bp |

DNA fragments based on their size. Capillary electrophoresis allows for precise and accurate determination of the DNA sequence by measuring the migration of the DNA fragments through the capillary. By analyzing the sequencing data obtained through capillary electrophoresis, the genetic variations in the ACE2 gene exons among the different study groups could be identified and analyzed.

### 2.4. Bioinformatic analysis

The obtained sequence results were analyzed using the web-based tool available at the following URL: https://www.ebi.ac.uk/Tools. To design the figures, Inkscape version 0.92.3 was utilized. On 13 February 2023, the sequence of ACE2 gene exons (1, 2, 8, and 9) was submitted to the National Center for Biotechnology Information (NCBI) and its database for single nucleotide polymorphisms (dbSNP) to acquire the corresponding accession numbers. Once the NCBI/dbSNP database staff verified the presence of variations in exons 8 and 9, the accession numbers were provided (Supplementary materials). As a result, the ACE2 gene sequence for the Iraqi Kurdish population is now publicly accessible to all researchers through NCBI/dbSNP at the following link: https://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=SUHAD_A_MUSTAFA.

### 2.5. Protein structure formation

In order to assess the three-dimensional (3D) structure of ACE2, the Swiss Model online tool (http://swissmodel.expasy.org) was utilized, as described by Guex and Peitsch in 1997. To ensure accuracy, a template search was performed using the Swiss Model, aiming to identify the most suitable template for predicting the ACE2 structure. The selection of the ACE2 protein model involved considering high sequence similarity with the template and favorable GMQE (Global Model Quality Estimate) and QSQE (Qualitative Model Energy Analysis) values. Furthermore, the protein-protein interaction (PPI) of the chosen template was carefully examined to ensure its relevance to ACE2. The resulting ACE2 model was subjected to analysis using the Swiss Model Structure Assessment feature, including the evaluation of its conformational quality using the Ramachandran plot. Additionally, the Local Quality Estimate and QMEAN (Qualitative Model Energy Analysis) score of the projected ACE2 model were assessed to provide further insight into its structural integrity and overall quality.

## 3. Results

### 3.1. ACE2 Exons Genotyping

We performed genotyping of the human ACE2 gene by conducting PCR and subsequent sequencing of exons 1, 2, 8, and 9. These specific exons were targeted as they contain the critical residues of the ACE2 peptidase domain (PD) that are important for the interaction with the SARS-CoV-2 virus. Figure (1) illustrates the expected sizes of the amplicons obtained from these ACE2 exons, providing valuable information about the genetic variations in the ACE2 receptor among our study population.

### 3.2. Natural variations in the ACE2 receptor

The analysis of Exons 1 and 2 revealed a complete match of the Kurdish population's sequences with the reference sequence from NCBI (Figure 2). Interestingly, we did not observe any of the single nucleotide variations (SNVs) reported in NCBI for other populations within the Kurdish population, regardless of the study groups (control, moderate, and severe).

In Exon 8, a significant distinction was observed between the Kurdish population and other populations documented in NCBI. While NCBI reported 48 SNVs for other populations, we found only one of these SNVs in the Kurdish population (Figure 3). Additionally, we identified 12 novel variations specific to the Kurdish population, including 8 SNVs and 4 insertions that have not been previously documented.Exon 9 exhibited substantial genetic diversity in the Kurdish population compared to other populations reported in NCBI. Out of the 65 SNVs reported in NCBI, only 9 SNVs were shared between the Kurdish population and other populations. Remarkably, we discovered a total of 129 novel variations among all participants, encompassing 123 new single nucleotide variations (NSVs), 3 deletions, and 3 insertions (Figure 4). These findings were consistent across individuals classified as control, moderate, and severe cases. These novel variations highlight the unique genetic landscape of the

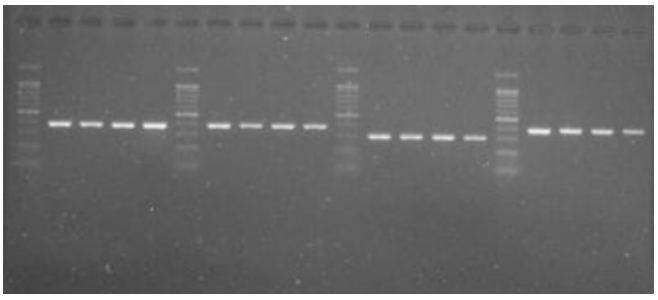

**Fig. 1.** Expected amplicon size of Human ACE2 gene exons (Exon1: 384bp, Exon 2: 443, Exon 8: 300bp and Exon 9: 384bp), M: 100bp molecular marker.

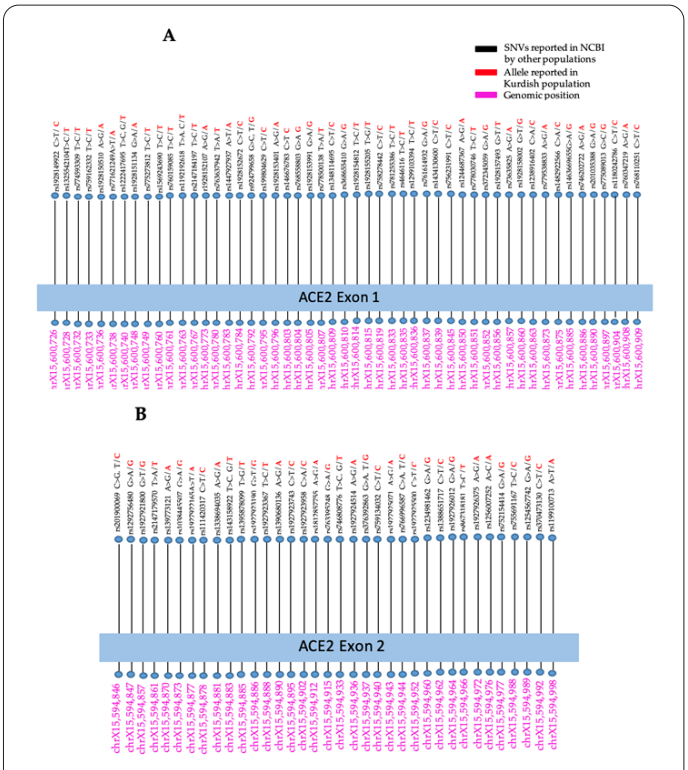


**Fig. 2.** Schematic representation of single nucleotide variations (SNVs) in ACE2 gene A: Exon 1(186 bp), and B: Exon 2(159bp).

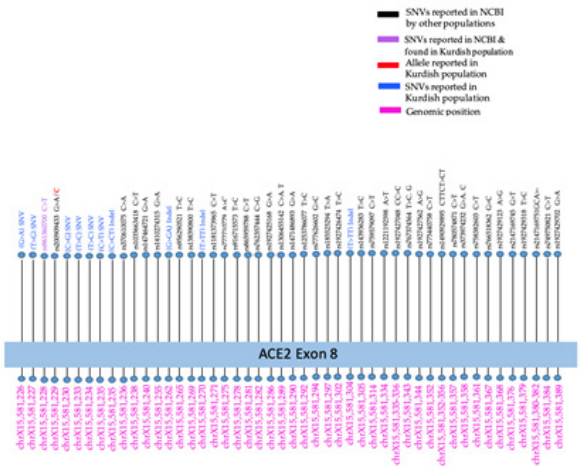


**Fig. 3.** Schematic representation of single nucleotide variations (SNVs) in ACE2 gene Exon 8 (170bp).

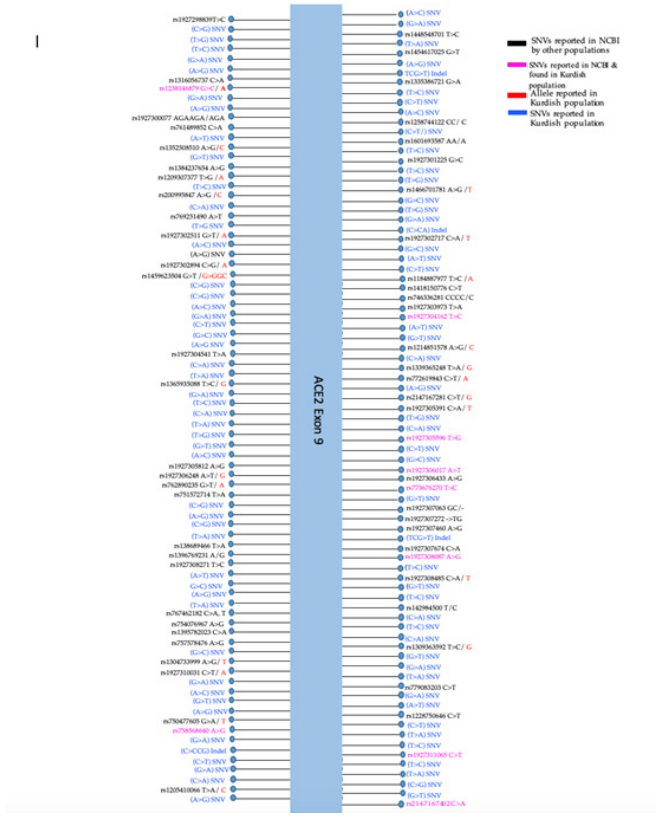


**Fig. 4.** Schematic representation of single nucleotide variations (SNVs) in ACE2 gene Exon 9 (227bp).

Kurdish population and suggest potential population-specific genetic factors that may influence disease susceptibility and progression. Further investigations are necessary to elucidate the functional implications of these novel variations and their relevance to disease outcomes in the Kurdish population.

### 3.3. Critical residues at the ACE2 S-protein-RBD interface

The genetic variations observed in the ACE2 gene have the potential to impact the structure and binding affinity of the ACE2 protein to the SARS-CoV-2 virus. Critical residue variations at positions N330/K330, K353/R353, R357/Q357, P389/H389, and R393/H393 (Figure 5), have the potential to influence the three-dimensional structure of the ACE2 protein, potentially altering its shape, electrostatic interactions, hydrogen bonding, side chain properties, charge, conformational adaptability, and overall stability and interaction between ACE2 and the SARS-CoV-2 virus. Understanding the impact of these variations is crucial for unraveling the molecular mechanisms of SARS-CoV-2 infection, susceptibility, and potential therapeutic interventions.

### 3.4.Model stability evaluation

The Swiss model structure analysis trait was used to validate the calculated, ACE2 model (Figure 6). Clash and Molprobity scores were 0.93 and 0.29 respectively. Ramachandran's favorite regions accounted for 96.74 % of the total, with outliers contributing 0.28%. The results of the model stability evaluation indicate that the calculated ACE2 model has a low clash score, a favorable Molprobity score, and a high percentage of residues in preferred conformations. These findings provide confidence in the reliability and accuracy of the model to study the impact of genetic variations on the interaction between ACE2 and the SARS-CoV-2 virus.

## 4. Discussion

Structural and computational studies demonstrated that SARS-CoV-2 has the ability to attach itself to the human receptor using a diverse set of ACE2 residues, these resi-

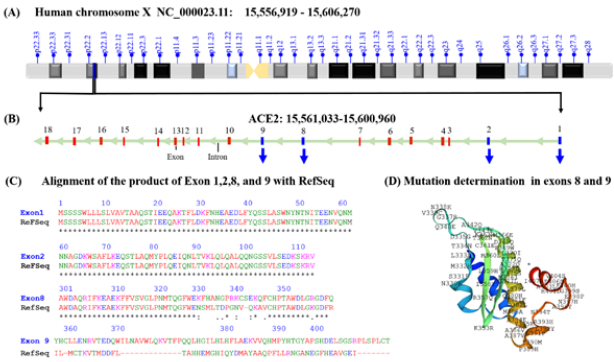


**Fig. 5.** Schematic representation of Human Chromosome X and ACE2 gene A: Chromosome X: NC_000023.11 B: 18 exons of ACE2 gene C: Alignment of amino acids sequences of exons (1,2,8 & 9) with RefSeq D: ACE2 crystal structure showing mutation in exons 8 and 9.

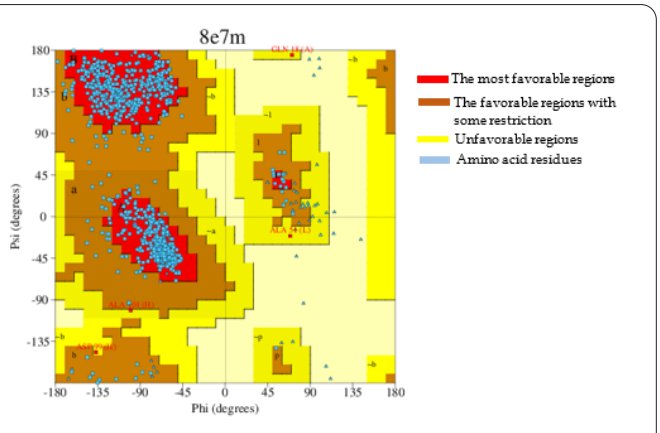


**Fig. 6.** Ramachandran plot for stability assessment of ACE2 Protein. Residues located in the red region generally indicate a stable and well-folded protein conformation, Residues falling within the brown region may still be acceptable, but with a lower degree of confidence compared to those in the red region, residues found in the yellow region indicate structural irregularities that could impact the stability of the protein.

dues play a critical role in facilitating the binding and entry of the virus into host cells [31-34]. Our study revealed significant genetic variations in the critical binding residues of the ACE2 receptor that interacts with the SARS-CoV-2 virus among the Kurdish population. Specifically, we observed substitutions in key residues such as N330, K353, R357, P389, and R393, where N330 was replaced with K330, K353 with R353, R357 with Q357, P389 with H389, and R393 with H393. These findings indicate a distinct genetic profile specific to the Kurdish population in terms of their ACE2-S protein interaction. Replacing multiple residues in the ACE2 receptor can have significant implications for the interface between the ACE2 receptor and the S protein of SARS-CoV-2. These substitutions introduce changes in charge, size, and hydrogen bonding capabilities at critical positions, potentially impacting the binding affinity, specificity, and stability of the ACE2-S protein interaction [35-38]. The substitution of N330 with K330 may disrupt existing hydrogen bonds and introduce new interactions. Similarly, substitutions like K353 to R353, R357 to Q357, P389 to H389, and R393 to H393 can alter electrostatic interactions, conformational adaptability, and hydrogen bonding patterns [39-41]. These substitutions collectively affect the molecular interactions at the ACE2-S protein interface, which can have consequences for viral entry, infectivity, and disease progression. However, it is important to note that the precise effects of these substitutions would need to be investigated through experimental studies involving structural analyses and binding assays [42-46]. The genetic variations we identified suggest a potential link between these changes in the ACE2 receptor and the susceptibility, severity, and clinical outcomes of COVID-19 cases within the Kurdish population. The alterations in critical binding residues can affect the strength and specificity of the interaction between the virus and the receptor, potentially influencing viral entry and disease progression. By highlighting these genetic variations, our study contributes to a better understanding of the geographic diversity in SARS-CoV-2 infection and its implications for disease outcomes. Further research is warranted to investigate the functional consequences of these genetic variations, their association with COVID-19 severity, and their potential implications for developing targeted therapeutic interventions and public health strategies tailored to the Kurdish population. To comprehensively address the functional implications of identified genetic variations within the ACE2 receptor on SARS-CoV-2 susceptibility in the Kurdish population, specific experimental studies should be pursued. Structural analyses, molecular dynamics simulations, and protein binding assays can illuminate alterations in the ACE2-S protein interaction, while employing recombinant ACE2 variants with observed substitutions in binding assays can offer insights into changes in affinity and specificity. Cell culture studies or animal models are crucial to understand the impact of these genetic variations on viral entry, infectivity, and disease progression. Moreover, utilizing structural techniques like X-ray crystallography or cryo-electron microscopy can unveil details about protein conformation and molecular interactions. These approaches collectively promise to unravel the mechanistic implications of genetic variations, thereby informing potential therapeutic interventions and tailored public health strategies specific to the Kurdish population. Understanding the impact of these variations on the ACE2-S protein interaction is crucial for unraveling the mechanisms of SARS-CoV-2 infection and developing targeted therapeutic strategies. Finding variations in the critical residues of the human ACE2 receptor that bind to the SARS-CoV-2 spike protein is crucial for understanding host susceptibility, disease severity, population genetics, evolution and transmission dynamics, as well as vaccine and therapeutic development. These variations can influence an individual's susceptibility to infection, disease outcomes, and transmission dynamics. They can also provide insights into population-specific vulnerabilities or protective factors, aid in monitoring viral evolution and transmission, and guide the development of targeted interventions. Additionally, understanding how these variations impact the interaction between the virus and the ACE2 receptor is vital for refining treatment strategies and vaccine design. Overall, studying these critical residues helps unravel the complex interplay between the virus and its host, leading to improved risk assessment and the development of effective interventions against SARS-CoV-2.

## 4. Conclusion

In conclusion, our study on the genetic variations in the critical binding residues of the ACE2 receptor among the Kurdish population sheds light on the potential implications for SARS-CoV-2 infection. The observed substitutions in key residues, such as N330K, K353R, R357Q, P389H, and R393H, suggest a distinct genetic profile specific to the Kurdish population in terms of ACE2-S protein interaction. These variations can significantly impact the binding affinity, specificity, and stability of the ACE2-S protein interaction, potentially influencing viral entry, infectivity, and disease progression. However, further experimental investigations are necessary to understand the precise effects of these substitutions. Our findings highlight the importance of considering geographic diversity in SARS-CoV-2 infection and its potential impact on disease outcomes. Such understanding can guide the development of targeted therapeutic interventions and public health strategies tailored to the Kurdish population, as well as contribute to vaccine and therapeutic development efforts.

## Supplementary Materials

The manuscript includes supplementary materials containing all the necessary information and resources used in this study.

## Author Contributions

The author of this study conducted the research and investigation described in the manuscript.

## Funding

This study was conducted without receiving any external funding or financial support.

## Institutional Review Board Statement

This study received ethical approval from the Salaheddin University-Erbil ethical committee (4e/198) for the use of SARS-CoV-2 nasal and oropharyngeal (N/OP) swabs. All research methods were conducted in accordance with the guidelines outlined in the Helsinki Declaration of 1964. A total of 90 nasopharyngeal/oropharyngeal swab samples were collected from the Rojawa Emergency Hospital. The presence of SARS-CoV-2 in the samples was confirmed

using the RT-PCR method. Prior to participation in the study, written informed consent was obtained from all patients and controls. The necessary authorization for publication of the study findings was also obtained.

**Informed Consent Statement**

Informed consent was obtained from all subjects involved in the study authorization to be published

**Data Availability Statement**

The ACE2 gene sequence for the Iraqi Kurdish population, specifically Exons 8 and 9, has been made publicly accessible to researchers through NCBI/dbSNP https://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?handle=SUHAD_A_MUSTAFA. This allows researchers worldwide to access and utilize the genetic information related to the ACE2 gene in the Iraqi Kurdish population. By making this data available, it promotes collaboration, further research, and a better understanding of the genetic variations within the ACE2 gene among the Kurdish population.

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**Conflicts of Interest**

We declare that there are no conflicts of interest associated with this study.

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