

Analysis of Three Mutations in Italian Strains of SARS-CoV-2: Implications for Pathogenesis

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Keywords

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped virus initially detected in Wuhan in December 2019, responsible for coronavirus disease 2019 (COVID-19), a respiratory syndrome currently affecting >220 countries around the world, with >80 million cases registered and >1.8 million deaths. **Objective:** As several vaccines are still being developed and 2 have been approved, it is particularly important to perform evolutionary surveillance to identify mutations potentially affecting vaccine efficacy. **Methods:** DynaMut server has been used to evaluate the impact of the mutation found on SARS-CoV-2 isolates available on GISAID. **Results:** In this article, we analyze whole genomes sequenced from Italian patients, and

we report the characterization of 3 mutations, one of which presents in the spike protein. **Conclusion:** The mutations analyzed in this article can be useful to evaluate the evolution of SARS-CoV-2.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) initially detected in Wuhan in December 2019 [1] is responsible for the coronavirus disease 2019 (COVID-19) [2], a respiratory syndrome, and currently, it is affecting >220 countries around the world, with >80 million cases registered and more than 1.8 million deaths. SARS-CoV-2 is an enveloped virus, belonging to the beta-CoV genus, with a positive-sense single-stranded RNA genome of approximately 30,000 bases, with 5'-cap structure and 3'-poly-A tail [3]. SARS-CoV-2 has 16 nonstruc-

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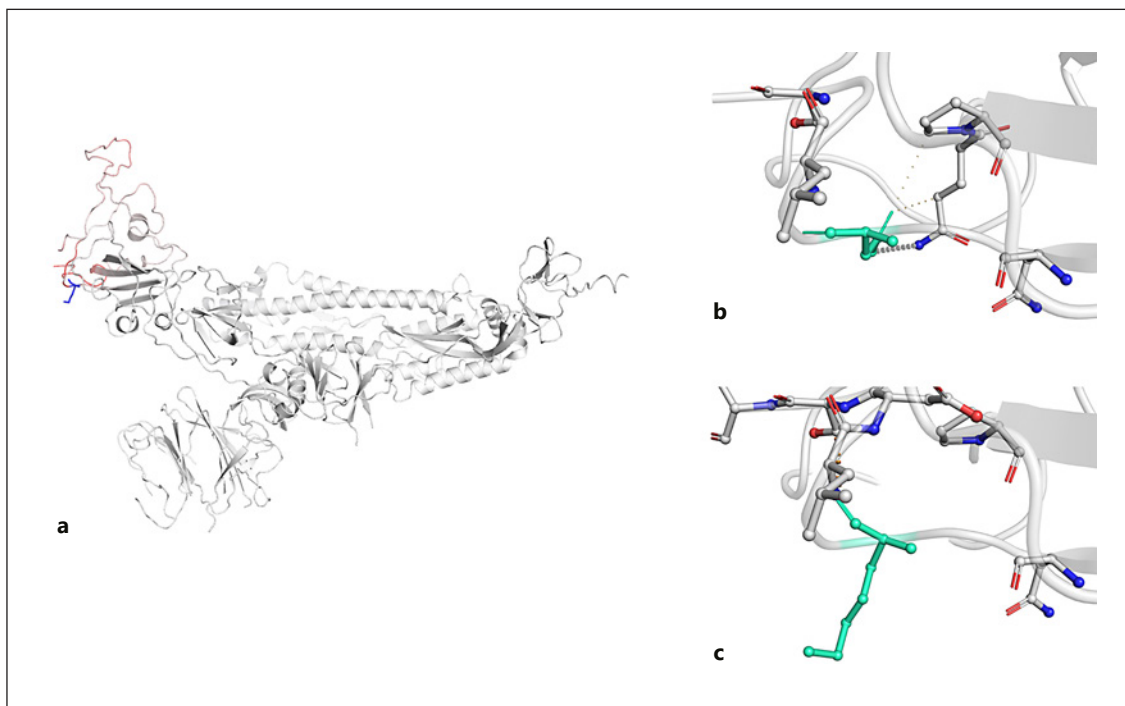


Fig. 1. **a** Model of S glycoprotein monomer displaying the amino acids colored according to the vibrational entropy change upon mutation. Red regions are those gaining in flexibility, whereas blue regions are those gaining stability; the top image shows the molecular interaction between the side chain of the wild-type amino acid and the side chains of the surrounding amino acid (**b**); the bottom image shows the molecular interaction between the side chain of the mutated amino acid and the side chains of the surrounding amino acid (**c**). S, spike.

tural proteins and, like other coronaviruses, 4 structural proteins, known as the spike (S), envelope (E), membrane, and nucleocapsid proteins [4, 5]. The main responsibility of the nucleocapsid protein is to hold the RNA genome, while the S, envelope, and membrane proteins together create the viral envelope. In particular, the S protein is responsible for allowing the virus to attach to and fuse with the membrane of a host cell by recognizing and interacting with a specific receptor, the angiotensin-converting enzyme 2 (ACE2) [6]. Italy is now experiencing the second epidemic wave of COVID-19, and as several vaccines are still being developed and 2 have been approved [7], it is particularly important to perform evolutionary surveillance to identify mutations potentially affecting vaccine efficacy [8]. Several reports have demonstrated how a single amino acidic substitution, first observed in Europe emerging from China [9], can affect the viral infectious phenotype [10–13], and several new mutations have been reported that have the possibility to influence the spreading of the SARS-CoV-2 [14–19]. In this article, we report 3 mutations possibly affecting its phenotypical characteristics.

Materials and Methods

A total of 870 COVID-19 whole genome sequences isolated from Italian patients from January 29 to November 10, 2020 have been downloaded from GISAID (<https://www.gisaid.org/>) database. The dataset has been aligned using multiple sequence alignment (MAFFT) online tool [20] and manually edited using BioEdit program v7.0.5 [21]. Sequence alignments and analyses were obtained through the Jalview editor [22], and structural models have been built relying on the website I-TASSER [23], protein data bank HHPred [24], and CUPSAT [25], and DynaMut [26] online server has been used to estimate the stability of potential mutations found using the selective pressure analysis. Three-dimensional structures have been analyzed and displayed using PyMOL.

Results

The analysis of the alignment has revealed the presence of 3 mutations on helicase, S, and papain-like protease (NSP3) proteins in COVID-19 sequences isolated from September to November 2020 from Italian patients for a total of 11 isolates. The helicase protein has shown nonsynonymous mutation from a histidine to a tyrosine

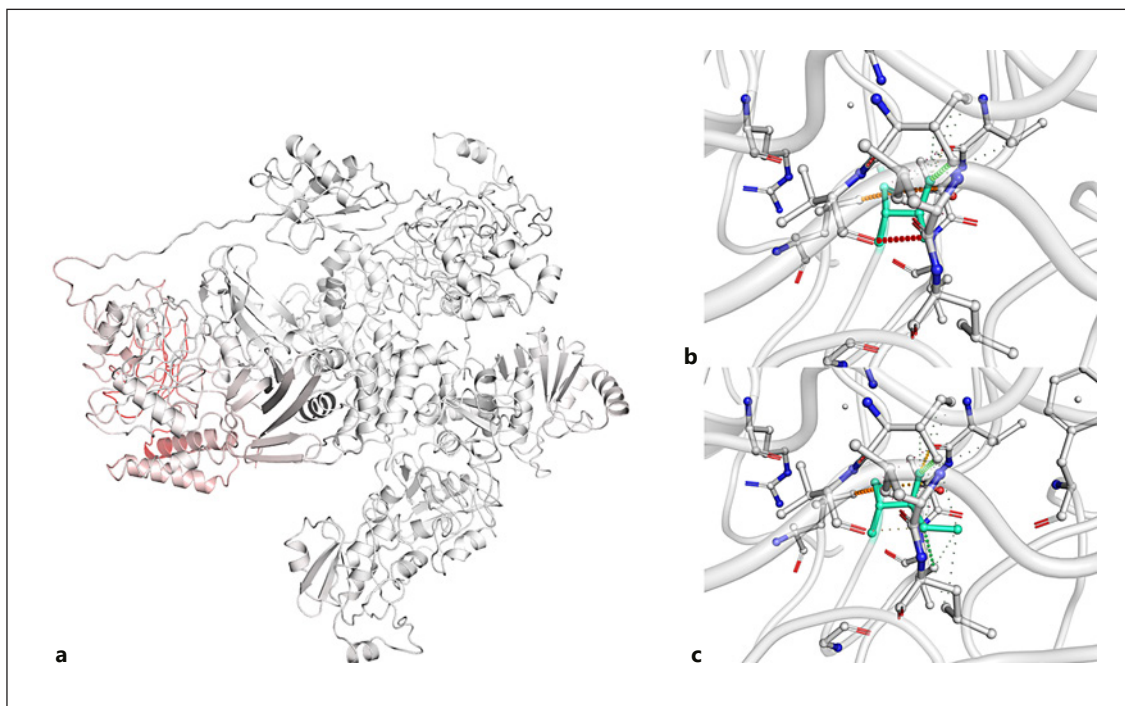


Fig. 2. **a** Model of papain-like protease protein displaying the amino acids colored according to the vibrational entropy change upon mutation. Red regions are those gaining in flexibility, whereas blue regions are those gaining stability; the top image shows the molecular interaction between the side chain of the wild-type amino acid and the side chains of the surrounding amino acid (**b**); the bottom image shows the molecular interaction between the side chain of the mutated amino acid and the side chains of the surrounding amino acid (**c**).

in the 39th amino acid position. They are both polar amino acid but with different side chain structures; the first one has an imidazole side chain, while the other has a phenol side chain. Using the 3-dimensional structure available on I-TASSER server, the implication of this mutation has been analyzed by DynaMut server. The results point out both mutations reduce the stability of the protein ($\Delta\Delta G$ [kcal/mol] 0.377), while the Δ vibrational entropy energy between wild-type and mutant papain-like protease protein has been calculated to be $\Delta\Delta S_{Vib} ENCoM$: $-0.472 \text{ kcal mol}^{-1} \text{ K}^{-1}$. In the S glycoprotein, the transition from an asparagine to a lysine occurs on the 439th amino acid position. Asparagine is a polar aliphatic amino acid which frequently occurs at the beginning of alpha helices, while lysine is a basic, chiral, charged, and aliphatic amino acid, and its ϵ -amino group often participates in hydrogen bonding, salt bridges, and covalent interactions. This mutation is located in the SD2 region of the RBD-containing S1 subunit and particularly in the most exposed region of the protein that interacts with the ACE2 receptor on human cells [13]. Using the crystallographic 3-dimensional structure of

the S protein, the implications of this mutation have been analyzed using CUPSAT and DynaMut servers. The results of these analyses have shown that the mutation from asparagine to lysine reduces the stability of the protein ($\Delta\Delta G$ [kcal/mol] -0.209) favoring the torsion potential. The Δ vibrational entropy energy between wild-type and mutant S protein has been calculated to be $\Delta\Delta S_{Vib} ENCoM$: $0.262 \text{ kcal mol}^{-1} \text{ K}^{-1}$ (Fig. 1). This mutation falls in one of the 2 N-terminal zinc-binding domain (1–99 a.a) [27]. Regarding the papain-like protease protein, the transition from an isoleucine to a threonine occurs on the 1,683th amino acid position. Isoleucine is a nonpolar, uncharged, branched chain, aliphatic amino acid, while threonine is a polar, uncharged amino acid and is susceptible to numerous posttranslational modifications. The homology modeling analysis performed using HHpred server has shown structural similarity of the subdomains where the mutation is with the ubiquitin-like protein domain of papain-like protease protein of MERS-CoV (Fig. 2). Using the crystallographic 3-dimensional structure of the helicase protein, the implications of this mutation have been analyzed using CUP-

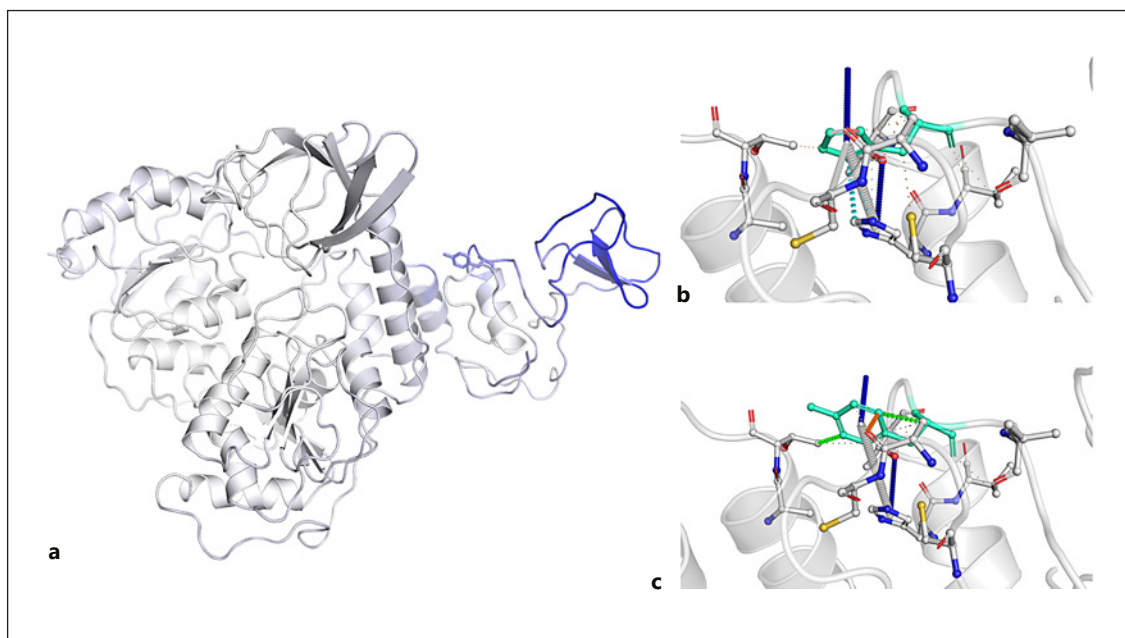


Fig. 3. **a** Model of helicase protein displaying the amino acids colored according to the vibrational entropy change upon mutation (**a**). Red regions are those gaining flexibility, whereas blue regions are those gaining stability; the top image shows the molecular interaction between the side chain of the wild-type amino acid and the side chains of the surrounding amino acid (**b**); the bottom image shows the molecular interaction between the side chain of the mutated amino acid and the side chains of the surrounding amino acid (**c**).

SAT and DynaMut servers. The results of these analyses have shown that the mutation from isoleucine to a threonine reduces the stability of the protein ($\Delta\Delta G$ [kcal/mol] -1.963) favoring the torsion potential. The Δ vibrational entropy energy between wild-type and mutant helicase protein has been calculated to be $\Delta\Delta S_{vib}^{ENC_{CoM}}$: $0.781 \text{ kcal mol}^{-1} \text{ K}^{-1}$ (Fig. 3).

Discussion

The presence of new mutations in Italian sequences isolated in October 2020 could partially clarify the higher spread of the virus reached in that month; moreover, nowadays a new strain from the UK probably responsible for a higher infection has been observed. The mutation analysis tool GISAID has also shown that the mutations evaluated in this article are highly prevalent in the UK. We have identified 3 nonsynonymous mutations, H39Y on helicase protein, N439K on S glycoprotein, and I1683T on papain-like protease. Previous studies have shown how single amino acidic substitutions could scientifically influence the COVID-19 characteristics [10–13] and how bioinformatics approach could be useful to predict the

impact of amino acidic mutation on the clinical features of the virus [28]. The structural analysis of the mutated helicase protein revealed an increase in stability of zinc-binding domain; moreover, the mutations affect the stability of the whole ZBD region. This could possibly make the structure more rigid and less capable of performing its normal activity and partially explain the presence of other mutations in these isolates. The N439K substitution on the S glycoprotein resides on the amino acidic region responsible for binding ACE2 on human cells. It is well known that lysine increases the ability of making new bindings, and our results suggests that this substitution has an effect on the structure of S glycoprotein, increasing its flexibility and potentially increasing the ability of the virus to bind cellular receptors, as already seen for the previous D614G S mutations. The last mutation on papain-like protease falls on a ZBD region. This specific region is involved in multiple molecular fundamental processes including autophagy and host cell regulation. The autophagy pathway seems to be crucial for SARS-CoV-2 since other mutations on this pathway have been found [14].

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Statement of Ethics

The paper is exempt from ethical committee approval because the dataset used for this analysis has been downloaded from a public open online repository and no human or animal has been involved in the research.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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Author Contributions

All authors wrote sections of this manuscript and approved the submitted version.