Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Gene Reports 29 (2022) 101705

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Whole exome sequencing identifies a rare variant in MAS1 gene in a subject with lethal COVID-19

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ARTICLE INFO

Edited by May Yin LEE

Keywords:
COVID-19
Whole exome sequencing
SARS-CoV-2
Renin-angiotensin system

ABSTRACT

COVID-19 may be considered a multifactorial disease caused by the interaction between the virus itself, as the environmental contribute, and the genetic background of the host. SARS-CoV-2 infection occurs through the interaction between the spike protein and ACE2, a receptor in the host cells. Clinically, COVID-19 is characterized by a high heterogeneity in symptomatology ranging from asymptomatic to severe symptoms, and even worsening to death. This variability relies on the host genomic profile and other individual comorbidities. We performed exome analysis in one family displaying a variable spectrum of SARS-CoV-2 infection despite a common exposure. After segregation analysis, we found that the c.446C>T p.(S149L) in *MAS1* gene was exclusively present in the individual with severe COVID-19, who died because of pneumonia and multiple thrombotic events. *MAS1* encodes a receptor for Ang1–7 in the renin-angiotensin system (RAS) with an anti-inflammatory, anti-fibrotic and anti-angiogenic effect. We hypothesize that downregulation of RAS, due to this rare variant, might impair the protective effect and concur to the clinical severity of the disease. Our results support the protective role of the ACE2/Ang-(1–7)/Mas1 axis and the potential danger of its dysregulation leading to severe COVID-19 disease; if further confirmed, these findings will be useful for management of critically ill patients.

1. Introduction

The Coronavirus disease-19 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has caused infection of >400 million people and over 5 million deaths worldwide. SARS-CoV-2 is an enveloped virus with a positive single-stranded RNA genome that belongs to the β-coronavirus subfamily (Hu et al., 2021). The viral entry into host cells is mediated by the interaction between the N-terminal domain of the receptor ACE2 (angiotensin-converting enzyme 2) and the SARS-CoV-2 spike (S) glycoprotein (Hoffmann et al., 2020; Li et al., 2003). ACE2 is a transmembrane protease enzyme with a full length of 805 amino acids, an N-terminal protease domain (PD) and a cytosolic tail supplied with a C-terminal collectrin-like domain (CLD) (Gheblawi et al., 2020; Zhang et al., 2001). Besides interacting with the virus S glycoprotein, ACE2 N-terminus has a zinc binding metalloprotease motif that plays a carboxypeptidase activity converting angiotensin I (Ang I) to Ang 1–9 and Ang II to Ang 1–7 (Vickers et al., 2002), thereby negatively regulating the renin-angiotensin system (RAS). An enzyme which has high homology with ACE2 is angiotensin-converting enzyme (ACE), that, in contrast, converts Ang I to Ang II (Khurana and Goswami, 2022). The ACE2-Ang(1–7)-Mas1 axis has been shown to have a vascular protective role and possibly prevent the worsening of clinical status of COVID-19 patients. The clinical spectrum of SARS-CoV-2 infection appears to be wide, ranging from asymptomatic phenotypes, or mild symptomatology, to severe cases with respiratory failure and even death. In addition, specific comorbidities seem to increase not only the risk of infection, but also cause the worsening of lung injury and multiorgan failure. According to the clinical and epidemiological data of COVID-19, the most common comorbidities reported with extreme disease manifestations are hypertension, cardiovascular

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https://doi.org/10.1016/j.genrep.2022.101705
Received 30 June 2022; Received in revised form 27 October 2022; Accepted 31 October 2022
Available online 4 November 2022

diseases, and diabetes (Zhou et al., 2020). The scale of genetic research in COVID-19 is extremely large. The use of Genome-Wide Association Studies (GWAS) and Next Generation Sequencing techniques (through exome and genome sequencing), has allowed to identify both polymorphisms and also rare variants associated with a predisposition to infection and severe forms of COVID-19. GWAS studies have identified chromosomal loci and SNPs in genes involved in immune function, antiviral cell defense mechanisms, and inflammatory damage mediators. These results are available in the database COVID-19 Host Genetic Initiative (COVID-19 HGI) and clustered on the basis of the positive test for SARS-CoV-2 and the symptomatology of the disease (Cappadona et al., 2021; Pairo-Castineira et al., 2021; The COVID-19 Host Genetics Initiative, 2020; The Severe Covid-19 GWAS Group, 2020). Exome/genome-studies about the contribution of rare coding genetic variations to SARS-CoV-2 infection severity and fatalities by Zhang et al. (2020) revealed inborn errors of type I interferon (IFN) and of innate immunity in patients with life-threatening COVID-19. Also Benetti et al. (2020) explored host genetics through whole exome sequencing analysis (WES) in a cohort of 35 hospitalized COVID-19 patients, defining a preliminarily combined model of rare and common variants impacting the clinical outcome.

In the present work, we performed WES in a family with wide clinical variability of COVID-19 with the goal of identifying genetic variants related to the severe outcome in one member who died from pneumonia and multiple thrombotic events.

2. Material and methods

2.1. Patients

We enrolled one Caucasian family from central Italy made up of five members who contracted the SARS-CoV-2 (lineage B.1.1.7) in November 2020, before the availability of any vaccines. They presented different COVID-19 clinical symptoms, despite an identical exposure, since they co-habited during the infection (Fig. 1). The most severe case in this family was individual I-1, a 53 y/o man who developed a COVID-19 related pneumonia. He was also affected by type-2 diabetes (controlled with diet only) and he was not obese or hypertensive. After seven days of high fever, he was hospitalized for oxygen desaturation ($pO_2 = 71$); the patient stayed in the Intensive Care Unit for seventeen days: in addition to a severe respiratory failure even in mechanical ventilation, he developed multiple thrombosis with high values of D-Dimer (up to 12,000) and hypernatremia. He had persistent tachycardia (110–125 bpm) but no sign of acute cardiac insufficiency. Bronchoscopy repeatedly detected intrabronchial bleeding, which was washed out. In the past three days, the patient developed a generalized edema and anuria, non-responsive to intravenous diuretic treatment. After seventeen days the patient died. His wife (I-2) was SARS-CoV-2 positive with a mild symptomatology (muscle pain); his three daughters were SARS-CoV-2 positive and asymptomatics. The family was enrolled through a collaboration with the Haematological Transfusion Medicine Unit upon subscription of an informed consent (Institutional Review Board approved n° 04.21). The family gave the consent for testing the DNA of the deceased father and they read and approved this manuscript.

2.2. Whole exome sequencing and Sanger sequencing

Genomic DNA was extracted from peripheral blood (I-1 patient) and from saliva of all the other family members using standard techniques and whole exome sequencing was performed on service at Dantelabs SRL (Aquila, Abruzzo), in individuals I-1*, I-2* and II-2*. An average coverage of 60× on an Illumina platform was reached. The bioinformatic analysis was performed on the online platform Galaxy (Goecks et al., 2010): the fastQ files were aligned using the Burrows-Wheeler Aligner (Li and Durbin, 2009) (Human GRCh37/hg19); duplicates were removed to perform the variant calling with FreeBayes and the variant annotation with wAnnovar (Yang and Wang, 2015).

All bioinformatic analyses were done following best practices recommendations (Depristo et al., 2011; Van der Auwera et al., 2013). We included in our analysis all variants identified within the coding/splicing regions and with a good coverage.

Sanger sequencing validated all the variants that passed the filtering steps. Primers flanking the variants were designed using Primer3 application on the UCSC genome browser. The 5′-3′ sequence of forward and reverse primers are "ctacaacacgggcctctatctg" and "gtgactctcttcttctctgtcg", respectively. Using manufactures guidelines, PCR products were cleaned up using a mixture of Exonuclease I and Shrimp Alkaline Phosphatase (ArticZymes, Tromsø, Norway), sequenced using BigDye terminator Kit (Applied Biosystems, Foster City, CA) and run on a 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA). The electropherograms were analysed by the Sequencing Analysis software (Applied Biosystems, Foster City, CA).

3. Results and discussion

As a first analysis, we wanted to check whether the variants already reported as risk/protection in the COVID-19 HGI database were present in our family members and whether any differences between asymptomatic, mild and severe positive subjects existed. Fig. 2 shows the number of variants per subject previously identified as risk (grey bars) or protection (black bars) in the COVID-19 HGI database.

We detected in all members two protective variants, whereas regarding risk variants we found that the severely affected subject (I-1) carriers fourteen risk variants, his wife (I-2), who had mild symptoms, has nine risk variants and the asymptomatic daughter (II-2) has thirteen risk variants. This suggests that the contribution of risk variants from the COVID-19 HGI database, it not significant for the serious condition of the patient I-1. In fact, both for the total of variants and for the presence of risk or protection ones there is no significant difference between the I-1 severe subject and his II-2 asymptomatic daughter.

As a second step we analysed rare variants in order to select potential "severity/lethal" variants with a role in the worsening of the clinical

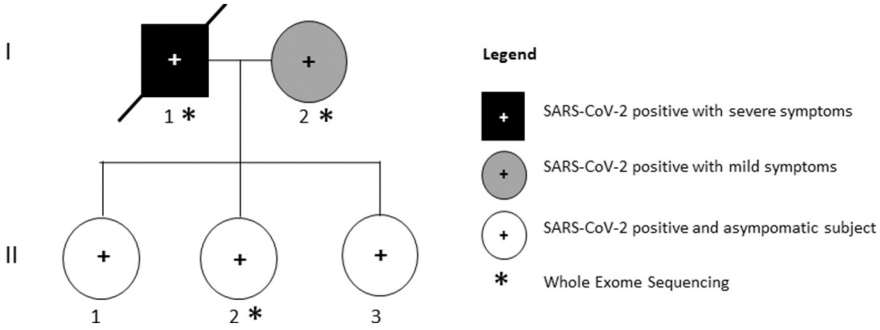

Fig. 1. Pedigree of the family.

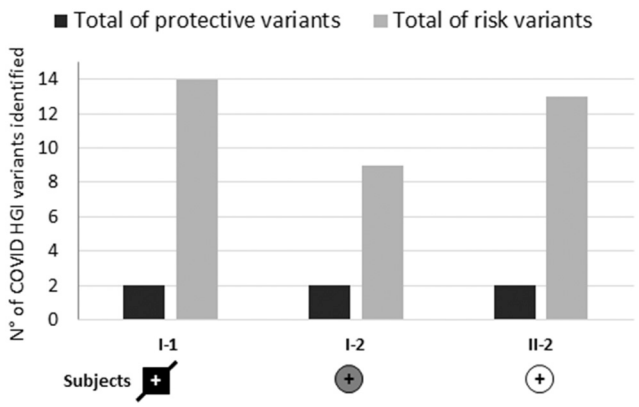

Fig. 2. Variants identified in our family and reported in COVID-19 HGI database.
Black and grey bars indicate the protective and the risk variants, respectively. White circles with the "plus" sign indicate SARS-CoV-2 positive and asymptomatic subjects. Grey circles with the "plus" sign indicate SARS-CoV-2 positive subjects with a mild phenotype. Dark squares with the "plus" sign indicate SARS-CoV-2 positive subjects with severe symptoms.

picture. For this reason, we selected the variants present only in individual I-1, not shared with his wife or his daughters and we included in our analysis all variants identified within the coding/splicing regions, with a good coverage, and with a minor allele frequency (MAF) < 0.001 in the gnomAD population. Subsequently, we prioritized variants using Varelect and Phenolyzer online tools with the following HPO terms: "COVID-19", "SARS-CoV-2", "virus". To evaluate the variants global impact ("damaging" or "tolerated"), we used VarSome (Kopanos et al., 2019). By using the above-mentioned HPO terms, we obtained a list of prioritized genes and all variants were individually inspected on the UCSC genome browser to confirm their presence on the BAM file and to exclude sequencing artefacts. Based on the MAF and according to "damaging" predictions from *in silico* tools, we selected six candidate variants in: *F5* gene (NM_000130, c.1128G>T, p.R376S), *MAS1* gene (NM_002377, c.446C>T, p.S149L), *SLC22A1* gene (NM_003057, c.113G>A, p.G38D), *SVOP* gene (NM_018711, c.1384C>T, p.R462X), *PIGS* gene (NM_033198 c.67_69del, p.F23del), *DDX52* gene (NM_007010, c.607C>G, p.R203G).

We validated and tested their segregation in all family members by Sanger sequencing and the only variant exclusively present in severe I-1 subject was c.446C>T p.(S149L) in *MAS1* gene. This missense variant shows an allele frequency of 0.00002124 (6/282494 heterozygous) in gnomAD database, it has a CADD score of 25.6 and is evaluated as

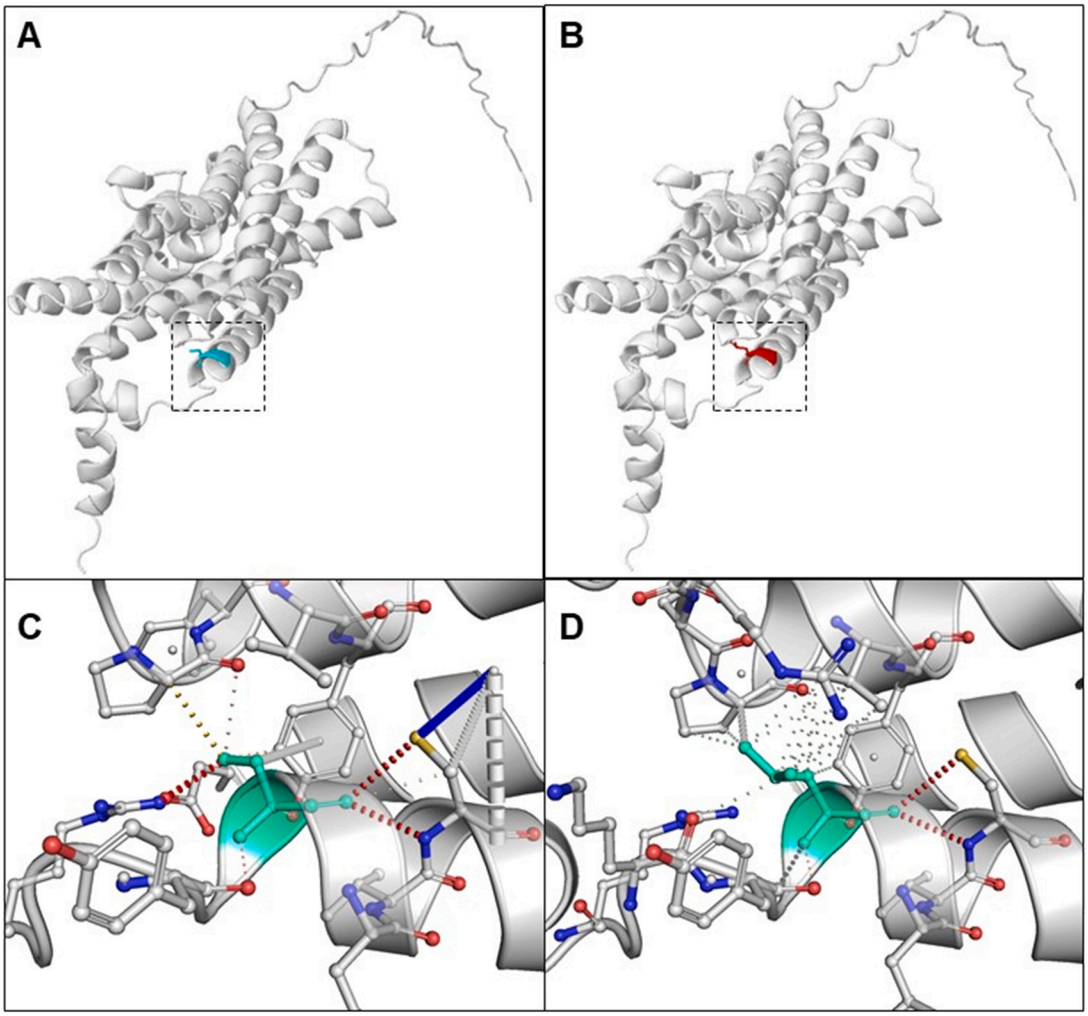

Fig. 3. Structure of MAS1 protein and localization of Ser149Leu change.
The models were created by Missense3D and DynaMut server. (A-B) Wild-type and mutated protein models with the wild-type and mutant residues colored in light blue and red respectively. (C-D) Wild-type and mutant residues are colored in light-green and are also represented as sticks alongside with the surrounding residues which are involved on interactions. The color of dashed lines defines the type of interaction: Ionic interactions in yellow; hydrogen bonds in red; halogen bonds in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

"damaging", based on eight pathogenic predictions tools (such as GERP score 5.65; DANN score 0.9991; SIFT deleterious) *vs* four tolerated predictions by VarSome. To date, only one variant in *MAS1* is reported in ClinVar, classified as benign.

The substitution Serine 149 to Leucine in MAS1 protein is located in the transmembrane domain, as reported in UniProt by Alphafold predicted model. Also, some structural protein predictions tools (I-Mutant2.0, Dynamut, mCSM-Membrane) evaluate the mutant structure as destabilizing and changing the dynamics of surrounding residues which are involved on any type of interactions (Fig. 3) (Capriotti et al., 2005; Pires et al., 2020; Rodrigues et al., 2021). We hypothesize that this missense variant might impair the correct insertion/conformation of the receptor or alter its global function, thus affecting the physiological balance in the pathway. This altered function of MAS1 can contribute to abolish or reduce the protective effect of the ACE2/Ang-(1–7)/Mas1 axis and cause a severe or even lethal COVID-19 disease due to cardiovascular and inflammatory complications.

MAS1 is a proto-oncogene but also a G-protein-coupled receptor for Ang1–7 (Jackson et al., 1988) and plays a key role in multiple processes related to the activation of the ACE2/Ang-(1–7)/Mas1 pathway, such as vasodilatory, anti-proliferative, anti-inflammatory and anti-fibrotic effects (Gheblawi et al., 2020).

These effects are mediated by the G protein coupled receptor MAS1, which signals through multiple pathways, including activation of Akt, increased Nitric Oxide generation, decreased ROS generation, and inhibition of Ang II-stimulated signaling pathways.

MAS1 plays a central role in RAS pathway and has recently been given a potential protective role in COVID-19 disease (Diaz, 2021; Kuriakose et al., 2021). RAS regulates the blood pressure and cardiovascular and renal health, in which the balance between ACE and ACE2 regulates the physiological homeostasis of these systems (Moon, 2013). The protective physiological role of ACE2 is to counterbalance ACE actions, by converting the vasoconstrictor angiotensin-II in to the vasodilator angiotensin-(1–7). The majority of ACE2 vascular effects are mediated by Ang-(1–7), which acts in opposition to Ang-II and promotes vasodilation. Ang-(1–7) is anti-proliferative, anti-fibrotic, anti-inflammatory and anti-angiogenic, and therefore maintains vascular integrity and health.

It is well known how ACE2 plays an important role related to COVID-19 because it allows SARS-CoV-2 to enter in host cells (Hoffmann et al., 2020), but, in addition, it may also contribute to the pathogenesis of severe acute respiratory distress syndrome or cardiovascular complications of the disease. To date, different variants related to COVID-19 susceptibility have been reported in *ACE2* gene (Martínez-Gómez et al., 2022), but no evidence has come out so far about other genes involved in the RAS.

4. Conclusion

The results of our study suggest that the severe clinical manifestation in individual I-1, particularly the multiple thrombotic event with high D-dimer values and the severe lung inflammation are compatible with an impairment of this axis. Since our study relates to only one family, these findings need to be confirmed in more individuals but they represent an important step forward in the knowledge related to COVID-19, also because the RAS pathway is increasingly targeted by novel therapeutic approaches. We are in the process of extending *MAS1* analysis to a cohort of patients admitted to our hospital, in the pre-vaccine period, to check for additional variants in *MAS1*.

If such studies will confirm that ACE2/Ang-(1–7)/MAS1 plays a protective role against complications of COVID-19 disease, they could offer an effective strategy of treatment for severe cases of COVID-19 (Kuriakose et al., 2021; Namsolleck and Moll, 2022; Vaduganathan et al., 2020).

Abbreviations

COVID-19 coronavirus disease-19
SARS-CoV-2 severe acute respiratory syndrome coronavirus-2
ACE2 angiotensin-converting enzyme 2
COVID-19 HGI COVID-19 Host Genetic Initiative
GWAS Genome-Wide Association Studies
WES whole exome sequencing
MAF minor allele frequency
HPO human phenotype ontology

Funding

ANIA Foundation and the Fundraising area of the Rome Bio-Medical Campus University funded this research work.

CRediT authorship contribution statement

A.A. was involved in the Data curation; Formal analysis, Methodology, original draft writing, revised manuscript review and editing. I.C. was involved in Methodology, original draft writing. C.L. was involved in Methodology. C.N., V.S, E.P. were involved in clinical work. M.C.T. was involved in final manuscript visualization. F.G. was involved in Conceptualization; revised manuscript review and editing, overall Supervision; Validation; Visualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgments

We thank the family for its collaboration for this study and the Rome Bio-Medical Campus University Foundation for the general support.

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