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Genetic, clinical and epidemiological characterization of Covid-19 patients in South Brazil

Caracterização genética, clínica e epidemiológica de pacientes com Covid-19 em uma região do Sul do Brasil

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ABSTRACT
Current paper characterizes the epidemiological, clinical and genetic profile of patients with Covid-19. An observational and cross-sectional study was carried out with volunteers diagnosed with Covid-19 between April 2021 and May 2021 in the municipality of Santa Cruz do Sul, Brazil; a blood sample also identified polymorphism in the *ACE2* gene. 87 patients were recruited; 6.7% required hospitalization, the majority being male. Although obesity was a more frequent co-morbidity, cardiovascular diseases, hypertension and diabetes were more significant when associated with hospitalizations. In the case of genetic characteristics, polymorphisms were found in the ACE2 gene among volunteers. Important research suggests male gender and co-morbidities are risk factors for the severity of Covid-19.

Keywords*:* COVID-19. SARS-CoV-2. Angiotensin converting enzyme. Genetic variation. Comorbidity.

RESUMO
Caracterizar o perfil epidemiológico, clínico e genético de pacientes com Covid-19. Realizou-se um estudo observacional e transversal com voluntários que tiveram diagnóstico de Covid-19 no período de abril de 2020 a maio de 2021 no município de Santa Cruz do Sul (RS, Brasil), no qual foram coletados dados clínicos e epidemiológicos, além de amostras de sangue para a identificação de polimorfismos no gene *ACE2*. Foram recrutados 87 indivíduos e destes, 16,7% necessitaram de internação hospitalar, sendo a maioria do sexo masculino. A obesidade foi a comorbidade mais frequente, no entanto, doenças cardiovasculares, hipertensão e diabetes apresentaram maior significância quando associadas às internações. Em relação à características genéticas, entre os voluntários não foram encontrados polimorfismos no gene *ACE2*. A pesquisa sugere que o sexo masculino e presença de comorbidades são importantes fatores de risco para a severidade da Covid-19.

Palavras-chave: COVID-19. SARS-CoV-2. Enzima conversora de angiotensina. Variação genética. Comorbidade.

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INTRODUCTION

SARS-CoV-2, the virus which caused the Covid-19, has already infected more than 460 million people during the last two years (March 2022) and killed more than 6 million people worldwide. Although the number of severe cases and hospitalizations has decreased due to intensive vaccine campaigns, Covid-19 is still a main item due to several lingering questions and issues.[1]

Featuring a similar infectious mechanism as its precedent virus (SARS-CoV), the SARS-CoV-2 penetrates human cells due to a link between spike, a spicula-type glycoprotein present on the virus´s surface, and the host´s main receptor, the angiotensin 2 (ACE2)-convertor enzyme.[2] On the other hand, ACE2 is associated to the functioning of renin-angiotensin-aldosterone system (RAAS) which consists of a synergic action of the sympathetic nervous system and aims at guaranteeing body homeostasis through the maintenance of arterial pressure and hydric equilibrium. However, the interaction between virus and receptor triggers a negative regulation of ACE2 and decreases the concentration of free cycling protein, compromising its biological activity.[3]

Gene *ACE2*, which causes the codification of the protein with its namesake, lies on chromosome X, at site Xp22, with size 39.98 kb, 18 exons and 20 introns.[4] *ACE2* is described as one of the most polymorphic genes. Variants are mostly associated with chronic diseases, such as hypertension, cardiopathies and dyslipidemias, featuring a risk factor or, in certain cases, due to the genotype, a protection factor.[5,6]

One may detect a wide variation in symptoms and degrees of severity of Covid-19 among diagnosed people with the disease. Consequently, genetic factors should be taken into account. Several studies have shown that polymorphisms in gene *ACE2* may affect the susceptibility to infection by the new coronavirus since such genetic changes may interfere in the affinity of the link between the receptor and SARS-CoV-2. Variants, such as rs4646116 (K26R), S16P, rs781255386 (T27A), rs1199100713 (N64K), rs1395878099 (Q102P), rs759579097 (G326E) and rs370610075 (G352V) may increase the interaction with the virus´s spike protein and, consequently, people with the polymorphisms in gene *ACE2* become more susceptible to infection. Other variants, such as rs758278442 (K31R), H34R, rs1348114695 (E35K), rs73635825 (S19P), rs146676783 (E37K), D38V, rs1569243690 (N51S), rs755691167 (K68E), rs1256007252 (F72V), N33I, H34R, rs751572714 (Q388L) and Y83H may weaken the link between protein and receptor, and, consequently, its interaction with the virus.[7–9]

The relationship between polymorphisms in gene *ACE2* and the link with Covid-19 is still uncertain and most studies are concentrated on *in silico*

analysis, with a dominance in Asian populations.[7–9] Current studies aims at characterizing the epidemiological, clinical and genetic profile of patients with Covid-19 in the municipality of Santa Cruz do Sul RS Brazil.

METHODOLOGY

A transversal study was undertaken with volunteers recruited through the social media, such as Internet, radio and newspapers. They had to be 18 years old or over and diagnosed for Covid-19 between April 2020 and May 2021, in the municipality of Santa Cruz do Sul RS, Brazil. All volunteers signed a Term of Free Consent and answered a clinical and epidemiological questionnaire. Current investigation was approved by the Committee for Ethics of the Universidade de Santa Cruz do Sul (n. 4.582.247 and CAAE: 41705121.4.0000.5343).

Further, 5 mL of blood from each person and their DNA retrieved by the Salting Out method, with modifications, were collected for molecular tests.[10] In the case of PCR, specific primers for the region were developed. They contained 17 polymorphisms of interest (rs781255386, rs4646116, rs73635825, rs142984500, rs758278442, rs1996225, rs2158082, rs2074192, rs146676783, rs778030746, rs756231991, rs1199100713, rs763395248, rs1348114695, rs1192192618, rs1569243690 and rs1325542104). Through the complete sequence of gene *ACE2* from Genbank, primers were designed by software Primer3Plus and the sequences were analyzed for specificity in Blastn. Conventional PCR was standardized by Platinum® Taq DNA Polymerase (Invitrogen®, USA) following instructions by manufacturer, namely, 5 uM of each primer (F: 5'-ACCGGTTTTGATTTGGCCAT-3'; R: 5'-CCCTTTTCAGTTTCACGGGC-3') were used. PCR products were then purified by ammonia acetate.

Sequencing of samples employed genetic analyzer 50 cm capillary ABI 3500 and POP7 polymer (Applied Biosystems, USA). Samples were stained with BigDye Terminatorv3.1 Cycle Sequencing Kit (Applied Biosystems, USA), following manufacturer´s instructions, precipitated with Ethanol-EDTA and denatured with formamide for electro-injection in the apparatus. Electropherograms were analyzed by ChromasTM 2.3 and sequences aligned by Geneious Prime 2021.2.2, employing complete original sequence of gene *ACE2* (NG_012575.2).

Data from genetic analysis and epidemiological data were fed to the statistical analysis program Statistical Package for the Social Sciences (SPSS 22.0) for descriptive statistical analysis. Result were given as means and standard deviation (x ± SD) or expressed by frequency (%). Test $\chi 2$ at $p<0.05$ was employed to evaluate categorical variables.

RESULTS

Eight-seven symptomatic people diagnosed with Covid-19 between April 2020 and May 2021 were recruited, of whom 80% stated that they did not live alone. Fifty percent of the above reported that people who shared the same premises during the period of infection failed to have any symptoms. Table 1 presents data on mean age, clinical symptoms, smoking, blood group, co-morbidities and hospitalization, and analyzed with regard to gender.

Table 1. Clinical and epidemiological characteristics of patients with Covid-19

	Female N (%)	**Male N (%)**	**Total N (%)**	***p***
Mean age	42.2 ± 14.14	49.1 ± 15.6	45.0 ± 15.0	-
Symptoms				
Fever	21 (38.2)	20 (62.5)	41 (41.1)	**0.028**
Dry coughing	29 (52.7)	22 (68.8)	51 (58.6)	0.143
Headache	39 (70.9)	21 (65.6)	60 (69.0)	0.607
Muscle ache	35 (63.6)	20 (62.5)	55 (63.2)	0.916
Coryza	29 (52.7)	19 (59.4)	48 (55.2)	0.548
Throat pain	24 (43.6)	20 (62.5)	44 (50.6)	0.090
Anosmia	31 (56.4)	13 (40.6)	44 (50.6)	0.157
Ageusia	27 (49.1)	13 (40.6)	40 (46.0)	0.445
Loss of appetite	33 (60.0)	17 (53.1)	50 (57.5)	0.532
Skin rash	10 (18.2)	3 (9.4)	13 (14.9)	0.267
Dyspnoea	19 (34.5)	20 (62.5)	39 (44.8)	**0.011**
Smoking				
Former smoker/Smoker	13 (23.6)	11 (34.4)	24 (27.6)	0.280
Never smoked	42 (76.4)	21 (65.6)	63 (72.4)	
ABO system				
A	24 (43.6)	15 (46.9)	39 (44.8)	
B	6 (10.9)	2 (6.3)	8 (9.2)	0.506
AB	2 (3.6)	0 (0.0)	2 (2.3)	
O	18 (32.7)	9 (28.1)	27 (31.0)	
Co-morbidities	35 (63.6)	37 (84.4)	62 (71.3)	**0.039**
Hospitalization at COVID ward	7 (12.7)	7 (21.9)	14 (16.1)	0.263

* obesity was classified by Body Mass Index (BMI), as BMI>25.

Further, 16.1% of interviewed were hospitalized in hospital wards, among whom more than 60% had at least one co-morbidity (Table 2). Days in hospital averaged 10.57. All patients hospitalized for a period over 10 (± 6.44) days had at least one co-morbidity. Another relevant datum revealed that obese patients had 1.45 more chances in being hospitalized, characterizing obesity as the main risk factor for Covid-19 severity in the population under analysis.

Table 2. Relationship between co-morbidity and hospitalization

	Hospitalized patients N (%)	Total N (%)	*p*
Heart condition	3 (21.4)	5 (5.7)	**0.006**
Hypertension	8 (57.1)	17 (19.5)	**<0.001**
Diabetes	4 (28.6)	6 (6.9)	**<0.001**
Dyslipidemia	4 (28.6)	11 (12.6)	**0.050**
Obesity	10 (71.4)	55 (63.2)	0.487
Chronic obstructive disease (COPD)	2 (14.3)	5 (5.7)	0.134
Tuberculosis	1 (7.1)	1 (1.1)	**0.022**

In the case of polymorphisms investigated in current analysis, the 86 interviewees did not reveal any polymorphism in the gene *ACE2* sequence analyzed.

DISCUSSION

Elderly people, mostly males, had the severe type of Covid-19.[11] Current study shows that 28.1% of males were over 60 years old. It may also be said that males frequently had severe symptoms featuring fever, dyspnoea, weakness and heaviness in the chest.[11] Symptoms related to the central or peripheral nervous system, such as headache and loss of smelling and taste, were more frequent in females, as reported in another study.[12] Neurological symptoms may be associated with high *ACE2* receptors since they are also found in glial and neuronal cells.[13] Further, skin rashes were rarely reported. It was the least frequent symptom in our study. However, females reported the symptoms more frequently and the symptom may be related to females who are prone to develop self-immune diseases.[14]

Smoking is a risk factor for viral respiratory infections and in certain cases it has been related to the development of the most severe symptoms of infection by SARS-CoV-2, such as fever, persistent coughing and dyspnoea. Although it has been reported as causing the de-regulation of the SRAA system,[15] significant data that associate the use of smoking with symptoms have not been detected in current study. In the case of blood groups, there is a high proportion of people infected by SARS-CoV-2 within type A and these are prone to be hospitalized and have heart conditions when compared to control. Although the mechanism is largely unknown, several hypotheses suggest that antibodies anti-A may interfere in the enzymatic activity of the protein ACE.[16] In current study, group A was prevalent and no significant association between patients with the blood group has been detected.

Several studies have reported that males are more prone to chronic diseases such as a heart condition, hypertension and diabetes.[17] One may observe that males are frequently associated with the occurrence of co-morbidities. It is a highly significant

difference when compared to the prevalence of co-morbidities in females. Frequency of patients infected by the new coronavirus with at least one previous disease was higher than that in other studies.[18] Further, 28.7% of the people under analysis had two or more co-morbidities. The most prevalent co-morbidity in current study was obesity followed by systemic arterial hypertension (SAH). However, most authors report that SAH and diabetes are the chronic disease most frequently associated with patients infected by SARS-CoV-2.[19] In the southern state of Rio Grande do Sul, Brazil, the most common co-morbidity was heart conditions, followed by diabetes mellitus in elderly groups and obesity in the younger group.[20]

Moreover, males are the group that most need hospitalization since they generally have severer symptoms and require professional aid.[18,21] As perceived in other research works, hospitalization was predominant in males even though difference was not significant. Several reasons are extant related to a greater severity of the disease in males, including hormonal factors, preexisting diseases or genetic factors which may be associated with ACE2. Gene *ACE2* is more dominant in the lungs of males. In fact, it may be detected in five different lung cells, whereas it in present in only two types of lung cells in females.[22] Further, several polymorphisms already described in *ACE2* may increase their gene load and make males more prone to the infection. In fact, the gene that codifies protein ACE2 lies in chromosome X and, consequently, homozygous polymorphisms are less frequent in females.[23]

Since the early days of the Covid-19 pandemic it has been disseminated that about one half of patients hospitalized have some type of co-morbidity and, consequently, co-morbidities are the main risk factors for the severer type of Covid-19.[19] It has not been different in current study. There has been a significant association between co-morbidities such as heart conditions, hypertension, obesity, dyslipidemia and diabetes in hospitalized patients. The above is corroborated with several studies that report a higher frequency rate of preexisting diseases in severer cases, also frequently associated to a longer hospitalization period.[18,21] Further, obese patients had 1.45 more chances of being hospitalized. This risk average is similar to that detected in another study with overweight patients.[24]

Different co-morbidities and severity of symptoms by Covid-19 may also be linked to *ACE2*, since the gene occurs less in healthy patients with no previous chronic disease. The above may underlie why these patients are more prone to infection and have higher severity rates.[24] Finally, when there is a SRAA imbalance caused by pathology, there is a decrease in the serum levels of protein ACE2, jeopardizing homeostasis and, consequently, decreasing the anti-inflammatory and antifibrotic response of the organism, causing heart and

hypertension conditions or intensifying these symptoms in people who already have them.[23]

Since the beginning of the Covid-19 pandemic, gene *ACE2* has been analyzed and investigated, highlighting polymorphisms and their influence in the disease´s proneness and severity.[27,25] *In silico* studies have shown that variants in *ACE2* may interfere in the linking capacity between the human receptor and the virus. Specialized literature claims that polymorphisms such as rs781255386, rs4646116, rs73635825, rs142984500, rs758278442, rs1996225, rs2158082, rs2074192, rs778030746 and rs756231991 increased the interaction between protein ACE2 and spike, facilitating the penetration and dissemination of the virus by the organism. The above may also occur with variants rs146676783, rs1199100713, rs763395248, rs1348114695, rs1192192618, rs1569243690 and rs1325542104 which decrease affinity by SARS-CoV-2 and, consequently, make difficult the link between the virus and the host.[8,25]

Polymorphisms analyzed in current research reveal a low frequency rate in populations in which they were studied, mainly restricted to people from Asia.[7–9] Polymorphisms of gene *ACE2*, rs781255386 and rs142984500, were detected in only 0.01% of Asian populations. They are thought to be very rare polymorphisms.[9] Variations rs73635825 and rs146676783 has a 0.3% frequency in studies that evaluated people from Africa and Asia, respectively.[3,26] However, other polymorphisms of *ACE2* are also related to Covid-19 and may feature a frequency varying from 0.5% in Europeans to 1.2% in Asians, as is the case of rs4646116.[26] Up to the time of writing, no analysis on polymorphisms in gene *ACE2* and its link with SARS-CoV-2 has been undertaken in Brazil. Current research is the first study in a Brazilian population. Further, the frequency of several polymorphisms evaluated in current research has never been described by *in vivo* genotype analysis, but in *in silico* analyses.[8,27,28] Moreover, several studies show that heterozygous polymorphisms have been detected in more than 90% of the analyzed samples, whereas homozygous polymorphisms are less predominant.[29] Consequently, due to the low frequency of these polymorphisms and to the low number of individuals participating in the research, one may account for the lack of polymorphisms detected.

Current study had several limitations: the small number of individuals under analysis, low index rates of patients for the severer type of disease who required hospitalization, and lack of a group exposed to SARS-CoV-2 with no symptoms but, in one way or another, would be interesting to evaluate the genetic issue with regard to clinic features of Covid-19 and its susceptibility.

Current research is relevant within the present context. In fact, it is the first to characterize in the region a portion of individuals infected by Covid-19,

evidencing the disease´s clinical difference between the sexes and highlighting the main risk factors with regard to the results. As a rule, current study may also generate themes on health promotion with special reference to lifestyles and behavior. The above is due to the fact that obesity, hypertension and diabetes are negatively associated with a prognosis of infected patients. It is well-known that healthy habits linked to food and physical activities are essential for the control and prevention of several chronic non-transmissible diseases. The promotion of healthy and active aging is necessary since life quality is a crucial factor for an improvement in the health elderly patients, especially those with a previous chronic disease and which together characterize this most vulnerable group.[30]

CONCLUSION

Males are more prone to be hospitalized and are predominant for co-morbidities. It has also been verified that obesity is an important risk factor for hospitalization followed by other chronic diseases, such as hypertension and diabetes. Males are also prone to have symptoms suggestiing Covid-19, whereas females have a trend for neurological signs and symptoms.

Regarding to the genetic issue evaluated, the relationship between polymorphisms of gene *ACE2* and the disease´s symptoms and severity caused by the new coronavirus was not identified. No variation was detected, perhaps due to the small number of participants in the study and the low frequency rate of the polymorphisms in populations. Current study is the first investigation in Brazil to research the genetic variability of *ACE2*, comparing it with Covid-19 clinical issues. Further research work should be undertaken in larger groups to better explore the relationship of the gene with the SARS-CoV-2-caused infection.

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