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Abstract

Background: Hypertension and type 2 diabetes increase the risk of severe SARS-CoV-2 infection. On the other hand, homozygous ACE deletion polymorphism (DD) has been associated with these two diseases and risk of acute respiratory distress syndrome.

The aim of the study was to conduct a metanalysis of the association between ACE gene I/D polymorphism (DD, II and DI) and severity of SARS-CoV-2 infection in hospitalized patients.

Material and methods: We searched PubMed, EMBASE and Google Scholar for studies published between January 2020 and April 2021. We included case-control studies evaluating the association between *ACE I/D* and severity of SARS-CoV-2 infection in hospitalized patients, were there was sufficient genotype or allele frequency data to calculate IRR (incidence rate ratio) and 95% confidence intervals (CIs).

Results: Five studies were included (mean age 58.5 years and 61% men), combining to a total of 786 patients. Three studies were conducted in Caucasians. Overall, patients who had homozygous co-dominance genotype DD were at 47% higher risk of severe COVID-19 compared with II or ID (IRR: 1.47; 95% CI: 1.15–1.89; p = 0.002).

Conclusions: The ACE DD genotype may confer a greater risk of severe COVID-19 in hospitalized patients. Further studies including more diverse ethnic groups are necessary to fully establish this association.

Key words: ACE gene, I/D polymorphism; SARS-CoV-2, COVID-19; meta-analysis

Introduction

The renin–angiotensin system (RAS) has an important role in regulating vascular physiology, directly or indirectly influencing functions of the lung, heart, kidney, brain and the immune system [1]. Angiotensin-converting enzyme 1 (ACE1) converts angiotensin-1 (Ang-I) to angiotensin-2 (Ang-II); Ang-II mediates its effects through activation of AT-1 and AT-2 receptors. On the other hand, angiotensin-converting enzyme 2 (ACE2) converts the potent vasoconstrictor angiotensin-II (Ang-II) into the vasodilator Ang1-7, which is crucial in controlling the local tissue homeostasis due to its anti-inflammatory, anti-coagulant, anti- proliferative and anti-fibrotic activity [2]. The normal physiological balance of RAS is based on the balance between the activity of the two axes or antagonist enzymes ACE1 and ACE2; that is, a local vasoconstrictor / proliferative axis (ACE1/Ang-

II/AT1 receptor), *versus* vasodilator/anti-proliferative axis (ACE2/Ang1-7/MAS receptor) [3].

The entry mechanism of SARS-CoV-2 into human cells is by binding its spike protein to the membrane receptor ACE2 and interacting with the transmembrane serine protease 2 (TMPRSS2) [4]. It has been postulated that during SARS-CoV-2 infection, a reduction in ACE2 expression/activity and, consequently, the balance between the two antagonist enzymes ACE1 and ACE2 is broken, in favor of the former; that is, the axis with vasoconstrictor/proliferative effects (ACE1/Ang-II/AT1 receptor) predominates, with deleterious action to organ functions including in the lung, kidney and heart [5]. Loss of ACE2 expression in mutant mice is associated with worse lung function and characterized by increases in vascular permeability, lung oedema and neutrophil accumulation [6].

The ACE1 gene maps on chromosome 17 (locus 17q23.3) and the ACE2 gene on chromosome X (locus Xp22.22) [3]. ACE1 has an insertion/deletion polymorphism that is characterized by an insertion (allele I) or deletion (allele D) of a 287 base pair marker in intron 16 that results in three different genotypes (DD and II homozygotes or ID heterozygotes) [3]. The DD genotype has been found to show the highest serum/tissue ACE1 activity, probably because it maintains the two active sites favoring Ang-I to Ang-II formation; on the other hand, the ID genotype shows intermediate levels, and the II genotype the lowest, probably because it only has one of the two enzyme active sites in the ACE1 I-allele. [7]

Five ecological studies have recently been published on the association of ACE-1 I/D polymorphism with COVID-19 incidence and mortality. Ecological studies consist of establishing a relationship between the geographical variation of the I/D polymorphism of the ACE1 gene, with the mortality and severity of COVID-19 reported by organizations such as the Center for Systems Science and Engineering at Johns Hopkins University (https: //coronavirus.jhu.edu/map.html). Four ecological studies found that the country level frequency of the D allele was associated with increased COVID-19 incidence and mortality [8–11]. One study considered that this relationship may be regarded as a confounder in the spread of COVID-19 and the outcome of the infection [12].

The present systematic study aimed to evaluate the association between ACE Gene I/D polymorphism and severity of SARS-CoV-2 infection in hospitalized patients.

Material and methods

This study was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [13].

Search strategy

Two independent reviewers performed a systematic review in PubMed, EMBASE, and Google Scholar for studies published between January 2020 and April 2021. The terms used were "*ACE* I/D", "polymorphism", "COVID-19", "SARS-CoV-2", "angiotensin converting enzyme insertion-deletion" and "COVID-19" as medical subject headings.

Eligibility criteria

The inclusion criteria were as follows: (i) case–control study design evaluating the association between *ACE* I/D and severity of SARS-CoV-2 infection in hospitalized patients and (ii) sufficient genotype or allele frequency data to allow calculation of odds ratios (ORs) and 95% confidence intervals (CIs). The exclusion criteria were as follows: a) studies without controls; b) studies with genotype or allele frequencies that were unusable or absent; c) articles that did not cover the polymorphism or disease in question, and d) reviews.

Data extraction and data distribution

The following data were extracted from each study: authors, study location, year of publication, study design, number of participants, sex, age at baseline, outcome definition (COVID-19 severity and mortality), country of origin, ethnicity, comorbidities, sample sizes and genotype data. Data distribution was assessed with the Shapiro- Wilks test [14]. The use of the mean ± standard deviation indicated a normal data distribution; otherwise, the choice of descriptive was the median with interquartile range.

Quality assessment and Hardy-Weinberg Equilibrium (HWE)

The methodological quality of observational studies (cohort and case control studies) was appraised according to the Clark-Baudouin scale [15]. Two investigators evaluated the quality of the studies independently. Conflicting results were resolved by discussion and involvement of a third reviewer if necessary. The Hardy-Weinberg Equilibrium (HWE) was assessed using the application in https://ihg.gsf.de/cgi-bin/hw/hwa1.pl

Outcome definition

High severity was defined as a composite of mortality and/or any of the following indicators: need for mechanical ventilation, critical care, severe pneumonia or pulmonary embolism. Survivors in the absence of any high severity indicators were considered to have disease without high severity.

Data synthesis

We conducted a metanalysis between the *ACE I/D* polymorphism and the risk of severity/mortality composite outcome, with allelic (I *versus* D allele), dominant (I/I versus I/D + D/D), and recessive (I/I + I/D versus D/D) models. The overall estimates in the pooled analysis were obtained using Stata 13 software (Stata Corp LP, College Station, TX). The pooled odds ratios (OR) were calculated using DerSimonian-Laird random-effects models [16] with 95% confidence intervals (CI) to measure the strength of the association. I^2 indicates the percentage variance in the pooled IRR (incidence rate ratio) that can be attributed to heterogeneity.

Results

After screening 308 citations, 5 case-control studies were included, with a total sample of 786 participants. Overall, mean (SD) age was 58.5 (6.26) years and 61% were men. The characteristics of included studies are summarized in Table 1. The studies were from Spain [17], India [18], Italy [19] and Turkey [20, 21]. Ethnicity was Caucasian in 4 studies, with the fourth study including patients of Indian ethnicity. Table 2 describes the distribution of the ACE polymorphisms in the included studies.

In all the included studies the outcome reported was severity, and none included mortality. The mean of Clark-Baudouin score was 6.4 (6–8).

Patients who had homozygous co-dominance genotype DD were at 47% higher risk of severe COVID-19 compared with II or ID (IRR: 1.47; 95% CI: 1.15–1.89; p = 0.002) (Figure 2). We did not detect significant heterogeneity (I^2 = 9.4%). In the II *versus* DD + ID model, a protective IRR of 0.72 (95% CI: 0.54–0.97; p = 0.028; I^2 = 0%) was obtained. In model DD vs II+ID, when the study of Gunal et al. was excluded (because, Hardy-Weinberg Equilibrium > 0.05), the value of IRR was 1.48; 95% CI: 1.10–1.97 (p = 0.009).

Discussion

The present study suggests that the DD genotype may confer an increased risk of severe COVID-19. However, results should be viewed with caution because this is only based on 5 observational studies, 4 of which were conducted in Caucasians and none of which included mortality as outcome.

Even though the present study is the first metanalysis on *ACE I/D* polymorphism and severity of SARS-CoV-2 infection in hospitalized patients, it is possible to compare it with other metanalyses on ACE genotype and risk of severity and mortality due to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), published before the COVID-19 pandemic. Matsuda et al. found a possible association between the DD polymorphism and the mortality risk of ALI/ARDS [22]. Hu et al. [23] found that Caucasian patients with ARDS had a significantly higher frequency of the DD genotype (OR: 1.65; 95% CI: 1.27–2.13) than controls. The metanalysis of Pabalan et al. published in 2021 found a significant association between DD (*vs.* II + ID) and a higher risk of mortality due to ALI/ARDS in Caucasians and children as well as in Asians [24].

On the other hand, prior to the COVID-19 pandemic, association studies between the ACE genotype and arterial hypertension and type 2 diabetes mellitus were published. A metanalysis found an association between the D allele and essential hypertension in Asian and Caucasian population [25]. Another metanalysis found that the D variant was associated with a 14% increased risk of type 2 diabetes mellitus relative to the I variant, in Caucasian and East Asians [26]. And another metanalysis found an association between the

D allele and the risk of essential hypertension compared to the carriers of the I allele in the African continent [27].

In the pathophysiology of COVID-19, the counterbalance between angiotensin converting enzyme (ACE) and ACE2 activities may play a role potentially determining the severity of the disease [19, 28–30]. However, putting the issue in context, the ACE gene is actually one of more than 30 SARS-CoV-2 susceptibility gene variants or haplotypes under investigation [31]. A recently Genome wide association analysis identified a 3p21.31 gene cluster as a genetic susceptibility locus in patients with Covid-19 with respiratory failure and confirmed a potential involvement of the ABO blood-group system [32]. Another study found significant associations with chromosome 12q24.13 in a gene cluster that encodes antiviral restriction enzyme activators (*OAS1*, *OAS2* and *OAS3*); chromosome 19p13.2 near the gene that encodes tyrosine kinase 2 (*TYK2*); chromosome 19p13.3 within the gene that encodes dipeptidyl peptidase 9 (*DPP9*); and chromosome 21q22.1 in the interferon receptor gene *IFNAR2 [33]*.

The present study has limitations especially due to the few published studies and most of them only in Caucasians. Similarly, the design of the included studies compares hospitalized patients and does not include non-hospitalized controls, nor does it analyze mortality. On the other hand, it would be important to study if the ACE gene influences the severity in patients without diabetes or arterial hypertension, and none of the included studies analyzed this group without these risk factors. It would also be important to study whether these results can be replicated in other ethnic groups. It should be noted that the relationship between hypertension and the ACE gene has not been found in studies in Latin American countries such as Peru [34] or Brazil [35].

In conclusion, with the limited studies evaluated predominantly in Caucasians, it appears that the ACE DD genotype may confer a greater risk of severe COVID-19 in hospitalized patients. Further studies at a global level including more diverse ethnic groups are necessary to fully establish this association.

Funding

None declared.

Conflict of interest

None declared.

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Table 1. Characteristics of the studies included in the metanalysis

Author	**Country**	**Ethnicity**	**Outcome**	**Mean age**		**Sex male (%)**		**Hypertension**		**Diabetes mellitus type 2**		**CBS**
				Severe	**No Severe**	**Severe**	**No Severe**	**Severe**	**No Severe**	**Severe**	**No Severe**	
Gómez et al. (2021)	Spain	Caucasian	Severity (need of critical care)	65.76	64.56	53 (79)	72 (53)	41 (61)	56 (41)	15 (22)	21 (15)	6
Celik et al. (2021)	Turkey	Caucasian	Severity (Pneumonia on chest CT /mechanical ventilation)	87.47	44.6	77 (49.7)	78 (50.3)	NR	NR	NR	NR	6

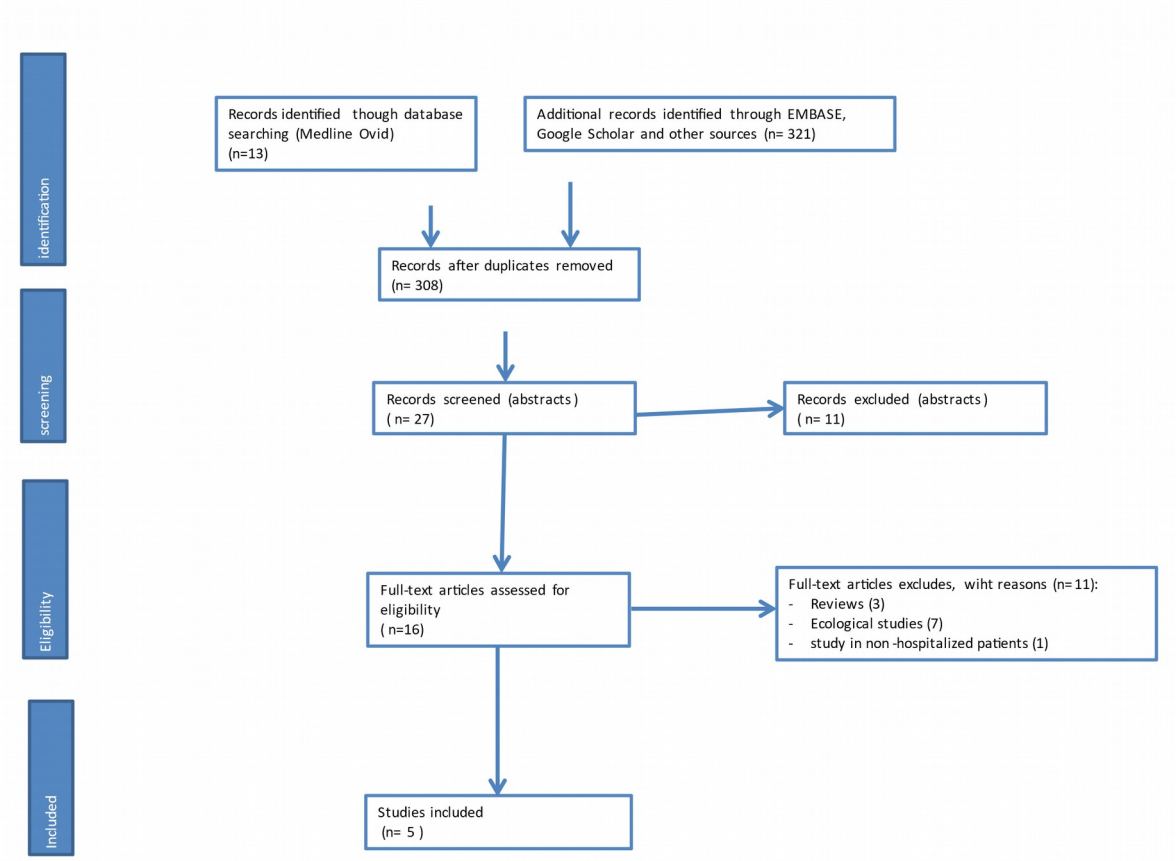
Verma et al. (2020)	India	Hindu	Severity (pneumonia)	NR	NR	73 (60.8)	97 (65.1)	20 (16.7)	7 (4.7)	34(28.3)	13 (87)	6
Calabrese et al. (2021)	Italy	Caucasian	Severity (pulmonary embolism)	62	57	19 (76)	29 (63.8)	13 (52)	21 (48)	3 (12)	7 (16.3)	8
Gunal et al. (2021)	Turkey	Caucasian	Severity (need of intensive care)	67	16.4	19 (63.3)	40 (66.67)	22 (73.3)	18 (30)	12 (12)	3 (5)	6

CBS — Clark-Baudouin scale; NR — not reported

Table 2. Hardy-Weinberg Equilibrium (HWE) for included studies

Author	Severe group			Non-severe group			HWE
	DD	ID	II	DD	ID	II	
Gómez et. al. (2021)	31	31	5	44	76	17	0.211
Celik et al. (2021)	24	42	10	24	37	17	0.65
Verma et al. (2020)	30	48	42	17	58	74	0.118
Calabrese et al. (2021)	18	4	3	20	21	2	0.703
Gunal et al. (2021)	19	2	9	26	12	22	> 0.05

Figure 1. Flowchart of included studies


identification
screening
Eligibility
Included
Records identified though database searching (Medline Ovid) (n=13)
Additional records identified through EMBASE, Google Scholar and other sources (n= 321)
Records after duplicates removed (n= 308)
Records screened (abstracts) (n= 27)
Records excluded (abstracts) (n= 11)
Full-text articles assessed for eligibility (n=16)
Full-text articles excludes, wiht reasons (n= 11):
- Reviews (3)
- Ecological studies (7)
- study in non -hospitalized patients (1)
Studies included (n= 5)

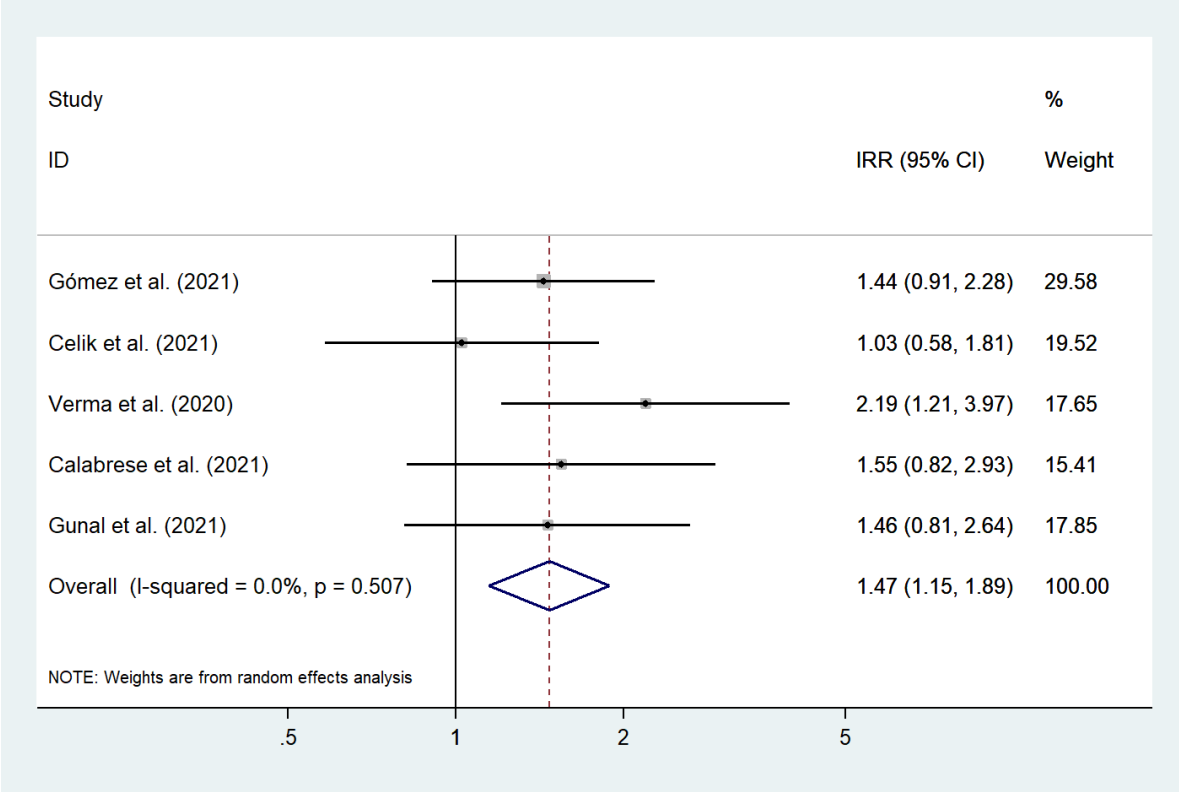


Figure 2. Forest plot for the effect of *ACE I/D* gene polymorphism on the association of Severity of SARS-CoV-2 infection in hospitalized patients (DD *vs.* II + ID model)