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Original Article

Insertion/deletion (I/D) polymorphisms of angiotensin-converting enzyme gene and their implications for susceptibility and severity of COVID-19: A systematic review and meta-analysis

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Abstract

The insertion or deletion polymorphisms of the *angiotensin-converting enzyme gene* (*ACE I/D*) have been the subject of significant research related to coronavirus disease 2019 (COVID-19). Despite this, the findings have remained uncertain and debatable. The aim of this study was to determine the associations between the *ACE I/D* polymorphisms and the susceptibility as well as the severity of COVID-19. A meta-analysis study (PROSPERO: CRD42022384562) was conducted by searching the articles published on PubMed, Scopus, and Embase as of May 15, 2023. Information regarding the impact of *ACE I/D* variant on the susceptibility to COVID-19 and its severity was collected and analyzed utilizing the Mantel-Haenszel method with a random effects model or fixed effects model, depending on the presence or absence of heterogeneity. Out of 3,335 articles, 21 articles were included, of which 13 investigated the association between *ACE I/D* and the risk of COVID-19 infection and 18 of them examined its influence on disease severity. The D allele of *ACE* increased risk of COVID-19 infection (OR: 1.41; 95%CI: 1.08–1.85; *p*-Egger: 0.0676; *p*-Heterogeneity: <0.001; *p*=0.0120), while *ACE* I allele (OR: 0.71; 95%CI: 0.54–0.93; *p*-Egger: 0.0676; *p*-Heterogeneity: <0.001; *p*=0.012) and II genotype (OR: 0.55; 95%CI: 0.34–0.87; *p*-Egger: 0.200; *p*-Heterogeneity: <0.001; *p*=0.011) decreased the risk of infection. Additionally, there was a notable association between the *ACE ID* genotype and an elevated likelihood of experiencing severe COVID-19 within the Asian population (OR: 1.46; 95%CI: 1.15–1.84; *p*-Egger: 0.092; *p*-Heterogeneity: 0.116; *p*=0.002). The presence of *ACE I/D* polymorphisms significantly influences the likelihood of being susceptible to and experiencing the severity of COVID-19.

Keywords: COVID-19, *ACE* intron/deletion, gene variant, severity, susceptibility

Introduction

Coronavirus disease 2019 (COVID-19) is a global health problem affecting millions of people worldwide. The high morbidity and mortality rates of COVID-19 have caused a significant impact on public health systems globally [1,2]. Despite several studies on COVID-19, the pathogenesis of this disease is complex and remains not fully understood [3-5]. The lack of understanding of the pathogenesis of COVID-19 poses a significant challenge in its management. Therefore, a

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comprehensive understanding of COVID-19 pathogenesis would provide broader insights into potential new treatment options. Within the context of COVID-19 pathogenesis, numerous biomarkers are involved, with *angiotensin-converting enzyme* (*ACE*) being one of them [6]. *ACE* has emerged as a potential biomarker for COVID-19, and its role in disease progression is currently being studied. Understanding the pathogenesis of COVID-19 is crucial for developing effective treatments and management.

ACE, a peptidyl dipeptidase, plays a vital function in controlling the renin-angiotensin system (RAS). *ACE* is responsible for the enzymatic transformation of angiotensin I into angiotensin II, which acts as a powerful vasoconstrictor involved in the control of blood pressure and maintenance of fluid balance [7]. *ACE* is involved in regulating cardiovascular and renal function, as well as electrolyte homeostasis [8]. In the case of COVID-19, *ACE* plays a crucial role in the disease pathogenesis by mediating the entry of the virus into host cells via the ACE2 receptor [9-12]. The interplay between ACE and ACE2 regulates the abundance of ACE2 receptors. When a person is infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the activity of ACE is much greater than that of ACE2 due to the impairment of ACE2 [13]. This indicates that *ACE* levels have an important impact on the pathogenesis of COVID-19. On the other hand, the levels of *ACE* in the blood were highly affected by the insertion/deletion (I/D) within *ACE* gene, a 287bp repeat sequence in intron 16 [14]. Investigations have produced contradictory outcomes regarding the association between *ACE I/D* gene variant and the susceptibility as well as severity of COVID-19. Some studies suggested that individuals carrying the D allele were at an increased likelihood of susceptibility to COVID-19 infection [15-19], while others reported no significant association [20-27]. Furthermore, meta-analysis studies also showed contradictory results. Aziz *et al.* [28] and Dieter *et al.* [29] reported no notable relationship between the genetic variation of *ACE I/D* and the risk of COVID-19 infection or disease severity, while Dobrijevic *et al.* [30] demonstrated that the *ACE ID* genotype was linked to increased severity of COVID-19. Given that these meta-analyses had certain limitations, such as limited sample size, inaccuracies in statistical analysis, and the evaluation of multiple genes rather than focusing specifically on the *ACE I/D* gene, a new meta-analysis study that addressed these weaknesses in previous studies was necessary. Therefore, to obtain a comprehensive overview of the relationship between *ACE I/D* genetic variations and COVID-19, a meta-analysis was conducted to address the existing knowledge gap. This would aid in identifying potential targets for therapy and tailoring treatment approaches for individuals affected by COVID-19.

Methods

Study designs

During the period from April 2023 to May 2023, a meta-analysis was conducted (PROSPERO: CRD42022384562) to determine the association between the genetic variation of *ACE I/D* and the risk of COVID-19 as well as its severity. In order to accomplish this goal, studies published in PubMed, Scopus, and Embase databases were searched and selected for analysis. To ensure a standardized and transparent approach, the systematic review adhered to the recommended guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The PRISMA checklist is a standardized framework guideline for meta-analyses and systematic reviews. This checklist includes points that must be followed in structuring a systematic review or meta-analysis study. It ensures that each procedure in the meta-analysis study is consistent and transparent, leading to more objective conclusions [31]. The comprehensive descriptions of the PRISMA protocol checklists of the systematic review were available in the supplementary file published online elsewhere [32].

Search strategy

Using specific search terms, a systematic search of published articles in PubMed, Scopus, and Embase was conducted. The search covered articles available up until May 15, 2023. The keywords "COVID-19" OR "coronavirus disease-19" OR “SARS-CoV-2 infection” OR “Severe Acute Respiratory Syndrome Coronavirus 2 infection” AND "angiotensin-converting enzyme

intron deletion" OR "*ACE I/D*" were used to retrieve relevant articles. The publications were limited to the English language only.

Eligibility criteria

The eligibility criteria of studies to be included in the systematic review were: (1) retrospective or prospective cohort studies, cross-sectional studies, randomized controlled trials (RCTs), controlled before-and-after studies, and cross-over studies; (2) studies that assessed the association between the genetic variation of ACE I/D rs1799752 and the susceptibility to COVID-19 or its impact on disease severity; (3) studies that supplied data for the calculation of the OR along with a corresponding 95%CI; (4) studies that conformed to the guidelines established by Rodriguez *et al.*, ensuring that the data reported were consistent with the Hardy-Weinberg equilibrium (HWE) criteria. Specifically, HWE was considered to be met if the Chi-squared value (χ^2) was less than 3.84 or the *p*-value for HWE was greater than 0.05 [33]; and (5) articles published in English language. Studies that had an obvious irrelevance in title and/or abstract, reviews or commentaries, or studies that provided incomplete data were excluded. In addition, studies involving patients in special conditions, such as pregnant patients, were also excluded from this study.

Quality assessment

A quality assessment of the included papers was conducted using the Newcastle-Ottawa scale (NOS) for cross-sectional and case-control studies to ensure consistent evaluation [34]. Studies were classified into three categories based on quality: poor, moderate, or high. Poor quality was assigned to studies with scores ranging from 0 to 3, moderate quality to studies with scores ranging from 4 to 6, and high quality to studies with scores ranging from 7 to 9. Articles with poor quality were excluded. A summary of the quality assessment for our study is provided in the supplementary files [32].

Data extraction

To ensure a comprehensive analysis, the pertinent information from each study were compiled and recorded by employing data extraction form covering: (1) the first author's name; (2) publication year; (3) the methodology employed in the study; (4) age of patients; (5) gene polymorphism; (6) outcomes; (7) the number of participants included in both the COVID-19 and control groups; (8) the frequencies and percentages of genotypes and alleles in individuals with COVID-19 and control; and (9) the country of origin. A comparison of genotype and allele frequencies of *ACE I/D* gene polymorphism between the COVID-19 and control groups was conducted.

Study covariates

The predictor in this study was genetic polymorphism of *ACE I/D*. The genetic polymorphism of *ACE I/D* consists of allele I, allele D, genotype II, genotype ID, and genotype DD. The comparisons between polymorphisms in this study were *ACE* allele I vs D, ACE allele D vs I, genotype II vs ID+DD, genotype ID vs II+DD, and genotype DD vs II+ID. There were two outcome variables of the study: (1) the probability or likelihood of contracting the disease (risk of infection); and (2) the degree or intensity of the illness experienced by individuals (severity of COVID-19) as described by World Health Organization [35]. Subgroup analysis categorized by ethnicity, dividing into Asian and non-Asian groups, was conducted. The choice of the term non-Asian was made due to the fact that the majority of studies conducted on non-Asian populations did not explicitly mention the ethnicity of the study populations.

Statistical analysis

An analysis to determine the association between *ACE I/D* gene polymorphism and susceptibility and severity of COVID-19 was conducted. This was achieved by calculating pooled ORs along with their corresponding 95%CIs. To assess the statistical significance of the pooled ORs, a Z test was used. A $p<0.05$ was regarded to indicate a statistically significant association between the variables under investigation. To evaluate the presence of heterogeneity, a Q test was performed. In cases where heterogeneity was observed (p-Heterogeneity <0.10), a random-effects model was

utilized, while if heterogeneity was not detected, a fixed-effects model was employed [36]. The Egger's test was employed to examine publication bias, and statistical significance was determined at a threshold of $p<0.05$ [37]. The R package (RStudio version 4.1.1, Boston, United States, RRID: SCR_000432) was utilized for all data analyses.

Results

Eligible studies

A total of 3,335 articles were identified during the search in PubMed, Scopus, and Embase. Eighteen articles were eliminated from the analysis due to duplication. Additionally, 3,262 articles were excluded due to irrelevance based on their titles and/or abstracts. Furthermore, after reviewing the full texts, an additional 22 articles were excluded because they were reviews and 12 articles because they had insufficient data [38-49]. The flowchart of the procedure of including or excluding studies in this study is presented in **Figure 1**. Subsequently, twenty-one articles were included in the meta-analysis [15-27,50-57].

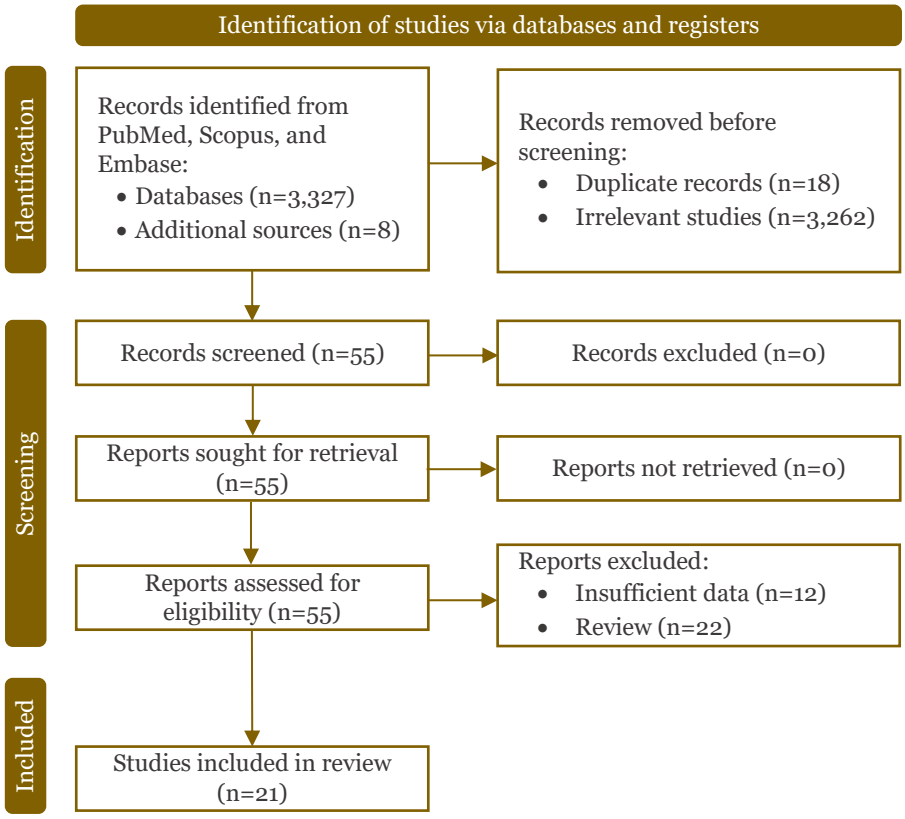

Figure 1. A flowchart of article selection in our study.

Characteristics of the included studies

Characteristics of the included studies are presented in **Table 1**. From the 21 articles included, four studies were conducted in Iran [20,23,55,56], three studies were conducted in Turkey [15,51,53], three studies were conducted in Italy [16,22,50], and one study each was conducted in Vietnam [21], Brazil [52], Spain [24], the Czech Republic [25], Pakistan [17], Mexico [54], Egypt [18], Saudi Arabia [19], Germany [26], Lebanon [27], and India [57]. Regarding study design, 12 studies used a cross-sectional design [15,16,20,24-27,50,51,54,55,57], and nine studies used a case-control design [17-19,21-23,52,53,56]. The smallest sample size was 39 patients [16], while the largest sample size was 2,987 patients [25]. Concerning the outcomes evaluated, 10 studies assessed infection risk and severity [15,17,19,20,22-27], three studies assessed infection risk [16,18,21], and eight studies assessed severity risk [50-57]. Regarding HWE assessment, we did not find evidence of HWE deviation in any of the studies. Regarding article quality, 14 articles were of high quality [16,17,19-21,24-26,51-56], and seven articles were of moderate quality [15,18,22,23,27,50,57].

Table 1. Baseline characteristics of articles included in our analysis

Study	Location	Design	Age	Sample size	Gene (s)	Outcomes	*p*-HWE	NOS
Akbari *et al.*, 2022 [20]	Iran	Cross-sectional	57.2±1.8	182	*ACE I/D* rs1799752	Infection & severity	0.2585	8
Aladag *et al.*, 2021 [15]	Turkey	Cross-sectional	48.2 (19–83)	412	*ACE I/D* rs1799752	Infection & severity	0.1544	6
Annunziata *et al.*, 2021 [16]	Italy	Cross-sectional	57.5±13.5	39	*ACE I/D* rs1799752	Infection	0.9174	7
Balzanelli *et al.*, 2022 [21]	Vietnam	Case-control	54.5±6.8	84	*ACE I/D* rs1799752	Infection	0.7168	7
Cafiero *et al.*, 2021 [50]	Italy	Cross-sectional	68.0±12.4	104	*ACE I/D* rs1799752	Severity	0.8625	6
Calabrese *et al.*, 2021 [22]	Italy	Case-control	58.5 (46.3–66.0)	179	*ACE I/D* rs1799752	Infection & severity	0.9999	5
Celik *et al.*, 2021 [51]	Turkey	Cross-sectional	52.3±17.5	154	*ACE I/D* rs1799752	Severity	0.6267	8
Dieter *et al.*, 2023 [29]	Brazil	Case-control	59.0±15.2	680	*ACE I/D* rs1799752	Severity	0.6420	7
Faridzadeh *et al.*, 2022 [23]	Iran	Case-control	45.3±13.3	526	*ACE I/D* rs1799752	Infection & severity	0.7818	5
Gomez *et al.*, 2020 [24]	Spain	Cross-sectional	64.9±NA	740	*ACE I/D* rs1799752	Infection & severity	0.9979	8
Gunal *et al.*, 2021 [53]	Turkey	Case-control	47.1±13.7	90	*ACE I/D* rs1799752	Severity	0.6712	8
Hubacek *et al.*, 2021 [25]	Czech Republic	Cross-sectional	48.0±11.0	2987	*ACE I/D* rs1799752	Infection & severity	0.1902	7
Kouhpayeh *et al.*, 2021 [17]	Pakistan	Case-control	50.2±14.8	504	*ACE I/D* rs1799752	Infection & severity	0.9404	8
Mart´ınez-Go´mez *et al.*, 2022 [54]	Mexico	Cross-sectional	51.0±43.6	481	*ACE I/D* rs1799752	Severity	0.6374	7
Marei *et al.*, 2023 [18]	Egypt	Case-control	63 (53–68)	90	*ACE I/D* rs1799752	Infection	0.2732	6
Mir *et al.*, 2021 [19]	Saudi Arabia	Case-control	NA	267	*ACE I/D* rs1799752	Infection & severity	0.0554	7
Möhlendick *et al.*, 2021 [26]	Germany	Cross-sectional	62.0 (18–99)	550	*ACE I/D* rs1799752	Infection & severity	0.6158	7
Najafi *et al.*, 2023 [55]	Iran	Cross-sectional	NA	146	*ACE I/D* rs1799752	Severity	0.7957	7
Rezaei *et al.*, 2022 [56]	Iran	Case-control	54.3±18.9	120	*ACE I/D* rs1799752	Severity	0.6002	8
Saad *et al.*, 2021 [27]	Lebanon	Cross-sectional	43.7±15.8	387	*ACE I/D* rs1799752	Infection & severity	0.5603	5
Verma *et al.*, 2021 [57]	India	Cross-sectional	NA	269	*ACE I/D* rs1799752	Severity	0.5615	5

ACE I/D: angiotensin-converting enzyme intron-deletion; AGT: angiotensinogen; CRP: C-reactive protein; DPP9: dipeptidyl peptidase 9; HWE: Hardy-Weinberg equilibrium; IFIH1: interferon induced with helicase C domain 1; IFNAR2: interferon alpha and beta receptor subunit 2; IFNL4: interferon lambda 4; IL1β: interleukin 1β; IFNγ: Interferon γ; IL1RN: interleukin 1 receptor antagonist; IL6: interleukin 6; IL6R: interleukin 6 receptor; IL10: interleukin 10; NA: not available; NOS: Newcastle–Ottawa scale; SERPINA3: serpin family A member 3; TLR3: toll-like receptor 3; TMPRSS2: transmembrane serine protease 2; TNF-α: tumor necrosis factor-alpha; TYK2: tyrosine kinase; VDRs: vitamin D receptors.
All articles diagnosed COVID-19 using RT-PCR and determined severity according to WHO criteria

The synthesis of quantitative data

A total of 13 papers [15-27], which comprised 2,326 cases and 4,584 controls, were included to analyze the association between the genetic variation of *ACE I/D* and the susceptibility to COVID-19. Our pooled calculation revealed that the *ACE* D allele (D vs I: OR: 1.41; 95%CI: 1.08–1.85; p=0.012) exhibited a significant association with an elevated risk of COVID-19 infection (**Figure 2**), while I allele (I vs D: OR: 0.71; 95%CI: 0.54–0.93; p=0.012) and II genotype (**Figure 3**) (II vs ID+DD: OR: 0.55; 95%CI: 0.34–0.87; p=0.011) were associated with a decreased risk of COVID-19 infection.

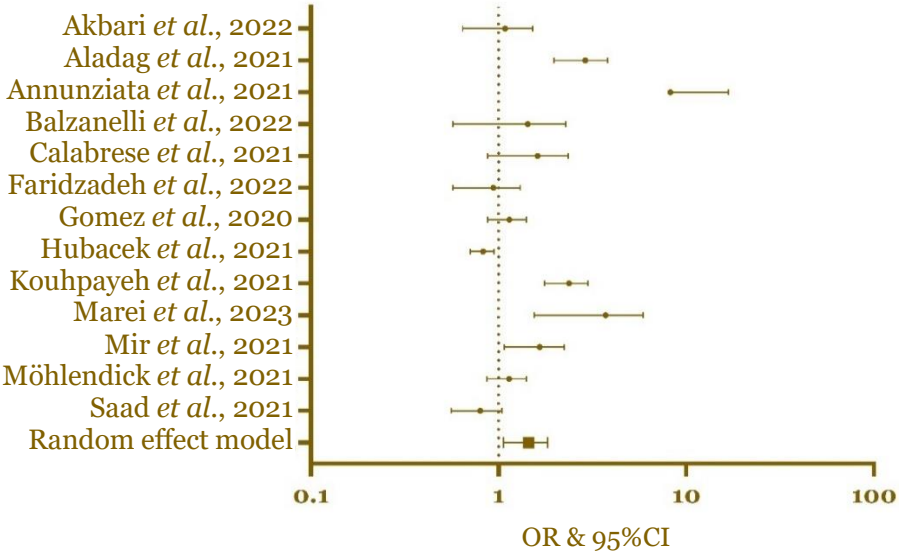

Figure 2. A forest plot of the association between D allele of *ACE I/D* gene rs1799752 (D vs I) polymorphism and the risk of COVID-19 infection.

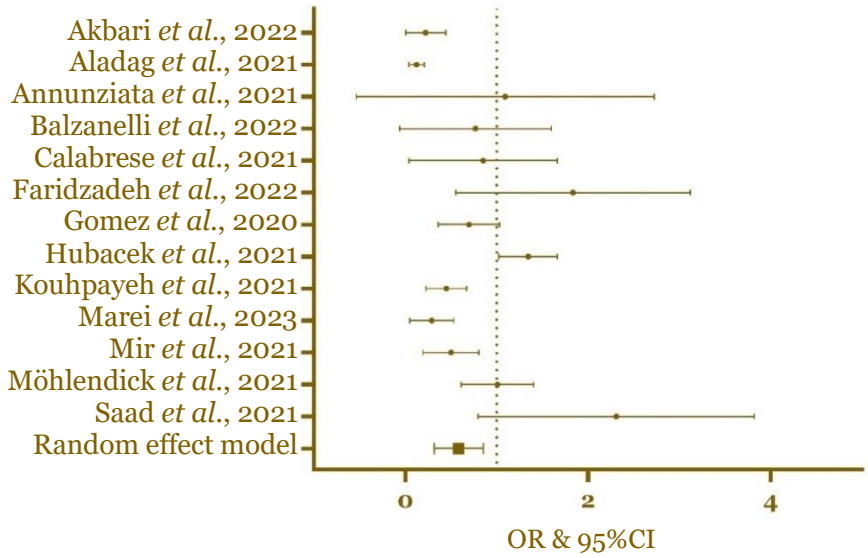

Figure 3. A forest plot of the association between II genotype of *ACE I/D* gene polymorphism rs1799752 (II vs ID+DD) and the risk of COVID-19 infection.

There was no significant association between ID (ID vs II+DD: OR: 1.09; 95%CI: 0.81–1.46; p=0.573) and DD genotypes (DD vs II+ID: OR: 1.32; 95%CI: 0.96–1.82; p=0.089) with the risk of COVID-19 infection. Subgroup analysis (Asian or non-Asian) indicated that the genetic variation of ACE I/D had no association with the risk of COVID-19 infection across all genetic models. **Table 2** presents a comprehensive summary of the *ACE I/D* variants observed in both the COVID-19 and control groups.

To assess the association between the genetic variation of *ACE I/D* and the risk of severe COVID-19, 18 papers were included, comprising a total of 1,891 cases and 2,147 controls [15,17,19,20,22-27,50-57]. Among the included papers, there was no conclusive evidence to support an association between the genetic variation of ACE I/D and the risk of severe COVID-19 in any of the genetic models analyzed. The subgroup analysis focusing on the Asian population found that the ID genotype (**Figure 4**) was associated with an elevated risk of severe COVID-19 (ID vs II+DD: OR: 1.46; 95%CI: 1.15–1.84; *p*=0.002). In the subgroup analysis specifically conducted among individuals of non-Asian ethnicity, there was no significant association found between the genetic variation of ACE I/D and the risk of severe COVID-19. The polymorphism of *ACE I/D* on the likelihood of severe COVID-19 is presented in **Table 2**.

Table 2. Summary of the association between the gene polymorphism of *ACE I/D* and the risk of infection and severity of COVID-19

ACE I/D	NS	OR	95%CI	*p*-Eg	*p*-Het	*P*-value
Risk of infection (all population)						
I vs D	13	0.71	0.54–0.93	0.0676	<0.0001	0.0120
D vs I	13	1.41	1.08–1.85	0.0676	<0.0001	0.0120
II vs ID+DD	13	0.55	0.34–0.87	0.2001	<0.0001	0.0110
ID vs II+DD	13	1.09	0.81–1.46	0.9513	<0.0001	0.5730
DD vs II+ID	13	1.32	0.96–1.82	0.2464	<0.0001	0.0890
Risk of infection (Asian)						
I vs D	6	0.71	0.46–1.11	0.9962	<0.0001	0.1330
D vs I	6	1.40	0.90–2.18	0.9962	<0.0001	0.1330
II vs ID+DD	6	0.54	0.26–1.13	0.4524	<0.0001	0.1020
ID vs II+DD	6	1.18	0.65–2.15	0.1329	<0.0001	0.5910
DD vs II+ID	6	1.28	0.69–2.38	0.7071	<0.0001	0.4330
Risk of infection (non-Asian)						
I vs D	7	0.70	0.49–1.01	0.1331	<0.0001	0.0570
D vs I	7	1.42	0.99–2.04	0.1331	<0.0001	0.0570
II vs ID+DD	7	0.55	0.29–1.04	0.3675	<0.0001	0.0650
ID vs II+DD	7	1.07	0.77–1.48	0.5480	0.0010	0.6810
DD vs II+ID	7	1.29	0.92–1.83	0.1331	0.0010	0.1420
Risk of severe COVID-19 (all population)						
I vs D	18	0.87	0.69–1.09	0.4487	<0.0001	0.2190
D vs I	18	1.15	0.92–1.45	0.4487	<0.0001	0.2190
II vs ID+DD	18	0.76	0.54–1.09	0.6495	<0.0001	0.1360
ID vs II+DD	18	1.11	0.87–1.40	0.8202	0.0010	0.4070
DD vs II+ID	18	1.11	0.79–1.56	0.4487	<0.0001	0.5440
Risk of severe COVID-19 (Asian)						
I vs D	8	1.00	0.66–1.53	0.5362	<0.0001	0.9900
D vs I	8	1.00	0.65–1.53	0.5362	<0.0001	0.9900
II vs ID+DD	8	0.64	0.33–1.22	0.7105	0.0010	0.1710
ID vs II+DD	8	1.46	1.15–1.84	0.0918	0.1160	0.0020
DD vs II+ID	8	0.80	0.42–1.49	0.9015	<0.0001	0.4760
Risk of severe COVID-19 (non-Asian)						
I vs D	10	0.78	0.59–1.03	0.2105	<0.0001	0.0800
D vs I	10	1.28	0.97–1.70	0.2105	<0.0001	0.0800
II vs ID+DD	10	0.87	0.58–1.32	0.8580	0.0110	0.5180
ID vs II+DD	10	0.85	0.63–1.14	0.1524	0.0160	0.2780
DD vs II+ID	10	1.41	0.94–2.10	0.1295	<0.0001	0.0950

ACE: angiotensin converting enzyme; CI: confidence interval; I/D: intron deletion; NS: number of studies; OR: odd ratio; *p*-Het: *p*-heterogeneity; *p*-Eg: *p*-Egger

Potential sources of heterogeneity and the likelihood of publication bias

A concise overview of the results pertaining to heterogeneity and publication bias observed in the study is presented in **Table 2**. We observed heterogeneity among all studies in the overall analysis, as indicated by p Heterogeneity values <0.10, except for the ID genotype of *ACE I/D* in the Asian subgroup. Therefore, a random effect model was used to analyze the data except for the *ACE I/D* ID genotype, where a fixed effect model was used. Additionally, Egger's test was conducted to identify potential publication bias and our analysis did not reveal any indications of bias.

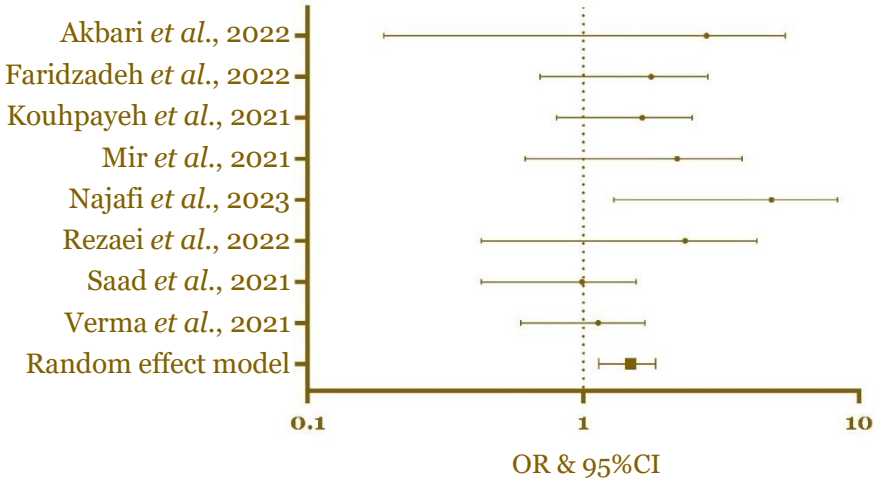

Figure 4. A forest plot of the association between ID genotype of *ACE I/D* gene polymorphism rs1799752 (ID vs II+DD) and the risk of severe COVID-19 among the Asian population.

Discussion

Our study demonstrated a notable association between the *ACE* D allele and an elevated risk of COVID-19 infection. Conversely, the *ACE* I allele and II genotype were found to be related to a reduced risk of COVID-19 infection. However, while we did not find a notable association between the genetic variation of *ACE* I/D and the severity of COVID-19 in our overall analysis, our subgroup analysis specifically conducted among individuals of Asian ethnicity revealed that the *ACE ID* genotype was associated with an elevated risk of severe COVID-19. Previous meta-analyses have explored the association between the genetic variation of *ACE I/D* and the vulnerability to COVID-19 infection as well as the severity of the disease [28-30]. However, our results differed from those of previous studies. Aziz *et al.* [28] and Dieter *et al.* [29] reported no notable relationship between the genetic variation of *ACE I/D* and the risk of COVID-19 infection or disease severity, while Dobrijevic *et al.* [30] demonstrated that the *ACE ID* genotype was linked to an increased severity of COVID-19.

The disparities observed in the findings between our study and previous investigations could be attributed to various factors, including sample size, statistical methods, and focus of the study. Aziz *et al.* involved only 11 papers [28], Dieter *et al.* involved five papers [29], and Dobrijevic *et al.* involved nine papers [30], while our study involved 21 papers. A greater number of papers might provide more accurate and comprehensive calculation. Moreover, Aziz *et al.* did not explain which results were related to the risk of infection and which were related to the risk of severity of COVID-19 [28]. This might be a question that needs clarification. Additionally, in Aziz *et al.*, all analysis models used a fixed-effect model, while the I-squared varied between 43% and 84% [28]. The mismatch between the analysis model and the level of heterogeneity could contribute to the final findings. In our study, the analysis model was adjusted for the level of heterogeneity, and therefore our results might provide more accurate calculations. Furthermore, Dieter *et al.* [29] and Dobrijevic *et al.* [30] evaluated many genes such as *ACE I/D*, *Transmembrane Serine Protease 2* (*TMPRSS2*), *Apolipoprotein E* (*APOE*), *Human Leukocyte Antigen* (*HLA*)-*A*30*, *HLA-A*33*, *HLA-B*38*, *HLA-C*06*, *C-C Chemokine Receptor Type 5* (*CCR5*), *ACE2*, *Interferon-induced Transmembrane Protein 3* (*IFITM3*), and *Vitamin D Receptor* (*VDR*). This might cause the focus of the study not to be only on *ACE I/D*, resulting in less detailed data collection and calculation.

The theoretical basis of our findings might not be well-defined, but we might still suggest some hypotheses. The impact of the genetic variation of *ACE I/D* on the severity of COVID-19 has been the focus of investigation in prior studies. This polymorphism affects the production of *ACE*, which plays a role in regulating blood pressure and inflammation [58-60]. *ACE* is involved in the physiological functioning of the lungs and has been implicated in the pathogenesis of COVID-19. The ACE2 receptor acts as a gateway for the SARS-CoV-2 virus, the pathogen behind COVID-19, as the virus's spike protein binds to this receptor to initiate cellular infection [61,62]. The

interaction between the virus and ACE2 downregulates its expression and results in an accumulation of angiotensin II, a vasoconstrictor, and pro-inflammatory peptide that can cause tissue damage [9,63,64]. The levels of ACE2 receptors are modulated by the balance of ACE/ACE2. In the context of infection caused by SARS-CoV-2, the activity of *ACE* is significantly higher than that of ACE2 due to ACE2 impairment, creating a substantial imbalance between the two [13]. Additionally, studies have shown that the levels of ACE in the blood can affect the severity of COVID-19 [65,66], and the ACE I/D gene variant can influence these levels. Higher levels of ACE were observed in subjects with the *ACE* D allele, while subjects with the *ACE* I allele exhibited lower levels of ACE [14]. This reason could potentially explain our results, which demonstrated that individuals carrying the D allele had a greater susceptibility to COVID-19 infection, while those carrying the I allele and II genotype of the *ACE I/D* gene variant had a reduced risk of COVID-19 infection. Furthermore, the prevalence of the genetic variation of *ACE I/D* varies among different populations. In the Asian population, the *ACE ID* genotype was more common than the II or DD genotype, and this genotype has been reported to be associated with an increased risk of hypertension among the Asian population [67]. In our prior investigation, we had uncovered an association between hypertension and an elevated risk of severe COVID-19 [68,69]. This theoretical explanation could potentially reconcile the findings of our study, which demonstrated that individuals with the *ACE ID* genotype had an increased risk of developing severe COVID-19. However, the relationship between the genetic variation of *ACE I/D* and COVID-19 is still an area of active research, and more studies are needed to understand the complex interplay between genetics and disease susceptibility.

Our study has successfully identified the contribution of the genetic variation of *ACE I/D* on the risk of COVID-19 infection and severity. We have also demonstrated that genetic factors of *ACE I/D* and ethnicity contribute to the variability in COVID-19 severity. These findings may serve as a foundation for understanding the involvement of *ACE I/D* in COVID-19 cases. Comprehensive knowledge of COVID-19 in the genetic context is expected to provide adequate information about COVID-19 pathogenesis, enabling the development of various new therapeutic options for COVID-19 cases in the future. On the other hand, our findings hold promise in resolving the debate surrounding the role of *ACE I/D* in the pathogenesis of COVID-19. However, it is premature to recommend *ACE I/D* as a predictive biomarker for COVID-19 at this stage. Further studies are warranted to obtain more precise results, taking into account factors such as the interplay between genes and the environment, interactions between different genes, and the influence of drugs on gene interactions.

There were several limitations to our meta-analysis. To begin with, we relied on an overall estimation of effects, which did not account for potential confounding variables such as age, sex, comorbidity, and history of ACE-related diseases that could impact COVID-19 risk. Additionally, the small sample size meant that false negative results were possible even when combining studies. Lastly, the unequal proportion of ethnicity in our analysis suggested a potential for bias that cannot be entirely ruled out.

Conclusion

Our findings suggest that individuals carrying the *ACE* D allele are at higher risk for COVID-19 infection, while those carrying the *ACE* I allele and II genotype have a lower risk of COVID-19 infection. Furthermore, our findings indicate that individuals with the *ACE ID* genotype have a heightened likelihood of developing severe COVID-19 in the Asian population.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

No new data were generated in this study.

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