ACE polymorphisms and COVID-19-related mortality in Europe

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged in China at the end of 2019 and has rapidly spread to Asia, Oceania, Europe, and America causing the coronavirus disease-19 (COVID-19) pandemic [1] and more than 700,000 deaths worldwide as of August 6, 2020. Epidemiology analyses have been showing higher mortality due to COVID-19 in Europe than in China [2]. In Table 1, we report the number of fatalities due to COVID-19 both in relation to the number of total cases (i.e., mortality) and the entire population of the indicated country (i.e., death/1 million population) as of August 5, 2020. The two parameters are differently influenced by several variables (e.g., the number and type of tests each country has used to confirm the clinical diagnosis, the access to hospitalization, or the parameters used to ascribe death to COVID-19). Considering that the mortality parameter strictly depends on the number of test performed in each country, and that testing has neither been homogeneously performed in the different countries [3] nor the entire populations of these countries have been screened, we focused on the death/1 million population. As reported in Table 1, COVID-19-related deaths are much less in China than in Europe. Furthermore, deaths related to COVID-19 are not equally distributed in Europe. Northern European countries, for example, Denmark, Germany, and Norway, have experienced rates of COVID-19-related deaths closer to China than Southern European countries like Italy, Spain, or France. There are several exceptions to this apparent rule. For example, the Belgian National Health Institute has been counting even suspected cases of COVID-19-related deaths, regardless of whether the deceased person was tested. Northern European countries like the UK and Sweden did not impose a lockdown, thus diverging from the politics of social containment to face the pandemic adopted by several other European nations. These considerations may apply to other countries worldwide.

Median age of the population (Table 1), social behaviors that are more distinctive of Southern European countries (e.g., intense social life in crowded places, warm greetings, apartments shared by youngsters and elders), or even air pollution [4–6] are additional factors that have been implicated in COVID-19-related mortality. As for the European countries analyzed in Table 1, median age of inhabitants does not appear to have a relevant impact on COVID-19-related mortality (Fig. 1a).

The renin-angiotensin-aldosterone system (RAAS) is under scrutiny in the coronavirus COVID-19 pandemic [7] because the angiotensin-converting enzyme 2 (ACE2) is the main receptor for the SARS-CoV-2 on alveolar epithelial cells [8]. ACE2 and the serine protease TMPRSS2, which is necessary for spike protein priming, are also expressed in several other tissues, including blood vessels, olfactory epithelium, brain, heart, kidney, and intestine, thus explaining the multiorgan dysfunction observed in COVID-19 patients [9, 10].

Increased expression of ACE2 in elder males has been put forward to explain the increased SARS-CoV-2 aggressiveness in this subpopulation [9]. However, treatment with ACE inhibitors or angiotensin receptor blockers, which may also cause increased ACE2 expression, does not associate with more severe COVID-19 [11–15]. Additionally, ACE2 counterbalances deleterious vasoconstrictive, proinflammatory, and profibrotic effects of angiotensin (Ang) II by generating downstream peptides such as the hypotensive metabolite Ang1–7 [7]. Indeed, targeted disruption of ACE2 in mice causes severe cardiac contractility defects, increased Ang II levels, and upregulation of hypoxia-induced genes in the heart [16]. ACE2 also exerts a protective effect by limiting leukocyte accrual during acute respiratory distress syndrome in mice, and recombinant ACE2 can protect mice from lung injury [17]. Finally, it has been recently reported that soluble human ACE2 can inhibit SARS-CoV-2 infection in human blood vessel organoids and human kidney organoids [18]. Thus, the role of the RAAS in COVID-19 is far from being elucidated.
Table 1  Summary of population median age, COVID-19*-related mortality, and ACE polymorphisms in the different countries under evaluation.

| Nation                      | Population median age** | Mortality, total deaths/total cases (%)** | Death/1M population** | ACE polymorphisms | Studied population age | Reference                                                                 |
|-----------------------------|-------------------------|-------------------------------------------|-----------------------|-------------------|------------------------|---------------------------------------------------------------------------|
| China                       | 38.4                    | 5.58                                      | 3                     | 0.39 0.43 0.18   | < 70                   | He Q et al. (2013) PLoS One 8: e75870                                      |
| Austria                     | 43.5                    | 3.34                                      | 80                    | 0.21 0.50 0.29   | < 75                   | Sunder-Plassmann G et al. (2002) Crit Care Med 30: 2236-2241              |
| Belgium                     | 41.9                    | 14.0                                      | 850                   | 0.17 0.48 0.35   | < 83                   | Tournoy KG et al. (1996) Clin Chim Acta 255: 39-55                        |
| Bosnia and Herzegovina      | 43.1                    | 2.90                                      | 114                   | 0.18 0.52 0.30   | < 55                   | Klupka-Saric I et al. (2011) Genet Test Mol Biomarkers 15: 835-838        |
| Croatia                     | 44.3                    | 2.89                                      | 38                    | 0.28 0.50 0.22   | < 60                   | Lovrecic L et al. (2006) Acta Neurol Scand 114: 374-377                   |
| Czechia                     | 43.2                    | 2.21                                      | 36                    | 0.19 0.48 0.33   | < 55                   | Hladikova M et al. (2011) J Neurol Sci 303: 31-34                         |
| Denmark                     | 42.3                    | 4.38                                      | 106                   | 0.23 0.51 0.26   | < 55                   | Panza F et al. (2003) Exp Gerontol 38: 1015-1020                          |
| Finland                     | 43.1                    | 7.33                                      | 60                    | 0.24 0.49 0.27   | NR                    | Pietinallo A et al. (1999) Eur Respir J 13: 723-726                      |
| France                      | 42.3                    | 15.84                                     | 464                   | 0.18 0.46 0.36   | < 55                   | Rigat B et al. (1990) J Clin Invest 86: 1343-1346                         |
| Germany                     | 45.7                    | 4.35                                      | 110                   | 0.21 0.52 0.27   | < 90                   | Huel T et al. (2009) Eur J Gastroenterol Hepatol 21: 1032-1035            |
| Greece                      | 45.6                    | 4.3                                       | 20                    | 0.17 0.48 0.35   | < 80                   | Karagiannis A et al. (2004) Eur Neurol 51: 148-152                       |
| Hungary                     | 43.3                    | 13.12                                     | 62                    | 0.28 0.49 0.23   | < 80                   | Szolnoki Z et al. (2001) J Neurol 248: 756-761                           |
| Northern Ireland            | 38.2                    | 6.72                                      | 357                   | 0.24 0.49 0.27   | < 65                   | Kee F et al. (2000) Eur J Clin Invest 30: 1076-1082                       |
| Italy                       | 47.3                    | 14.17                                     | 582                   | 0.13 0.47 0.40   | < 70                   | Panza F et al. (2003) Exp Gerontol 38: 1015-1020                          |
| Italy                       | 47.3                    | 14.17                                     | 582                   | 0.13 0.47 0.40   | < 70                   | Panza F et al. (2003) Exp Gerontol 38: 1015-1020                          |
| Lithuania                   | 45.1                    | 3.74                                      | 29                    | 0.26 0.46 0.28   | < 75                   | Kupcinskas J (2011) BMC Med Genet 12: 112                                 |
| Montenegro                  | 39.8                    | 1.58                                      | 84                    | 0.19 0.53 0.28   | NR                    | Kostic M et al. (2004) Pediatr Nephrol 19: 853-857                        |
| Netherlands                 | 43.3                    | 10.99                                     | 359                   | 0.24 0.50 0.26   | < 65                   | van der Sman-de Beer F et al. (2005) Kidney Int 68: 2237-2243             |
| Norway                      | 39.8                    | 2.74                                      | 47                    | 0.23 0.51 0.26   | < 70                   | Tronvik E, et al. (2008) BMC Neurol 8: 4                                 |
| Poland                      | 41.7                    | 3.59                                      | 46                    | 0.35 0.43 0.22   | < 50                   | Zak I et al. (2003) Acta Biochim Pol 50: 527-534                          |
| Portugal                    | 46.2                    | 3.36                                      | 171                   | 0.17 0.47 0.36   | < 70                   | Pereira da Silva A et al. (2019) Mol Cell Biochem 455: 61-71             |
| Russia                      | 39.6                    | 1.67                                      | 99                    | 0.25 0.46 0.29   | < 92                   | Farrer LA et al. (2000) Arch Neurol 57: 210-214                          |
| Serbia                      | 41.6                    | 2.26                                      | 69                    | 0.17 0.53 0.29   | < 65                   | Zivkovic M et al. (2016) J Neurol Sci 363: 29-32                          |
| Slovenia                    | 44.5                    | 5.61                                      | 60                    | 0.26 0.53 0.21   | < 59                   | Salobir B et al. (2007) Med Sci Monit 13: CR538-542                       |
| Spain                       | 44.9                    | 8.14                                      | 609                   | 0.14 0.51 0.35   | NR                    | Martinez E et al. (2000) J Hum Hypertens 14: 131-135                      |
| Sweden                      | 41.1                    | 7.08                                      | 569                   | 0.22 0.51 0.27   | > 18                   | Kurland L et al. (2001) J Hypertens 19: 1783-1787                         |
| United Kingdom              | 40.5                    | 15.12                                     | 682                   | 0.24 0.50 0.26   | < 55                   | Marshall RP et al. Am J Respir Crit Care Med 166: 646-650                 |

*COVID-19 coronavirus disease 19, NR not reported, Ref reference, 1M one million, I insertion, D deletion

** Data source: https://www.worldometers.info/coronavirus/ updated to August 5, 2020.
ACE2 polymorphisms have been investigated with no evidence of an association with the aggressiveness of severe acute respiratory syndrome [19]. The ACE gene contains an insertion/deletion (Ins/Del) polymorphism (rs4646994; ref. [20]), which associates with higher serum ACE levels [20], obesity [21], hypertension [22], increased cardiovascular risk [23], and thrombophilia [24]; all clinical conditions correlated with more aggressive COVID-19 [1]. Additionally, the ACE Del/Del polymorphism has been associated with mortality in acute respiratory distress syndrome (ARDS) [25]. Our hypothesis is that increased availability of ACE due to ACE Del/Del polymorphism might help explain why SARS-CoV-2 is hitting so hard in Southern Europe.

We found that the distribution of ACE polymorphisms varied among populations, and Del/Del was much more represented in Italy, especially in the eldest, than in China. A gradient in Del/Del polymorphism was also apparent moving from the south to the north of Europe (Table 1). As reported in Fig. 1b, a simple linear regression showed an association between Del/Del polymorphism and COVID-19-related deaths in 25 European countries for which we were able to retrieve comparable information on ACE polymorphisms (e.g., gender distribution, age, samples analyzed). Because data appeared bimodally distributed, they were also analyzed by Spearman’s correlation. The association between ACE Del/Del polymorphism and COVID-19-related deaths remained significant ($r = 0.4024$ (95% CI $0.005720$ to $0.6896$), $p$ (two tailed) = 0.0416).

A potential association between ACE polymorphisms and COVID-19 was already investigated by Delanghe and colleagues, who compared the Del-allele frequency of ACE with the mortality of COVID-19 in 25 different European countries [26]. They found a significant correlation between COVID-19-related deaths and the prevalence of the ACE D-allele (Spearman $r = -0.510$, $p = 0.01$). However, focusing on D-allele, the authors aggregated Ins and Del polymorphisms in the analysis, thus diluting the negative effect of Del/Del into the positive effects of Ins/Ins polymorphisms. Indeed, in our analysis, Ins/Ins polymorphism inversely correlated with COVID-19-related deaths (Fig. 1c, Spearman: $r = -0.4898$ (95%, $-0.7427$ to $-0.1145$), $p$ (two tailed) = 0.0111). A correlation between Ins/Del polymorphism and COVID-19-related deaths was not found (Table 1; $p = 0.9061$).

Altogether, these findings suggest a pathogenic role of ACE in COVID-19. This finding might also help explain why in Southern Europe COVID-19-associated mortality has been so high. ACE polymorphisms remain one of probably many genetic and non-genetic factors influencing COVID-19 outcomes.

Abundance of ACE in the blood of Del/Del COVID-19 patients might favor the generation of Ang II. Additionally, ACE2 receptor downregulation caused by SARS-Cov-2 engagement increases Ang II availability, and its deleterious effects downstream of the Ang II type 1 receptor [17]. ACE2 downregulation might also facilitate neutrophil recruitment in the lungs, eventually leading to increased tissue damage. Additionally, Ang II is involved in platelet activation and aggregation [27], which may occur in COVID-19 patients [1]. Dysregulation of RAAS due to excessive ACE might also explain the increased susceptibility of elders to COVID-19 [28], in which the Del/Del polymorphism is particularly high

Fig. 1 Prevalence of COVID-19-related deaths (number of deaths/10^6 inhabitants) versus median age of the population of the specified country (a), ACE Del/Del polymorphisms expressed in percentage (b), and ACE Ins/Ins polymorphisms expressed in percentage (c). Numbers in panels refer to $R$ squared ($r$) and $p$ value ($p$) for simple linear regression. Nations are reported in color code: Austria, black; Belgium, cantaloupe; Bosnia and Herzegovina, grape; China, mercury; Czechia, blueberry; Croatia, iron; Denmark, eggplant; Finland, asparagus; France, carnation; Germany, moss; Greece, aqua; Hungary, maroon; Ireland, tangerine; Italy, salmon; Lithuania, cayenne; Montenegro, mocha; Netherlands, lemon; Poland, nickel; Portugal, turquoise; Russia, strawberry; Serbia, magenta; Slovenia, magnesium; Spain, maraschino; Sweden, lime; UK, sea foam.
Thus, serum ACE levels and/or Del/Del polymorphism might be predictive of more aggressive disease.

Based on these premises, ACE Del/Del polymorphism and serum ACE levels should be investigated as predictive biomarkers of COVID-19 aggressiveness, as it has been already proposed in ARDS patients [29], and ACE and Ang II might be potential therapeutic targets in COVID-19 patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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