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Association of Apolipoprotein e polymorphism with SARS-CoV-2 infection

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ABSTRACT

Coronavirus 2019 (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus-2 (SARS CoV-2). The disease resulted in global morbidity and mortality that led to considering as pandemic. The human body response to COVID-19 infection was massively different from being asymptomatic to developing severe symptoms. Host genetic factors are thought to be one of the reasons for these disparities in body responses. Few studies have suggested that Apolipoprotein Epsilon (Apo E) is a candidate gene for playing roles in the development of the disease symptoms. This work aims to find an association between different Apo E genotypes and alleles to COVID-19 infection comparing a general population and a group of COVID-19 patients. For the first time, the results found that Apo E4 is associated with COVID-19 disease in a Kurdish population of Iraq. Further study is required to reveal this association in different ethnic backgrounds all over the world.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection was declared a pandemic by WHO in March 2020 (WHO, 2020). It has been observed that SARS-CoV-2 infected individuals show massive differences in their response to the infection. The human body response was ranging from being asymptomatic in a large proportion of infected individuals (Yanes-Lane et al., 2020), to being mild, or to develop severe symptoms (Wu and McGoogan, 2020). The reasons behind these disparities in the severity of coronavirus disease 2019 (COVID-19) infection are yet unknown. Early at the beginning of the pandemic, it has been revealed that demographic factors like older age and male gender are risk factors for acquiring the infection and developing more severe symptoms (Jin et al., 2020). Then different pre-existing comorbidities are suggested as risk factors; these were including cardiovascular diseases (CVD), chronic respiratory disease (CRD), obesity, Cancer (Bajgain et al., 2021; Gasmi et al., 2021; Zheng et al., 2020), Alzheimer's, dementia (Wang et al., 2021a), type 2 diabetes (DM), chronic obstructive pulmonary disease (COPD), pneumonia, atrial fibrillation (AF), chronic kidney disease (CKD) and hypertension (HTN) (Atkins et al., 2020).

Despite the demographic factors and pre-existing comorbidities the basic question regarding the differential susceptibility to COVID-19 infection and the development of symptoms has remained unanswered. However, one possible answer could be the host genetic background, therefore; the host genetic risk factors for acquiring infection and developing symptoms should be considered. Recently, a large number of studies have been directed toward finding genes that confer differences in susceptibility for COVID-19 infection (Schurr, 2020). Some of the genetic association studies are performed based on the comparison of biobank genetic data (Kuo et al., 2020a). Most of these studies have revealed genetic risk factors, however, each study found different genes from other studies (Hu et al., 2020). Several genes have been suggested for possible association to COVID-19 acquisition and severity; these was including; Angiotensin converting enzyme 2 (ACE2), trans-membrane protease serine 2 (TMPRSS2) (Hou et al., 2020), interferon-induced transmembrane protein 3 (IFITM3) (YC and BH, 2020), ABO blood type (Cordero et al., 2021), and Toll-like receptor 7 (Fallerini et al., 2021).

One of the genetic risk factors, found to be associated with COVID-19 infection in a community cohort study, was Apolipoprotein E (Apo E) (Kuo et al., 2020a). Apo E is a lipoprotein that is related to the metabolism of lipids in the human body and it is also associated with both cardiovascular and neurodegenerative diseases including Alzheimer's disease (Poirier et al., 1993). Apo E gene is polymorphic having three different alleles, E2, E3 and E4, with different frequencies (5–10%, 65–70% and 15–20%, respectively) among the general population (Mahley, 2016). The combination of these alleles gives rise to three

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homozygous and three heterozygous genotypes including E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4 (Mahley, 2016). Apo E is found to be related to susceptibility to parasitic, bacterial and viral infections. Apo E4 allele has been associated with the greater risk of acquisition and severity of viral infections, like hepatitis C, Herpes simplex, and HIV (Burt et al., 2008; Kuhlmann et al., 2010). It was shown that the Apo E deficiency caused immune response impairment to certain bacterial infections (De Bont et al., 2000; Roselaar and Daugherty, 1998). There are also a growing number of studies showing that the Apo E gene is associated with pulmonary inflammation and oxidative stress. For instance, the E4 allele is found to be related to increasing pro-inflammatory cytokines, while Ape E3 has immune-modulatory roles in inflammation, (Figueroa et al., 2018) which is a major problem in COVID-19 patients.

Interestingly, Apo E polymorphism is also a genetic risk factor in most of the comorbidities related to SARS-COV-2 severity. Apo E polymorphism is found associated with cardiovascular diseases, type-2 Diabetes (Liu et al., 2019), Alzheimer's disease, and Dementia (Wang et al., 2021a).

Before the COVID-19 pandemic happened, we had been studying genetic polymorphisms of Apo E alleles in a general population in Sulaymaniyah province, Kurdistan Region of Iraq (Al-Jaf, 2021). In an attempt to find a possible guilty gene for COVID-19 infection, we have designed a cohort study to seek an association between Apo E polymorphism and COVID-19 infection.

2. Materials and methods

2.1. Study subjects

Previously we have investigated the frequency of Apo E alleles in one hundred fourteen healthy individuals in the same area (Al-Jaf, 2021); the data from this study was used to represent the control group for the current study. From July to December/2020, One-hundred and five patients who visited COVID-19 clinics in Kalar district (Sulaimaniyah province, Iraq) were selected to represent COVID-19 cases. All the cases were confirmed by RT-PCR from nasopharyngeal swabs. Signed consent forms were filled for each patient. Venous blood samples were taken from each subject and kept in a refrigerator at 4 °C. The study was performed under the University of Garmian ethical committee that was adhered to the Declaration of Helsinki.

A detailed questionnaire was designed to collect all the necessary data from the patient's records such as age, sex, and the clinical history for comorbidities like; asthma, diabetes, hypertension, ischemic heart disease and stroke. Acute-phase parameters including CRP, ESR, D-dimer and serum ferritin were also collected. PCR positive test, CT scan and SPO_2 were also documented in the questionnaire.

2.2. DNA extraction

Total genomic DNA was extracted from whole venous blood by mixing 200 μl of each blood sample with 20 μl proteinase K and incubated at 56 °C for 10 min, then the manufacturer's (GENET BIO CO., Daejeon, KR) instructions were followed. The genomic DNA was eluted with a 200 μl elution buffer. The extracted genomic DNA samples were kept at −30 °C until used.

2.3. Apo E genotyping

In this study, a sequence-specific primer method was used for Apo E genotyping. This method is a PCR based method in which four allele-specific primers (Macrogen Co., Seoul, KR) were used for the identification of Apo E isoforms (Forward Primer-1; CGG ACA TGG AGG ACG TGT for Apo E-112cys, Reverse Primer-2; CTG GTA CAC TGC CAG GCG for Apo E-158arg, Reverse Primer-3 CTG GTA CAC TGC CAG GCA for Apo E-158cys, Forward Primer-4 CGG ACA TGG AGG ACG TGC for Apo E-112arg, these primers were developed and validated previously (Pantelidis et al., 2003), and recetly used in our lab for Apo E genotyping in a general population (Al-Jaf, 2021). Combinations of two allele-specific sequence-specific primers were used to determine each Apo E haplotype: primers F1 and R3 for E2, primers F1 and R2 for E3 and primers R2 and F4 for E4 haplotypes. Three PCR reactions were required to confirm the genotyping of each DNA sample. In each PCR reaction, in addition to Apo E primers, a pair of other primers were added to amplify a 406 bp region on chromosome 9 (Niranji, 2020), this was used as a positive control to ensure that the PCR reactions are working. A thermal cycler (Nexus, Eppendorf AG, Germany) was used to perform PCR reactions. A volume of 11 μl of a reaction mixture was prepared as follow: 5 μl of mastermix (Addbio, South Korea), 5 μl of sample DNA, and 0.8 μl (10 pmol)of allele-specific primers (0.4 μl of each forward and reverse primer) and 0.1 (10 pmol) μl of each control primers. The negative control was performed for each experiment by adding 5 μl of water instead of sample DNA. To avoid non-specific binding, the PCR reactions were carried out in high-stringency touch-down conditions: 1 cycle of initial denaturation at 95 °C for 5 min. This was followed by 5 cycles of denaturation at 96 °C for 20 s, annealing at 70 °C for 45 s, and extension at 72 °C for 25 s; then 21 cycles at 96 °C for 25 s, 65 °C for 50 s, and 72 °C for 30 s; and finally 6 cycles at 96 °C for 30 s, 55 °C for 60 s, and final extension was at 70 °C for 2 min.

Ten (10) μl of the PCR products were run on 1.5% Tris-borate-EDTA/ethidium bromide Agarose gel (Simply, South Korea). The gel images were captured using Syngene Imaging System (Synoptics Ltd., Surrey, United Kingdom).

2.4. Statistical analysis

The frequency of genotypes and alleles compared between the general population and COVID-19 patients for significance, using χ^2 (Fisher's exact test). The odds ratio (OR) and 95% confidence intervals (95% CI) were used to verify the effects. Graphpad prism 9.1.1 software (GraphPad Software, San Diego, California USA) was used to perform statistical analysis. *p*-value less than 0.05 was considered as significant.

3. Results

The patients' clinical information has been shown in Table 1. The data contained age groups from below 20 to over 70 years old. Out of 105 patients, 48 (45.71%) were male and 57 (54.29%) were female. CT scan performed for 31 patients included; moderate, 17 (16.19%) and severe 14 (13.33%). SpO_2 of 62 (59.05%) patients were higher than 93 and of 43 (40.95%) were lower than 93. Acute-phase reactant parameters were high including positive CRP 98 (93.33%), high Ferritin 39 (37.14%), high D-dimer 39 (37.14%) and high ESR 86 (81.90%). Comorbidities includes: obesity 23 (21.90%), hypertension 36 (34.28%), Asthma 3 (2.85%), Diabetes 11 (10.47%), IHD 10 (9.52%), and Stroke 1 (0.95%).

Three PCR reactions were needed for the genotyping of each DNA sample. The presence of a 173 bp band in PCR reactions (E2, E3 and E4) indicated the presence of a corresponding allele. The presence of a 406 bp band in every PCR reaction was necessary as it was indicated successful amplification produced by control primers. The absence of both allele-specific amplicon (173 bp) and control amplicon (406 bp) in any reaction is regarded as a failure.

The frequency of six different genotypes and three different Apo E alleles were calculated for COVID-19 patients and compared to the Apo E frequency data in a general population which we previously obtained in a separate study (Al-Jaf, 2021). All the three alleles E2, E3 and E4 have been found in both groups. However, E2/E2 and E4/E4 genotypes were not found in both healthy and patient groups, and E2/E4 genotype has not been found in the healthy group. The frequency results from both healthy controls and SARS-CoV-2 infected patients are summarized in Table 2.

Table 1
Acute-phase reactant parameters, comorbidities and demographic data of studied COVID-19 cases.

Parameters		Comorbidities		Demographic data			
Parameter	No. (%)	Comorbidity	No. (%)	Age		Sex	
High ESR	86 (81.90)	Obesity	23 (21.90)	Age groups	No.	Male No.	Female No.
Positive CRP	98 (93.33)			1–20 years	6	2	4
High D-Dimer	39 (37.14)	Asthma	3 (2.85)	21–30 years	11	5	6
High Ferritin	39 (37.14)			31–40 years	21	9	12
CT scan	31	Diabetes	11 (10.47)	41–50 years	19	9	10
Moderate	17 (16.19)	Hypertension	36 (34.28)	51–60 years	13	7	6
Severe	14 (13.33)						
SpO_2	62 (59.05)	Ischemi heart disease	10 (9.52)	Over 60 years	35	16	19
Higher than 93	43 (40.95)						
Lower than 93		Stroke	1 (0.95)	Total	105	48 (45.71%)	57 (54.29%)

Table 2
Apo E genotypes and alleles frequency in both COVID-19 patients and general population with calculated *p*-values, odds ratio (OR) and 95% confidence intervals (95% CI).

Genotypes and alleles	General population N (%) $N = 114$	SARS-CoV-2 Patients N (%) $N = 105$	p-value	OR (95% CI)
E2/E2	0 (0.0)	0 (0.0)	NA	
E2/E3	14 (12.3)	10 (9.5)	0.8277	0.8534 (0.3584 to 2.035)
E2/E4	0 (0.0)	3 (2.86)	NA	
E3/E3	92 (80.7)	77 (73.33)	0.6820	0.9087 (0.6075 to 1.355)
E3/E4	8 (7.0)	15 (14.23)	0.1180	2.240 (0.9004 to 5.389)
E4/E4	0 (0.0)	0 (0.0)	NA	
E2	14 (6.10)	13 (6.19)	>0.9999	1.069 (0.5021 to 2.226)
E3	206 (90.40)	179 (85.24)	0.1243	0.8003 (0.6077 to 1.052)
E4	8 (3.50)	18 (8.57)	0.0268*	2.589 (1.092 to 5.746)

N = Sample number, NA = Not applicable; because of zero values, OR = Odds ratio, CI = Confidence interval.
* Significant.

The statistical analysis of the data showed non-significant differences between the frequency of the six genotypes between the general population and COVID-19 patients, despite the relative difference in the frequency of E3/E4 genotype ($P = 0.1180$) between both groups. However, regarding the frequency of alleles, the Apo E4 allele is significantly increased ($P = 0.0268$) in COVID-19 patients compared to the general population. While the differences between Apo E2 and Apo E3 alleles were not significant comparing the two groups.

4. Discussion

Attempting to find the causes of heterogeneity in COVID-19 clinical manifestation, and severity, a large number of studies have been performed. The epidemiological studies identified several risk factors for developing severe disease. More recently, studies have focused on host genetic factors which lead to the identification of possible genetic variants that may be associated with disparities in COVID-19 disease outcome (Ellinghaus, 2020). ApoE protein is a multi-faceted protein that has been found associated with many diseases (Smith, 2000). Several studies have been performed to reveal a possible association between Apo E polymorphism and COVID-19 acquisition and severity (Kuo et al., 2020b); including few experimental studies (Hubacek et al., 2021) and the current study would be one of that few.

In the current study, none of both homozygous E2/E2 and E4/E4 genotypes have been detected in the COVID-19 infected individuals, and this might be due to the low frequency of both Apo E2 and E4 alleles among the general population in this area, as it was suggested in our previous study (Al-Jaf, 2021). The frequency of both Apo E2/E3 and E3/E3 genotypes was lower and that of Apo E3/E4 was higher in the patient group compared to the general population, however, none of these differences were statistically significant but the results were comparable. Regarding the Apo E alleles, the frequency of Apo E2 remained somewhat constant and the frequency of Apo E3 was lower in the patient group but it was not significant. These findings suggest the neutral role of Apo E2 and the possible protective role of the Apo E3 allele. Interestingly, the frequency of the Apo E4 allele among the COVID-19 patient group is more than twice its frequency compared to the general population and it was statistically significant. These data suggest that the Apo E4 allele might play role in the COVID-19 infection. Previous studies have shown that the Apo E4 allele directly plays roles in inducing pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) while Apo E3 regulates these cytokines (Figueroa et al., 2018). It seemed that the increase in the frequency of the E4 allele is at the expense of a decrease in the E3 allele as its frequency decreased by the same ratio in COVID-19 infected individuals. This finding suggests the possible protective role of E3 and predisposing role of E4 alleles. Given that Apo E polymorphism is associated with most of the comorbidities related to COVID-19 infection like cardiovascular diseases, diabetes, hypercholesterolemia, and dementia. The effect of the E4 isoform could be indirect as it is predisposing to these comorbidities. However, a recent UK Biobank cohort study showed that the Apo E4 allele increases the risk of severe COVID-19 infection even after excluding the role of comorbidities like dementia, cardiovascular disease, and type-2 diabetes (Kuo et al., 2020b).

The results obtained in this study are similar to the findings by Kuo and colleagues as they suggested a 2.2 fold increase in acquiring COVID-19 infection among Apo E4 homozygotes compared to Apo E3 homozygotes (Kuo et al., 2020a). However, other studies, utilizing UK Biobank data, have found that the role of Apo E4 was weakened after normalization of the results to associated comorbidities like AD, and CAS, suggesting the adding effect of these comorbidities to the role of Apo E4 allele (Kosmicki et al., 2020).

In another study performed among Czeck subjects, Hubacek and colleagues found no significant difference in the frequency of Apo E alleles in 408 SARS CoV-2 PCR-positive subjects compared to the general population. However, when they compared symptomatic and asymptomatic COVID-19 subjects separately with the control population, they found a significant difference in the frequency of the Apo E4 allele between symptomatic patients and the control groups (Hubacek et al., 2021). Given that our COVID-19 subjects were symptomatic patients the results obtained by Hubacek and colleagues might not be in real contradiction to our results, as they found a significant difference when compared the symptomatic group of their COVID-19 subjects to the general population. In a cohort of 913 older volunteers in Spain, aged 75–90 years, similar results were achieved as it has been found that

the Apo E4 allele was associated with the presence of symptoms and clinical severity of COVID-19 infection (Del Ser et al., 2021).

A recent *in vitro* study also supported the harmful association of Apo E4 allele with the severity of COVID-19 infections. The study has found that neurons and astrocytes which expressed Apo E4 allele are more susceptible to SARS-CoV-2 infection than those expressed Apo E3, and more severe response are exhibited by Apo E4 carried astrocytes to SARS-CoV-2 infection than those which carried Apo E3 allele (Wang et al., 2021b). Thus, both *in vitro* and association studies support that Apo E4 plays detrimental roles in the clinical outcomes of COVID-19 patients.

Revealing genetic predisposition to COVID-19 infection is important for identifying high-risk individuals for the purpose of health care and preventive measure prioritizations like vaccination and monoclonal antibody treatment, it is also important for the development of drugs as it might guide the treatment targets, in the similar way that CR5 variation and HIV infection, helped in identifying drug targets and therapeutic strategies (Kosmicki et al., 2020).

The limitation of this study was that the sample numbers were not enough to perform statistical analyses between comorbid and non-comorbid patients to observe any roles of comorbidities in the severity of the disease.

In conclusion, this work found an association of Apo E4 allele in COVID-19 patients compared with the general population in a cohort study in Iraq for the first time. Also, Apo E3 was suggested to have protective roles; however, larger samples are required for future study to confirm this suggestion. There were no associations in the Apo E2 allele between both patient and general population groups. Future work should also focus on various ethnic backgrounds and comorbidities using a large number of samples to see whether this association can be generalized to the entire world.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Al-Jaf, S.M.A., 2021. Frequencies of Apolipoprotein E polymorphism in Iraqi Kurdish population. Meta Gene 28, 100867. https://doi.org/10.1016/j.mgene.2021.100867.

Atkins, J.L., Masoli, J.A.H., Delgado, J., Pilling, L.C., Kuo, C.L., Kuchel, G.A., Melzer, D., 2020. Preexisting comorbidities predicting COVID-19 and mortality in the UK biobank community cohort. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 75, 2224–2230. https://doi.org/10.1093/gerona/glaa183.

Bajgain, K.T., Badal, S., Bajgain, B.B., Santana, M.J., 2021. Prevalence of comorbidities among individuals with COVID-19: a rapid review of current literature. Am. J. Infect. Control. https://doi.org/10.1016/j.ajic.2020.06.213.

Bont, De, Netea, Demacker, Kullberg, Van Der Meer, Stalenhoef, 2000. Apolipoprotein E-deficient mice have an impaired immune response to *Klebsiella pneumoniae*. Eur. J. Clin. Investig. 30, 818–822. https://doi.org/10.1046/j.1365-2362.2000.00715.x.

Burt, T.D., Agan, B.K., Marconi, V.C., He, W., Kulkarni, H., Mold, J.E., Cavrois, M., Huang, Y., Mahley, R.W., Dolan, M.J., Mccune, J.M., Ahuja, S.K., 2008. Apolipoprotein (Apo) E4 Enhances HIV-1 Cell Entry in Vitro, and the APOE 4/4 Genotype Accelerates HIV Disease Progression.

Cordero, A.I.H., Li, X., Milne, S., Yang, C.X., Bossé, Y., Joubert, P., Timens, W., van den Berge, M., Nickle, D., Hao, K., Sin, D.D., 2021. Multi-omics highlights ABO plasma protein as a causal risk factor for COVID-19. Hum. Genet. 140, 1. https://doi.org/10.1007/S00439-021-02264-5.

Del Ser, T., Fernández-Blázquez, M.A., Valentí, M., Zea-Sevilla, M.A., Frades, B., Alfayate, E., Saiz, L., Calero, O., García-López, F.J., Rábano, A., Medina, M., Calero, M., 2021. Residence, clinical features, and genetic risk factors associated with symptoms of COVID-19 in a cohort of older people in Madrid. Gerontology. https://doi.org/10.1159/000513182.

Ellinghaus, D., F, D., L, B., M, B., A, A., P, I., J, F., D, P., G, B., R, A., MM, G., C, M., F, A., J, K., S, M., M, W., L, W., F, U.-W., T, Z., X, Y., R, de P., AG, C., Pa, K.T., 2020. Genomewide association study of severe covid-19 with respiratory failure. N. Engl. J. Med. 383, 1522–1534. doi:https://doi.org/10.1056/nejmoa2020283.

Fallerini, C., Daga, S., Mantovani, S., Benetti, E., Picchiotti, N., Francisci, D., Paciosi, F., Schiaroli, E., Baldassarri, M., Fava, F., Palmieri, M., Ludovisi, S., Castelli, F., Quiros-Roldan, E., Vaghi, M., Rusconi, S., Siano, M., Bandini, M., Spiga, O., Capitani, K., Furini, S., Mari, F., Study, G.-C.M., Renieri, A., Mondelli, M.U., Frullanti, E., 2021. Association of Toll-like receptor 7 variants with life-threatening COVID-19 disease in males: findings from a nested case-control study. Elife 10. https://doi.org/10.7554/ELIFE.67569.

Figueroa, D.M., Gordon, E.M., Yao, X., Levine, S.J., 2018. Apolipoproteins as context-dependent regulators of lung inflammation. In: Mechanisms and Manifestations of Obesity in Lung Disease. Elsevier, pp. 301–326. https://doi.org/10.1016/B978-0-12-813553-2.00013-0.

Gasmi, A., Peana, M., Pivina, L., Srinath, S., Gasmi Benahmed, A., Semenova, Y., Menzel, A., Dadar, M., Bjørklund, G., 2021. Interrelations between COVID-19 and other disorders. Clin. Immunol. https://doi.org/10.1016/j.clim.2020.108651.

Hou, Y., Zhao, J., Martin, W., Kallianpur, A., Chung, M.K., Jehi, L., Sharifi, N., Erzurum, S., Eng, C., Cheng, F., 2020. New insights into genetic susceptibility of COVID-19: an ACE2 and TMPRSS2 polymorphism analysis. BMC Med. 18 https://doi.org/10.1186/S12916-020-01673-Z.

Hu, J., Li, C., Wang, S., Li, T., Zhang, H., 2020. Genetic variants are identified to increase risk of COVID-19 related mortality from UK Biobank data. medRxiv. https://doi.org/10.1101/2020.11.05.20226761.

Hubacek, J.A., Dlouha, L., Dusek, L., Majek, O., Adamkova, V., 2021. Apolipoprotein E4 allele in subjects with COVID-19. Gerontology 67, 1–3. https://doi.org/10.1159/000516200.

Jin, J.M., Bai, P., He, W., Wu, F., Liu, X.F., Han, D.M., Liu, S., Yang, J.K., 2020. Gender differences in patients with COVID-19: focus on severity and mortality. Front. Public Health 8. https://doi.org/10.3389/fpubh.2020.00152.

Kosmicki, J.A., Horowitz, J.E., Banerjee, N., Lanche, R., Marcketta, A., Maxwell, E., Sun, D., Backman, J., Sharma, D., Yadav, A., Mansfield, A.J., Li, A., Watanabe, K., Gurski, L., McCarthy, S., Locke, A., Khalid, S., Chazara, O., Huang, Y., Nadkar, A., Nioi, P., Parker, M.M., Petrovski, S., Runz, H., Szustakowski, J.D., Wang, Q., Jones, M., Salerno, W., Shuldiner, A., Marchini, J., Overton, J., Habegger, L., Cantor, M.N., Reid, J., Baras, A., Abecasis, G.R., Ferreira, M.A., 2020. Genetic association analysis of SARS-CoV-2 infection in 455,838 UK Biobank participants 1. medRxiv. https://doi.org/10.1101/2020.10.28.20221804, 2020.10.28.20221804.

Kuhlmann, I., Minihane, A.M., Huebbe, P., Nebel, A., Rimbach, G., 2010. Apolipoprotein e genotype and hepatitis C, HIV and herpes simplex disease risk: a literature review. Lipids Health Dis. https://doi.org/10.1186/1476-511X-9-8.

Kuo, C.L., Pilling, L.C., Atkins, J.L., Masoli, J.A.H., Delgado, J., Kuchel, G.A., Melzer, D., 2020a. APOE e4 genotype predicts severe COVID-19 in the UK biobank community cohort. J. Gerontol. Ser. A Biol. Sci. Med. Sci. https://doi.org/10.1093/gerona/glaa131.

Kuo, C.L., Pilling, L.C., Atkins, J.L., Masoli, J.A.H., Delgado, J., Kuchel, G.A., Melzer, D., Newman, A.B., 2020b. ApoE e4e4 genotype and mortality with COVID-19 in UK biobank. J. Gerontol. Ser. A Biol. Sci. Med. Sci. https://doi.org/10.1093/gerona/glaa169.

Liu, S., Liu, J., Weng, R., Gu, X., Zhong, Z., 2019. Apolipoprotein e gene polymorphism and the risk of cardiovascular disease and type 2 diabetes. BMC Cardiovasc. Disord. 19, 1–6. https://doi.org/10.1186/s12872-019-1194-0.

Mahley, R.W., 2016. Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders. J. Mol. Med. https://doi.org/10.1007/s00109-016-1427-y.

Niranji, Sherko, 2020. Genetic Polymorphism of Toll-Like Receptor 4 Thr399Ile Variant in Iraqi Kurdish Population: Sulaymaniyah Province. Passer. https://doi.org/10.24271/psr.08.

Pantelidis, P., Lambert-Hammill, M., Wierzbicki, A.S., 2003. Simple sequence-specific-primer-PCR method to identify the three main apolipoprotein E haplotypes. Clin. Chem. https://doi.org/10.1373/clinchem.2003.021683.

Poirier, J., Bertrand, P., Poirier, J., Kogan, S., Gauthier, S., Poirier, J., Gauthier, S., Davignon, J., Bouthillier, D., Davignon, J., 1993. Apolipoprotein E polymorphism and Alzheimer's disease. Lancet. https://doi.org/10.1016/0140-6736(93)91705-Q.

Roselaar, S.E., Daugherty, A., 1998. Apolipoprotein E-deficient mice have impaired innate immune responses to *Listeria monocytogenes* in vivo. J. Lipid Res. 39, 1740–1743. https://doi.org/10.1016/s0022-2275(20)32160-x.

Schurr, T.G., 2020. Host genetic factors and susceptibility to SARS-CoV-2 infection. Am. J. Hum. Biol. https://doi.org/10.1002/ajhb.23497.

Smith, J.D., 2000. Apolipoprotein E4: an allele associated with many diseases. Ann. Med. https://doi.org/10.3109/07853890009011761.

Wang, Q.Q., Davis, P.B., Gurney, M.E., Xu, R., 2021a. COVID-19 and dementia: analyses of risk, disparity, and outcomes from electronic health records in the US. Alzheimers Dement. https://doi.org/10.1002/alz.12296.

Wang, C., Zhang, M., Garcia, G., Tian, E., Cui, Q., Chen, X., Sun, G., Wang, J., Arumugaswami, V., Shi, Y., 2021b. ApoE-isoform-dependent SARS-CoV-2 Neurotropism and cellular response. Cell Stem Cell 28. https://doi.org/10.1016/j.stem.2020.12.018, 331-342.e5.

WHO, 2020. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19 - 11 March 2020 [WWW Document]. URL. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020.

Wu, Z., McGoogan, J.M., 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. J. Am. Med. Assoc. https://doi.org/10.1001/jama.2020.2648.

Yanes-Lane, M., Winters, N., Fregonese, F., Bastos, M., Perlman-Arrow, S., Campbell, J. R., Menzies, D., 2020. Proportion of asymptomatic infection among COVID-19 positive persons and their transmission potential: a systematic review and meta-analysis. PLoS One 15, e0241536. https://doi.org/10.1371/journal.pone.0241536.

YC, K., BH, J., 2020. Strong correlation between the case fatality rate of COVID-19 and the rs6598045 single nucleotide polymorphism (SNP) of the interferon-induced transmembrane protein 3 (IFITM3) gene at the population-level. Genes (Basel) 12, 1–9. https://doi.org/10.3390/GENES12010042.

Zheng, Z., Peng, F., Xu, B., Zhao, J., Liu, H., Peng, J., Li, Q., Jiang, C., Zhou, Y., Liu, S., Ye, C., Zhang, P., Xing, Y., Guo, H., Tang, W., 2020. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. J. Inf. Secur. https://doi.org/10.1016/j.jinf.2020.04.021.