



Mendelian Randomization Study on Causal Association of Pyroglutamine with COVID-19

Wenting Su¹ · Shan Zhou¹ · Gaizhi Zhu¹ · Yaqi Xu¹ · Ran Gao¹ · Min Zhang¹ · Qi Zeng¹ · Renxi Wang¹

Received: 8 August 2022 / Accepted: 5 October 2022
© The Author(s) 2022

Abstract

Background Glutamine family amino acids such as glutamate, pyroglutamate, and glutamine have been shown to play important roles in COVID-19. However, it is still unclear about the role of pyroglutamate in COVID-19. Thus, we use a two-sample Mendelian randomization (MR) study to identify the genetic causal link between blood pyroglutamine levels and COVID-19 risk.

Methods Pyroglutamine genetic instrumental variables (IVs) were chosen from the largest pyroglutamine-associated genome-wide association studies (GWAS). The largest COVID-19 GWAS dataset was employed to evaluate the causal link between blood pyroglutamine levels and COVID-19 risk using two-sample MR analysis.

Results We found no significant pleiotropy or heterogeneity of pyroglutamine-associated genetic IVs in COVID-19 GWAS. Interestingly, we found that as pyroglutamine genetically increased, the risk of COVID-19 decreased using inverse variance weighted (IVW) (Beta = - 0.644, $p = 0.003$; OR = 0.525, 95% CI [0.346–0.798]) and weighted median (Beta = - 0.609, $p = 0.013$; OR = 0.544, 95% CI [0.337–0.878]).

Conclusion Our analysis suggests a causal link between genetically increased pyroglutamine and reduced risk of COVID-19. Thus, pyroglutamine may be a protective factor for patients with COVID-19.

Keywords Pyroglutamine · Genetic variants · COVID-19 · Genome-wide association study · Mendelian randomization

Abbreviations

IVs	Instrumental variants
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Corona virus disease 2019
GWAS	Genome-wide association study
MR	Mendelian randomization
SNP	Single-nucleotide polymorphism
IVW	Inverse variance weighted

1 Introduction

In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused humans mild to severe acute respiratory syndrome called as corona virus disease (COVID-19) [1]. The ongoing COVID-19 pandemic have affected millions of people and caused a serious public health threat worldwide [2]. To effective control of its spread, it is important to understand the risk and protective factors involved in this disease.

The major COVID-19-associated metabolic risks could predispose certain groups to severe COVID-19 complications [3]. Compare with healthy controls, COVID-19 and COVID-19-like patients have enriched serum L-glutamic acids [4]. L-pyroglutamic acids, a cyclized derivative of L-glutamic acids, have been proposed as potential antiviral candidates to treatment of COVID-19 based on calculations and molecular docking [5]. Deficiency of glutamine, made via the action of glutamine synthetase from glutamate and ammonia, has been proposed to be the central metabolic characteristic of COVID-19 and its high-risk groups [3]. The usage of glutamine supplementation has been suggested to

✉ Renxi Wang
renxi_wang@ccmu.edu.cn

¹ Beijing Institute of Brain Disorders, Laboratory of Brain Disorders, Ministry of Science and Technology, Collaborative Innovation Center for Brain Disorders, Capital Medical University, No. 10 Xitoutiao, You An Men, Beijing 100069, China

prevent the development of severe COVID-19 pneumonia [3]. However, it is still unclear about the role of pyroglutamate, a cyclic amino acid formed as a result of dehydration of glutamate, in COVID-19.

Many factors including reverse causation and confounding bias observational studies and result in the absence of high-quality randomized controlled trials (RCTs) [6–14]. Mendelian randomization (MR) studies highly similar to RCTs, using genetic variants independent of many factors that bias observational studies such as RCTs, have many advantages over RCTs in assessing the causal link between an exposure and an outcome [8–14]. Thus, pyroglutamine genetic variants were used as instrumental variables (IVs) to identify the causal association of pyroglutamine with COVID-19 in two-sample MR analysis.

2 Materials and Methods

2.1 Pyroglutamine Genetic Instrumental Variables (IVs)

Pyroglutamine genetic IVs were chosen from the largest pyroglutamine GWAS. This GWAS was reported by So-Youn Shin et al. in 2014 [15]. Its primary aim is to provide unprecedented insights into how genetic variation influences metabolism and complex disease using genome-wide association scans with high-throughput metabolic profiling. Pyroglutamine GWAS has 7800 European participants. The summary statistics for genetic associations of pyroglutamine are available at <https://gwas.mrcieu.ac.uk/datasets/met-a-501>. Four independent pyroglutamine genetic IVs were obtained based on the following three criteria: (1)

genome-wide significance threshold p value $< 5 \times 10^{-8}$; (2) $r^2 < 0.001$, indicating no linkage disequilibrium between SNPs by the Linkage disequilibrium (LD) analysis using LDlink (<https://ldlink.nci.nih.gov/?tab=ldmatrix>, CEU); (3) no effects on other potential risk factors including body mass index, smoking, and blood pressure. Detailed information about these IVs is shown in Table 1. R^2 is the proportion of pyroglutamine variance explained for each independent SNP and estimated based on beta, standard error, and sample size. F -statistic was used to assess the strength of relationship between IVs and phenotype, and calculated using the following equation: $F = R^2 * (N-2)/(1-R^2)$ [16], where R^2 is the proportion of pyroglutamine variance, k is the number of instruments used in the model and n is the sample size.

2.2 COVID-19 GWAS Dataset

The largest GWAS for COVID-19 (RELEASE 4) was described by the COVID-19 Host Genetics Initiative in 2020 [17]. This GWAS dataset is based on 14,134 cases and 1,284,876 controls with European ancestry. The profile of this GWAS is provided in Table 2. The summary dataset is available at <https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST010780>.

2.3 Association of Pyroglutamine Genetic Instrumental Variables (IVs) in COVID-19 GWAS

Potential proxy SNPs were identified by the LD proxy Tool ($r^2 > 0.8$) when pyroglutamine IVs could not be found in COVID-19 summary statistics. However, we were able to successfully extract four independent pyroglutamine genetic IVs from the COVID-19 GWAS summary dataset. The

Table 1 Pyroglutamine genetic instrumental variables (IVs)

SNP	EA	NEA	EAF	Beta	SE	p val	Sample size	R^2 (%)	F -statistics
rs715	C	T	0.286	-0.036	0.004	2.46E-16	7354	0.91	67.31
rs17279437	A	G	0.095	0.059	0.006	1.25E-20	7354	1.18	87.70
rs11613331	A	G	0.553	0.037	0.004	2.23E-25	7354	1.45	107.93
rs1600760	A	T	0.663	-0.022	0.004	5.12E-09	7354	0.46	34.08

R^2 the proportion of pyroglutamine variance explained by the selected genetic variants, F -statistics the strength of relationship between IVs and phenotype

IVs instrumental variables, SNP single-nucleotide polymorphism, EA effect allele, NEA non-effect allele, EAF effect allele frequency, Beta the regression coefficient based on the pyroglutamine effect allele, SE standard error

Table 2 Corona virus disease 2019 (COVID-19) genome-wide association study (GWAS)

GWAS ID	Year	Trait	ncase	ncontrol	nsnp	Population	PMID
ebi-a-GCST010780	2020	COVID-19 (RELEASE 4)	14,134	1,284,876	12,508,741	European	32404885

COVID-19 corona virus disease 2019, GWAS genome-wide association study, GWAS ID GWAS identity, ncase the number of COVID-19 case, ncontrol the number of the control, nsnp the number of single-nucleotide polymorphism, PMID PubMed unique identifier

association of four independent pyroglutamine genetic IVs with COVID-19 GWAS is shown in Table 3.

2.4 Pleiotropy and Heterogeneity Test

MR-egger_intercept and MR-pleiotropy residual sum and outlier (MR-PRESSO) tests have previously been described to test the pleiotropy [18]. MR_Egger is based on the same regression model with inverse variance weighted (IVW), but allows and accounts for the potential pleiotropy using the MR-Egger intercept test [18–20]. If the selected genetic variants are not pleiotropic, then the MR_Egger intercept term should tend to zero as the sample size increases [20]. MR-PRESSO could detect and correct for the horizontal pleiotropy via outlier removal (the MR-PRESSO outlier test) [18]. MR-egger_intercept and PRESSO methods were used to test the pleiotropy of independent pyroglutamine genetic IVs in COVID-19 GWAS dataset. MR_egger and IVW in Cochran’s *Q* statistic have been broadly used to examine the heterogeneity [21, 22]. Cochran’s *Q* statistic could provide evidence of heterogeneity due to pleiotropy or other causes.

[21]. Thus, MR_egger and IVW in Cochran’s *Q* statistic were used to test the heterogeneity of independent pyroglutamine genetic IVs in COVID-19 GWAS dataset. Table 4 demonstrates the results about pleiotropy and heterogeneity test. When $p > 0.05$, there is no significant pleiotropy or heterogeneity of four independent pyroglutamine genetic IVs in COVID-19 GWAS.

2.5 MR Analysis

Two methods including IVW and weighted median were used to analyze the causal association of blood pyroglutamine levels with COVID-19. The IVW was selected as the main MR analysis method to combine the variant-specific Wald estimators by taking the inverse of their approximate variances as the corresponding weights [20]. In addition, we also selected the weighted median that could produce consistent estimates even up to 50% of selected genetic variants are not valid [18–20]. Table 5 demonstrates the results about MR analysis. When $p < 0.05$, there is the causal association of pyroglutamine with COVID-19.

Table 3 Association of pyroglutamine genetic instrumental variables (IVs) with COVID-19 GWAS

SNP	Exposure (pyroglutamine) GWAS			Outcome (COVID-19) GWAS		
	Beta	SE	<i>p</i> val	Beta	SE	<i>p</i> val
rs11613331	0.037	0.004	2.23E-25	− 0.024	0.013	0.055
rs1600760	− 0.022	0.004	5.12E-09	− 0.002	0.013	0.891
rs17279437	0.059	0.006	1.25E-20	− 0.067	0.023	0.004
rs715	− 0.036	0.004	2.46E-16	0.015	0.014	0.289

IVs instrumental variables, COVID-19 corona virus disease 2019, GWAS genome-wide association study, SNP single-nucleotide polymorphism, Beta the regression coefficient based on pyroglutamine raising effect allele, SE standard error

Table 4 Pleiotropy and heterogeneity test of pyroglutamine genetic instrumental variables (IVs) in COVID-19 GWAS

Pleiotropy test				Heterogeneity test					
MR_Egger			Presso	MR Egger			IVW		
Intercept	SE	<i>p</i> val	<i>p</i> val	<i>Q</i>	<i>Q</i> _df	<i>Q</i> _p val	<i>Q</i>	<i>Q</i> _df	<i>Q</i> _p val
0.043	0.025	0.224	0.454	0.266	2	0.876	3.285	3	0.350

p val > 0.05 represent no significant pleiotropy. Q _p val > 0.05 represents no significant heterogeneity
IVs instrumental variables, COVID-19 corona virus disease 2019, GWAS genome-wide association study, IVW inverse variance weighted, SE standard error

Table 5 The causal association of blood pyroglutamine levels with COVID-19

Method	nsnp	Beta	SE	<i>p</i> val	OR	OR_lci95	OR_uci95
IVW	4	− 0.644	0.213	0.003	0.525	0.346	0.798
Weighted median	4	− 0.609	0.244	0.013	0.544	0.337	0.878

COVID-19 corona virus disease 2019, IVW, inverse variance weighted, nsnp the number of single-nucleotide polymorphism, Beta the regression coefficient based on pyroglutamine raising effect allele, SE standard error, $p < 0.05$ represents the causal association of the increased pyroglutamine levels with COVID-19, OR odds ratio, OR_lci95 lower limit of 95% confidence interval for OR, OR_uci95 upper limit of 95% confidence interval for OR

2.6 Each SNP Effect Analysis

To analyze each SNP effect, three analysis methods including individual causal effect, each SNP effect size, and SNPs leave-one-out effect were used to explore the effect of pyroglutamine SNP on COVID-19 and shown in Figs. 1, 2, and 3, respectively.

3 Results

3.1 Four Pyroglutamine Genetic Instrumental Variables (IVs) Have no Significant Pleiotropy or Heterogeneity

To identify the causal association of pyroglutamine with COVID-19, we first chose four independent pyroglutamine genetic variants as potential IVs from pyroglutamine GWAS (Table 1). All these selected four genetic variants could explain 3.99% variance of blood pyroglutamine levels (Table 1). The F -statistics of the selected four IVs were all above the threshold of weak instruments of F -statistic < 10 [23], indicating strong IVs for this MR study (Table 1).

We successfully extracted four independent pyroglutamine genetic variants (Table 1) from COVID-19 GWAS dataset (Table 2). The association of pyroglutamine genetic variants within COVID-19 GWAS dataset is shown (Table 3). We found no significant pleiotropy or

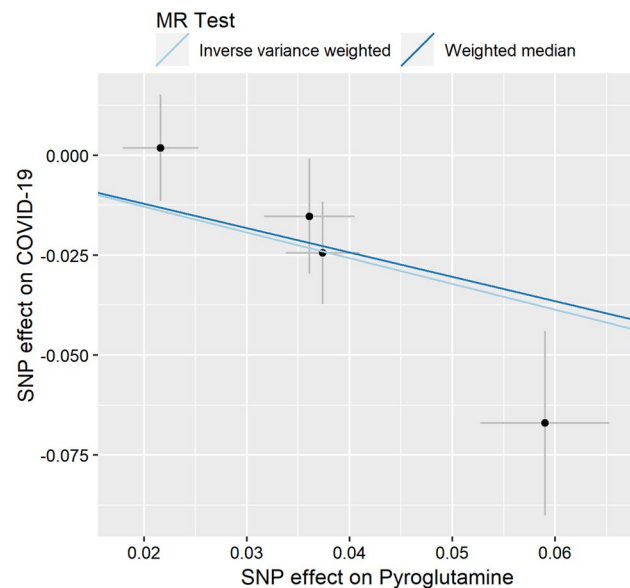


Fig. 1 Individual estimates about the causal effect of pyroglutamine on COVID-19. The x-axis shows the SNP (single-nucleotide polymorphism) effect and SE (standard error) on each of pyroglutamine. The y-axis shows the SNP effect and SE on COVID-19. The regression line for inverse variance weighted (IVW) and weighted median is shown

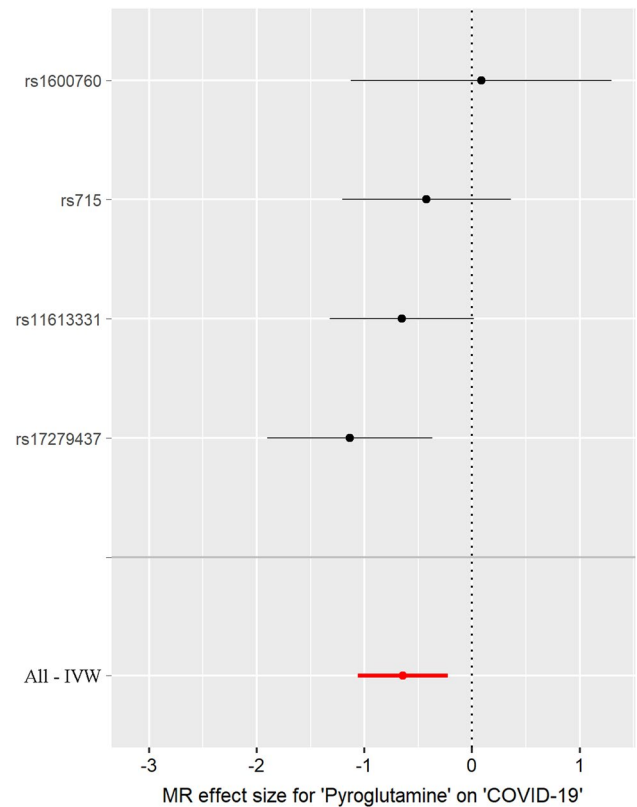


Fig. 2 Forest plot of pyroglutamine-associated with risk of COVID-19. The x-axis shows MR effect size for pyroglutamine on COVID-19. The y-axis shows the analysis for each of SNPs

heterogeneity of four independent pyroglutamine genetic variants in COVID-19 GWAS dataset (Table 4). Therefore, all selected pyroglutamine genetic variants can be taken as the effective IVs in our MR study to explore the causal association of pyroglutamine with COVID-19.

3.2 Pyroglutamine Genetically Reduces COVID-19 Risk

Interestingly, we found that as pyroglutamine genetically increased, the risk of COVID-19 decreased using IVW (Beta = -0.644 , $p = 0.003$; OR = 0.525, 95% CI [0.346–0.798]) and weighted median (Beta = -0.609 , $p = 0.013$; OR = 0.544, 95% CI [0.337–0.878]) (Table 5). Collectively, our data suggested a causal association of genetically increased pyroglutamine levels with the reduced risk of COVID-19.

3.3 Single SNP Effect of Pyroglutamine on COVID-19 is Robust Without Obvious Bias

The individual MR estimates demonstrated that as the effect of each SNP on pyroglutamine increased, the suppressive

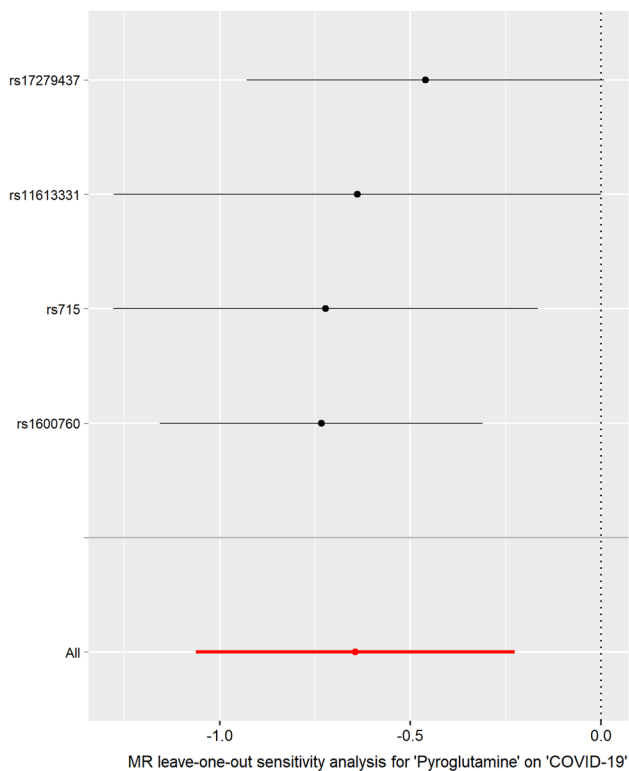


Fig. 3 MR leave-one-out sensitivity analysis for the effect of pyroglutamine SNPs on COVID-19. The x-axis shows MR leave-one-out sensitivity analysis for pyroglutamine on COVID-19. The y-axis shows the analysis for the effect of leave-one-out of SNPs on COVID-19

effect of each SNP on COVID-19 increased using IVW and weighted median (Fig. 1). All effect size analyses suggest that each effect of pyroglutamine SNPs on COVID-19 was robust (Fig. 2). MR leave-one-out sensitivity analysis suggested that removing a specific SNP of the four pyroglutamine SNPs did not change the results (Fig. 3). Altogether, these results indicate that our data were robust without obvious bias.

4 Discussion

Previous studies have shown that glutamine family amino acids such as glutamate, pyroglutamate, glutamine have important roles in COVID-19 [3–5, 24–26]. In the present study, we used two-sample MR study and found a causal link between genetically increased pyroglutamine levels and reduced risk of COVID-19. Our findings showed that genetic predisposition to a higher pyroglutamine level may be genetically associated with lower risk of COVID-19.

Serum pyroglutamine has been reported to be inversely associated with overall prostate cancer (OR = 0.53, 95% CI [0.36–0.78], $p = 0.0013$) [25]. The metabolomics profiles of severe COVID-19 patients and patients with advanced

cancer are similar and SARS-CoV-2 infection promotes a cancer-like metabolism [3]. These researches propose that pyroglutamine may be inversely associated with COVID-19. As expected, our results proved the proposal.

The concentrations of pyroglutamine in the blood serum were increased in patients who took antihypertensives such as beta-blockers [26]. Hypertension has emerged as significant risk factors for COVID-19 [27]. Antihypertensive drugs have been implicated in COVID-19 susceptibility and severity [28]. These studies suggest that pyroglutamine may be positively associated with anti-COVID-19 therapy.

Pyroglutamine is a cyclic derivative of glutamine related to pyroglutamic acid [29]. Pyroglutamate (or pyroglutamic acid) is an intermediate in the glutathione metabolism and a marker of glutathione deficiency [30]. Glutathione is one of the most potent anti-oxidants in the human body. In fact, glutathione plays an important role in cell proliferation [31]. An increase of intracellular oxidative stress likely leads to its cytotoxicity, inhibition of cell proliferation, and induction of cell death [32]. It also suggests that the glutamic acid rather than the cysteine released from glutathione is responsible for the cell proliferation [33]. The virus-host-specific interactions, molecular targets on host cell deaths, and the involved signaling are crucial issues, which become potential targets for treatment [34]. Anti-COVID-19 action of puerarin was associated with the suppression of oxidative stress and inflammatory cascades, and cell apoptosis [35]. Thus, it is possible that pyroglutamine suppresses oxidative stress to reduce host cell apoptosis in patients with COVID-19.

This study has several strengths. First, pyroglutamine genetic IVs are chosen from the largest pyroglutamine GWAS reported by So-Youn Shin et al. in 2014 [15]. Second, we used the largest GWAS for COVID-19 described by the COVID-19 Host Genetics Initiative in 2020 [17]. Third, both pyroglutamine genetic IVs and COVID-19 GWAS are from European ancestry. Thus, it removed the influence of population stratification. Fourth, four independent pyroglutamine genetic IVs were successfully extracted from COVID-19 GWAS. Fifth, we used four different analysis methods demonstrated no significant pleiotropy or heterogeneity of pyroglutamine genetic IVs as the effective IVs. Sixth, two MR analysis including IVW and weighted median proved the causal link between genetically increased pyroglutamine levels and reduced risk of COVID-19. Finally, all three methods demonstrated that each effect of pyroglutamine SNPs on COVID-19 was robust without obvious bias.

This study has several limitations. First, because pyroglutamine genetic IVs and COVID-19 GWAS are from European ancestry, similar results in other ancestries need be proven. Second, it is necessary to clarify whether pyroglutamine could reduce the risk of COVID-19 by randomized controlled trials. Third, it is still unclear about the underlying mechanism by which pyroglutamine genetically reduced

COVID-19 risk that is worth to be explored in the future. Finally, further research on pyroglutamine is needed since too little is known so far about its physiological role [26].

In conclusion, our analysis suggested a causal link between genetically increased pyroglutamine and reduced risk of COVID-19. Thus, pyroglutamine may be a protective factor for patients with COVID-19.

Acknowledgements We thank IEU OpenGWAS project (<https://gwas.mrcieu.ac.uk/datasets/>) for providing summary results data for these analyses.

Author Contributions RW conceived and initiated the project, analyzed the data, and wrote the manuscript. All authors contributed to the interpretation of the results and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

Funding This study was supported by grants from National Natural Science Foundation of China (82071758 and 32270933) and R&D Program of Beijing Municipal Education Commission (KZ202210025035).

Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author [Renxi Wang], on reasonable request.

Declarations

Conflict of Interest The authors declare they have no conflicts of interest.

Ethical Approval and Consent to participate Not applicable.

Consent for Publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Rai P, Kumar BK, Deekshit VK, Karunasagar I, Karunasagar I. Detection technologies and recent developments in the diagnosis of COVID-19 infection. *Appl Microbiol Biotechnol*. 2021;105:441–55.
- Majumder J, Minko T. Recent developments on therapeutic and diagnostic approaches for COVID-19. *AAPS J*. 2021;23:14.
- Matsuyama T, Yoshinaga SK, Shibue K, Mak TW. Comorbidity-associated glutamine deficiency is a predisposition to severe COVID-19. *Cell Death Differ*. 2021;28:3199–213.
- Shi D, Yan R, Lv L, Jiang H, Lu Y, Sheng J, et al. The serum metabolome of COVID-19 patients is distinctive and predictive. *Metabolism*. 2021;118: 154739.
- Sagaama A, Brandan SA, Ben Issa T, Issaoui N. Searching potential antiviral candidates for the treatment of the 2019 novel coronavirus based on DFT calculations and molecular docking. *Heliyon*. 2020;6: e04640.
- Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–63.
- Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, et al. Vitamin D and risk of multiple sclerosis: a mendelian randomization study. *PLoS Med*. 2015;12: e1001866.
- Zhu G, Zhou S, Xu Y, Gao R, Li H, Zhai B, et al. Mendelian randomization study on the causal effects of omega-3 fatty acids on rheumatoid arthritis. *Clin Rheumatol*. 2022;41:1305–12.
- Zhu G, Zhou S, Xu Y, Gao R, Li H, Su W, et al. Mendelian randomization study on the causal effects of COVID-19 on childhood intelligence. *J Med Virol*. 2022;94:3233–9.
- Zhou S, Zhu G, Xu Y, Gao R, Li H, Han G, et al. Mendelian randomization study on the putative causal effects of omega-3 fatty acids on low back pain. *Front Nutr*. 2022;9: 819635.
- Xu Y, Gao R, Zhu G, Zhou S, Li H, Su W, et al. Genetic variation of allergic disease is associated with the susceptibility to COVID-19. *J Infect*. 2022;84:e92–3.
- Wang R. Genetic variation of interleukin-1 receptor type 1 is associated with severity of COVID-19 disease. *J Infect*. 2022;84:e19-21.
- Wang R. Mendelian randomization study updates the effect of 25-hydroxyvitamin D levels on the risk of multiple sclerosis. *J Transl Med*. 2022;20:3.
- Gao R, Xu Y, Zhu G, Zhou S, Li H, Han G, et al. Genetic variation associated with COVID-19 is also associated with endometrial cancer. *J Infect*. 2022;84:e85–6.
- Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, et al. An atlas of genetic influences on human blood metabolites. *Nat Genet*. 2014;46:543–50.
- Zhang W, Wu P, Yin R, Sun M, Zhang R, Liao X, et al. Mendelian randomization analysis suggests no associations of herpes simplex virus infections with multiple sclerosis. *Front Neurosci*. 2022;16: 817067.
- COVID-19 Host Genetics Initiative. The COVID-19 host genetics initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic. *Eur J Hum Genet*. 2020;28:715–8.
- Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50:693–8.
- Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304–14.
- Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-egger method. *Eur J Epidemiol*. 2017;32:377–89.
- Greco MF, Minelli C, Sheehan NA, Thompson JR. Detecting pleiotropy in mendelian randomisation studies with summary data and a continuous outcome. *Stat Med*. 2015;34:2926–40.
- Liu G, Zhang S, Cai Z, Ma G, Zhang L, Jiang Y, et al. PICALM gene rs3851179 polymorphism contributes to Alzheimer's disease in an Asian population. *Neuromolecular Med*. 2013;15:384–8.
- Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in mendelian randomization studies. *Int J Epidemiol*. 2011;40:755–64.
- Mogensen KM, Lasky-Su J, Rogers AJ, Baron RM, Fredenburgh LE, Rawn J, et al. Metabolites associated with malnutrition in the intensive care unit are also associated with 28-day mortality. *JPEN J Parenter Enteral Nutr*. 2017;41:188–97.

25. Huang J, Mondul AM, Weinstein SJ, Koutros S, Derkach A, Karoly E, et al. Serum metabolomic profiling of prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Br J Cancer*. 2016;115:1087–95.
26. Altmaier E, Fobo G, Heier M, Thorand B, Meisinger C, Romisch-Margl W, et al. Metabolomics approach reveals effects of antihypertensives and lipid-lowering drugs on the human metabolism. *Eur J Epidemiol*. 2014;29:325–36.
27. Perez A, Naljayan M, Shuja I, Florea A, Reisin E. Hypertension, OBESITY, and COVID-19: a collision of pandemics. *Curr Hypertens Rep*. 2021;23:36.
28. Rezel-Potts E, Douiri A, Chowiecnyk PJ, Gulliford MC. Antihypertensive medications and COVID-19 diagnosis and mortality: population-based case-control analysis in the United Kingdom. *Br J Clin Pharmacol*. 2021;87:4598–607.
29. Liu YD, Goetze AM, Bass RB, Flynn GC. N-terminal glutamate to pyroglutamate conversion in vivo for human IgG2 antibodies. *J Biol Chem*. 2011;286:11211–7.
30. Roesel RA, Hommes FA, Samper L. Pyroglutamic aciduria (5-oxoprolinuria) without glutathione synthetase deficiency and with decreased pyroglutamate hydrolase activity. *J Inher Metab Dis*. 1981;4:89–90.
31. Pallardo FV, Markovic J, Garcia JL, Vina J. Role of nuclear glutathione as a key regulator of cell proliferation. *Mol Aspects Med*. 2009;30:77–85.
32. Ma B, Guo S, Nishina Y, Bianco A. Reaction between graphene oxide and intracellular glutathione affects cell viability and proliferation. *ACS Appl Mater Interfaces*. 2021;13:3528–35.
33. Kang YJ, Feng Y, Hatcher EL. Glutathione stimulates A549 cell proliferation in glutamine-deficient culture: the effect of glutamate supplementation. *J Cell Physiol*. 1994;161:589–96.
34. Yapasert R, Khaw-On P, Banjerdpongchai R. Coronavirus infection-associated cell death signaling and potential therapeutic targets. *Molecules*. 2021;26(24):7459.
35. Qin X, Huang C, Wu K, Li Y, Liang X, Su M, et al. Anti-coronavirus disease 2019 (COVID-19) targets and mechanisms of puerarin. *J Cell Mol Med*. 2021;25:677–85.