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Association between regional selenium status and reported outcome of COVID-19 cases in China

Dear Editor:

Potentially relevant to the recent appearance of COVID-19 in China is the fact that there is a belt of selenium deficiency running from northeast to southwest in the country and, indeed, China has populations that have both the lowest and the highest selenium status in the world (1). A set of interesting studies published by the Beck laboratory in the 1990s showed that host selenium deficiency increased the virulence of RNA viruses such as coxsackievirus B3 and influenza A (2, 3). Passage through a selenium-deficient animal that was unable to produce sufficient antioxidant selenoproteins for its own protection resulted in the virus mutating to a virulent form that caused more severe pathology (2, 3). Those findings shed light on a human selenium-deficiency disease, a cardiomyopathy known as Keshan disease, named after the area in northeast China where it was endemic. The disease showed a seasonal variation, suggesting a viral cofactor that was later identified as coxsackievirus B3 (2). When the population was supplemented with selenium, the incidence of Keshan disease decreased dramatically (1, 2).

Significant clinical benefits of selenium supplementation have also been demonstrated in other viral infections, as reviewed previously (4, 5), including HIV-1 [where a negative correlation between selenium status and mortality has been established (1, 6)]; in liver cancer linked to hepatitis B; and in patients with "epidemic hemorrhagic fever" that was successfully treated with oral sodium selenite, giving an overall 80% reduction in mortality (4, 7). As such, selenium appears relevant to a number of evolutionarily distinct viruses, via potential immunomodulatory effects that are fully consistent with the many essential roles of selenium in the immune system (2) and its ability (especially in deficiency) to influence viral mutation and evolution (3). These and other studies prompted us to hypothesize that selenium status was associated with COVID-19 disease outcome in China.

In this population-based, retrospective analysis, we collected real-time data from the Baidu website, a nongovernmental website that provides daily updates of the reports of the health commissions of each province, municipality, or city on numbers of COVID-19 confirmed cases, numbers cured, and numbers who died (8). [According to the National Health Commission of China, cured patients are those in whom temperature has returned to normal for >3 d, respiratory symptoms are significantly improved, lung imaging shows significant reduction of inflammation, and there is a negative nucleic acid test of respiratory pathogen on 2 consecutive occasions with a sampling interval of at least 1 d (9).] Cure rate and death rate were defined as percentage of patients cured or who died, respectively, from infection with SARS-CoV-2. We tracked the course of the outbreak from 14 February and chose data from 18 February as a "snapshot" of the progress of the outbreak to that date. We included provinces or municipalities with >200 cases and cities with >40 cases (**Supplemental Table 1**).

The largest data sets available on selenium status in China are on hair selenium concentration (**Supplemental Table 2**), which, in a previous study, was found to be highly correlated with selenium intake in different Chinese districts ($R^2 = 0.74$) (10). Data on hair selenium are generally more available for cities. Seventeen cities outside Hubei Province included in the study had documented hair selenium data (Supplemental Table 2).

We compared cure rate and death rate using the Stata prtest to compare 2 proportions (StataCorp 2019 Stata Statistical Software: Release 16). The *prtest* of the difference of 2 proportions uses an asymptotically normally distributed test statistic derived from the proportions and the SE of the difference. Associations between cure rates and mean regional or city hair selenium concentration were analyzed by fitting weighted linear regression models, weighted by the number of cases. *P* values (2-sided tests) from the *F* test of overall significance are presented.

The cure rate inside Hubei Province, of which Wuhan is the capital, was significantly lower than that in all other provinces combined (designated outside-Hubei): 13.2% compared with 40.6%, respectively ($P < 0.0001$; Supplemental Table 1). Correspondingly, the death rate inside Hubei Province was significantly higher than the death rate in provinces outside-Hubei: 3.0% compared with 0.6%, respectively ($P < 0.0001$; Supplemental Table 1). These analyses show that the outcome data for Hubei and outside-Hubei are statistically distinct, necessitating the separate treatment of Hubei (where mortality was much higher) and outside-Hubei in our subsequent analyses.

On inspection of the Hubei data, it is notable that the cure rate in Enshi city, at 36.4%, was much higher than that of other Hubei cities, where the overall cure rate was 13.1% (Supplemental Table 1); indeed, the Enshi cure rate was significantly different from that in the rest of Hubei ($P < 0.0001$). Enshi is renowned for its high selenium intake and status [mean ± SD hair selenium: 3.13 ± 1.91 mg/kg for females and 2.21 ± 1.14 mg/kg for males (11)]—compare typical levels in Hubei of 0.55 mg/kg (10)—so much so that selenium toxicity was observed there in the 1960s (11, 12). Selenium intake in Enshi was reported as 550 μg/d in 2013 (11).

Similar inspection of data from provinces outside Hubei shows that Heilongjiang Province in northeast China, a notoriously low-selenium region in which Keshan is located, had a much higher death rate, at 2.4%, than that of other provinces (0.5%; $P < 0.0001$). The selenium intake was recorded as only 16 μg/d in a 2018 publication (13), while hair selenium in the Songnen Plain of Heilongjiang was measured as only 0.26 mg/kg (Supplemental Table 2) (10, 13).

Finally, we found a significant association between cure rate and background selenium status in cities outside Hubei ($R^2 = 0.72$, *F* test $P < 0.0001$; **Figure 1**, Supplemental Table 2). No correlation analysis was done for cities inside Hubei because selenium status was only available for 2 cities.

Our results show an association between the reported cure rates for COVID-19 and selenium status. These data are consistent with the

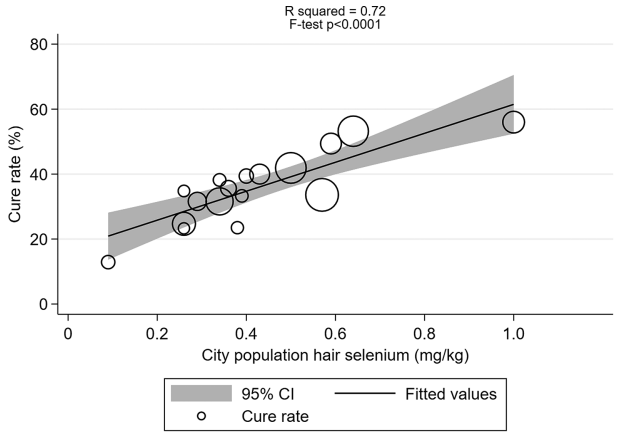

FIGURE 1 Correlation between COVID-19 cure rate in 17 cities outside Hubei, China, on 18 February, 2020 and city population selenium status (hair selenium concentration) analyzed using weighted linear regression (mean ± SD = 35.5 ± 11.1, $R^2 = 0.72$, F test $P < 0.0001$). Each data point represents the cure rate, calculated as the number of cured patients divided by the number of confirmed cases, expressed as a percentage. The size of the marker is proportional to the number of cases.

evidence of the antiviral effects of selenium from previous studies (2, 5–7, 14). Indeed, multiple cellular and viral mechanisms involving selenium and selenoproteins could influence viral pathogenicity, including virally encoded selenium-dependent glutathione peroxidases [reviewed in (14, 15)]. Such viral mechanisms could contribute to the well-documented oxidative stress associated with many RNA virus infections (2, 5, 6, 14, 15); increased viral replication (hence increased mutation rate); and observed higher pathogenicity or mortality under selenium deficiency, as reported here for SARS-CoV-2.

As with most ecological studies, our study has several important limitations. The association between hair selenium and COVID-19 cure rate that we note is based on city population selenium status data, mostly dating from 2011, although some data are considerably older. Furthermore, we were unable to collect city- or patient-level data for the following likely confounders: age and comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancer (16). We also lack information on variation in medical facilities and therapy protocols (including the use of traditional Chinese medicine or anti-viral therapies). Clearly, we were not able to adjust for these possible confounders in the analysis.

We are fully aware, therefore, that the association shown is far from being robust to criticisms of confounding. At best, it points towards the need for further research, particularly when viewed in the context of associations between selenium status and disease outcome found with other viruses (3, 5–7). In due course, more individual-level data will emerge, and the association between the severity of COVID-19 and many factors, including selenium, can be explored.

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Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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References

1. Rayman MP. Selenium and human health. Lancet 2012;379:1256–68.
2. Hoffmann PR, Berry MJ. The influence of selenium on immune responses. Mol Nutr Food Res 2008;52:1273–80.
3. Beck MA, Handy J, Levander OA. Host nutritional status: the neglected virulence factor. Trends Microbiol 2004;12:417–23.
4. Taylor EW, Ruzicka JA, Premadasa L, Zhao L. Cellular selenoprotein mRNA tethering via antisense interactions with Ebola and HIV-1 mRNAs may impact host selenium biochemistry. Curr Top Med Chem 2016;16:1530–5.
5. Steinbrenner H, Al-Quraishy S, Dkhil MA, Wunderlich F, Sies H. Dietary selenium in adjuvant therapy of viral and bacterial infections. Adv Nutr 2015;6:73–82.
6. Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB. High risk of HIV-related mortality is associated with selenium deficiency. J Acquir Immune Defic Syndr Hum Retrovirol 1997;15:370–4.
7. Hou JC. Inhibitory effect of selenite and other antioxidants on complement-mediated tissue injury in patients with epidemic hemorrhagic fever. Biol Trace Elem Res 1997;56:125–30.
8. Baidu. Epidemic real-time big data report [Internet] [cited 18 February, 2020]. Available from: https://voice.baidu.com/act/newpneumonia/newpneumonia/?from = osari_pc_3.
9. Baidu. Commission answered the questions of covered journalists: the discharge standard of patients with new coronary pneumonia is unified nationally [Internet] [cited 18 February, 2020]. Available from: https://baijiahao.baidu.com/s?id=1657688134616589632&wfr=spider&for=pc.
10. Li S, Banuelos GS, Wu L, Shi W. The changing selenium nutritional status of Chinese residents. Nutrients 2014;6:1103–14.
11. Huang Y, Wang Q, Gao J, Lin Z, Banuelos GS, Yuan L, Yin X. Daily dietary selenium intake in a high selenium area of Enshi, China. Nutrients 2013;5:700–10.
12. Yang GQ, Xia YM. Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. Biomed Environ Sci 1995;8:187–201.
13. Dinh QT, Cui Z, Huang J, Tran TAT, Wang D, Yang W, Zhou F, Wang M, Yu D, Liang D. Selenium distribution in the Chinese environment and its relationship with human health: a review. Environ Int 2018;112:294–309. See also Online Supplementary Material, Table S5, Human daily dietary Se intake in China.
14. Guillin OM, Vindry C, Ohlmann T, Chavatte L. Selenium, selenoproteins and viral infection. Nutrients 2019;11:2101.

15. Zhao L, Cox AG, Ruzicka JA, Bhat AA, Zhang W, Taylor EW. Molecular modeling and in vitro activity of an HIV-1-encoded glutathione peroxidase. Proc Natl Acad Sci USA 2000;97:6356–61.
16. Epidemiological Group of Emergency Response Mechanism of New Coronavirus Pneumonia, Chinese Center for Disease Control and Prevention. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China [J]. Chinese J Epidemiol 2020;41(2):145–51.

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Application and interpretation of Mendelian randomization approaches in exploring the causality between folate and coronary artery disease

Dear Editor:

It has long been disputed whether folate deficiency plays a causal role in cardiovascular disease and whether folate fortification should be promoted worldwide. Recently we read with great interest the article by Long et al. (1) that aimed at investigating the association, possibly causation, between serum folate and coronary artery disease (CAD) in a Chinese population. We applaud the authors for rigorously completing observational epidemiological analyses based on a nested case-control study. Nevertheless, we wish to comment on their subsequent findings through Mendelian randomization (MR) approaches.

Firstly, there seemed to be several numeric errors when they conducted the 2-sample MR using publicly shared summary statistics. The authors selected 3 genome-wide significant single nucleotide polymorphisms (SNPs) as instrumental variables for genetically predicted folate concentrations. As shown in their Table 4, the allelic effects (β) from the genome-wide association study of European ancestry (2), however, were exactly the same as those (Supplemental Table 5) from the Chinese Dongfeng-Tongji cohort. The fraction of folate variance explained failed to agree with the allele frequency and effect size according to the formula $R^2 = 2 \times \text{MAF} \times (1 - \text{MAF}) \times (\beta/\text{SD})^2$ in their Table 4, also. The authors might as well check their data sets and 2-sample MR results in a meticulous fashion.

Furthermore, 1 essential assumption underlying 2-sample MR is violated in their studies, that is, that instrumental SNPs are not independently associated with the exposure. Linkage disequilibrium within multiple instrumental variables should be avoided in case of biased results when using inverse-variance weighted MR yields the summarized statistic from individual regression. There should be no high intercorrelation within single SNPs, which is just like the case of the no multicollinearity assumption in classic multiple linear regression. Using LDlink 3.8 (3) with 1000 Genomes Phase 3 samples as the reference panel, however, rs17421511 and rs1801133 are obviously in linkage disequilibrium in the European population ($r^2 = 0.13$, D' = 1). With rs1801133 and rs652197 retained and their effect size corrected, we performed an inverse-variance weighted MR using R software, version 3.5.3 (R Foundation for Statistical Computing) and the "TwoSample MR" package (4). MR results suggested a weak causality of folate on CAD (OR: 1.23; 95% CI: 1.00, 1.51; $P = 0.049$). Given a sample size of 184,305, the proportion of CAD cases at 0.33, and the estimated folate variance explained equal to 0.68%, our 2-sample MR was well-powered (0.95) to detect an OR of 1.23 (5). Notably, instead of an inverse association, genetically predicted higher concentrations of serum folate seemed to increase the risk of CAD in the European population.

Thirdly, we deem that their instrument selection for 1-sample MR analysis was not appropriate. The Chinese cohort was genotyped for 3 candidate SNPs from the largest European-ancestry meta-analysis of folate concentrations. As shown in Supplemental Table 5, rs652197 even failed to reach Bonferroni-corrected significance at $P = 0.05/3$ and further analysis incorporating rs652197 as 1 instrumental variable obviously violated the MR assumption. As for rs17421511, it seems to be in weaker linkage disequilibrium with rs1801133 in East Asians ($r^2 = 0.05$, D' = 1) than in Europeans; however, the effect of allele frequency in their cohort (G = 0.07) diverged greatly from both the European and East Asian panels (Supplemental Table 1). The inclusion of rs17421511 should also be scrutinized. Although they did acknowledge the low power to test a significant association in Chinese, we would go so far as to say that it was seriously inadequate; it was estimated likewise that the power was merely 0.37 (5) when employing the 1-sample MR to detect an OR of 0.71 as their observational analyses suggested. The conclusion that there was no genetic evidence to support a causal association between serum folate and CAD in the Chinese population, therefore, was relatively unconvincing.

Lastly, we should be cautious with the interpretation of MR results. Exploring causality with the MR approaches presented here is specifically trying to ascribe a linear causal role for circulating folate on the risk of CAD. Just as their restricted cubic spline indicated, the relation between folate and CAD may be too nonlinear to be efficiently determined by current statistical models. Previous studies also suggested the causation and protective effect of folate supplementation in reducing the risk of stroke in populations with low folate exposure status, yet not in those with higher folate intake amounts (6). Hence, it may be a reasonable hypothesis that the possibly protective effect of folate on CAD is predominantly or merely in populations with poor folate status. Indeed, rs1801133 (*MTHFR* 677C→T) has long been recognized as a functional variant influencing the activity of methylenetetrahydrofolate reductase and should be an ideal genetic proxy for the methyl cycle, in which folate is extensively involved (7). Leveraging the instrumental variable so as best to elucidate the relation between circulating folate and cardiovascular disease, further genetic epidemiological studies allowing for populations with different folate intake status and comprehensive statistical analyses stratified by it are warranted.

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Abbreviations used: CAD, coronary artery disease; MR, Mendelian randomization; SNP, single nucleotide polymorphism.