Genetically Predicted Circulating Concentrations of Alanine and Alanine Aminotransferase Were Associated with Prostate Cancer Risk

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Object: Prostate cancer is one of the leading malignancies in men worldwide. Previous observational studies have linked amino acids and transaminase with altered risk of prostate cancer. However, whether these associations were causal remained unclear. Therefore, we conducted a Mendelian randomization (MR) to assess their potential causal associations.

Methods: Summary-level data for prostate cancer were obtained from a meta-analysis of genome-wide association studies (GWAS) including 79,148 prostate cancer cases and 61,106 controls of European descent. Instrumental variables (IVs) of amino acids and alanine aminotransferase (ALT) were obtained from a GWAS of 86,507 European individuals and a GWAS of 312,572 participants from the UK Biobank, respectively. MR analyses were performed using inverse-variance-weighted (IVW), likelihood-based, MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test and MR-Egger regression.

Results: Genetically predicted circulating concentrations of alanine were associated with an increased risk of prostate cancer (odds ratio (OR): 1.16, 95% confidence interval (CI): 1.01–1.33, \(P=0.037\) by IVW). Consistently, genetically predicted ALT was inversely associated with the risk of prostate cancer (OR: 0.43, 95% CI: 0.27–0.68, \(P=3.28 \times 10^{-4}\) by IVW). MR-Egger regression did not indicate evidence of directional pleiotropy and sensitivity analyses yielded consistent associations.

Conclusion: Our study revealed that genetically predicted circulating alanine and ALT levels were associated with an altered risk of prostate cancer, suggesting their potential roles in the development of prostate cancer. Whether targeting alanine, ALT or its downstream effectors are helpful in reducing prostate cancer incidence warrants further investigation.

Keywords: alanine, alanine aminotransferase, amino acids, Mendelian randomization, prostate cancer

Introduction
Prostate cancer is the second most frequently diagnosed cancer type and the fifth leading cause of cancer death among men, with approximately 1.4 million new cases and 375,000 deaths worldwide in 2020.1–2 Incidence rates of prostate cancer vary from 6.3 to 83.4 per 100,000 men across regions, with the highest rates found in Northern and Western Europe, Australia/New Zealand and Northern America, and the lowest rates in Asia and Northern Africa.3 For a disease with a heavy burden such as prostate cancer, its etiology and pathogenesis remain largely unknown. Established risk factors for prostate cancer are limited to advanced age, family history of cancer and genetic factors.3–5 Therefore, it is necessary to search for additional biomarkers, so as to aid in the prevention, diagnosis and treatment of prostate cancer.

Since the metabolic characteristics of cancer cells are different from those of normal cells, determination of metabolites, such as free amino acids (AAs), transaminase, in biological fluids may be valuable in the prevention,
diagnosis or treatment of cancer. Previous observational studies have linked the associations of amino acids with the risk of prostate cancer. For example, a prospective study of 523 prostate cancer patients and 523 matched controls found that serum levels of cysteine were associated with a reduced risk of prostate cancer, while levels of dipeptide leucyl glycine and glutamyl amino acids were associated with an increased risk of prostate cancer. Another case–control study including 400 participants also found statistically significant differences in free amino acids profiles between patients with prostate cancer and controls. In addition, transaminases, such as alanine aminotransferase (ALT), were found to be markedly elevated in serum samples from patients with prostate cancer, compared with controls. However, Zhou et al reported that there were no differences in the serum levels of ALT in patients with prostate cancer compared with those diagnosed with benign prostatic hyperplasia. Since the above findings were from traditional observational epidemiological studies, which may be vulnerable to bias such as confounding and reverse causation, it remains unclear whether these AAs and transaminases are causally associated with the risk of prostate cancer.

Mendelian randomization (MR) study is a genetic approach designed to overcome the biases of traditional epidemiological research by using genetic variants associated with exposure as instrumental variables (IV). Since genotypes are presumed to be randomly assigned during the process of gamete formation, MR study can effectively avoid the interference of reverse causality and are less susceptible to confounding factors. Therefore, in the present study, we implemented a MR design to investigate the potential causal effects of AAs and related transaminases on the risk of prostate cancer, so as to provide genetic evidence on the potential roles of AAs and transaminases in the development of prostate cancer.

**Methods**

**Data Sources**

The overall design of this study is shown in Figure 1. MR analyses were performed based on summary-level data from publicly available genome-wide association studies (GWASs) of AAs, transaminases and prostate cancer, respectively. The detailed information of related GWASs is summarized in Supplementary Table 1. Briefly, summary-level data for prostate cancer were obtained from the Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome (PRACTICAL) Consortium, which includes 79,148 prostate cancer cases and 61,106 controls of European descent. The effect estimate of each single-nucleotide polymorphism (SNPs) was obtained using fixed-
effects meta-analysis combining the summary statistics from OncoArray analysis and seven previous prostate cancer GWAS or high-density SNP panels of European ancestry imputed to the 1000 Genomes Project.

Selection of Instrumental Variables
Amino acid-associated SNPs used as genetic instruments were obtained from a GWAS of 86,507 adult individuals with European ancestry from the Fenland cohort with those run in the EPIC-Norfolk and INTERVAL studies. A total of 119 independent SNPs achieving genome-wide association threshold \( (P<5 \times 10^{-8}) \) and with linkage disequilibrium (LD) \( r^2<0.1 \) were identified for common AAs (16 for alanine, 9 for arginine, 3 for aspartate, 15 for glycine, 10 for glutamine, 8 for histidine, 4 for isoleucine, 6 for leucine, 9 for lysine, 1 for methionine, 7 for phenylalanine, 3 for proline, 5 for serine, 7 for threonine, 2 for tryptophan, 6 for tyrosine, and 6 for valine). In addition, a total of 237 independent SNPs \( (r^2<0.1) \) associated with serum ALT were obtained from two GWASs of circulating biomarkers, including 315,572 participants from the UK Biobank (UKBB). Association estimates were obtained assuming an additive genetic model on log 10-transformed ALT values, with adjustment for age, sex, and genetic principal components.

Statistical Analysis
We first calculated \( F \) statistics for SNPs used as IVs to measure the strength of the instruments, since IVs with an \( F \) statistic less than 10 were frequently labeled “weak instruments” and thus excluded from the MR analyses.

The potential causal associations between common AAs and the risk of prostate cancer were assessed using the inverse variance weighted (IVW) method as the primary analysis. This method first obtains the causal effect estimates based on a single genetic instrumental variable to calculate the Wald estimates, and then meta-analyzed using a fixed- or random-effects model to generated a combined causal effect estimate which provide a constant estimate of the causal association between AAs and the risk of prostate cancer. Cochran’s Q test was used to test whether the estimate of the association between exposure and outcome was consistent across each individual SNP. A fixed-effects model was utilized when no statistically significant heterogeneity was presented, otherwise the random-effects model was used to provide more conservative estimates.

We further conducted several sensitivity analyses to evaluate the robustness of our main analyses. The likelihood-based method was used to evaluate the potential linear relationship between the exposure and the outcome, and the likelihood-based estimator expresses the causal increase in the outcome per unit change in the risk factor assuming a linear association between the risk factor and the outcome variables. We also performed the Robust Adjusted Profile Score (MR-RAPS) with Huber loss function to estimate the causal effects, which considers the measurement error in SNP-exposure effects and is robust to systematic and idiosyncratic pleiotropy. In addition, we conducted MR-Egger regression and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) to identify the potential horizontal pleiotropic effects of the SNPs. The intercept term from MR-Egger regression analysis represents the average pleiotropic effect of a genetic variant. An intercept different from zero suggests evidence of directional pleiotropy. MR-PRESSO analysis conducts a global test of heterogeneity by regressing the SNPs-outcome associations on the SNPs-exposure associations and comparing the observed distance of each SNP from the regression with the distance expected under the null hypothesis of no pleiotropy.

Moreover, we performed “leave-one-out” analyses to identify potential influential SNPs, by sequentially removing of each SNP from the IV set and rerunning MR analyses. Finally, considering the potential influence of pleiotropy on the association results, SNPs used as IVs were manually scanned for related secondary phenotypes using the GWAS Catalog, last accessed on April 15, 2022). SNPs associated with other traits at the genome-wide significance level were excluded from the IV list and MR analyses were further performed using the remaining SNPs.

All statistical analyses were performed using R (version 4.1.0) with packages “MendelianRandomization” and “MR-PRESSO”, unless otherwise noted. \( P \)-values <0.05 were considered statistically significant.

Results
The detailed information of the SNPs used as IVs for AAs and ALT and their association estimates with the risk of prostate cancer are listed in Supplementary Tables 2 and 3. We first calculated F-statistics to quantify the strength of the IVs by using
the following equation: \( F = R^2 \times (N - 1 - K)/(1-R^2) \times K \), in which \( R^2 \) represents the variance explained by the IVs and \( N \) indicates the sample size. \( R^2 \) was estimated according to minor allele frequency (MAF) and \( \beta \)-value.\(^{21}\) The \( F \)-statistics for AAs and ALT ranged from 30.24 to 1035.80, satisfying the threshold of \( >10 \) (Supplementary Table 4).

As shown in Table 1, genetically predicted higher circulating levels of alanine were nominally associated with the risk of prostate cancer (odds ratio (OR) per 1 SD = 1.16, 95% confidence interval (CI): 1.01–1.33, \( P = 0.037 \)). Sensitivity analyses using maximum-likelihood methods (OR = 1.16, 95% CI: 1.01–1.34, \( P = 0.034 \)) and MR-RAPS (OR = 1.16, 95% CI: 1.05–1.28, \( P = 0.005 \)) supported a similar association as the main analysis. No outlier SNPs were detected using MR-PRESSO test, and the causal effect estimate between alanine and prostate cancer was similar (OR:1.16, 95% CI: 1.01–1.33, \( P = 0.037 \)). MR-Egger regression did not suggest evidence of potential directional pleiotropy (\( P \) for intercept =0.508) (Figure 2).

In addition, genetically predicted higher ALT levels were associated with a decreased risk of prostate cancer (OR=0.43, 95% CI: 0.27–0.68, \( P = 3.28 \times 10^{-4} \), by IVW). The effect estimate was consistent in sensitivity analyses using the maximum likelihood (OR = 0.41, 95% CI: 0.26–0.66, \( P=2.57 \times 10^{-4} \)) method and MR-RAPS (OR=0.53, 95% CI: 0.41–0.69, \( P=3.11 \times 10^{-6} \)). MR-Egger regression did not suggest evidence of directional pleiotropy (\( P \) for intercept=0.054) (Figure 3). Fourteen outliers were detected using the MR-PRESSO test. After excluding these SNPs, subsequent MR analyses showed that the effect between ALT and prostate cancer did not change markedly (OR = 0.52, 95% CI: 0.36–0.77, \( P = 9.70 \times 10^{-4} \)). Moreover, we manually searched the SNPs used as IVs for ALT in the GWAS Catalog, and found 37 SNPs were associated with secondary traits (Supplementary Table 5). Using the remaining SNPs as IVs, the MR analysis suggested an inverse association between ALT and prostate cancer (OR = 0.40, 95% CI: 0.24–0.67, \( P = 5.04 \times 10^{-4} \), by IVW) (Supplementary Table 6). Finally, we performed “leave-one-out” analyses to identify potential influencing SNPs. We found that the effect

| Table 1 | Associations Between Genetically Predicted Serum Concentrations of AAs and ALT with the Risk of Prostate Cancer |
|---------|--------------------------------------------------------------------------------------------------------|
|         | No. of SNPs | OR (95% CI)                                             | \( P \) for Association | \( P \) value for Cochran Q test | \( P \) Intercept from MR-Egger Regression |
| Alanine |             |                                                        |                           |                                  |                                         |
|         | Inverse-variance weighted | 16 | 1.16 (1.01–1.33) | 0.037 | 0.009 |
|         | Maximum-likelihood | 16 | 1.16 (1.01–1.34) | 0.034 |                                  |
|         | MR-RAPS | 16 | 1.16 (1.05–1.28) | 0.005 |                                  |
|         | MR-PRESSO | 16 | 1.16 (1.01–1.33) | 0.037 |                                  |
|         | MR-Egger | 16 | / | / | 0.508 |
| ALT     | Inverse-variance weighted | 237 | 0.43 (0.27–0.68) | 3.28 \times 10^{-4} | 3.16 \times 10^{-55} |
|         | Maximum-likelihood | 237 | 0.41 (0.26–0.66) | 2.57 \times 10^{-4} |                                  |
|         | MR-RAPS | 237 | 0.53 (0.41–0.69) | 3.11 \times 10^{-6} |                                  |
|         | MR-PRESSO | 223 | 0.52 (0.36–0.77) | 9.70 \times 10^{-4} |                                  |
|         | MR-Egger | 237 | / | / | 0.054 |
| Arginine| Inverse-variance weighted | 9 | 0.96 (0.89–1.03) | 0.368 | 0.061 |
|         | Maximum-likelihood | 9 | 0.96 (0.87–1.05) | 0.363 |                                  |
|         | MR-RAPS | 9 | 0.97 (0.90–1.04) | 0.407 |                                  |
|         | MR-PRESSO | 9 | 0.96 (0.87–1.05) | 0.394 |                                  |
|         | MR-Egger | 9 | / | / | 0.355 |
| Aspartate| Inverse-variance weighted | 3 | 0.99 (0.92–1.07) | 0.874 | 0.480 |
|         | Maximum-likelihood | 3 | 0.99 (0.92–1.10) | 0.874 |                                  |
|         | MR-RAPS | 3 | 0.99 (0.92–1.08) | 0.877 |                                  |
|         | MR-Egger | 3 | / | / | 0.231 |

(Continued)
Table 1 (Continued).

| Amino Acid  | Method                   | No. of SNPs | OR (95% CI)       | P for Association | P value for Cochran Q test | P Intercept from MR-Egger Regression |
|-------------|--------------------------|-------------|-------------------|-------------------|-----------------------------|-------------------------------------|
| Glutamine   | Inverse-variance weighted| 10          | 0.94 (0.82–1.08)  | 0.405             | 1.94×10^{-4}                |                                     |
|            | Maximum-likelihood       | 10          | 0.95 (0.82–1.08)  | 0.414             |                             |                                     |
|            | MR-RAPS                  | 10          | 0.95 (0.88–1.02)  | 0.153             |                             |                                     |
|            | MR-PRESSO                | 9           | 0.92 (0.82–1.04)  | 0.22              |                             |                                     |
|            | MR-Egger                 | 10          | /                 | /                 |                             | 0.892                               |
| Glycine     | Inverse-variance weighted| 14          | 1.00 (0.90–1.12)  | 0.991             | 4.01×10^{-18}               |                                     |
|            | Maximum-likelihood       | 14          | 1.00 (0.90–1.12)  | 0.991             |                             |                                     |
|            | MR-RAPS                  | 14          | 1.01 (0.95–1.07)  | 0.791             |                             |                                     |
|            | MR-PRESSO                | 11          | 1.00 (0.90–1.11)  | 0.971             |                             |                                     |
|            | MR-Egger                 | 14          | /                 | /                 |                             | 0.124                               |
| Histidine   | Inverse-variance weighted| 8           | 0.95 (0.86–1.05)  | 0.323             | 0.118                       |                                     |
|            | Maximum-likelihood       | 8           | 0.95 (0.83–1.09)  | 0.441             |                             |                                     |
|            | MR-RAPS                  | 8           | 0.97 (0.87–1.08)  | 0.530             |                             |                                     |
|            | MR-PRESSO                | 8           | 0.95 (0.83–1.08)  | 0.466             |                             |                                     |
|            | MR-Egger                 | 8           | /                 | /                 |                             | 0.01                                |
| Isoleucine  | Inverse-variance weighted| 4           | 1.07 (0.86–1.34)  | 0.533             | 0.370                       |                                     |
|            | Maximum-likelihood       | 4           | 1.07 (0.86–1.35)  | 0.540             |                             |                                     |
|            | MR-RAPS                  | 4           | 1.06 (0.84–1.34)  | 0.620             |                             |                                     |
|            | MR-PRESSO                | 4           | 1.07 (0.86–1.34)  | 0.585             |                             |                                     |
|            | MR-Egger                 | 4           | /                 | /                 |                             | 0.432                               |
| Leucine     | Inverse-variance weighted| 6           | 1.07 (0.83–1.38)  | 0.612             | 0.035                       |                                     |
|            | Maximum-likelihood       | 6           | 1.07 (0.82–1.39)  | 0.606             |                             |                                     |
|            | MR-RAPS                  | 6           | 1.07 (0.90–1.27)  | 0.449             |                             |                                     |
|            | MR-PRESSO                | 6           | 1.07 (0.83–1.38)  | 0.633             |                             |                                     |
|            | MR-Egger                 | 6           | /                 | /                 |                             | 0.256                               |
| Lysine      | Inverse-variance weighted| 9           | 1.05 (0.99–1.11)  | 0.145             | 0.386                       |                                     |
|            | Maximum-likelihood       | 9           | 1.05 (0.98–1.11)  | 0.153             |                             |                                     |
|            | MR-RAPS                  | 9           | 1.05 (0.98–1.12)  | 0.104             |                             |                                     |
|            | MR-PRESSO                | 9           | 1.05 (0.98–1.11)  | 0.195             |                             |                                     |
|            | MR-Egger                 | 9           | /                 | /                 |                             | 0.849                               |
| Methionine  | Inverse-variance weighted| 1           | 0.92 (0.70–1.21)  | 0.571             | /                           |                                     |
|            | Maximum-likelihood       | 1           | 0.92 (0.70–1.21)  | 0.572             |                             |                                     |
|            | MR-RAPS                  | 1           | 0.92 (0.69–1.24)  | 0.595             |                             |                                     |
| Phenylalanine| Inverse-variance weighted| 7           | 0.99 (0.89–1.11)  | 0.937             | 0.454                       |                                     |
|            | Maximum-likelihood       | 7           | 1.00 (0.84–1.11)  | 0.936             |                             |                                     |
|            | MR-RAPS                  | 7           | 0.99 (0.89–1.12)  | 0.986             |                             |                                     |
|            | MR-PRESSO                | 7           | 0.99 (0.90–1.11)  | 0.938             |                             |                                     |
|            | MR-Egger                 | 7           | /                 | /                 |                             | 0.328                               |

(Continued)
estimates of genetically predicted ALT with the risk of prostate cancer did not change substantially after excluding one single SNP at a time (Supplementary Figure 1).

**Discussion**

To the best of our knowledge, this study is the first MR analysis which assessed the associations of circulating AAs and ALT with the risk of prostate cancer using MR approach based on large-scale genetic data. We provided suggestive evidence that higher circulating alanine was associated with an increased risk of prostate cancer and higher ALT was associated with a decreased risk of prostate cancer, suggesting a potential causal role of alanine and ALT in the development of prostate cancer.

Amino acid metabolism is a crucial metabolic pathway for regulating growth, reproduction and immunity. Inflammatory stimulation in patients with cancer may lead to changes in the collective demand for protein and energy,
Figure 2 Association between genetically predicted alanine levels and risk of prostate cancer based on different MR methods.

Figure 3 Association between genetically predicted ALT levels and risk of prostate cancer based on different MR methods.
accompanied by metabolic disorders. A growing body of evidence has suggested that amino acids are potential biomarkers that could be used in cancer screening, however, there was only a handful of studies with small sample sizes in prostate cancer. Our study used an MR design and provided evidence for potential causal associations of genetically predicted circulating levels of alanine with the risk of prostate cancer. This association was in the same direction as those observed in conventional epidemiological analyses. For example, in a case–control study, statistically significant differences in serum alanine levels were found in patients with prostate cancer compared to the control group (median (range): 384.8 (281.9–604.1) vs 479.9 (207.6–782.2), P<0.001). Moreover, May-Britt et al measured alanine concentrations in 82 benign and 16 malignant prostate biopsies by spectroscopy and found that the concentrations of alanine were higher in prostate cancer biopsies than that in the benign prostate (mean ± SD: 0.26±0.07 mmol/kg vs 0.14 ±0.06 mmol/kg, P<0.001). Our results, together with these observational findings, suggested a potential causal role of alanine in the development of prostate cancer.

In addition, we also found that ALT, one of the most common transaminases, which catalyzes the reversible conversion of alanine and 2-oxoglutaric acid to pyruvate and glutamate, was negatively associated with the risk of prostate cancer. Previous studies have indicated that ALT is involved in the conversion of alanine to pyruvic acid so as to promote gluconeogenesis. Transaminases have been reported to be associated with the development of hepatocellular cancer, intrahepatic and extrahepatic cholangiocarcinoma. Epidemiological studies have examined the association of ALT with the risk of prostate cancer, but the findings have been conflicting. For example, a case-cohort study found that ALT levels were not associated with prostate cancer, but the findings have been conflicting. For example, a case-cohort study found that ALT levels were not associated with the risk of prostate cancer (hazard ratio (HR): 0.89, 95% CI: 0.73–1.07, P=0.212). A case–control study including 194 patients with prostate cancer and 210 controls did not find evidence for an association between ALT and risk of prostate cancer (mean ± SD: 20.08±10.87 vs 21.71±13.21), whereas another cross-sectional study reported that prostate patients with poorly differentiated tumors had a higher ALT level (0.35ukat/l vs 0.39ukat/l) than had patients with well-differentiated clinical prostate cancer, and Govand et al indicated that the activity of ALT was higher in patients with prostate cancer compared with controls (median and range, 16.25:11.38–29.05 vs 17.1:11.33–31.75). These conflicting findings from conventional observational studies may because that these studies are susceptible to biases, such as reverse causation and residual confounding (eg, cancer staging, dietary, or molecular factors), which may confound the observational association estimates between ALT and prostate cancer. Nevertheless, evidence from a meta-analysis reported an inverse association between circulating levels of ALT and risk of overall cancer in European populations (RR per 1 SD=0.96, 95% CI: 0.94–0.99), suggesting that ALT may be involved in carcinogenesis.

In the current study, we found evidence that higher ALT levels were associated with a decreased risk of prostate cancer using the IVW method. It has been suggested that lower ALT levels may reflect impaired synthesis in the liver. Since detoxification and lipid metabolism are closely related to hepatocytes, a sharp decrease in functional hepatocytes may increase the susceptibility of the body to toxins and metabolic disorders, and hepatocyte injury also affects the metabolism of androgens in the liver, which may lead to the occurrence and development of prostate cancer. These findings support the rationale for using alanine and ALT as a promising target for prostate cancer screening and prevention. Further large-scale studies with a longitudinal design as well as in vitro and in vivo experiments are required to validate the causal nature of the associations between ALT and the risk of prostate cancer.

MR is a genetic epidemiological approach which uses SNPs that strongly associated with the exposure as IVs to estimate the potential causal relationship between the exposure and the outcome. Since genotypes are presumed to be randomly allocated in the process of gamete formation, the introduction of IV model to a large extent solves the problem of confounding in observational studies, especially the bias effect of unmeasured confounders on causal inference. The strong instruments we used in our study (ie, F-statistic much >10) should make it possible to reduce the potential bias caused by weak instrument as much as possible. Whether our results could apply to other ethnic groups remains to be confirmed, because the participants in this study involved only those of European descent. However, this greatly reduces the impact of demographic stratification bias on the results. Furthermore, for the result that was not statistically significant in this study, we cannot completely negate the association between these exposures and prostate cancer risk, since it may be caused by the small sample size and insufficient statistical power (<80%).
Conclusion
To sum up, our study suggested that alanine and ALT may play a causal role in the development of prostate cancer. Further studies are warranted to validate the observed associations and to elucidate the underlying mechanisms of alanine and ALT in risk of prostate cancer.

Data Sharing Statement
The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Statement of Ethics
Our study was approved by the Ethical Committee of Zhejiang Chinese Medical University on Nov 16, 2021 (No. AF-20211116-1). Because the project was mainly based on statistical analyses of publicly accessible databases and published studies, in which informed consents were obtained and ethical review were completed separately in each study, we have received ethical waiver for the research project.

Acknowledgment
The author sincerely thanks the researchers and participants of the original GWAS for their collection and management of large-scale data resources, as well as those who actively participated in this study.

Funding
This work was jointly supported by grants from National Natural Science Foundation of China (82103936, 82174208, and 81973663) and Natural Science Foundation of Zhejiang Province (LQ20H260008 and LQ21H260001); Zhejiang Medical and Health Science and Technology Plan (2021KY081 and 2022KY671. The funder had no role in the study design, data analysis, interpretation of data, or preparation of the manuscript.

Disclosure
The authors declare that they have no competing interests.

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