TOENAIL ZINC AND RISK OF PROSTATE CANCER IN THE MCC-SPAIN CASE-CONTROL STUDY

Gutiérrez-González E, Pastor-Barriuso R, Castelló A, Castaño-Vinyals G, Fernández de Larrea-Baz N, Dierssen-Sotos T, Jiménez-Moleón JJ, Molina-Barceló A, Fernández-Tardón G, Zumel-Marne Á, Moreno V, Gómez-Ariza JL, Sierra MÁ, García-Barrera T, Espinosa A, Plans-Beriso E, Gómez-Acebo I, Aragonés N, Kogevinas M, Pollán M, Pérez-Gómez B. Toenail zinc and risk of prostate cancer in the MCC-Spain case-control study. Environ Res. 2024 Mar 15;245:118065. doi: 10.1016/j.envres.2023.118065. Epub 2023 Dec 28. PMID: 38159663.

Toenail zinc and risk of prostate cancer in the MCC-Spain case-control study

Abstract

Background

Some researchers have suggested that zinc (Zn) could reduce the risk of prostate cancer (PC). However, research from observational studies on the relationship between PC risk and biomarkers of Zn exposure shows conflicting results.

Objectives

To evaluate the association between toenail Zn and PC, considering tumour extension and aggressiveness, along with a gene-environment approach, exploring the interaction of individual genetic susceptibility to PC in the relationship between toenail Zn and PC.

Methods

In MCC-Spain study we invited all incident PC cases diagnosed in the study period (2008-2013) and recruited randomly selected general population controls. In this report we included 913 cases and 1,198 controls with toenail Zn determined by inductively coupled plasma mass spectrometry. To measure individual genetic susceptibility, we constructed a polygenic risk score based on known PC-related single nucleotide polymorphisms. The association between toenail Zn and PC was explored with mixed logistic and multinomial regression models.

Results

Men with higher toenail Zn had higher risk of PC (OR quartile 4 vs.1: 1.41; 95% CI: 1.07–1.85). This association was slightly higher in high-grade PC [(ISUP≤2 Relative risk ratio (RRR) quartile 4 vs.1: 1.36; 1.01–1.83) vs. (ISUP3-5 RRR quartile 4 vs.1: 1.64; 1.06–2.54)] and in advanced tumours [(cT1-cT2a RRR quartile 4 vs.1: 1.40; 95% CI: 1.05–1.89) vs. (cT2b-cT4 RRR quartile 4 vs.1: 1.59; 1.00–2.53)]. Men with lower genetic susceptibility to PC were those at higher risk of PC associated with high toenail Zn (OR quartile 4 vs.1: 2.18; 95% CI: 1.08–4.40).

Discussion

High toenail Zn levels were related to a higher risk for PC, especially for more aggressive or advanced tumours. This effect was stronger among men with a lower genetic susceptibility to PC.

Keywords: toenail zinc; prostatic neoplasms; biomarkers; exposure; genetic predisposition to disease

Introduction

Prostate cancer (PC) is, worldwide, the second most frequent cancer in men [1] and the third leading cause of cancer deaths in men in Europe [2]. The etiology of PC is still uncertain, with only limited evidence on some environmental, occupational and dietary risk factors, that may differ depending on the aggressiveness of the tumour [3]. For PC in general, age, family history of PC and African-American race are well-established risk factors, while obesity, physical inactivity, tobacco consumption and some dietary factors (calcium intake, dairy products) have been associated with an increased risk of advanced and/or lethal PC [3]. On the other hand, PC is one of the tumours for which genetic susceptibility may play a greater role [4]. However, very few studies have adopted a gene-environment interaction approach, evaluating whether the relationship of these risk factors with PC is different depending on the individual susceptibility to develop this cancer.

One of the essential elements involved in prostate function is zinc (Zn). Zn inhibits the Krebs cycle, leading to the formation of large amounts of citrate, a key element of prostatic fluid. It also contributes to the inhibition of proliferation and induction of apoptosis in prostate cells [5] and is a component of superoxide dismutase type 1 (SOD1), crucial for its function in combating oxidative stress [6]. Inside the organism, the highest Zn concentrations are found in the prostate gland, especially in the epithelial cells of the peripheral zone, the main site where cancer develops [7]; however, PC cells have markedly lower Zn levels than healthy ones, due to a reduced expression of zinc uptake transporters [7]. Low levels of Zn allow PC cells to be more energetically efficient, while the apoptogenic effect of this element is lost, favoring cell proliferation [7]. In this context, some authors have suggested that Zn might play a relevant role in the etiology of PC and could even have a preventive effect against PC development [8], supporting the use of Zn supplements in men [9]. Surprisingly, when researchers have assessed the relationship of PC with dietary Zn -the main source of exposure in general population [10]-, the results seem to point more towards a non-linear positive association between Zn intake and PC risk, which may differ depending on individual genetic risk for PC [11,12].

Whether dietary estimates reflect the real exposure to zinc is unknown. For this reason, some authors have tried to disentangle the complex relationship between Zn and PC using Zn biomarkers. Among them, toenails are commonly used as biomarkers of long-term exposure to essential trace elements [13]; however, for Zn, toenail levels might not only represent external exposures, but could also be a reflect of Zn homeostasis [13,14]. Thus, exploring whether toenail Zn (tZn) levels are related to PC risk could provide additional clues to understand the role of this element in PC. Up to date, the relationship between Zn in nails and PC has been poorly explored in the literature, with conflicting results and limitations (small sample size or type of nail not specified) [15,16].

In this work, we try to better understand the role of Zn in PC through the evaluation of the association between tZn and the risk of PC, taking into account tumour grade or extension as well as the individual genetic susceptibility to PC, estimated by a polygenic risk score.

Subjects and methods

Study population and design

MCC-Spain (www.mccspain.org) is a population-based multicase-control study designed to explore the influence of environmental and genetic factors associated with five cancers (breast, colorectal, prostate, stomach and chronic lymphocytic leukemia) in 12 Spanish provinces between 2008 and 2013 [17]. Participants had to be aged 20-85 years, able to answer an epidemiological questionnaire to have resided ≥6 months in the study areas. Eligible cases were all subjects with an initial diagnosis of any of the included tumours -histologically confirmed incident cases- and living in the catchment area of recruiting hospitals. General population controls were randomly selected from general practitioner lists of selected primary healthcare centers within the catchment areas of the collaborating hospitals, and were frequency-matched to all tumour cases distribution by sex, age (5-year age groups) and geographical area. Response rates reached 72% in PC cases and 53% in controls.

Study protocol was approved by the recruiting centers' Ethical and Research Committees, and each participant provided written informed consent. The study was performed in accordance with the Declaration of Helsinki. For this study we included histologically-confirmed incident PC cases (International Classification of Diseases 10th Revision: C61, D07.5), with a Gleason grade ≥6 (i.e., International Society of Urological Pathology grading (ISUP) 1-5)[18]. In controls, we excluded those with personal history of PC or from provinces that had not recruited PC cases; within each geographical area, we also excluded those ≥ 5 years younger than the youngest PC case. Finally, 913 PC cases and 1 198 controls with available tZn were included for the analyses. Among these, 633 cases and 938 controls had genetic data (Supplementary Figure S1).

Data collection

The epidemiological questionnaire, collected through face-to-face interviews by trained personnel, included sociodemographic characteristics, anthropometric measures, physical activity, family and personal history of cancer, and smoking status one year before recruitment. Dietary habits were explored through a food frequency questionnaire including more than 140 food items [19]. Clinical information was obtained from medical records.

Toenail sampling, laboratory analyses and calibration

Big toes' clippings (both feet) were collected with stainless steel nail clippers within 2 weeks of recruitment and stored in paper envelopes at room temperature. Toenail clippings were cleaned twice by washing for 5 minutes in Triton 5% solution, Mili-Q water and acetone in a sonicator. The samples were then digested in nitric acid and hydrogen peroxide mixture solution at 4: 1 ratio using a microwave digestion system and then diluted to 5ml using MiliQ water. The determination of tZn was carried out by inductively coupled mass spectrometry (ICP-MS) (XSeries 2, Thermo Scientific) at the Environmental and Bioanalytical Chemistry Group, Chemistry Department of the University of Huelva (Spain). Analyses were performed blindly from case-control status. The concentrations measured by the equipment were adjusted considering the sample's weight and dilution factor. The limit of detection for Zn was 0.27 ng/g.

Several quality control procedures were performed during the analyses, including: (a) analysis of hair reference material NSC DC73347a (LGC Standards), in each sample batch with a medium accuracy of 90 %; (b) monitoring of ICP-MS response over time by measurement of control concentrations of the different elements at a point on the calibration curve (5 ng ml-1), every 20 samples; (c) instrumental drift correction by addition of rhodium (Rh) (100 ng ml-1), as internal standard, and analyzing again those samples whose response differs ± 10% with respect to the internal standard; (d) analysis every 5 samples of reagents blanks containing 5% HNO3 (Suprapur quality) and 100 ng ml-1 of Rh; (e) analysis of duplicate samples every 2.5 hours; (f) spike sample analysis, spiking the reference materials with the analytes under study (50 ng ml-1).

Reproducibility analyses were also carried out using toenail samples from non-eligible patients from MCC-Spain in two different laboratories (University of Oviedo and University of Huelva, Spain), achieving an intraclass correlation coefficient for Zn of 0.983 (95% CI: 0.973-0.989).

The concentrations measured by the equipment were adjusted taking into account the sample's weight and dilution factor, according to the following formula: [Real](ppb)= [Equipment](ppb)·(dilution factor (g))/(sample weight(g)). Small toenail clippings may represent a challenge for ICP-MS as their signal can be out of the optimal measurement range of the calibration line [20]. In preliminary analyses, we observed that very small toenail samples showed the highest geometric mean (GM) Zn concentrations. This bias has been previously reported in other studies, and the sample weight has been taken into account when measuring trace elements concentrations [13]. Additionally, there was variability in measured metal concentrations across laboratory batches. For these reasons, toenail Zn concentrations were calibrated for sample mass heterogeneity and

between-batch variability by means of a heteroscedastic spline mixed model [21], with fixed effects for the average bias in log-transformed Zn concentrations as a spline function of log-transformed toenail sample weight, random effects for between-batch variation in this mass-related bias, and heterogeneous within-batch error variance in log-transformed Zn concentrations as a spline function of log-transformed weight. From this model, we derived the calibrated Zn concentrations that would have been observed had all toenail specimens been analyzed in the same average batch and sample masses been set to the GM for all participants, conditional on sex, five-year age group, and province.

Genetic susceptibility: Polygenic Risk Score

Peripheral blood was collected from participants during recruitment. The genotyping of participants was performed at the Centro Nacional de Genotipado (CEGEN-ISCIII) using the Infinium Human Exome BeadChip (Illumina, San Diego, USA). We built a polygenic risk score (PRS) to explore the genetic polymorphic susceptibility to PC. For this purpose, we used Genomebrowser to identify those SNPs associated with PC through genetic wide association studies (GWAS) in the population with European ancestry, of which 56 were included in our data. The PRS score was obtained by means of logistic regression analyses, adding the number of copies of the risk allele of each SNP, weighted by their beta coefficients. The procedure is explained in detail elsewhere [22]. Briefly, compared with individuals with low risk (decile 1), those with an intermediate risk (decile 5) had an increased risk of PC (OR 3.65, 95% CI 2.26-5.91).

Statistical analysis

We described the main characteristics of cases and controls using absolute figures, percentages and means with standard deviations (SD). To test differences between cases and controls, Pearson chi-square for categorical variables and Student's t-test for continuous variables were used. Subsequently, we categorized toenail zinc in quartiles based on the distribution among controls.

We explored the association of tZn with PC risk by fitting mixed logistic regression models: a first model included design-related variables (age, education as fixed effects) as well as province of residence as random effect term; the second model added also selected risk factors for PC as fixed effects (body mass index (BMI) one year before diagnosis; family history of PC; smoking status one year before diagnosis; and physical activity –metabolic equivalents of task (METS)/week–). To test for linear trend, quartiles of tZn were treated as continuous. Additionally, we evaluated a potential non-linear association between tZn and PC risk by including restricted cubic splines of log-transformed toenail zinc with two internal knots at the 35th and 65th percentiles and boundary knots at the 5th and 95th percentiles among controls in the fully adjusted model.

Subsequently, we explored the relationship between tZn and PC risk considering tumour characteristics with mixed multinomial logistic regression models, also adjusted by the variables previously mentioned and calculated relative risk ratios (RRR) with 95% CI. PC cases were classified by aggressiveness into low (ISUP≤ 2) and high grade (ISUP 3-5) and by clinical local extension at diagnosis (cT1-cT2a vs cT2b-cT4). Additionally, we explored other high-grade definitions (Gleason=6 vs. Gleason>6; AJCC 8th ed I-IIA vs. IIB-IV; and PSA<10 vs. PSA≥10). Heterogeneity of tZn effects by tumour aggressiveness was tested by means of likelihood ratio tests comparing nested multinomial models with and without homogeneous zinc coefficients.

Lastly, we explored the possible modifying effect of genetic susceptibility in the association of tZn and risk of PC by including main effects and interaction terms between toenail zinc quartiles and PRS tertiles among controls in mixed logistic regression models. Heterogeneity of zinc effects by PRS tertiles was evaluated by joint Wald test of all interaction coefficients. We performed all analyses with Stata, version 16 (Stata Corp).

Results

The main characteristics of cases and controls (all participants and genotyped subsample) are shown in Table 1. Details on the clinical profile of PC cases are presented in Supplementary Table S1. Toenail Zn distribution in cases and controls is detailed in Supplementary Table S2. Controls showed higher educational level, higher physical activity and greater nail sample weight than cases, while family history of PC was more frequent among cases. Toenail Zn concentrations of controls and cancer cases by main characteristics are summarized in Supplementary Table S3.

Table 2 presents the risk (odds ratios, ORs) of PC according to tZn. In both, basic and fully adjusted models, men with tZn levels in the second, third and fourth quartiles had higher risk of PC than those with the lowest tZn levels.

PC risk was higher among men with higher tZn in all PC ISUP grades or tumour extension categories (Table 3); however, for those men with tZn in the highest quartile, ORs were always slightly higher in more aggressive or advanced PC cases. Similar results were observed with other PC classifications (Gleason grade; AJCC 8th edition; PSA at diagnosis) (Supplementary Table S4).

When we evaluated non-lineal dose-response relationships between toenail zinc and PC risk, we observed an initial increase in PC risk with medium tZn concentrations, followed by a subsequent decline (Figure 1). Again, the increase of risk with tZn levels was more intense in high grade tumours (ISUP 3-5) than in low grade malignancies. We performed a sensitivity analysis excluding those participants that have received Zn supplements at least during the last year, with identical results.

Additionally, we explored whether the association between tZn and PC was modified by genetic susceptibility to PC, estimated by a PRS (the distribution of cases and controls by genetic susceptibility, ISUP grade and stage are summarized in Supplementary Table S5). There were differences in this association according to PRS tertiles (p-heterogeneity: <0.001), with a stronger relationship in those men in the lower PRS tertile (OR quartile 4 vs. 1: 2.18; 95% CI: 1.08–4.40*; p*-trend:0.021) than in those in the higher tertile (OR quartile 4 vs. 1: 1.32; 95% CI: 0.82–2.13) (Table 4). Also, we evaluated whether age modified the association between tZn and PC, not observing relevant differences (Supplementary Table S6).

Discussion

Even though Zn is an essential element for the prostate gland [8], its role in the development of PC remains unclear. In our study, higher tZn levels are associated with an increased risk of PC, at least up to a certain tZn level, both in localized, low-grade tumours and especially in more advanced, high-grade malignancies. In addition, the observed association is more evident among men with lower genetic susceptibility to PC.

While it seems clear that PC cells have lower Zn levels than healthy cells [8], researchers have tried different approaches to test whether Zn exposure could reduce PC risk. Unfortunately, available studies have yielded quite conflicting results. As diet is the main source of Zn in humans [10], a logic strategy has been to investigate the relationship between Zn intake -either through diet or in supplements- and PC, with contradictory results [11]. Within MCC-Study, we also estimated Zn dietary intake, and observed a direct relationship with risk of PC [12], although, in this case, the effect was more evident in those participants with higher genetic susceptibility to PC. This difference could be due to the fact that dietary fluctuations do not always imply differences in Zn availability in the body, as Zn homeostasis is tightly controlled [23] to maintain metabolic functions, and could suggest that dietary Zn and toenail Zn could be participating in different pathways of PC pathogenesis.

However,. Hence, another approach has been to study the relationship between PC and Zn directly measured in plasma/serum –the most commonly used biomarker for this element. Again, data on differences in serum Zn levels between PC cases and healthy men are inconsistent [24], while those

studies that have assessed PC risk have reported, in line with our results, a positive association with elevated serum Zn levels [25,26], in some cases restricted to selected ethnic groups [27].

An alternative option is to use tZn as a biomarker, as we do in the present work. Due to their slow growth rate, toenails clippings may reflect past serum status of the individual (6-12 months)[13]. However, the only previous study that has used tZn is a nested case-control study in the USA , found a non-significant inverse association between big toe tZn and PC risk, especially for advanced tumours, although the sample size was relatively small and all participants had higher tZn than ours [15]. On the other hand, another small study reported higher PC risk on men with higher zinc levels in nails (without specifying toe or finger), with similar results for Zn in hair [16].

Our results support that high levels of tZn -proxy of serum Zn levels in the previous year [13]- are associated to higher PC risk. Even though evidence supports a protective role of Zn against PC through induction of cell apoptosis, suppression of cell proliferation and DNA protection [8], recent studies suggest that long-term zinc exposure might also be associated with malignancy in prostate cells [28,29]. While metallothioneins and Zn transporters play an important role in short-term Zn buffering, KRAS, NF-kB and PI3K are relevant for long-term modulation [30]. Long-term high Zn concentrations may shift cell metabolism to a more malignant type, increasing invasiveness and expression of genes associated with treatment resistance and malignancy [28]. This hypothesis is consistent with the stronger effect we found in advanced/aggressive tumours. It is also in line with the findings in Health Professionals Follow-up cohort study, es which recently reported an increased risk of being diagnosed of lethal/aggressive cases among men using Zn supplements [31]. Nevertheless, further results of the same study showed that that low-dose zinc supplement use among nonmetastatic prostate cancer patients was associated to an increased survival [32], highlighting the complexity of the relationship between PC and Zn .

On the other hand, a striking finding, not previously studied, is the differential effect of tZn depending on genetic susceptibility to PC. The association in men with lower genetic susceptibility may point to a higher need of other contributing risk factors (i.e., Zn) for PC development. Of note, changes in Zn transport in prostate cells (upregulation of Zn exporters and downregulation of Zn importers) have been described as an early event during prostate tumorigenesis [29], with zinc resistance associated with a worse, more aggressive phenotype [28].

Some strengths of this study are its population-based design, the recruitment of incident cases and a relatively large sample size. Furthermore, the availability of histopathological information allowed stratified analysis by tumour extension and aggressiveness, and the genetic information, the evaluation of the interaction with genetic susceptibility to PC. Another novel aspect is the use of toenails as a biomarker of Zn exposure, which might reflect past Zn status. Also, we calibrated tZn taking into account the variability associated with the samples' weight, as well as batch-related variability, to avoid a systematic bias associated with toenail mass [13].

Among the limitations of our work, we cannot rule out a possible selection bias (higher participation rates and lower educational status in cases than in controls). We have tried to limit this problem by adjusting by educational level in all models. In addition, since toenail samples were collected at time of recruitment, we have to consider possible reverse causation bias. On the one hand, it is not expected that dietary Zn intake could be affected by PC. On the other hand, since Zn transporters are downregulated in PC cells, this could lead to an increase in serum Zn levels, and consequently, a higher deposition on toenails among cases; however, it seems unlikely that local tissue specific changes may result in changes in body serum levels. At least in breast cancer prediagnostic tZn levels correlate well with levels after diagnosis [33], and another study in pancreatic cancer showed that toenail Zn concentrations were not affected by the development of the disease before toenail

collection [34]. Finally, unmeasured, residual confounding due to the non-experimental design or the lack of control for other metals cannot be discarded.

Conclusions

High tZn levels were associated with a higher risk for PC, especially for more advanced tumours. This effect was clearer among men with a lower genetic susceptibility to PC. Our results show an increased risk of prostate cancer associated to higher Zn exposure (toenail Zn) and do not support Zn supplementation as a preventive strategy against prostate cancer. These findings need to be confirmed in new studies and the possible mechanisms of this association need to be further investigated.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. mayo de 2021;71(3):209-49.

2. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer Oxf Engl 1990. noviembre de 2018;103:356-87.

3. Pernar CH, Ebot EM, Wilson KM, Mucci LA. The Epidemiology of Prostate Cancer. Cold Spring Harb Perspect Med. diciembre de 2018;8(12):a030361.

4. Hjelmborg JB, Scheike T, Holst K, Skytthe A, Penney KL, Graff RE, et al. The heritability of prostate cancer in the Nordic Twin Study of Cancer. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. noviembre de 2014;23(11):2303-10.

5. Franklin RB, Costello LC. Zinc as an anti-tumor agent in prostate cancer and in other cancers. Arch Biochem Biophys. 15 de julio de 2007;463(2):211-7.

6. Skrajnowska D, Bobrowska-Korczak B. Role of Zinc in Immune System and Anti-Cancer Defense Mechanisms. Nutrients. 22 de septiembre de 2019;11(10):2273.

7. Franz MC, Anderle P, Bürzle M, Suzuki Y, Freeman MR, Hediger MA, et al. Zinc transporters in prostate cancer. Mol Aspects Med. junio de 2013;34(2-3):735-41.

8. Costello LC, Franklin RB. A comprehensive review of the role of zinc in normal prostate function and metabolism; and its implications in prostate cancer. Arch Biochem Biophys. 1 de diciembre de 2016;611:100-12.

9. Sauer AK, Vela H, Vela G, Stark P, Barrera-Juarez E, Grabrucker AM. Zinc Deficiency in Men Over 50 and Its Implications in Prostate Disorders. Front Oncol. 2020;10:1293.

10. Simon-Hettich B, Wibbertmann A, Wagner D, Tomaska L, Malcolm H, Organization WH, et al. Zinc [Internet]. World Health Organization; 2001 [citado 20 de marzo de 2022]. Disponible en: https://apps.who.int/iris/handle/10665/42337

11. Mahmoud AM, Al-Alem U, Dabbous F, Ali MM, Batai K, Shah E, et al. Zinc Intake and Risk of Prostate Cancer: Case-Control Study and Meta-Analysis. PloS One. 2016;11(11):e0165956.

12. Gutiérrez-González E, Castelló A, Fernández-Navarro P, Castaño-Vinyals G, Llorca J, Salas D, et al. Dietary Zinc and Risk of Prostate Cancer in Spain: MCC-Spain Study. Nutrients. 20 de diciembre de 2018;11(1):E18.

13. Gutiérrez-González E, García-Esquinas E, de Larrea-Baz NF, Salcedo-Bellido I, Navas-Acien A, Lope V, et al. Toenails as biomarker of exposure to essential trace metals: A review. Environ Res. diciembre de 2019;179(Pt A):108787.

14. Gutiérrez-González E, Fernández-Navarro P, Pastor-Barriuso R, García-Pérez J, Castaño-Vinyals G, Martín-Sánchez V, et al. Toenail zinc as a biomarker: relationship with sources of environmental exposure and with genetic variability in MCC-Spain study. Environ Int. 14 de septiembre de 2022;107525.

15. Platz EA, Helzlsouer KJ, Hoffman SC, Morris JS, Baskett CK, Comstock GW. Prediagnostic toenail cadmium and zinc and subsequent prostate cancer risk. The Prostate. 1 de septiembre de 2002;52(4):288-96.

16. Karimi G, Shahar S, Homayouni N, Rajikan R, Abu Bakar NF, Othman MS. Association between trace element and heavy metal levels in hair and nail with prostate cancer. Asian Pac J Cancer Prev APJCP. 2012;13(9):4249-53.

17. Castaño-Vinyals G, Aragonés N, Pérez-Gómez B, Martín V, Llorca J, Moreno V, et al. Population-based multicase-control study in common tumors in Spain (MCC-Spain): rationale and study design. Gac Sanit SESPAS. 2015;29(4):308-15.

18. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol. febrero de 2016;40(2):244-52.

19. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol. junio de 1993;22(3):512-9.

20. Harris DC. Quantitative Chemical Analysis. Macmillan Learning; 2020. 193 p.

21. Pinheiro JC, Bates DM, editores. Extending the Basic Linear Mixed-Effects Model. En: Mixed-Effects Models in S and S-PLUS [Internet]. New York, NY: Springer; 2000 [citado 9 de mayo de 2022]. p. 201-70. (Statistics and Computing). Disponible en: https://doi.org/10.1007/0-387-22747-4_5

22. Gómez-Acebo I, Dierssen-Sotos T, Fernandez-Navarro P, Palazuelos C, Moreno V, Aragonés N, et al. Risk Model for Prostate Cancer Using Environmental and Genetic Factors in the Spanish Multi-Case-Control (MCC) Study. Sci Rep. 21 de 2017;7(1):8994.

23. Virgili F, Ambra R, McCormack J, Simpson EEA, Ciarapica D, Barnaba L, et al. Genetic Polymorphisms and Zinc Status: Implications for Supplementation in Metabolic Diseases. Curr Pharm Des. 2018;24(35):4131-43.

24. Zhao J, Wu Q, Hu X, Dong X, Wang L, Liu Q, et al. Comparative study of serum zinc concentrations in benign and malignant prostate disease: A Systematic Review and Meta-Analysis. Sci Rep. 12 de mayo de 2016;6:25778.

25. Białkowska K, Marciniak W, Muszyńska M, Baszuk P, Gupta S, Jaworska-Bieniek K, et al. Association of zinc level and polymorphism in MMP-7 gene with prostate cancer in Polish population. PloS One. 2018;13(7):e0201065.

26. Lim JT, Tan YQ, Valeri L, Lee J, Geok PP, Chia SE, et al. Association between serum heavy metals and prostate cancer risk - A multiple metal analysis. Environ Int. noviembre de 2019;132:105109.

27. Park SY, Wilkens LR, Morris JS, Henderson BE, Kolonel LN. Serum zinc and prostate cancer risk in a nested case-control study: The multiethnic cohort. The Prostate. 15 de febrero de 2013;73(3):261-6.

28. Holubova M, Axmanova M, Gumulec J, Raudenska M, Sztalmachova M, Babula P, et al. KRAS NF-κB is involved in the development of zinc resistance and reduced curability in prostate cancer. Met Integr Biometal Sci. julio de 2014;6(7):1240-53.

29. Kratochvilova M, Raudenska M, Heger Z, Richtera L, Cernei N, Adam V, et al. Amino Acid Profiling of Zinc Resistant Prostate Cancer Cell Lines: Associations With Cancer Progression. The Prostate. mayo de 2017;77(6):604-16.

30. Li D, Stovall DB, Wang W, Sui G. Advances of Zinc Signaling Studies in Prostate Cancer. Int J Mol Sci. 19 de enero de 2020;21(2):E667.

31. Zhang Y, Song M, Mucci LA, Giovannucci EL. Zinc supplement use and risk of aggressive prostate cancer: a 30-year follow-up study. Eur J Epidemiol. diciembre de 2022;37(12):1251-60.

32. Zhang Y, Stopsack KH, Wu K, Song M, Mucci LA, Giovannucci E. Post-diagnostic Zinc Supplement Use and Prostate Cancer Survival Among Men With Nonmetastatic Prostate Cancer. J Urol. marzo de 2023;209(3):549-56.

33. O'Brien KM, White AJ, Sandler DP, Jackson BP, Karagas MR, Weinberg CR. Do Post-breast Cancer Diagnosis Toenail Trace Element Concentrations Reflect Prediagnostic Concentrations? Epidemiol Camb Mass. enero de 2019;30(1):112-9.

34. Pumarega J, Camargo J, Gasull M, Olshan AF, Soliman A, Chen Y, et al. Timing of Toenail Collection and Concentrations of Metals in Pancreatic Cancer. Evidence Against Disease Progression Bias. Expo Health. 2022;14(3):581-93.

374

375 **Figure captions**

376

377 Figure 1. Odds ratio for overall prostate cancer (A) and relative risk ratios for low-grade (ISUP 1-2)(B)
378 and high-grade (ISUP 3-5) prostate cancer (C) as a smooth function of toenail zinc concentration.
379 Curves represent adjusted odds ratios and relative risk ratios (solid lines) and their 95% confidence
380 intervals (dashed lines) based on restricted cubic splines of log-transformed toenail zinc with two
381 internal knots at the 35th and 65th percentiles and boundary knots at the 5th and 95th percentiles
382 among controls. The reference value (odds ratio = relative risk ratio = 1) was set at the 12.5th
383 percentile of toenail zinc among controls (74.8 ug/g). Odds ratios were obtained from mixed logistic
384 models and relative risk ratios from mixed multinomial logistic models adjusted for age, education,
385 body mass index, family history of prostate cancer, smoking status and physical activity as fixed
386 effects, and province of residence as a random effect. Histograms represent toenail zinc distribution
387 (stacked bars) among controls (shaded bars) and among all, low-grade, and high-grade prostate
388 cancer cases (white bars).

Table 1. Main characteristics of controls and prostate cancer cases in the MCC-Spain Study.

	All participants			Participants with genetic data		
	Controls	**Cases**	**p-value**	**Controls**	**Cases**	**p-value**
	n=1198	n=913		n=938	n=633	
Age (years), mean (SD)	66.3 (8.5)	65.9 (7.3)	0.296	66.3 (8.3)	66.0 (7.4)	0.499
Education, n (%)			0.001			0.001
Primary School	583 (48.7%)	576 (63.1%)		464 (49.5%)	390 (61.6%)	
Secondary School	346 (28.9%)	204 (22.3%)		271 (28.9%)	149 (23.5%)	
University or more	269 (22.5%)	133 (14.6%)		203 (21.6%)	94 (14.8%)	
Ethnicity, n (%)			0.148			0.779
European	1188 (99.2%)	901 (98.7%)		937 (99.9%)	632 (99.8%)	
Non-European	8 (0.7%)	12 (1.3%)		1 (0.1%)	1 (0.2%)	
Unknown	2 (0.2%)	0 (0.0%)		.	.	
Smoking status, n (%)			0.444			0.148
Never smoker	334 (27.9%)	272 (29.8%)		259 (27.6%)	194 (30.6%)	
Former smoker	598 (49.9%)	433 (47.4%)		464 (49.5%)	303 (47.9%)	
Current smoker	264 (22.0%)	204 (22.3%)		214 (22.8%)	132 (20.9%)	
Unknown	2 (0.2%)	4 (0.4%)		1 (0.1%)	4 (0.6%)	
Toenail zinc (µg/g), mean (SD)	115.7 (74.9)	118.3 (61.8)	0.403	114.4 (71.1)	118.8 (62.2)	0.205
Toenail weight (mg), mean (SD)	29.6 (20.1)	27.2 (14.7)	0.003	28.5 (16.2)	27.5 (15.1)	0.225
BMI (kg/m2), mean (SD)	27.5 (3.8)	27.6 (3.8)	0.516	27.5 (3.7)	27.5 (3.8)	0.975
Physical activity, (METs/week)			0.001			0.357
0	454 (37.9%)	347 (38.0%)		379 (40.4%)	254 (40.1%)	
0.1-7.9	123 (10.3%)	119 (13.0%)		61 (6.5%)	51 (8.1%)	
8.0-15.9	141 (11.8%)	116 (12.7%)		79 (8.4%)	64 (10.1%)	
>=16	462 (38.6%)	331 (36.3%)		419 (44.7%)	264 (41.7%)	
Unknown	18 (1.5%)	0 (0.0%)				
Province, n (%)			0.001			0.001
Madrid	297 (24.8%)	271 (29.7%)		283 (30.2%)	217 (34.3%)	
Barcelona	428 (35.7%)	328 (35.9%)		296 (31.6%)	194 (30.6%)	
Asturias	94 (7.8%)	15 (1.6%)		57 (6.1%)	0 (0.0%)	
Huelva	55 (4.6%)	22 (2.4%)		17 (1.8%)	0 (0.0%)	
Cantabria	155 (12.9%)	152 (16.6%)		137 (14.6%)	139 (22.0%)	
Valencia	62 (5.2%)	70 (7.7%)		55 (5.9%)	50 (7.9%)	
Granada	107 (8.9%)	55 (6.0%)		93 (9.9%)	33 (5.2%)	
Family history of PC, n (%)			0.001			0.001
No	1 113 (92.9%)	722 (79.1%)		865 (92.2%)	501 (79.1%)	
2nd degree	10 (0.8%)	26 (2.8%)		9 (1.0%)	20 (3.2%)	
One 1st degree member	71 (5.9%)	138 (15.1%)		60 (6.4%)	93 (14.7%)	
More than one of 1st degree	4 (0.3%)	27 (3.0%)		4 (0.4%)	19 (3.0%)	
ISUP classification, n (%)			N/A			N/A
Control	1198 (100.0%)	N/A		938 (100.0%)	N/A	
ISUP 1-2	N/A	681 (74.6%)		N/A	466 (73.6%)	
ISUP 3-5	N/A	216 (23.7%)		N/A	155 (24.5 %)	
Unknown	N/A	16 (1.8%)		N/A	12 (1.9%)	
Stage, n (%)			N/A			N/A
Control	1198 (100.0%)	N/A		938 (100.0%)	N/A	
cT1-cT2a	N/A	660 (72.3%)		N/A	461(72.8%)	
cT2b-cT4	N/A	196 (21.5%)		N/A	148(23.4%)	
Unknown	N/A	57 (6.2%)		N/A	24 (3.8%)	

SD: standard deviation; BMI: body mass index; MET: Metabolic Equivalent of Task; PC: prostate cancer; N/A: Not applicable; n=number.

Table 2. Toenail zinc and prostate cancer risk in the MCC-Spain case control study

	Basic model			Fully adjusted model		
	Controls	**Cases**	**OR[1] (95% CI)**	**Controls**	**Cases**	**OR[2] (95% CI)**
Zinc (µg/g)						
Q1 (<86.2)	299	182	1.00	290	179	1.00
Q2 (86.2-104.4)	300	225	1.29 (0.99;1.68)	285	221	1.32 (1.00;1.74)
Q3 (104.5-127.0)	300	262	1.53 (1.18;1.99)	286	257	1.49 (1.13;1.96)
Q4 (>127.0)	299	244	1.38 (1.06;1.79)	284	240	1.41(1.07;1.85)
p-trend			0.009			0.011

Q: quartile; OR[1]: Odds ratio for prostate cancer adjusted by age and education as fixed effects and province of residence as random effect; OR[2]: Odds ratio for prostate cancer adjusted by age, education, BMI, family history of prostate cancer, physical activity and smoking status as fixed effects, and province of residence as a random effect; CI: confidence interval; µg/g: microgram/gram.

Table 3. Association between toenail zinc and prostate cancer by ISUP classification and local tumor extension

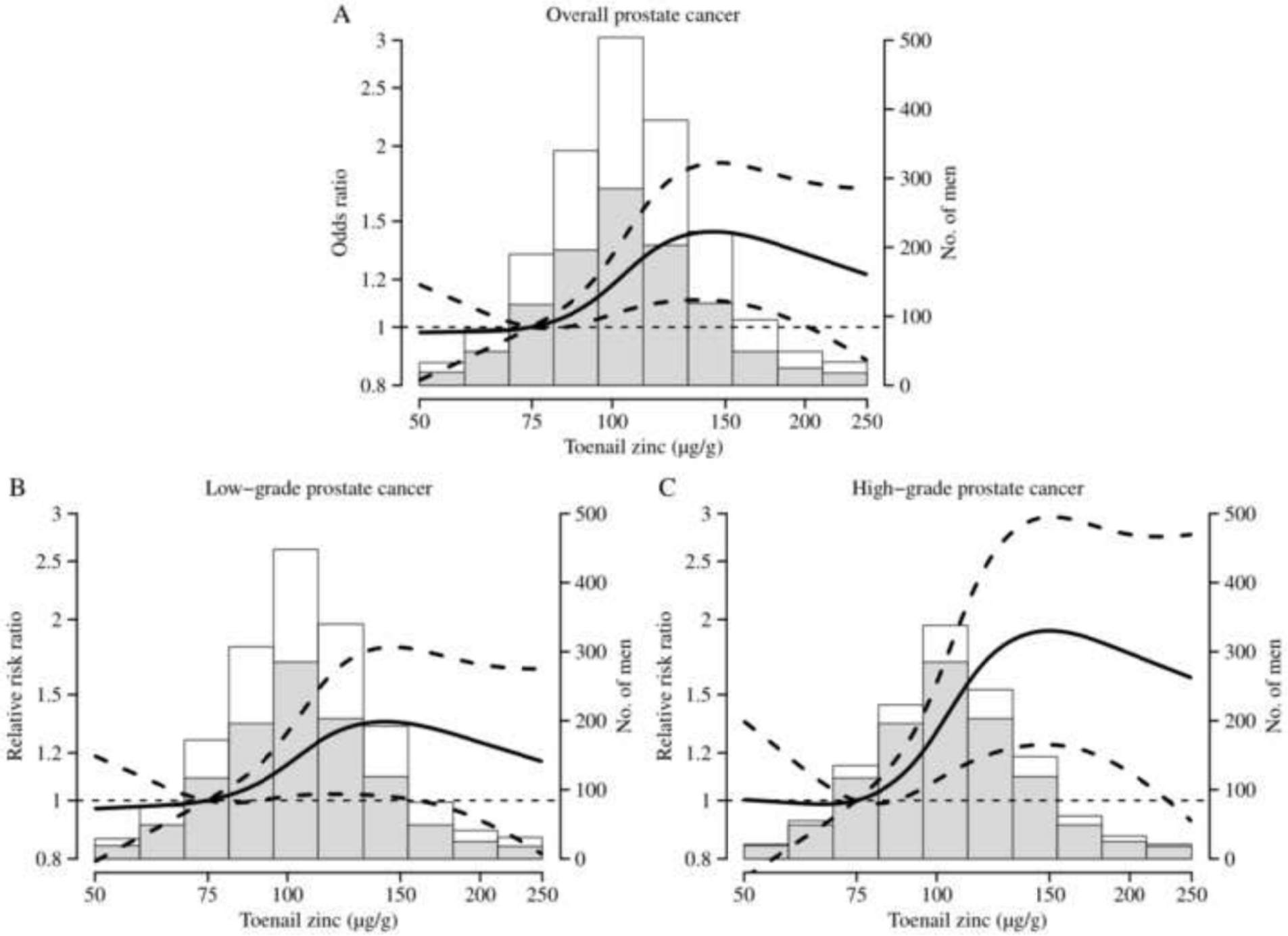
	ISUP≤2			ISUP 3-5				cT1-cT2a			cT2b-T4			
	Co	**Ca**	**RRR (95% CI)**	**Co**	**Ca**	**RRR (95% CI)**	**p-het**	**Co**	**Ca**	**RRR (95% CI)**	**Co**	**Ca**	**RRR (95% CI)**	**p-het**
Zinc (µg/g)							0.540							0.842
Q1 (<86.2)	290	132	1.00	290	43	1.00		290	130	1.00	290	43	1.00	
Q2 (86.2-104.4)	285	167	1.34 (0.99;1.80)	285	47	1.18 (0.74;1.88)		285	157	1.26 (0.93;1.70)	285	51	1.55 (0.97;2.48)	
Q3 (104.5-127.0)	286	197	1.51 (1.13;2.03)	286	57	1.45 (0.93;2.29)		286	185	1.41 (1.04;1.89)	286	46	1.48 (0.92;2.39)	
Q4 (>127.0)	284	173	1.36 (1.01;1.83)	284	65	1.64 (1.06;2.54)		284	177	1.40 (1.05;1.89)	284	52	1.59 (1.00;2.53)	
p-trend			0.037			0.016				0.021			0.080	

Q: quartile; RRR: Relative risk ratio for prostate cancer subtypes adjusted by age, education, BMI, family history of prostate cancer, physical activity, smoking status and province of residence; Co: controls; Ca: cancers; CI: confidence interval. p-het: P value for heterogeneity of relative risk ratios across categories of ISUP classification and tumor extension obtained from likelihood ratio tests; ISUP: International Society of Urological Pathology; µg/g: micrograms/gram; cT: clinical tumor size from the TNM staging system.

Table 4. Association between toenail zinc and prostate cancer by genetic susceptibility to prostate cancer, measured with tertiles of the polygenic risk score (PRS).

	ALL			PRS Tertile 1			PRS Tertile 2			PRS Tertile 3			
	Co	Ca	OR (95% CI)	Co	Ca	OR (95% CI)	Co	Ca	OR (95% CI)	Co	Ca	OR (95% CI)	p-het
Zinc (µg/g)													0.001
Q1 (<86.2)	231	124	1.00	83	19	1.00	83	33	1.00	65	72	1.00	
Q2 (86.2-104.4)	207	166	1.58 (1.15;2.17)	68	17	1.12 (0.52;2.39)	63	60	2.56 (1.46;4.49)	76	89	1.15 (0.71;1.86)	
Q3 (104.5-127.0)	213	159	1.46 (1.06;2.02)	73	25	1.53 (0.75;3.11)	70	51	1.98 (1.12;3.50)	70	83	1.15 (0.70;1.88)	
Q4 (>127.0)	210	174	1.65 (1.21;2.27)	60	29	2.18 (1.08;4.40)	75	46	1.69 (0.96;2.99)	75	99	1.32 (0.82;2.13)	
p-trend			0.011			0.021			0.200			0.291	

PRS: polygenic risk score; Q: quartile; OR: Odds ratio for prostate cancer adjusted by age, education, BMI, family history of prostate cancer, physical activity and smoking status as fixed effects, and province of residence as a random effect; Co: controls; Ca: cancers; CI: confidence interval. p-het: P value for heterogeneity of odds ratios across tertiles of PRS obtained from Wald test; µg/g: micrograms/gram.



Click here to access/download
Supplementary Material
Supplementary Material.docx

CREDIT Author Statement

Enrique Gutiérrez-González: Data curation, Formal analysis, Investigation & Methodology; Validation & Visualization; Writing - original draft; ; Writing - review & editing; **Roberto Pastor-Barriuso:** Data curation; Formal analysis, Investigation & Methodology, exposure assessment, Writing - original draft; Writing - review & editing; **Adela Castelló:** Data curation, Formal analysis, Investigation & Methodology;; Writing - review & editing **Gemma Castaño-Vinyals** Participants' recruitment and data acquisition; Writing - review & editing; **, Nerea Fernández de Larrea-Baz** Participants' recruitment and data acquisition; ; Writing - review & editing; **Trinidad Dierssen-Sotos** Participants' recruitment and data acquisition; Writing - review & editing; **José Juan Jiménez-Moleón** Participants' recruitment and data acquisition; Writing - review & editing; **Ana Molina-Barceló** Participants' recruitment and data acquisition; Writing - review & editing; **Guillermo Fernández-Tardón** Participants' recruitment and data acquisition; Writing - review & editing; **Ángela Zumel-Marne** Participants' recruitment and data acquisition; Writing - review & editing; **Victor Moreno** Participants' recruitment and data acquisition; Writing - review & editing; **José Luis Gómez-Ariza** Investigation & Methodology; Laboratory analyses; Writing - review & editing; **M. Ángeles Sierra** Participants' recruitment and data acquisition; Writing - review & editing; **Tamara García-Barrera** Investigation & Methodology; Laboratory analyses; Writing - review & editing; **Ana Espinosa** Participants' recruitment and data acquisition; Writing - review & editing; **Elena Plans Beriso** Participants' recruitment and data acquisition; Writing - review & editing; **Inés Gómez-Acebo** Participants' recruitment and data acquisition; Writing - review & editing; **Nuria Aragonés** Participants' recruitment and data acquisition; Writing - review & editing; **Manolis Kogevinas:** Funding acquisition, Participants' recruitment and data acquisition; Writing - review & editing; **Marina Pollán:** Funding acquisition, Participants' recruitment and data acquisition; Investigation & Methodology Writing - review & editing; **Beatriz Pérez-Gómez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Validation & Visualization, Exposure assessment, Writing - original draft Writing - review & editing

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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

bstract

Toenail zinc and risk of prostate cancer in population-based MCC-Spain case-control study

oduction

Zinc (Zn) has a relevant role in prostate function, inhibiting the Krebs cycle.

It has been suggested that Zn could reduce the risk of prostate cancer (PC).

esearch on the relationship risk of PC vs xposure to Zn shows conflicting results.

ods

MCC-Spain study

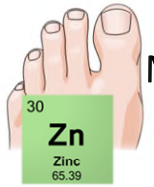
913 histologically confirmed incident **PC cases**

1,198 randomly-selected general population **controls**

Toenail Zn (Zn_t) determined by **inductively coupled plasma mass spectrometry (quartiles).**

Genetic susceptibility to PC: **Polygenic Risk Score (tertiles)**

Results



Men with **higher Zn_t levels had higher risk of PC**

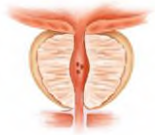
OR_{Q4} vs. OR_{Q1}: 1.41; 95% CI: 1.07–1.85

Higher association Zn_t-PC

a) In high-grade PC

→ ISUP≤2: RRR_{Q4} vs $_{Q1}$: 1.36; 1.01–1.83

→ ISUP3-5: RRR_{Q4} vs $_{Q1}$: 1.64; 1.06–2.54



b) In advanced tumours

→ cT1-cT2a: RRR_{Q4} vs $_{Q1}$: 1.40; 1.05–1.89

→ cT2b-cT4: RRR_{Q4} vs $_{Q1}$: 1.59; 1.00–2.53

c) Among those men with **lower genetic susceptibility to PC**

Lower

T1: OR_{Q4} vs. OR_{Q1}: 2.18; 1.08–4.40

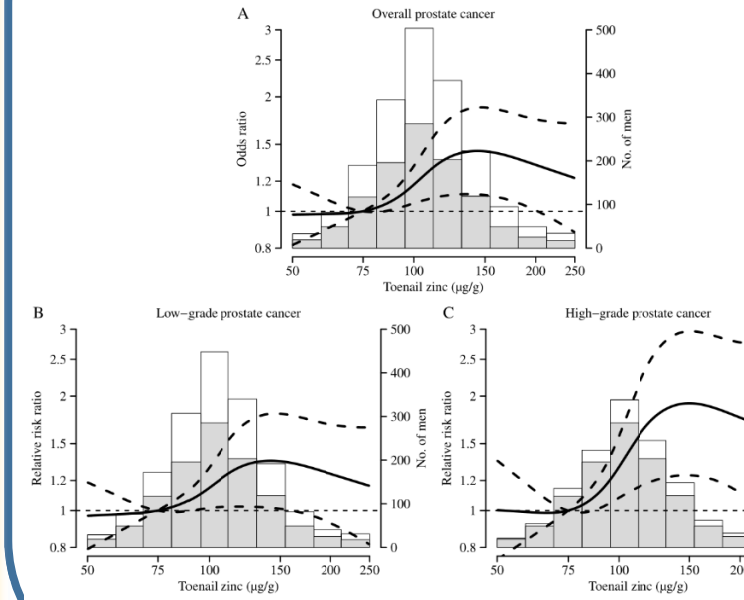
T2: OR_{Q4} vs. OR_{Q1}: 1.69; 0.96–2.99

T3: OR_{Q4} vs. OR_{Q1}: 1.32; 0.82–2.13

Higher

OR: Odds ratio; RRR: relative risk ratio; Q: quartile; T: tertile
ISUP: International Society Urological Pathology

Odds ratio for overall prostate cancer (A) and relative risk ratios by cancer grade (B)



Conclusions

- **High toenail Zn** levels were related **higher risk for PC**, especially for r aggressive or advanced tumours.
- This effect was stronger among with a lower genetic susceptibility to

Supplementary Table 1: Clinical profile of PC cases: Total number and percentages of tumour classification according to Gleason grade (biopsy), ISUP grading, PSA at diagnosis and AJCC stage (8th edition).

GLEASON grade (biopsy)		n	%
	6	397	43.5
	7	364	39.9
	8	71	7.8
	9	50	5.5
	10	1	0.1
	Unknown	30	3.3
Clinical T Stage		n	%
	cT1a	3	0.3
	cT1b	3	0.3
	cT1c	566	62.0
	cT2a	88	9.6
	cT2b	51	5.6
	cT2c	99	10.8
	cT3a	35	3.8
	cT3b	10	1.1
	cT4	1	0.1
	Unknown	57	6.2
ISUP Grading		n	%
	1	411	45.0
	2	270	29.6
	3	94	10.3
	4	71	7.8
	5	51	5.6
	Unknown	16	1.8
PSA at diagnosis		n	%
	<10	667	73.0
	10-20	174	19.1
	>20	65	7.1
	Unknown	7	0.8
AJCC stage (8th edition)		n	%
	I	335	36.7
	IIA	69	7.6
	IIB	237	26.0
	IIC	125	13.7
	IIIA	34	3.7
	IIIB	26	2.9
	IIIC	47	5.2
	IV A	3	0.3
	IV B	9	1.0
	Unknown	28	3.1

[a] The number of missing values on clinical stage, ISUP, PSA and AJCC stage (8th Edition) reported here is higher than the numbers reported in Table 3 and Table S2, because such tables only consider cases with complete information on all the covariables included in the models.

Supplementary Table 2. Distribution of toenail Zn (µg/g) in cases and controls

	Controls	**Cases**
p1	28.5	31.9
p5	60.3	64.8
p10	70.5	73.6
p25	86.2	89.9
p50	104.4	108.4
p75	127.0	129.3
p90	156.7	164.2
p95	192.5	202.8
p99	412.7	376.7

Supplementary Table 3: Geometric mean toenail Zn concentrations (µg/g) of controls and prostate cancer cases in MCC-Spain study

	All participants				Participants with genetic data			
	Controls		Cases		Controls		Cases	
	N	GMean	N	GMean	N	GMean	N	GMean
Total	1198	104.9	913	108.3	938	103.7	633	109.0
Age (years)								
≤60	253	105.5	204	111.4	196	105.3	145	112.4
61-70	565	107.4	453	108.0	452	106.3	306	108.7
≥71	380	100.8	256	106.4	290	98.5	182	106.8
Education								
Primary School	583	105.9	576	109.3	464	104.5	390	111.3
Secondary School	346	103.1	204	107.5	271	101.7	149	105.8
University or more	269	105.0	133	105.1	203	104.3	94	104.6
Ethnicity, n (%)								
European	1188	104.9	901	108.5	937	103.7	632	109.0
Non-European	8	112.5	12	94.6	1	78.4	1	96.1
Smoking status, n (%)								
Never smoker	334	97.6	272	111.1	259	95.3	194	110.8
Previous smoker	598	107.3	433	104.8	464	106.2	303	106.3
Current smoker	264	108.9	204	111.3	214	108.8	132	111.3
Body mass index (kg/m^2)								
<25	301	100.8	233	107.4	230	98.8	161	109.8
25-30	595	106.3	463	110.6	477	105.1	331	111.0
>30	268	104.8	204	103.6	209	104.4	134	103.3
Physical activity, (METs/week)								
0 METs/week	454	105.3	347	111.3	362	103.5	236	111.0
0.1-7.9 METs/week	123	103.5	119	107.7	100	101.8	73	110.0
8.0-15.9METs/week	141	101.4	116	103.0	106	101.0	80	106.0
≥16 METs/week	462	105.9	331	107.3	370	105.1	244	107.7
Province, n (%)								
Madrid	297	108.2	271	112.1	283	108.7	217	114.1
Barcelona	428	110.6	328	109.2	296	110.2	194	107.4
Asturias	94	108.0	15	107.2	57	102.7	-	-
Huelva	55	114.0	22	94.9	17	104.8	-	-
Cantabria	155	84.7	152	104.6	137	84.9	139	104.2
Valencia	62	96.2	70	105.2	55	94.4	50	109.2
Granada	107	104.2	55	104.9	93	104.9	33	105.3
Previous history of PC, n (%)								
No	1113	104.5	722	108.1	865	103.3	501	109.2
2nd Degree	10	107.8	26	100.6	9	108.0	20	96.2
One of 1st degrees	71	109.4	138	110.1	60	106.8	93	109.8
More than one of 1st degree	4	116.3	27	113.1	4	116.3	19	113.8
Stage								
cT1-cT2a	-	-	660	109.1	-	-	461	109.0
cT2b-cT4	-	-	196	106.6	-	-	148	108.5
Gleason grade								
Gleason=6	-	-	397	108.0	-	-	273	109.8
Gleason>6	-	-	486	110.2	-	-	338	110.5
ISUP grade								
ISUP≤2	-	-	681	108.1	-	-	466	108.9
ISUP 3-5	-	-	216	110.7	-	-	155	110.9
AJCC 8th classification								
I-IIa	-	-	404	107.7	-	-	288	107.1
IIb-IV	-	-	481	109.0	-	-	337	110.7
PSA at diagnosis								

<10	-	-	667	108.9	-	-	459	108.3
≥10	-	-	239	106.6	-	-	170	110.8
PSA screening								
Yes	825	106.4	878	108.1	657	104.7	609	108.8
No	317	99.7	29	116.5	245	99.2	19	118.8

GMean: Geometric mean; PC: prostate cancer; PSA: prostate specific antigen; MET: Metabolic Equivalent of Task; AJCC: American Joint Committe on Cancer; ISUP: International Society of Urological Pathology.

Supplementary Table 4. Association between toenail zinc and prostate cancer according to Gleason grade, AJCC classification and PSA at diagnosis

	GLEASON=6			GLEASON>6			
	Co	**Ca**	**RRR (95% CI)**	**Co**	**Ca**	**RRR (95% CI)**	**p-het**
Zinc (µg/g)							0.818
Q1 (<86.2)	290	130	1.00	290	43	1.00	
Q2 (86.2-104.4)	285	157	1.22 (0.85;1.76)	285	51	1.46 (1.05;2.04)	
Q3 (104.5-127.0)	286	185	1.51(1.06;2.15)	286	46	1.57 (1.12;2.19)	
Q4 (>127.0)	284	177	1.45 (1.01;2.06)	284	52	1.51 (1.08;2.10)	
p-trend			0.022			0.019	
	AJCC I-IIa			**AJCC IIb-IV**			
Zinc (µg/g)	**Co**	**Ca**	**RRR (95% CI)**	**Co**	**Ca**	**RRR (95% CI)**	**p-het**
Q1 (<86.2)	290	78	1.00	290	97	1.00	0.870
Q2 (86.2-104.4)	285	93	1.21 (0.84;1.73)	285	122	1.41 (1.01;1.97)	
Q3 (104.5-127.0)	286	121	1.44 (1.02;2.05)	286	127	1.47 (1.05;2.05)	
Q4 (>127.0)	284	106	1.35 (0.95;1.92)	284	126	1.44 (1.03;2.00)	
p-trend			0.062			0.041	
	PSA<10 ng/mL			**PSA≥ 10 ng/mL**			
Zinc (µg/g)	**Co**	**Ca**	**RRR (95% CI)**	**Co**	**Ca**	**RRR (95% CI)**	**p-het**
Q1 (<86.2)	290	125	1.00	290	54	1.00	0.427
Q2 (86.2-104.4)	285	165	1.40 (1.03;1.89)	285	53	1.11 (0.72;1.70)	
Q3 (104.5-127.0)	286	197	1.58 (1.17;2.13)	286	58	1.25 (0.82;1.91)	
Q4 (>127.0)	284	167	1.38 (1.02;1.86)	284	71	1.47 (0.98;2.21)	
p-trend			0.031			0.055	

Q: quartile; PSA: prostate specific antigen; RRR: Relative risk ratio of prostate cancer adjusted by age, education, BMI, family history of prostate cancer, physical activity, smoking status and province of residence; Co: controls; Ca: cancers; CI: confidence interval. p-het: Effect heterogeneity comparing OR of toenail Zn across categories of Gleason, AJCC and PSA.

Supplementary Table 5. Frequency table of cases and controls by genetic susceptibility, grade and stage

	Controls	ISUP ≤2	ISUP 3-5
PRS T1	312	70	18
PRS T2	313	135	58
PRS T3	313	261	79
	Controls	**cT1-cT2a**	**cT2b-T4**
PRS T1	312	73	16
PRS T2	313	134	52
PRS T3	313	254	80

PRS: Polygenic Risk Score; ISUP: International Society of Urological Pathology

Supplementary Table 6. Association between toenail zinc and prostate cancer by age

	ALL			≤ 60 YEARS			>60 YEARS			
	Co	**Ca**	**OR (95% CI)**	**Co**	**Ca**	**OR (95% CI)**	**Co**	**Ca**	**OR (95% CI)**	**p-het**
Zinc (µg/g)										0.389
Q1 (<86.2)	290	179	1.00	57	32	1.00	233	147	1.00	
Q2 (86.2-104.4)	285	221	1.33 (1.01;1.74)	54	50	1.99 (1.08;3.69)	231	171	1.20 (0.89;1.63)	
Q3 (104.5-127.0)	286	257	1.51 (1.15;1.98)	74	60	1.81 (1.00;3.25)	212	197	1.45 (1.07;1.97)	
Q4 (>127.0)	284	240	1.42 (1.08;1.86)	54	58	2.02 (1.11;3.70)	230	182	1.30 (0.96;1.76)	
p-trend			0.009			0.052			0.054	

Q: quartile; OR: Odds ratio for prostate cancer adjusted by education, BMI, family history of prostate cancer, physical activity and smoking status as fixed effects, and province of residence as a random effect; Co: controls; Ca: cancers; CI: confidence interval. p-het: P value for heterogeneity of odds ratios across categories of age obtained from Wald test.

Supplementary Figure 1. Flow diagram of participants in the present study