Osteoporosis International (2019) 30:2343–2348
https://doi.org/10.1007/s00198-019-05118-z

SHORT COMMUNICATION

Health effects associated with serum calcium concentrations: evidence from MR-PheWAS analysis in UK Biobank

A. Zhou[1] · H. A. Morris[2] · E. Hyppönen[1,3,4]

Received: 14 May 2019 / Accepted: 30 July 2019 / Published online: 8 August 2019

Abstract

Summary We conducted a phenome-wide Mendelian randomization analysis (MR-PheWAS) to survey health effects associated with high normal serum calcium. We found causal evidence for conditions related to renal function, bone and joint health, and cardiovascular risk. These conditions collectively suggest that tissue calcification may be a key mechanism through which serum calcium influences health.

Introduction Calcium is essential for the normal functioning of the cardiovascular system, muscles, and nerves. In this MR-PheWAS study, we sought to capture the totality of health effects associated with high normal serum calcium.

Methods We used data from up to 337,535 UK Biobank participants, and tested for associations between calcium genetic score (calcium-GS) and 925 disease outcomes, with follow-up analyses using complementary MR methods.

Results Calcium-GS was robustly associated with serum calcium concentration (F statistics = 349). After multiple testing correction ($P < 1.62E-4$), we saw genetic evidence for an association between high serum calcium and urinary calculus (OR per 1 mg/dl 3.5, 95%CI 1.3–9.2), renal colic (9.1, 95%CI 2.5–33.5), and allergy/adverse effect of penicillin (2.2, 95%CI 1.5–3.3). Secondary analyses with independent replication from consortia meta-analyses suggested further effects on myocardial infarction and osteoarthrosis.

Conclusion We found causal evidence for effects of high normal serum calcium with conditions related to renal function, bone and joint health, and cardiovascular risk, which may collectively reflect influences on tissue calcification and immune function.

Keywords Calcium genetic score · Mendelian randomization · Phenome-wide · Serum calcium

Introduction

Although less than 1% of the total body calcium is in blood, the circulating ionized component is essential for the normal functioning of the cardiovascular system, muscles, and nerves. While circulating calcium concentrations are typically tightly regulated [1], a genome-wide association study (GWAS) has identified common variants explaining some of the variations in concentrations [2]. In this phenome-wide association study (PheWAS), we use the information on these genetic calcium variants to obtain causal evidence for the association between serum calcium level and multiple disease outcomes.

Our study combines two relatively new approaches, namely Mendelian randomization (MR) and PheWAS, for disease screening. There has been a growing recognition that MR can be used to prioritize or complement randomized controlled trials (RCT) for assessing evidence on causality [3]. In MR, we use genetic variants as proxy markers or instruments for the exposure of interest (here, serum calcium). As genetic

H.A. Morris is deceased. This paper is dedicated to his memory.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00198-019-05118-z) contains supplementary material, which is available to authorized users.

✉ E. Hyppönen
Elina.Hypponen@unisa.edu.au

[1] Australian Center for Precision Health, University of South Australia Cancer Research Institute, GPO Box 2471, Adelaide, SA 5001, Australia

[2] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

[3] Population, Policy and Practice, UCL Institute of Child Health, London, UK

[4] South Australian Health and Medical Research Institute, Adelaide, Australia

variants are randomly allocated at conception, resembling the random allocation process in a RCT, analyses on disease association using the MR approach are better able to overcome limitations such as confounding and reverse causality that affect findings from conventional studies [3]. The benefits of combining the MR analyses with the hypothesis-free PheWAS approach, which screens for associations of a variant or a risk score across multiple disease outcomes, include the ability to capture novel associations for which no prior evidence is available. In this study, we used information from up to 337,535 UK Biobank participants, and tested for associations between calcium genetic score (calcium-GS) and 925 disease outcomes, with follow-up analyses using multiple complementary MR methods to interrogate the causal nature of the signals.

Materials and methods

Participants UK Biobank is a large prospective cohort study with over 500,000 participants aged 37–73 years recruited from 22 assessment centers across the UK between 2006 and 2010 [4]. The study had the genome-wide genotyping data and also collected a wide range of health and lifestyle information, with health outcome data enhanced by electronic health records and mortality registrations until March 2016. We restricted our analyses to participants who were unrelated and confirmed as White-British ancestry based on self-report and genetic information [5] ($N = 337{,}535$).

Genetic instrument We used seven genome-wide significant variants identified from the large scale discovery study to proxy serum calcium concentration, including *CASR* (rs1801725), *DGKD* (rs1550532), *GCKR* (rs780094), *GATA 3* (rs10491003), *CARS* (rs7481584), *DGKH/KIAA0564* (rs7336933), and *CYP24A1* (rs1570669) [2]. Each variant was coded as 0, 1, and 2 to reflect the number of calcium-increasing alleles. We derived a weighted genetic score for the serum calcium concentration (calcium-GS) by first computing the weighted average of number of calcium-increasing alleles (with weights being the variant-calcium effect estimates in the original GWAS [2]) and then multiplying it by the number of available variants.

Serum calcium concentration (mg/dL) was measured using Arsenazo III analysis on a Beckman Coulter AU5800 (http://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=30680).

Phenome construction Phenome (or disease phenotypes) were constructed using linked Hospital Episode Statistics and Mortality data, which were recorded using the ICD coding system, versions 9 and 10. We mapped the ICD codes to phecodes, which have been shown to provide disease groupings which are closely aligned with diseases commonly mentioned in clinical practice and genomic studies [6]. Participants with the phecode of interest were recorded as cases, while controls were those without the phecode or directly related codes. We restricted analyses to the phecodes with more than 200 cases, suggested in an earlier simulation study to reflect the threshold with reasonable power for detecting variant outcome associations in PheWAS [7].

Statistical analyses As instrument validation, we first examined the association between calcium-GS and serum calcium concentration by fitting a linear regression model. Our MR-PheWAS analyses involved two stages, with the initial PheWAS to screen for disease outcomes associated with calcium-GS and 2-sample MR analyses to follow up the signals and interrogate their causal nature. In the PheWAS analysis, a logistic model was fitted, adjusting for age, sex, SNP array, assessment center, and top 15 genetic principal components, with threshold lower than 5% false discovery rate (FDR) used to account for multiple testing (1.62E-4) [8]. In the 2-sample MR analyses, variant-calcium and variant-disease outcome effects were retrieved from the GWAS for serum calcium concentration and from the initial PheWAS analysis in the UK Biobank, respectively. We computed the conventional inverse-variance-weighted (IVW) MR estimates and complemented it with pleiotropy-robust methods, including MR Egger regression [9], weighted median MR [10], and weighted mode MR [11], each with largely independent assumptions on pleiotropy and consistent estimates across multiple approaches that strengthen causal evidence [3]. We also performed leave-one-out analysis and MR pleiotropy residual sum and outlier (MR-PRESSO) test [12] to examine if IVW estimates were distorted by outliers. Where possible results were replicated by consortia data from the MR-Base [13] including outcomes identified under nominal significance ($p < 0.01$). Instrument validation was performed in STATA (v14), and all other analyses were conducted in R using the PheWAS [14], Two-sample MR [13], and MR-PRESSO [12] package.

Results

Instrument validation In the UK Biobank, mean serum calcium concentration is 9.5 mg/dl (SD 0.38), with 1.5% of participants outside the normal range of 8.5–10.5 mg/dL [15] and 1.2% above 10.5 mg/dL. Calcium-GS was robustly associated with serum calcium concentration with F statistic of 349, explaining 0.94% of variation ($P_{\text{trend}} < 1.0\text{E-}300$, Supplementary Fig. 1); this provided assurance that our MR analyses were less likely to be affected by weak instrument bias (threshold F statistic = 10) [16].

MR-PheWAS We observed associations with three phecodes at the 5% FDR corrected threshold, representing two distinct diseases conditions (Fig. 1). Calcium-GS was associated with the risk of urinary calculus, renal colic, and allergy/adverse effect of penicillin ($P \leq 6.2E\text{-}05$ for all; Fig. 1), with odds ratios (OR) from IVW MR of 3.5 (95%CI 1.3–9.2), 9.1 (95%CI 2.5–33.5), and 2.2 (95%CI 1.5–3.3) per 1 mg/dL higher serum calcium, respectively (Table 1). For all three diseases, MR estimates using different methods were broadly consistent. We observed no evidence for unbalanced horizontal pleiotropy ($P_{\text{pleiotropy}} \geq 0.09$ for all, Table 1) and leave-one-out analysis and MR-PRESSO outlier test did not suggest influential outliers (Supplementary Table 1). In the secondary analysis, of 22 nominally significant signals ($p < 0.01$), four phenotypes (or their proxies) were identified in the MR-Base [13]. Independent replication was suggested for myocardial infarction, and osteoarthrosis (Table 1), with an inverse association seen for osteoarthrosis.

Discussion

This is the first MR-PheWAS examining the totality of health effects associated with high normal serum calcium concentrations. We found evidence for a possible causal association for higher serum calcium levels with an increased risk for renal colic, urinary calculus, and allergy/adverse effect of penicillin, while independent confirmation for association was obtained for myocardial infarction and osteoarthrosis which were nominally significant in our analyses.

Our study provides causal evidence for an adverse effect of high normal serum calcium on the risk for urinary calculus and renal colic. This is consistent with kidney stone formation being a key complication of hypercalcemia, a condition in which serum calcium level is above the normal range. It also supports findings from the calcium supplementation trial, in which 36,000 postmenopausal women were followed-up for 7 years, and individuals who received daily calcium and vitamin D supplements had 17% increased risk for kidney stone formation compared to those who received placebo [17]. We also observed an association with allergy/adverse effects of penicillin, which may reflect a consequence of compromised renal function associated with high normal calcium level given that adverse antibiotic effects are often observed among people with renal insufficiency [18]. Further, also supporting previous studies [19, 20], serum calcium was associated with an increased cardiovascular risk but a decreased risk for osteoarthrosis in our secondary analyses. Taken together, these observed genetic evidence may all reflect downstream consequences of tissue calcification. That said, the association with allergy/adverse effects of penicillin and with osteoarthrosis/osteoarthritis may also be related to the role of calcium in immune function [21], although the underlying mechanism is likely to be complex given the opposing direction of the association for the two conditions. Independent cohort studies and functional studies are warranted to validate and characterize these relatively novel associations.

The current study, based on a large prospective cohort, is the first MR-PheWAS to study the totality of health effects associated with higher serum calcium levels. To ensure robust

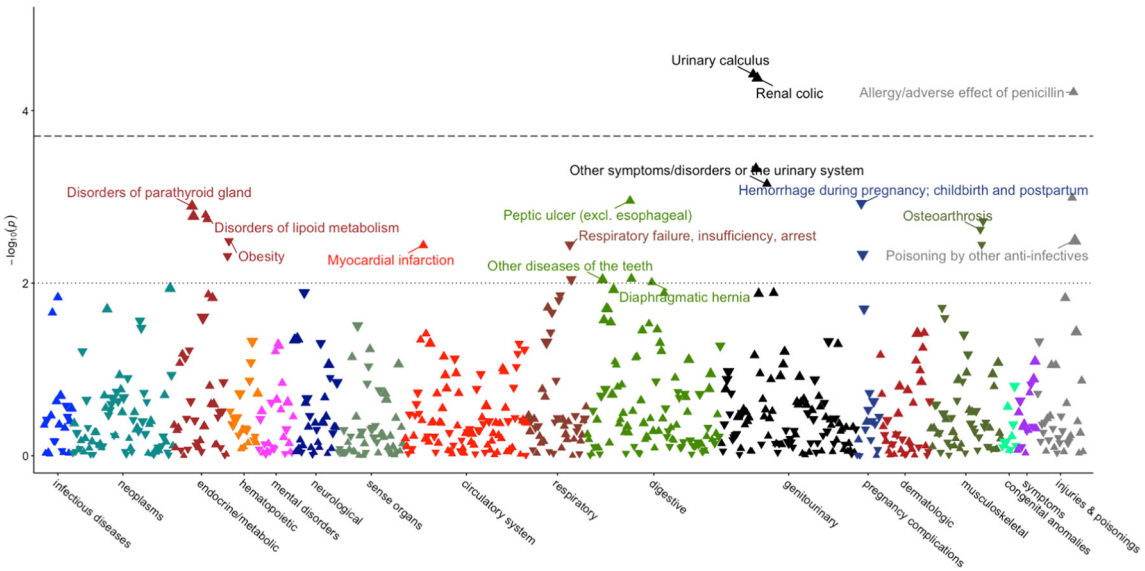

Fig. 1 Manhattan plot for the phenome-wide association analysis using calcium-GS. Dashed line = 5% FDR threshold ($P = 1.62E\text{-}4$); dotted line = nominal significant threshold ($P = 0.01$); upward triangles indicate OR ≥ 1; downward triangles indicate OR < 1; size of the triangle is proportional to OR; among nominally significant and correlated outcomes ($P < 0.01$), labels are only shown for the ones in the highest level. For each calcium-GS-outcome association, a logistic model was fitted adjusting for age, sex, SNP array, assessment center, and top 15 genetic principal components. A higher calcium-GS indicates a higher serum calcium concentration

Table 1 Two-sample MR analyses for PheWAS signals and for replication of nominal PheWAS associations

	Stage I: UK biobank PheWAS, *P*	Stage II: two-sample MR						
		Source of SNP-outcome effects	[a]*N*/ Cases:controls	MR IVW		WMedian	WMode	MR Egger
				[b]OR/β (95%CI)	*P*	[b]OR/β (95%CI)	[b]OR/β (95%CI)	[b]OR/β (95%CI)
PheWAS signals								
Urinary calculus	3.82E-05	UK Biobank	5641:330763	3.51 (1.34, 9.17)	0.011	2.19 (1.04, 4.63)	1.85 (0.87, 3.96)	0.99 (0.24, 4.11)
								$P_{pleiotropy} = 0.93$
Renal colic	4.24E-05	UK Biobank	1689:330763	9.11 (2.48, 33.5)	8.82E-04	7.45 (2.03, 27.4)	7.26 (1.76, 30.1)	5.52 (0.43, 70.5)
								$P_{pleiotropy} = 0.66$
Allergy/adverse effect of penicillin	6.20E-05	UK Biobank	13,152:313986	2.21 (1.49, 3.30)	9.36E-05	2.38 (1.48, 3.84)	2.38 (1.43, 3.96)	2.38 (1.07, 5.29)
								$P_{pleiotropy} = 0.84$
[c]Nominal associations ($P < 0.01$) with replication through the MR-Base								
Musculoskeletal system								
Osteoarthrosis	2.40E-03	UK Biobank	36,434:301101	0.67 (0.51, 0.88)	4.40E-03	0.63 (0.47, 0.85)	0.62 (0.45, 0.85)	0.55 (0.33, 0.91)
								$P_{pleiotropy} = 0.38$
Replication: knee and hip osteoarthritis[d]	–	arcOGEN	3498:11009	0.34 (0.17, 0.7)	3.70E-03	0.31 (0.14, 0.73)	0.31 (0.13, 0.76)	0.29 (0.08, 1.1)
								$P_{pleiotropy} = 0.79$
Cardiovascular system								
Myocardial infarction	3.66E-03	UK Biobank	9828:311419	1.99 (1.17, 3.39)	0.011	2.03 (1.17, 3.51)	2.01 (1.10, 3.67)	1.95 (0.67, 5.65)
								$P_{pleiotropy} = 0.96$
Replication: myocardial infarction	–	CARDIoGRAMplusC4D	43,676:128199	1.48 (1.08, 2.02)	0.015	1.50 (1.04, 2.16)	1.52 (1.03, 2.24)	1.66 (0.95, 2.92)
								$P_{pleiotropy} = 0.65$
Obesity and lipid profile								
Obesity	3.26E-03	UK Biobank	8840:328554	0.48 (0.29, 0.78)	3.13E-03	0.51 (0.28, 0.94)	0.51 (0.28, 0.94)	0.48 (0.18, 1.28)
								$P_{pleiotropy} = 0.99$
Replication: obesity class I	–	GIANT	32,858:65839	1.33 (0.84, 2.13)	0.23	1.56 (0.99, 2.46)	1.56 (0.97, 2.51)	1.94 (0.83, 4.53)
								$P_{pleiotropy} = 0.35$
Disorders of lipoid metabolism	1.63E-03	UK Biobank	29,411:308124	1.55 (0.71, 3.39)	0.27	1.28 (0.92, 1.80)	1.29 (0.90, 1.84)	0.89 (0.21, 3.85)
								$P_{pleiotropy} = 0.41$
Replication: HDL cholesterol[d] (SD, 1 SD = 15.5 mg/dL)	–	GLGC	187,167	0.07 (−0.18, 0.32)	0.57	0.10 (−0.03, 0.23)	0.10 (−0.03, 0.24)	0.17 (−0.29, 0.63)
								$P_{pleiotropy} = 0.64$
Replication: LDL cholesterol[d] (SD, 1 SD = 38.7 mg/dL)	–	GLGC	173,082	0.21 (−0.09, 0.50)	0.17	0.10 (−0.04, 0.24)	0.08 (−0.06, 0.22)	−0.14 (−0.51, 0.24)
								$P_{pleiotropy} = 0.091$
Replication: triglycerides[d] (SD, 1 SD = 90.7 mg/dL)	–	GLGC	177,861	0.56 (−1.07, 2.19)	0.50	−0.0016 (−0.14, 0.13)	0.006 (−0.12, 0.13)	−1.02 (−3.48, 1.43)
								$P_{pleiotropy} = 0.19$
Replication: total cholesterol[d]	–	GLGC	187,365	0.38 (−0.35, 1.10)	0.31	0.08 (−0.06, 0.22)	0.09 (−0.06, 0.23)	−0.44(−1.40, 0.51)

Table 1 (continued)

	Stage I: UK biobank PheWAS, *P*	Stage II: two-sample MR						
		Source of SNP-outcome effects	[a]*N*/ Cases:controls	MR IVW		WMedian	WMode	MR Egger
				[b]OR/β (95%CI)	*P*	[b]OR/β (95%CI)	[b]OR/β (95%CI)	[b]OR/β (95%CI)
(SD, 1 SD = 41.8 mg/dL)								$P_{pleiotropy} = 0.11$

OR, odds ratio per 1 mg/dL increase in genetically instrumented serum calcium concentration; *IVW*, inverse variance weighted method; *Egger*, MR Egger regression; *WMedian*, weighted median method; *WMode*, weighted mode method

[a] *N* for continuous traits; cases:controls for binary traits

[b] OR for binary traits; *β* for continuous traits, including HDL cholesterol, LDL cholesterol, triglycerides, and total cholesterol

[c] Nominally significant ($P < 0.01$) associations with no replication data included other symptoms/disorders or the urinary system, peptic ulcer (excl. esophageal), hemorrhage during pregnancy, childbirth and postpartum, disorders of parathyroid gland, poisoning by other anti-infectives, respiratory failure, insufficiency, arrest, other diseases of the teeth, and diaphragmatic hernia

[d] Harmonized SNPs included rs1570669, rs7336933, rs7481584, rs10491003, rs1801725, and rs780094

findings, we applied different MR methods and sensitivity analyses to evaluate potential bias due to pleiotropic effects of genetic instruments. We also performed replication for outcomes with calcium variant-outcome results available in independent resources. It is important to note that our study also has some limitations. Despite its large sample size, our analyses are likely to be underpowered for rare diseases and/or modest effects, which may explain why only three outcomes (out of 925) survived the 5% FDR threshold, although the low discovery rate may also be attributed to the tightly regulated calcium homeostasis (such that serum calcium is maintained in a stable and narrow range) [1]. Also as MR assumes a linear effect, any non-linear effects using this approach would have been imprecisely captured (even though they could still be statically significant) or missed. Further, as our calcium-GS can only proxy serum calcium concentration within the normal range [2], effects of high serum calcium (i.e., above the normal range) are likely to be underestimated. For example, there is evidence that elevated serum calcium level (> 10.2 mg/dL) is associated with an increased risk of heart failure [22], while our data had little power to detect this association that operates at high end of the distribution (observed OR 1.01, 95% CI 0.98–1.03, $P = 0.35$). Genetic instruments, such as ours, approximate the average effects over the life course, while the true biological association for serum calcium could vary by life stage and potentially be more complex than that indexed in our study. We restricted our analysis to participants of White-British descent, which may limit its inference in other ethnic groups. Furthermore, collider bias may arise due to selection in the UK Biobank which only had 5% participation rate, although related effects are likely to be modest [23]. Finally, MR approach only estimates the population average effect of serum calcium; gene-environment interaction studies are warranted to follow-up the observed associations examining if they vary across subpopulations defined by genetic/phenotypic profiling. Moreover, a two-step epigenetic Mendelian randomization strategy [24] can be employed to evaluate any involvement/contribution of epigenetics providing mechanistic insights to the observed associations, although this study design relies on the availability of epigenetic data.

Conclusion

In conclusion, high normal serum calcium may reflect increases in tissue calcification and alternation in immune function, with influences on renal function, bone and joint health, and cardiovascular risk.

Funding This study was funded by the Australian National Health and Medical Research Foundation (NHMRC), grant number 1123603.

Compliance with ethical standards

Ethical approval UK Biobank was approved by the National Information Governance Board for Health and Social Care and North West Multicentre Research Ethics Committee (11/NW/0382). The present study was conducted under application number 20175.

Conflict of interests None.

References

1. Mundy GR, Guise TA (1999) Hormonal control of calcium homeostasis. Clin Chem 45:1347–1352
2. O'Seaghdha CM, Wu H, Yang Q, Kapur K, Guessous I, Zuber AM, Köttgen A, Stoudmann C, Teumer A, Kutalik Z, Mangino M, Dehghan A, Zhang W, Eiriksdottir G, Li G, Tanaka T, Portas L, Lopez LM, Hayward C, Lohman K, Matsuda K, Padmanabhan S, Firsov D, Sorice R, Ulivi S, Brockhaus AC, Kleber ME, Mahajan A, Ernst FD, Gudnason V, Launer LJ, Mace A, Boerwinckle E, Arking DE, Tanikawa C, Nakamura Y, Brown MJ, Gaspoz JM, Theler JM, Siscovick DS, Psaty BM, Bergmann S, Vollenweider P, Vitart V, Wright AF, Zemunik T, Boban M, Kolcic I, Navarro P, Brown EM, Estrada K, Ding J, Harris TB, Bandinelli S, Hernandez D, Singleton AB, Girotto G, Ruggiero D, d'Adamo AP, Robino A, Meitinger T, Meisinger C, Davies G, Starr JM, Chambers JC, Boehm BO, Winkelmann BR, Huang J, Murgia F, Wild SH, Campbell H, Morris AP, Franco OH, Hofman A, Uitterlinden AG, Rivadeneira F, Völker U, Hannemann A, Biffar R, Hoffmann W, Shin S–Y, Lescuyer P, Henry H, Schurmann C, The SUNLIGHT consortium, The GEFOS consortium, Munroe PB, Gasparini P, Pirastu N, Ciullo M, Gieger C, März W, Lind L, Spector TD, Smith AV, Rudan I, Wilson JF, Polasek O, Deary IJ, Pirastu M, Ferrucci L, Liu Y, Kestenbaum B, Kooner JS, Witteman JCM, Nauck M, Kao WHL, Wallaschofski H, Bonny O, Fox CS, Bochud M (2013) Meta-analysis of genome-wide association studies identifies six new loci for serum calcium concentrations. PLoS Genet 9:e1003796
3. Davies NM, Holmes MV, Davey Smith G (2018) Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ 362:k601
4. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R (2015) UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 12:e1001779
5. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J (2018) The UK biobank resource with deep phenotyping and genomic data. Nature 562:203–209
6. Wei WQ, Bastarache LA, Carroll RJ, Marlo JE, Osterman TJ, Gamazon ER, Cox NJ, Roden DM, Denny JC (2017) Evaluating phecodes, clinical classification software, and ICD-9-CM codes for phenome-wide association studies in the electronic health record. PLoS One 12:e0175508
7. Verma A, Bradford Y, Dudek S, Lucas AM, Verma SS, Pendergrass SA, Ritchie MD (2018) A simulation study investigating power estimates in phenome-wide association studies. BMC Bioinformatics 19:120
8. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289–300
9. Bowden J, Davey Smith G, Burgess S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 44:512–525
10. Bowden J, Davey Smith G, Haycock PC, Burgess S (2016) Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 40:304–314
11. Hartwig FP, Davey Smith G, Bowden J (2017) Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol 46:1985–1998
12. Verbanck M, Chen CY, Neale B, Do R (2018) Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 50:693–698
13. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, Tan VY, Yarmolinsky J, Shihab HA, Timpson NJ, Evans DM, Relton C, Martin RM, Davey Smith G, Gaunt TR, Haycock PC (2018) The MR-Base platform supports systematic causal inference across the human phenome. eLife 7
14. Carroll RJ, Bastarache L, Denny JC (2014) R PheWAS: data analysis and plotting tools for phenome-wide association studies in the R environment. Bioinformatics 30:2375–2376
15. Bove-Fenderson E, Mannstadt M (2018) Hypocalcemic disorders. Best Pract Res Clin Endocrinol Metab 32:639–656
16. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G (2008) Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 27:1133–1163
17. Jackson RD, LaCroix AZ, Gass M et al (2006) Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 354: 669–683
18. Manian FA, Stone WJ, Alford RH (1990) Adverse antibiotic effects associated with renal insufficiency. Rev Infect Dis 12:236–249
19. Larsson SC, Burgess S, Michaelsson K (2017) Association of genetic variants related to serum calcium levels with coronary artery disease and myocardial infarction. Jama 318:371–380
20. Li H, Zeng C, Wei J, Yang T, Gao SG, Li YS, Luo W, Xiao WF, Xiong YL, Lei GH (2016) Serum calcium concentration is inversely associated with radiographic knee osteoarthritis: a cross-sectional study. Medicine 95:e2838
21. Vig M, Kinet JP (2009) Calcium signaling in immune cells. Nat Immunol 10:21–27
22. Lutsey PL, Alonso A, Michos ED, Loehr LR, Astor BC, Coresh J, Folsom AR (2014) Serum magnesium, phosphorus, and calcium are associated with risk of incident heart failure: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr 100:756–764
23. Gkatzionis A, Burgess S (2018) Contextualizing selection bias in Mendelian randomization: how bad is it likely to be? Int J Epidemiol 48:691–701
24. Relton CL, Davey Smith G (2012) Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. Int J Epidemiol 41:161–176