Health effects of high serum calcium levels

Health effects of high serum calcium levels: updated phenome-wide Mendelian randomisation investigation and review of Mendelian randomisation studies

Running head: Serum calcium and health

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Abstract

Background Calcium plays a role in a wide range of biological functions. Here we conducted a phenome-wide Mendelian randomisation (MR-PheWAS) analysis and a systematic review for MR studies to comprehensively investigate the health effects of serum calcium.

Methods One-hundred and thirty genetic variants strongly associated with serum calcium levels were used as instrumental variables. A phenome-wide association analysis (PheWAS) was conducted to examine the associations of genetically predicted serum calcium with 1473 distinct phenotypes in the UK Biobank including 339,197 individuals. Observed associations in PheWAS were further tested for replication in two-sample MR replication analysis. A systematic review for MR studies on serum calcium was performed to synthesize the published evidence and compare with the current MR-PheWAS findings.

Findings Higher genetically predicted calcium levels were associated with decreased risk of 5 diseases in dermatologic and musculoskeletal systems and increased risk of 17 diseases in circulatory, digestive, endocrine, genitourinary and immune systems. Eight associations were replicated in two-sample MR analysis. These included decreased risk of osteoarthritis and increased risk of coronary artery disease, myocardial infarction, coronary atherosclerosis, hyperparathyroidism, disorder of parathyroid gland, gout, and calculus of kidney and ureter with increased serum calcium. Systematic review of 25 MR studies provided supporting evidence on five out of the eight disease outcomes, while the increased risk of gout, hyperparathyroidism and disorder of parathyroid gland were novel findings.

Interpretation This study found wide-ranged health effects of high serum calcium, which suggests that the benefits and adversities of strategies promoting calcium intake should be assessed.

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Key words: calcium; health; Mendelian randomisation; PheWAS; systematic review

Research in context

Evidence before this study

Calcium is an essential nutrient that can be merely obtained to the body through diet and dietary supplement. Calcium plays a role in a wide range of biological functions as the calcium signalling system regulates divergent cellular processes. The current dietary guideline recommends a calcium intake of 200 to 1300 mg/d depending on age and sex. However, randomized controlled studies did not found a protective effect of calcium on fracture. A previous phenome-wide Mendelian Randomisation analysis using a few genetic instruments revealed several adversities of high serum calcium levels.

Added value of this study

This updated phenome-wide Mendelian Randomisation analysis found that higher genetically predicted serum calcium levels were associated with decreased risk of psoriasis, skin cancer, fasciitis, osteoarthrosis, and Dupuytren's disease, and increased risk of hypertension, coronary atherosclerotic diseases, liver cirrhosis, nonalcoholic fatty liver disease, parathyroid and endocrine gland diseases, diabetes, gout, calculus of kidney and ureter, poisoning by antibiotics, and allergy/adverse effect of penicillin. The associations for coronary disease, hyperparathyroidism, disorder of parathyroid gland, gout, calculus of kidney and ureter, and osteoarthritis were successfully replicated in two-sample Mendelian Randomisation analyses. The systematic review additionally found that higher genetically predicted serum calcium levels were associated with increased risk of migraine and chronic pancreatitis, and lower risk of colorectal cancer, glioma, and Alzheimer’s disease as well as reduced bone mineral density and JT and QT intervals.

Implications of the all the available evidence

Our findings suggest that the benefits and adversities of strategies promoting calcium intake should be assessed.

1. Introduction

Calcium is an essential nutrient that can be merely obtained to the body through diet and dietary supplement. The current dietary guideline recommends a calcium intake of 200 to 1300 mg/d depending on age and sex (1). Except for influencing skeletal mineralization (2), calcium plays a role in a wide range of other biological functions as the calcium signalling system regulates divergent cellular processes (3). Randomized controlled studies have unexpectedly found that calcium or calcium plus vitamin D supplements cannot prevent fracture among healthy community-dwelling adults but instead increased the risk of kidney stones (4) and cardiovascular disease (5, 6). The positive association between calcium and cardiovascular disease risk has also been observed in Mendelian randomisation (MR) and observational studies (7-9). In addition, higher calcium levels may shorten longevity (10) and elevate the risk of other health conditions, like migraine (11) and chronic pancreatitis (12). These findings question the overall benefit of calcium supplementation over its adversity and indicate the need of a systematic appraisal of the health effects of calcium.

A phenome-wide Mendelian Randomisation analysis (MR-PheWAS) has been proposed as a hypothesis-searching method to comprehensively examine the causality between an exposure and a broad range of outcomes (13). The approach can minimize confounding and diminish reverse causality by using genetic variants as instrumental variables for an exposure (e.g., serum calcium) (14). A previous MR-PheWAS in the UK Biobank including 337,535 individuals found that genetically predicted serum calcium levels were associated with risk of urinary stones, allergy/adverse effect of penicillin, osteoarthrosis, and myocardial infarction (15). However, the

study utilized 7 genetic instruments that explain less than 1% of phenotypic variance of serum calcium levels (16) and might therefore have overlooked weak-to-moderate associations due to inadequate power. Here, we conducted an updated PheWAS in the UK Biobank and two-sample MR analysis with an improved genetic instrument to robustly assess the health outcomes in relation to serum calcium levels. We also conducted a systematic review of MR studies on serum calcium to comprehensively synthesize the evidence to validate any possible health effects.

2. Method

2.1 Study design

Figure 1 shows the study design overview. We firstly performed an updated PheWAS of serum calcium in the UK Biobank study (**Supplementary Figure 1**). We then tested identified associations for replication in external data sources using two-sample MR approach (**Supplementary Figure 1**). To comprehensively assess the health effects of serum calcium, we further conducted a systematic review of published MR studies on serum calcium. There are three assumptions for MR analysis (14). The first assumption is that the genetic variants used as instrumental variables should be strongly associated with the exposure; the second assumption is that the utilized genetic variants should not be associated with any confounders; and the third assumption is that the selected genetic variants should affect the outcome merely through the risk factor, not via other pathways.

2.2 Genetic instrument selection

Genetic variants (i.e., single nucleotide polymorphisms, SNPs) strongly associated with serum calcium levels ($p<5\times10^{-8}$) were identified from a genome-wide association analysis adjusted for age, sex, the first ten genetic principal components, assessment centre, genotyping batch, and month of assessment, in 325,659 individuals in the UK Biobank (17). We pruned SNPs without linkage disequilibrium (r^2 <0.001) and selected 130 SNPs as instrumental variables for serum calcium. These SNPs explain about 3.72% of variance of serum calcium levels. We rescaled the unit of these instrumental variables to one standard deviation (SD, ~0.5 mg/dL) increase. Detailed information on genetic instruments is presented in **Supplementary Table 1**.

2.3 PheWAS analysis

PheWAS analysis was conducted in the UK Biobank study including a total of 339,197 unrelated White British individuals aged between 40-69 years in 2006-2010 after removal of participants of other ancestries to minimize population bias. The study collected information on germline genotype and a wide range of health outcomes with diagnostic data from national medical records (e.g., inpatient hospital episode records, cancer registry, and death registry). We used the PheCODE schema (18) to define phenotypes based on an integrative application of 10,750 unique ICD-10 codes and 3,113 ICD-9 codes. Detailed information on genotyping and quality control in UK Biobank is described in previous studies (19, 20).

2.4 Two-sample MR analysis

Two-sample MR analysis was based on the summary-level data from the R5 release of FinnGen consortium (21) and international genetic consortia (22-28). The R5 FinnGen consortium is a

project combining genotype data from Finnish biobanks and digital health record data on clinically defined outcomes from Finnish health registries in ~269,000 individuals. Detailed information on FinnGen and used international genetic consortia is displayed in **Supplementary Table 2**.

2.5 Statistics

In the PheWAS analysis, we constructed a weighted genetic risk score with selected genetic instruments associated with serum calcium levels by summing up the number of calcium-increasing alleles for each SNP weighted by effect size on calcium levels and then adding this weighted score for all used SNPs. As suggested by power calculation (29), outcomes with more than 200 cases were included in the analysis. The associations of genetically proxied serum calcium levels with diseases were estimated using logistic regression models adjusted for age, sex, body mass index (BMI), assessment centre, and the first ten genetic principal components. To reduce the influence of calcium supplements on serum calcium levels, we conducted a sensitivity analysis in individuals without calcium supplementation. In addition, we performed a sensitivity analysis stratified by vitamin D status with consideration that vitamin D plays an important role in calcium absorption. We used a false discovery rate (FDR) correction with the method by Benjamini-Hochberg to account for multiple comparisons in phenome-wide analysis (30).

In two-sample MR analysis, the inverse variance weighted (IVW) method under multiplicative random effects was used as the main analysis. Estimates for one association from different sources were combined using fixed-effect meta-analysis. Four sensitivity analyses were

performed, including the weighted median, MR-Egger, contamination mixture, and MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier) methods. The weighted median analysis can provide consistent causal estimates on the prerequisite that more than a half of instrumental variables are valid (31). MR-Egger regression can generate estimates after correcting for horizontal pleiotropy; however, corresponding associations are usually unpowered (32). Contamination mixture method excels at the analysis with hundreds of instrumental variables and can provide causal estimates with absence of invalid instruments (33). MR-PRESSO analysis can detect out-lying SNPs and provide the causal estimate after removal of these outliers (34). The embedded distortion test can be used to examine the difference in estimates before and after outlier removal (34). We performed multivariable MR analyses to detect possible mediation effects of blood pressures and urate. We used Cochrane's Q value to assess the heterogeneity in estimates of SNPs for each association and the *p* value for MR-Egger intercept to assess the horizontal pleiotropy ($p<0.05$). The power for two-sample MR analyses was estimated using an online tool (**Supplementary Table 3**) (35). The association with a $p<0.05$ was deemed significant in the two-sample MR analysis for replication. All tests were two-sided and conducted using a R package by Carroll et al (36), and TwoSampleMR, MendelianRandomziation and MR-PRESSO packages (34, 37, 38) in R Software 4.0.2.

2.6 Systematic review for MR studies on serum calcium

We performed a systematic review of MR studies on serum calcium to complement our findings in PheWAS and two-sample MR analysis. We identified articles by a search in the PubMed database up to 8 September 2021 with the following search strategy: "Mendelian Randomization

Analysis"[Mesh] OR mendelian[tiab] AND "Calcium"[Mesh] OR calcium[tiab] (**Supplementary Table 4**). Information on the first author, year of publication, used SNPs, outcomes, numbers of cases and controls, and the association estimates in main statistical method was extracted. Studies on dietary calcium intake, weak instrumental variables, and offspring health were excluded. The literature search, review process, and data extraction were done in parallel by two authors (S.Y and L.Y.).

2.7 Ethics approval

The UK Biobank received ethical permits from the North West Multi-centre Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. All participants provided written informed consent.

2.8 Role of funding source

The funding sources had no role in the design of this study and did not have any role in the study design, data collection, data analyses, interpretation, writing of report, or decision to submit results.

3. Results

3.1 PheWAS analysis

The characteristics of included participants (182,072 females and 157,125 males) are shown in **Supplementary Table 5**. The mean age of the studied population was 56.9 (SD: 8.0) years and the mean levels of serum calcium was 9.5 (SD: 0.4) mg/dL at the time of recruitment. The F statistic of used genetic instruments was >1000. We defined a total of 1853 distinct phenotypes in PheWAS analysis. After exclusion of outcomes with cases <200, the remaining 1473 phenotypes classified into 18 disease categories were examined in relation to genetically predicted levels of serum calcium (**Supplementary Table 6**). Genetically proxied calcium levels were associated with 22 distinct outcomes in 7 disease categories after correcting for multiple testing (FDR-adjusted *p* value <0.05) (**Table 1 and Figure 1**). Among these outcomes, higher genetically predicted calcium levels were associated with decreased risk of 5 diseases related to dermatologic system and musculoskeletal system (psoriasis, skin cancer, fasciitis, osteoarthrosis, and Dupuytren's disease) and increased risk of 17 diseases in circulatory (hypertension and four coronary atherosclerotic diseases), digestive (liver cirrhosis and nonalcoholic fatty liver disease), endocrine (three parathyroid gland diseases, benign neoplasm of other endocrine glands, diabetes, and gout), and genitourinary (calculus of urinary tract) systems, and injuries and immune system (poisoning by antibiotics and allergy/adverse effect of penicillin) (**Table 1 and Figure 1**). The associations remained generally consistent in individuals without calcium supplementation (**Table 1**) and in participants with different levels of circulating vitamin D (**Supplementary Table 7**).

3.2 Two-sample MR analysis

Eight out of 22 associations were replicated in two-sample MR analysis (**Figure 2**). Per one SD increase in genetically predicted serum calcium levels, the odds ratios were 1.14 (95% confidence

interval (CI), 1.02, 1.26; *p*=0.019, IVW) for coronary artery disease, 1.18 (95% CI, 1.05, 1.32; *p*=0.004, IVW) for myocardial infarction, 1.20 (95% CI, 1.01, 1.42; *p*=0.042, IVW) for coronary atherosclerosis, 2.40 (95% CI, 1.73, 3.33; *p*<0.001, IVW) for hyperparathyroidism, 2.08 (95% CI, 1.53, 2.83; *p*<0.001, IVW) for disorder of parathyroid gland, 1.34 (95% CI, 1.10, 1.65; *p*=0.004, IVW) for gout, 1.53 (95% CI, 1.08, 2.18; *p*=0.018, IVW) for calculus of kidney and ureter, and 0.77 (95% CI, 0.61, 0.98; *p*=0.032, IVW) for osteoarthritis of the hip and knee. In addition, a positive association between genetically predicted calcium levels and serum urate levels was observed (change, 0.18; 95% CI, 0.08, 0.31; *p*=0.001, IVW). The associations were consistent in sensitivity analyses and no horizontal pleiotropy was detected by MR-Egger intercept test even though moderate to high heterogeneity was observed in certain analyses (**Supplementary Table 8**). The associations for coronary artery events, type 2 diabetes, gout, and allergic disease became stronger in the MR-PRESSO analyses after removal of outliers (**Supplementary Table 9**).

In multivariable MR analyses, the associations of genetically predicted serum calcium levels with coronary artery disease and myocardial infarction did not change after adjusting for genetically predicted blood pressures (**Supplementary Table 10**). The associations for gout and calculus of kidney and ureter attenuated after adjusting for genetically predicted urate levels (**Supplementary Table 10**).

3.3 Systematic review of MR studies on serum calcium

A total of 193 studies were identified. After exclusion of review articles and irrelevant studies, 29 studies were included for full article screening. Four out of 29 studies were excluded after full article screening due to analysis on dietary calcium intake (n=1), weak instrumental variables

(n=2), and offspring health (n=1). Information on 25 included studies is presented in **Supplementary Table 10**. By reviewing these studies, we found that the associations between genetically predicted serum calcium levels and several disease outcomes (i.e., coronary artery disease, myocardial infarction, urinary calculus, osteoporosis) have been consistently reported in previous MR studies; while the increased risk of gout, hyperparathyroidism and disorder of parathyroid gland have not been previously reported. The systematic review of MR studies also identified a number of health outcomes that were not captured in PheWAS analysis (**Figure 3**). In detail, one standard deviation increase in genetically predicted serum calcium levels were associated with elevated risk of migraine (OR, 1.34, 95% CI, 1.14, 1.57; p<0.001, IVW) and chronic pancreatitis (OR, 1.27; 95% CI, 1.08, 1.50; p=0.004, IVW) and lower risk of colorectal cancer (OR, 0.85, 95% CI, 0.74, 0.96; p=0.021, IVW), glioma (OR, 0.84, 95% CI, 0.71, 0.98; p=0.042, IVW), and Alzheimer's disease (OR, 0.57; 95% CI, 0.35, 0.95; p=0.024, IVW). The associations were directionally consistent for migraine (OR, 1.13, 95% CI, 0.90, 1.43; p=0.292, IVW), chronic pancreatitis (OR, 1.48, 95% CI, 0.81, 2.67; p=0.200, IVW), and Alzheimer's disease (OR, 0.76, 95% CI, 0.52, 1.09; p=0.138, IVW), but not for colorectal cancer (OR, 1.35, 95% CI, 0.53, 3.44; p=0.534, IVW) in our PheWAS. There were no data for glioma. In addition, higher genetically predicted serum calcium levels were associated with reduced levels of bone mineral density and JT and QT intervals (**Figure 3**).

4. Discussion

This updated PheWAS in the UK Biobank found that higher genetically predicted serum calcium levels were associated with decreased risk of psoriasis, skin cancer, fasciitis, osteoarthrosis, and Dupuytren's disease, and increased risk of hypertension, coronary atherosclerotic diseases, liver cirrhosis, nonalcoholic fatty liver disease, parathyroid gland diseases, benign neoplasm of other endocrine glands, diabetes, gout, calculus of kidney and ureter, poisoning by antibiotics, and allergy/adverse effect of penicillin. The associations for coronary artery disease, myocardial infarction, coronary atherosclerosis, hyperparathyroidism, disorder of parathyroid gland, gout, calculus of kidney and ureter, and osteoarthritis were successfully replicated in two-sample MR analyses. Urate appeared to mediate the associations for gout and possibly for calculus of kidney and ureter. The systematic review additionally found that higher genetically predicted serum calcium levels were associated with increased risk of migraine and chronic pancreatitis, and lower risk of colorectal cancer, glioma, and Alzheimer's disease as well as reduced bone mineral density and JT and QT intervals.

Certain associations identified in our PheWAS and MR analysis were consistent with results of previous studies, including the associations of higher calcium levels with increased risk of coronary atherosclerotic diseases (6, 7, 9) and urinary stones (4), and a decreased risk of osteoarthritis (39). Thus, our findings further strengthened the evidence of causality for these associations. Nevertheless, the associations with calcium are uncertain or scarcely investigated for other identified outcomes. In detail, we observed consistent positive associations of genetically predicted calcium levels with hyperparathyroidism and parathyroid gland disorder in PheWAS and two-sample MR analysis. However, these findings cannot completely determine whether the high calcium level at the pre-disease stage is a causal risk factor for these outcomes

since these diseases are usually asymptomatic and these diseases per se cause an increase in serum calcium levels (40). Our findings on the other side implied the utility of serum calcium levels as a diagnostic biomarker for parathyroid disorders, which is widely acknowledged and used in clinical settings (40). In addition, we observed positive associations of genetically predicted calcium levels with serum urate levels and gout risk, which was examined in few observational studies. A large population-based case-control study found that calcium channel blocker treatment was associated with a lower risk of incident gout among people with hypertension (41). A cross-sectional study observed a positive association between high serum calcium levels and the prevalence of hyperuricemia (42).

The high levels of serum calcium caused by excessive intestinal calcium absorption (like calcium supplementation and high dietary calcium intake), decreased renal tubular calcium reabsorption, and decreased bone mineralization reflect high concentrations of activators of calcification. High serum calcium levels can reduce calcitriol, increase serum levels of fibroblast growth factor 23 (43), and suppress vitamin D, which results in hypertension and higher levels of proinflammatory cytokines and thereby facilitating coronary atherosclerosis, carotid artery intima medial thickness, and impaired endothelial function (44). High levels of serum calcium can lead to increased supersaturation for calcium oxalate or phosphate, which accelerates urinary stone formation (45). In addition, this MR study found a positive association between calcium and urate levels, which is the underlying mechanism for the increased risk of gout as well as urinary stones (45) in individuals with high levels of serum calcium. Calcium is involved in several biological processes related to chondrocyte, such as matrix synthesis, cytoskeletal remodeling, cell hyperpolarization, and cell death, and therefore may play a role in the development of osteoarthritis (46).

Even though we observed associations for psoriasis, skin cancer, fasciitis, Dupuytren's disease, hypertension, liver cirrhosis, nonalcoholic fatty liver disease, benign neoplasm of endocrine glands, diabetes, poisoning by antibiotics, and allergy/adverse effect of penicillin in PheWAS, these associations were not replicated in two-sample MR analysis. The null findings of our two-sample MR investigation might be due to inadequate power, especially for certain outcomes with a few cases, like melanoma. A large-scale randomized controlled trial including 36,282 women found that the supplementation of 1,000 mg of calcium plus 400 IU of vitamin D3 reduced melanoma risk in women with a history of nonmelanoma skin cancer (47). Even though the inconsistent findings between PheWAS and two-sample MR analysis make the causality of certain associations inconclusive, our findings provide suggestions for future research.

Findings of the systematic review of MR studies on serum calcium identified most associations that we observed (except for the association for gout) in our MR analysis and expanded the health effects of serum calcium to migraine (11), chronic pancreatitis (12), colorectal cancer (48), glioma (49), Alzheimer's disease (50), and ventricular repolarization (JT and QT intervals) (51). In addition, several studies found an inverse association between calcium and bone mineral density (52-54). These associations confirmed our finding on the inverse association between calcium and osteoarthritis since lower bone mineral density has been associated with a decreased risk of osteoarthritis (55).

The major advantage of this study is the MR-PheWAS design, which examined the associations of genetically predicted calcium levels with a wide range of diseases. We used more SNPs that explain a good variance of serum calcium as instrumental variables compared to previous studies

(15). Thus, we had more power to detect associations even though we might still have overlooked weak associations for outcomes with a small number of cases. In addition, we performed a systematic review of MR studies on serum calcium to detect any other potential health effects that were not captured in our MR-PheWAS. Our analyses were confined to individuals of European ancestry, to minimize the population structure bias. However, this population confinement limited the generalizability of our findings to other populations.

There are several limitations. Pleiotropy is an important issue; however, this bias should be minimal due to the consistency of results in sensitivity analyses and no indication of horizontal pleiotropy in MR-Egger analysis. The summary-level data for the exposure and outcomes in PheWAS were both from the UK Biobank, which might bias the causal estimates towards observational associations (56). Nevertheless, the F statistic >10 of genetic instruments indicated that the bias caused by sample overlap should be minimal. Certain SNPs might be used as inappropriate instrumental variable due to the violation of the third assumption of MR that used SNPs should not be strongly associated with the studied outcome (14). In PheWAS analysis including 1473 phenotypes, it is unlikely to examine whether calcium-associated SNPs were strongly associated with each outcome. However, given that our analysis was based on 130 SNPs sharing phenotypic variance in serum calcium, which means that each SNP contributed a small proportion of variance, even though certain SNPs might be strongly associated with certain outcomes, it is less likely that the observed associations were driven by these SNPs. In the two-sample MR analysis, we observed strong influences of a few calcium-associated SNPs on certain studied outcomes. However, the observed associations remained consistent in the analyses with exclusion of these SNPs (not shown). In addition, the observed associations in the two-sample

MR analysis were overall consistent across different sensitivity analyses, especially in MR-PRESSO analysis where outlier SNPs were removed. All these indicate that our findings were less likely to be steered by a few unappropriated genetic instruments. In PheWAS analysis, most cases were identified from the inpatient hospital records, which might compromise the coverage of case ascertainment, especially for the diseases that do not usually need hospitalization. For hyperparathyroidism and disorder of parathyroid gland, the reverse MR analysis could not be performed due to lack of data. Serum levels of calcium might not exactly reflect the dietary calcium intake since osteoporotic calcium loss and physical inactivity increase serum calcium levels (57, 58), although our previous meta-analysis found that calcium and calcium plus vitamin D supplementation increased fasting serum calcium levels over one to four years of use (10). Thus, whether our findings can imply the health effects of calcium supplementation needs to be confirmed. Only eight out of twenty-two associations revealed in PheWAS analysis in the UK Biobank were replicated in two-sample MR analysis using data from the FinnGen consortium (21) and international genetic consortia (22-28). The possible reasons explaining the divergence might be noncausal associations established by PheWAS analysis, inadequate power in two-sample MR analysis, and different features of studied populations.

In summary, this study observed wide-ranged health effects related to different systems of high serum calcium levels. These findings suggest that the benefits and adversities of strategies promoting calcium intake should be carefully assessed.

Contributors

S.Y. and X.L had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. S.Y., S.C.L., X.L., and E.T. conceived and designed the study. S.Y. and X.L. undertook the statistical analyses. S.Y. and X.L. made figures. All authors advised on statistical analyses and visualization. S.Y. wrote the first draft of the manuscript. All authors made critical revisions of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Declaration of Interests

All authors declare no competing interest.

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Data Sharing Statement

Data from UK Biobank can be obtained via application (https://www.ukbiobank.ac.uk/). The UK Biobank is an open access resource and bona fide researchers can apply to use the UK Biobank dataset by registering and applying at http://ukbiobank.ac.uk/register-apply/. This research was conducted using the UK Biobank study under Application Number 66354. Data used in two-

sample MR analysis and review of MR studies can be obtained by a reasonable request to corresponding author.

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Table 1. Disease outcomes associated with the weighted polygenic risk score of serum calcium in phenome-wide association analysis in the UK Biobank

Outcome	Phecode	All participants					Participants without calcium supplementation				
		Cases	Controls	OR	95% CI	P	Cases	Controls	OR	95% CI	P
Circulatory system											
Hypertension	401	96 779	241 393	1.17	1.11, 1.24	1.23E-07	94 089	232 745	1.19	1.11, 1.28	7.25E-08
Ischemic heart disease	411	37 856	298 797	1.19	1.09, 1.30	4.00E-05	37 063	288 306	1.21	1.11, 1.31	1.42E-05
Myocardial infarction	411.2	13 363	298 797	1.29	1.13, 1.48	1.91E-04	13 108	288 306	1.31	1.14, 1.50	1.34E-04
Coronary atherosclerosis	411.4	25 046	298 797	1.19	1.08, 1.31	7.29E-04	24 571	288 306	1.21	1.09, 1.33	3.01E-04
Angina pectoris	411.3	18 919	298 797	1.22	1.08, 1.39	7.80E-04	18 551	288 306	1.22	1.09, 1.37	5.87E-04
Dermatologic system											
Psoriasis	696.4	3416	321 350	0.63	0.49, 0.81	5.01E-04	3315	310 748	0.64	0.49, 0.84	6.67E-04
Skin cancer	172	20 709	317 294	0.84	0.75, 0.93	1.22E-03	19 834	306 837	0.84	0.75, 0.93	1.80E-03
Digestive system											
Chronic liver disease and cirrhosis	571	6943	323 056	1.41	1.17, 1.69	1.89E-04	6713	312 215	1.43	1.19, 1.71	1.31E-04
Chronic nonalcoholic liver disease	571.5	5435	323 056	1.43	1.16, 1.76	6.43E-04	5271	312 215	1.43	1.17, 1.73	6.16E-04
Endocrine system											
Hyperparathyroidism	252.1	1296	332 624	4.20	2.79, 6.30	4.26E-12	1221	321 527	4.20	2.75, 6.40	1.79E-11
Disorders of parathyroid gland	252	1472	332 624	3.13	2.14, 4.58	5.09E-09	1380	321 527	3.29	2.22, 4.88	3.25E-09
Benign neoplasm of parathyroid gland	227.2	545	335 829	4.74	2.53, 8.87	1.00E-06	515	324 598	4.40	2.30, 8.41	6.29E-06
Benign neoplasm of other endocrine glands	227	1362	335 829	2.45	1.64, 3.65	1.05E-05	1310	324 598	2.34	1.57, 3.50	3.70E-05
Diabetes mellitus	250	24 824	312 261	1.21	1.08, 1.35	5.01E-04	24 362	301 415	1.19	1.08, 1.31	8.85E-04
Gout	274.1	4486	333 174	1.48	1.17, 1.86	6.95E-04	4403	321 939	1.50	1.21, 1.86	4.17E-04
Genitourinary system											
Urinary calculus	594	7604	329 898	1.92	1.62, 2.28	9.35E-14	7426	318 760	1.94	1.64, 2.30	4.81E-14
Calculus of lower urinary tract	594.2	1072	329 898	2.45	1.57, 3.82	9.17E-05	1053	318 760	2.47	1.57, 3.89	8.87E-05
Injuries & immune system											
Poisoning by antibiotics	960	25 606	299 009	1.21	1.09, 1.33	1.68E-04	24 434	289 435	1.21	1.09, 1.33	2.03E-04
Allergy/adverse effect of penicillin	960.2	21 474	299 009	1.21	1.09, 1.33	3.18E-04	20 557	289 435	1.21	1.08, 1.35	6.94E-04
Musculoskeletal system											
Fasciitis	728.7	3680	301 930	0.57	0.44, 0.72	6.54E-06	3680	301 930	0.57	0.44, 0.72	6.54E-06
Osteoarthritis	740	55 332	282 840	0.85	0.79, 0.91	1.08E-05	53 374	273 460	0.85	0.79, 0.91	1.20E-05
Dupuytren's disease	728.71	3423	312 377	0.60	0.47, 0.78	9.65E-05	3340	301 930	0.60	0.46, 0.80	9.82E-05

CI, confidence interval; OR, odds ratio.

Figure legends

Figure 1. Study design overview. MR, Mendelian randomization; PheWAS, phenome-wide association analysis.

Figure 2. Results of the phenome-wide association study on serum calcium for clinical outcomes in the UK Biobank. The x-axes correspond to the logarithms of the *p* values derived from the phenome-wide association analyses. The red lines correspond to the statistical significance level (false discovery rate<0.05). Associations surviving the significance criteria are labelled by name.

Figure 3. Associations of genetically predicted higher levels of serum calcium with disease outcomes in two-sample Mendelian randomisation analysis (inverse-variance weighted method). CARDIoGRAMplusC4D, Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics; CI, confidence interval; DIAGRAM, DIAbetes Genetics Replication And Meta-analysis; GEFOS, GEnetic Factors for OSteoporosis Study; GWAS, genome-wide association study; OR, odds ratio; UKB, UK Biobank.

Figure 4. Associations for higher genetically predicted serum calcium levels (1 SD increase) from systematic review for Mendelian randomisation studies (inverse-variance weighted method). CI, confidence interval; OR, odds ratio; SD, standard deviation; SNPs, single nucleotide polymorphisms.