Osteoarthritis and Cartilage xxx (xxxx) xxx

Osteoarthritis and Cartilage

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Genetically predicted circulating levels of copper and zinc are associated with osteoarthritis but not with rheumatoid arthritis

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ARTICLE INFO

Article history:
Received 10 September 2020
Accepted 18 February 2021

Keywords:
Mendelian randomization
Copper
Zinc
Minerals
Osteoarthritis
Rheumatoid arthritis

SUMMARY

Objective: Osteoarthritis (OA) and rheumatoid arthritis (RA) are both debilitating diseases that cause significant morbidity and disability globally. This study aims to investigate the causal effects of varying blood levels of five minerals – iron, zinc, copper, calcium, and magnesium, on OA and RA.

Design: We performed two-sample Mendelian randomization (MR) analyses to assess the associations of five circulating minerals with OA and RA. Single nucleotide polymorphisms (SNPs) serving as genetic instruments for the circulating mineral levels were selected from large genome-wide association studies of European-descent individuals. The associations of these SNPs with OA and RA were evaluated in UK Biobank participants. Multiple sensitivity analyses were applied to detect and correct for the presence of pleiotropy.

Results: Genetically determined copper and zinc status were associated with OA, but not with RA. Per standard deviation (SD) increment in copper increases the risk of OA (OR = 1.07, 95% CI: 1.02−1.13) and one of its subtypes, localized OA (OR = 1.08, 95% CI: 1.03−1.15). Per SD increment in zinc is positively associated with risks of OA (OR = 1.07, 95% CI: 1.01−1.13), generalized OA (OR = 1.18, 95% CI: 1.05−1.31), and unspecified OA (OR = 1.21, 95% CI: 1.11−1.31). Additionally, per SD increment in calcium decreases the risk of localized OA (OR = 0.83, 95% CI: 0.69−0.98).

Conclusions: Genetically high zinc and copper status were positively associated with OA, but not with RA. Given the modifiable nature of circulating mineral status, these findings warrant further investigation for OA prevention strategies.

Introduction

Osteoarthritis (OA) and rheumatoid arthritis (RA) are two different types of arthritis, both manifested with inflammation. OA is the most common joint disease worldwide, affecting an estimated 3.8% of the global population[1]. It is characterized by degraded cartilage, moderate synovial inflammation, alteration of bony structure, pain, and impaired mobility[2]. RA is estimated to affect approximately 0.24–1% of the population and is twice as common in women than men[3]. It is a chronic autoimmune disease characterized by inflammatory polyarthritis that preferentially affects the small joints[4]. The clinical symptoms of OA and RA are similar and sometimes difficult to distinguish, although there are significant differences in their pathogenesis. A variety of genetic and environmental factors have been linked to the development and progression of these two diseases. While many of these risk factors are difficult to change, some may be more amenable to medical and behavioral interventions (e.g., nutrition). Poor nutrient status in OA or RA patients has been reported, and minerals may affect these two diseases through their cofactor roles in immune functions and different metabolic processes in joint tissues[5,6].

Minerals are a diverse group of substances constituting enzyme complexes and participating in a variety of fundamental physiological activities[7]. Naturally occurring minerals, such as iron (Fe),

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https://doi.org/10.1016/j.joca.2021.02.564
1063-4584/

calcium (Ca), magnesium (Mg), copper (Cu), and zinc (Zn), have been implicated in different inflammatory effects in both animal and human studies[8–11]. Zinc can function as an anti-inflammatory agent, and clinical manifestations of zinc deficiency include growth retardation, immune dysfunctions, increased oxidative stress, and enhanced production of proinflammatory cytokines[12,13]. Chronic intake of a large quantity of zinc may interfere with copper absorption, reduce the levels of high-density lipoprotein cholesterol, and impair immune responses[14]. Furthermore, copper is essential for iron absorption, and thus copper deficiency can impair iron metabolism[15]. Iron is indispensable for the immune cell proliferation, particularly that of lymphocytes, in response to infection[16]. Likely due to their close interactions, different minerals are usually found to be associated with similar diseases. Elevated blood iron and copper levels are positively with the risk of OA[17], while those of calcium and magnesium are inversely associated its risk[18,19]. Copper and zinc have been reported to be positively correlated with RA[20]. Due to the fact that most studies were observational and may suffer from reverse causality and residual confounding, it remains uncertain whether these associations indicate causal relationships.

Mendelian randomization (MR), a complementary approach to epidemiological observations, uses genetic variants as instrumental variables and allows the investigation of whether the effect of an exposure (i.e., circulating mineral level) on a clinical outcome is likely to be causal. Genetic variants, which affect the absorption, distribution, or excretion of essential minerals, may influence their systematic status and subsequently lead to health effects related to sub-clinical deficiency or excess. In contrast to the observed circulating mineral levels that are results of ion–ion interactions, genetic backgrounds, socioeconomic status, existing medical conditions, and many other confounding factors, genetically predicted circulating levels of a specific mineral represent the lifelong mineral status that is not influenced by other minerals or existing diseases[21]. In this work, we performed a two-sample MR study to explore the causal associations of five circulating minerals with the risks of OA and RA. Genetic instruments for mineral status were obtained from existing large genome-wide association studies (GWAS) of blood minerals in participants of European descent, while the associations of these genetic instruments with OA and RA were evaluated in the UK Biobank[22]. Additionally, sex-stratified MR analysis was performed to examine sex differences in the causal effects.

Method

This study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (S1 STROBE-MR Checklist). UK Biobank has ethics approval from the North West Multi-Centre Research Ethics Committee (11/NW/0382). Appropriate informed consent was obtained from participants. Data for this work were obtained from UK Biobank under approved application (ID 48818). This study utilizes a subset of unrelated White British individuals with high-quality genotype data in order to minimize the influence of diverse population structure within UK Biobank.

Genetic instruments for the blood minerals

Single nucleotide polymorphisms (SNPs), serving as genetic instrumental variables for each blood mineral status (i.e., iron, copper, zinc, calcium, and magnesium), were selected from recent large GWAS in samples of European descent. SNPs included are independent of each other ($r^2 < 0.01$). We selected three genetic instruments for systemic iron status. These three genetic instruments are related to all four biomarkers of systemic iron status in a consistent manner: elevated blood iron, ferritin, and transferrin saturation, and decreased transferrin levels[23,24]. All three of these SNPs were independent and explained approximately 3.8% of the variation in blood iron, and the mean F-statistic was 629[25]. We used six calcium-associated genetic variants, which altogether explained 0.37% of the variance for blood calcium levels, and the mean F-statistic was 67[26]. We selected six SNPs that were significantly associated ($P < 5 \times 10^{-8}$) in published GWAS with blood magnesium levels in the joint analysis of the discovery ($N = 15{,}366$ individuals) and replication ($N = 8{,}463$ individuals) cohorts[27]. These six SNPs explained 1.62% of the variance in blood magnesium levels, and the mean F-statistic was 64. The genetic variants for erythrocyte copper and zinc levels were derived from the Queensland Institute of Medicine's twins and their families ($N = 2{,}603$)[28]. Collectively, we selected two significant SNPs ($P < 10^{-10}$) associated with blood copper concentration, which accounted for 5% of the phenotypic variance for copper, and the mean F-statistic was 62. For zinc, two SNPs were selected as instrumental variables, explaining 4.59% variance for its concentration, and the mean F-statistic was 61. We excluded X-linked SNP rs4826508 to avoid invalid instruments because its different allele frequencies in male (0.89) and female (0.79) are statistically significant. Summary-level data (beta coefficients and standard errors) for the associations of the selected SNPs with each mineral from different GWAS cohorts were listed in Table I.

Ascertainment of osteoarthritis and rheumatoid arthritis

The associations of selected genetic instruments with OA and RA were estimated in UK Biobank. Diagnosed diseases status was recorded in the hospital episode statistics as the International Classification of Diseases (ICD) codes. We mapped these ICD codes to the phecode grouping system, which merges related codes into relatively independent and clinically meaningful groups. The phecode system provides a scheme to automatically define case and control status for each phecode by excluding participants with similar or potentially overlapping disease states from the corresponding control group[29]. The phecode 740 was used for the identification of OA ($N = 36{,}612$ cases; 274,387 controls), while the phecode 714.1 was used for RA ($N = 2{,}547$ cases; 308,452 controls). In addition, the phecodes of 740.1, 740.2, and 740.9 were used for identification of OA subcategories: localized ($N = 30{,}741$ cases; 280,258 controls), generalized ($N = 5{,}617$ cases; 305,382 controls), and unspecified ($N = 9{,}949$ cases; 301,050 controls), respectively[29].

All analyses were restricted to participants of genetically European descent to maintain consistency with the European samples used to obtain genetic instruments for circulating mineral status. We quality-controlled and filtered the UK Biobank dataset by removing individuals whose genetic ancestry is not Caucasian, or who are not included in the genetic principal component analysis, or who have sex chromosome aneuploidy. To avoid bias from related individuals, one participant from each pair of relatives was excluded based on a kinship coefficient of >0.0884. Logistic regression analysis was performed for each instrument SNP separately across all OA and RA phecodes, adjusting for age, sex, genotyping array, assessment center, and the first ten genetic principal components. Genetic principal components were included to explicitly account for possible population stratification. They have been pre-calculated based on selected genome-wide genotype markers[22].

Statistical analysis for MR estimates

The estimates of the causal effect were obtained using the inverse variance-weighted (IVW) method. Effect estimates were

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SNP	Effect allele	Baseline allele	Chr	Closest gene	% variance explained	F-statistic	EAF	Beta*	SE	*P*
3 SNPs for iron status from GWAS by Gill, Dipender, *et al.* (N† = 48,972)										
rs1800562	A	G	6	HFE	1.30	645	0.067	0.328	0.016	2.72×10^{-97}
rs1799945	G	C	6	HFE	0.90	445	0.15	0.189	0.010	1.10×10^{-81}
rs855791	G	A	22	TMPRSS6	1.60	796	0.554	0.181	0.007	1.32×10^{-139}
8 SNPs for calcium status from GWAS by O'Seaghdha, Conall M., *et al.* (N† = 61,079)										
rs1801725	T	G	3	CASR	0.50	307	0.15	0.071	0.004	9×10^{-86}
rs1570669	G	A	20	CYP24A1	0.06	37	0.66	0.018	0.003	9×10^{-12}
rs1550532	C	G	2	DGKD	0.06	37	0.31	0.018	0.003	8×10^{-11}
rs780094	T	C	2	GCKR	0.05	31	0.42	0.017	0.003	1×10^{-10}
rs7481584	G	A	11	CARS	0.06	37	0.3	0.018	0.003	1×10^{-10}
rs7336933	G	A	13	RPS28P8, VWA8-AS1	0.05	31	0.15	0.022	0.004	9×10^{-10}
rs10491003	T	C	10	LINC00709	0.05	31	0.09	0.027	0.005	5×10^{-9}
rs17711722	T	C	7	GTF2IP5, RNU6-912P	0.04	24	0.47	0.015	0.003	8×10^{-9}
6 SNPs for magnesium status from GWAS by Meyer, Tamra E., *et al.* (N† = 23,829)										
rs4072037	T	C	1	MUC1	0.57	136	0.54	0.01	0.001	2.0×10^{-36}
rs7965584	A	G	12	ATP2B1	0.25	60	0.71	0.007	0.001	1.1×10^{-16}
rs3925584	T	C	11	DCDC5	0.25	60	0.55	0.006	0.001	5.2×10^{-16}
rs11144134	C	T	9	TRPM6	0.23	55	0.08	0.011	0.001	8.2×10^{-15}
rs13146355	A	G	4	SHROOM3	0.19	45	0.44	0.005	0.001	6.3×10^{-13}
rs448378	A	G	3	MDS1	0.13	30	0.53	0.004	0.001	1.3×10^{-8}
2 SNPs for copper status from GWAS by Evans, David M., *et al.* (N† = 2,603)										
rs1175550	G	A	1	SMIM1	1.45	38		0.198	0.032	5.03×10^{-10}
rs2769264	G	T	1	SELENBP1	3.15	85		0.313	0.034	2.63×10^{-20}
2 SNPs for zinc status from GWAS by Evans, David M., *et al.* (N† = 2,603)										
rs1532423	A	G	8	CA1	1.77	47		0.178	0.026	6.40×10^{-12}
rs2120019	T	C	15	PPCDC	2.82	75		0.287	0.033	1.55×10^{-18}

SNP single nucleotide polymorphism, Chr: chromosome, EAF: effect allele frequency, SE: standard error.

* The beta coefficient of the effect-increasing allele. Their units are μmol/L for iron, mg/dl for calcium, mmol/L for magnesium, copper, and zinc.

† The sample size of the initial GWAS from which the genetic variants were selected.

Table I Blood minerals-associated SNPs used as genetic instruments in the Mendelian randomization analyses

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expressed as odds ratios (OR) per one standard deviation (SD) increment in the blood mineral level. Several sensitivity analyses were performed to check and correct for the presence of pleiotropy among genetic instruments. First, we calculated Cochran's Q statistic, with which a *P*-value ≤ 0.05 indicates the presence of heterogeneity[30,31]. If there was no evidence of heterogeneity based on the Q statistic, a fixed-effects model was used in the IVW MR estimation. If there was substantial heterogeneity, we moved to a random-effects IVW method that allows all SNPs to exhibit balanced horizontal pleiotropy. Moreover, for minerals that have three or more genetic instruments, the weighted median (WM) MR and MR-Egger methods were applied. The WM MR approach is able to provide consistent effect estimates when more than half of the genetic instruments are valid[32]. The MR-Egger method provides an intercept test for the presence of unbalanced horizontal pleiotropy, and it also offers an unbiased estimate of the causal effects while taking into account the pleiotropy[33]. All the analyses were conducted using the MendelianRandomisation[34] and TwoSampleMR[35] packages and the R programming language.

Results

Genetically predicted higher copper and zinc status was positively associated with OA, but not with RA (Fig. 1). The ORs of OA were 1.07 (95% CI, 1.02–1.13; $P = 0.01$) and 1.07 (95% CI, 1.01–1.13; $P = 0.02$), per one SD increment in erythrocyte copper and zinc levels, respectively. Cochran Q tests did not find evidence of heterogeneity (Supplementary Table 1). The associations of calcium, iron, and magnesium levels with OA did not reach the significance level at $P < 0.05$. On the other hand, genetic predisposition to high circulating levels of all these blood minerals showed no significant associations with RA. Detailed analysis on the subcategories of OA, including localized, generalized, and unspecified OA, unraveled evidence of causal associations with copper, zinc, and calcium (Fig. 2, Supplementary Table 2). Per SD increment in copper is associated with increased risks of localized OA (OR = 1.08, 95% CI: 1.03–1.15, $P = 0.003$). Per SD increment in zinc is positively associated with risks of generalized OA (OR = 1.18, 95% CI: 1.05–1.31, $P = 0.014$), and unspecified OA (OR = 1.21, 95% CI: 1.11–1.31, $P = 0.0002$). In contrast to the risk-increasing effect of copper and zinc, per SD increment in calcium decreases the risk of localized OA (OR = 0.83, 95% CI: 0.69–0.98, $P = 0.021$). For all these estimates, Cochran Q tests did not find evidence of heterogeneity. Additionally, for the effect of calcium on localized OA, the intercept test in MR-Egger did not find evidence of unbalanced pleiotropy, and the WM MR also revealed a significant estimate (OR = 0.84, 95% CI: 0.71–0.98, $P = 0.042$).

We further performed sex-stratified IVW MR analysis to identify sex-specific causal effects of these five minerals. That is, their MR estimates are significant only in one sex group, but not in the other or the sex-combined sample. In males, per SD increment in serum iron is associated with increased risk of unspecified OA (OR = 1.27, 95% CI: 1.05–1.54, $P = 0.013$), but serum calcium is negatively associated with the risk of OA (OR = 0.67, 95% CI: 0.46–0.98, $P = 0.038$), RA (OR = 0.35, 95% CI: 0.14–0.86, $P = 0.02$) and generalized OA (OR = 0.35, 95% CI: 0.13–0.93, $P = 0.035$). No sex-specific effects were found for copper, zinc, and magnesium. All these sex-specific causal estimates do not have indications of

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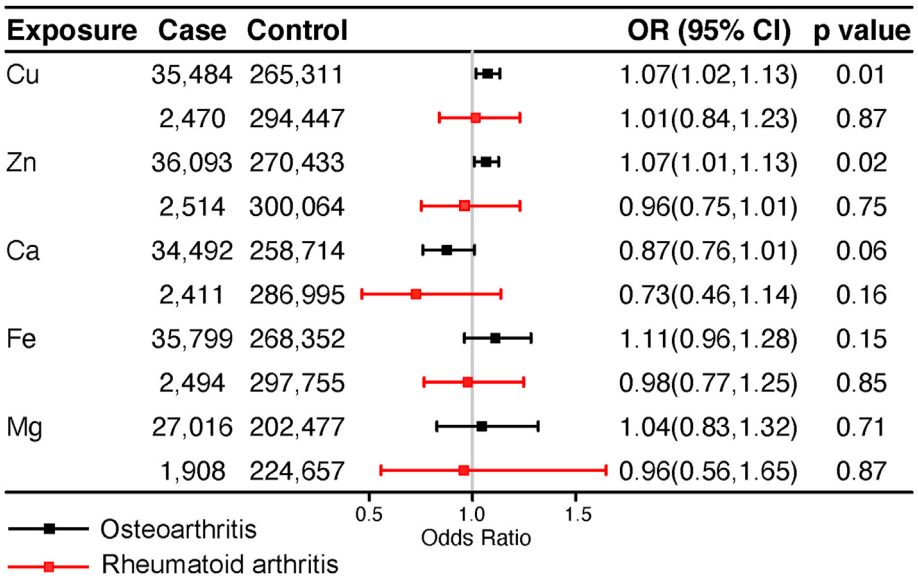

Fig. 1

A forest plot showing associations between genetically determined levels of minerals and OA or RA based on IVW MR analysis. The odds ratios (ORs) with their 95% confidence intervals (CIs) are scaled to 1-SD increase in blood mineral level. The case and control numbers include only individuals with complete genotype data for all genetic instruments of a mineral, and therefore they vary slightly across minerals. Complete MR results are provided in Table S1.

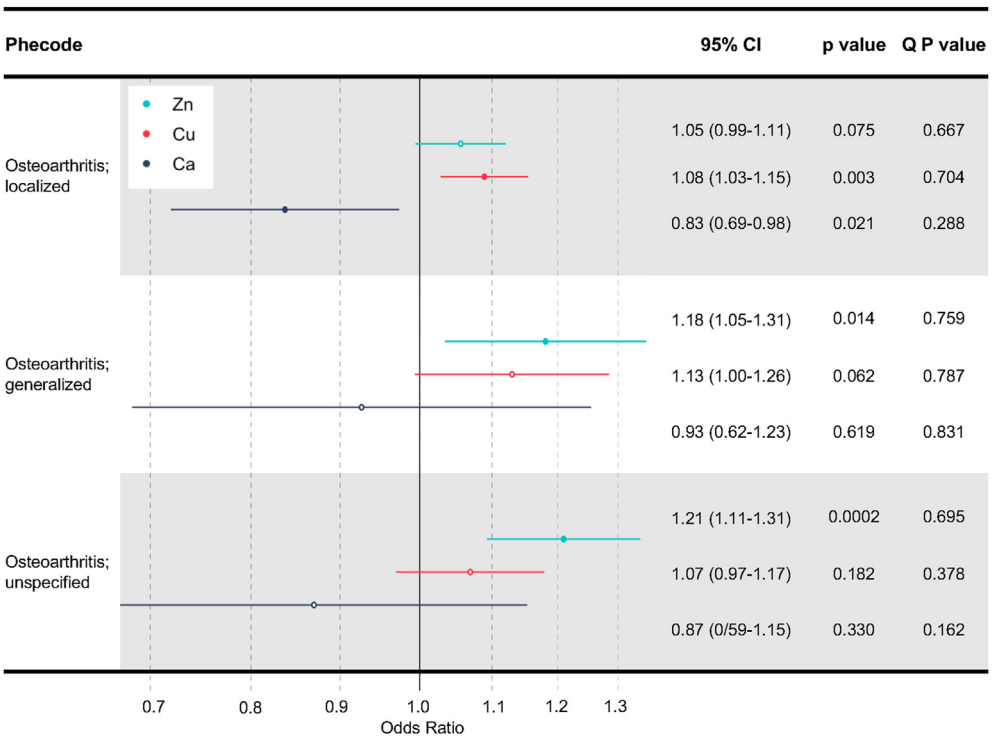

Fig. 2

A forest plot showing associations between three minerals and three OA subcategories based on MR analysis. The causal estimates are from IVW MR and have no indications of pleiotropy. The odds ratios (ORs) with their 95% confidence intervals (CIs) are scaled to 1-SD increase in blood mineral level. Complete MR results between all five minerals and the three OA subcategories are provided in Table S2. No significant associations were found for iron or magnesium.

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heterogeneity across genetic instruments (Supplementary Table 3). We additionally performed a sensitivity analysis without adjustment for sex, and the results showed no difference in the significance of MR associations when compared to that with adjustment for sex.

Discussion

In this study examining the causality of five circulating minerals (i.e., iron, calcium, magnesium, zinc, copper) on OA and RA, we found that genetically predicted high levels of copper and zinc were positively associated with OA, but not RA. There was some evidence supporting a causal role of iron and calcium on OA, especially in males. No causal associations were found for RA, except a suggestive protective effect of calcium in males.

Across combined and sex-stratified analyses, we found that copper, zinc, and iron increase, while calcium decreases the risks of OA and/or its subtypes. Of note, the effects of iron and calcium on OA were also reported in two independent MR studies[36,37]. Among these minerals, zinc has the best-elucidated mechanism[38]. OA is primarily characterized by cartilage destruction, with the extracellular matrix degraded by zinc-dependent matrix-degrading enzymes (e.g., MMP3, MMP9, MMP12, MMP13, and ADAMTS5). In human cell lines and mouse models, the influx of zinc into chondrocytes upregulates a zinc-activated transcription factor, MTF1, which then induces expression of matrix-degrading enzymes, causing cartilage destruction[38]. For iron, a longitudinal cohort study observed that increased serum ferritin is associated with symptomatic knee OA incidence and severity in males[39]. Mechanistically, iron overload in a murine model of hereditary hemochromatosis enhances the expression of MMP3 and is associated with accelerated OA progression under mechanical stress[40]. These are consistent with our male-specific MR result of iron on OA. For copper, few published studies examined its effects on OA. We only found one small study reporting elevated plasma and synovial copper concentrations in OA patients when compared to healthy controls[17], but their pathological roles are still elusive. In contrast to the risk-increasing effect of the other three minerals, calcium is protective against OA in our MR results, especially in men. In a previous cross-sectional study, serum calcium concentration was inversely associated with knee OA[18], but a null association was also reported[41]. A randomized controlled trial showing the benefit of vitamin D supplements on knee OA also found that the serum calcium level increased after treatment[42]. All of these existing studies suggest that blood minerals play important roles in the etiology of OA. Our study supports some minerals as targets of intervention for preventing OA, especially zinc, copper and iron. It also demonstrates that not all minerals associated with OA in observational research are causal, advising against their usage in clinical prevention. Overall, our study pinpoints the prominent roles of blood minerals in the pathogenesis of OA, calling for further confirmative and mechanistic studies.

RA is an autoimmune disease of unknown etiology, and it is mostly characterized by symmetric polyarticular inflammation of the synovial membrane that affects the joints of the hands, wrists, and feet[4,43]. Inflammatory processes in the bone near the joints, as well as in bone erosions and large cysts around the joint, can decrease the mineral content of the bone. Intakes of vitamin D, calcium, and other nutrients, including iron and zinc, which are needed to build healthy bones, prevent the development of RA[44]. In recent years, many studies have investigated the possible roles of micronutrients in the etiology and pathogenesis of RA. However, findings on the association between different serum mineral status and the RA risk are still conflicting and inconclusive[17,45]. A recent meta-analysis showed that increased serum level of copper and decreased serum level of zinc are associated with the disease activity in RA patients[46]. In light of potential residual confounding and reverse causation bias, large MR studies are needed to determine whether mineral status might influence the risk of RA. Nevertheless, our present MR findings do not support the causal roles of these minerals in the development of RA. They probably support the hypothesis that minerals are altered by some immunocytokines as a defense response against RA[47]. Consistently, several studies reported that the causes of plasma mineral changes in RA might not be a result of a specific deficiency/excess from dietary imbalances, but a result of the inflammatory status regulated by immunoregulatory cytokines[48]. Our genetically informed approach for causal inference may help distinguish the etiology of OA and RA, offering distinct and effective means of prevention. While dietary or clinical interventions to modify circulating mineral levels are promising prevention strategies for OA, they may not be effective for RA.

This study, based on a large prospective cohort, evaluated the causality of five circulating minerals on OA and RA. In order to obtain unbiased estimates of causality, we applied multiple MR methods and sensitivity analyses to evaluate potential bias due to pleiotropic effects of genetic instruments. For all our findings, there is no evidence of heterogeneity or pleiotropy. However, we recognized that a full verification of the assumptions for genetic instruments is difficult, particularly in assessing canalization and pleiotropic effects. It is important to note that our study also has some limitations. One limitation is about statistical power. We performed a power calculation for MR analysis based on the current case and control numbers and the amount of variance explained by selected genetic instruments (Supplementary Table 4)[49]. Since the number of RA cases is much smaller than that of OA cases, our statistical power to detect an effect on RA is much lower. On the other hand, the genetic instruments for each mineral explain a relatively small amount of phenotypic variance, ranging from 8% for zinc to 0.37% for calcium. Our analysis for magnesium and calcium are under power. Future studies with larger case sizes are needed for RA, magnesium, and calcium. Another limitation is that the MR approach only estimates the population average effect of blood minerals. Gene-environment interaction studies are warranted to examine if their effects vary across population subgroups defined by genetic or phenotypic profiles. Furthermore, collider bias may arise due to sample selection in the UK Biobank, which only had a 5% participation rate, although related effects are likely to be modest[50]. Genetic instruments approximate the average effect of an exposure over the life course, while the true biological effects of blood minerals could vary by life stages and be more complex than that indexed in our study. Finally, it is important to note that UK Biobank is an older cohort of European ancestry, so results may differ in younger populations or in other ethnic backgrounds.

Our MR study suggests that genetic predisposition to physiologically higher circulating copper and zinc status may increase the risk of OA but has no effects on RA. High levels of blood calcium decrease the risk of localized OA. The associations between high iron status and OA risk were only significant in males. Given that mineral status is a modifiable trait, these results may have clinical and public health implications but need confirmation by further large MR studies and clinical trials.

Author contributions

JZ and KY conceived and designed the study. JZ, CL, and YS performed statistical analyses. MF managed and curated the UK Biobank dataset. All authors interpreted the results. JZ and KY drafted the manuscript. All authors edited the manuscript for

Please cite this article as: Zhou J et al., Genetically predicted circulating levels of copper and zinc are associated with osteoarthritis but not with rheumatoid arthritis, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2021.02.564

intellectual content. All authors take responsibility for the integrity of the study.

Funding

AG is supported by the United States Department of Agriculture, National Institute of Food and Agriculture Hatch Funds. MSR is supported by the Allen Foundation Inc., and the United States Department of Agriculture, National Institute of Food and Agriculture Hatch Funds. KY is supported by the University of Georgia Research Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability statement

Genetic and phenotypic data from UK Biobank could be accessed following an application and approval process. Summary statistics generated in this project are provided in the supplementary files.

Declaration of competing interest

The authors have declared that no competing interests exist.

Acknowledgments

This study was conducted using the UK Biobank Resource under project 48818. The authors would like to thank all UK Biobank participants. We also want to thank other members of the Ye lab for stimulating discussions.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2021.02.564.

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Please cite this article as: Zhou J et al., Genetically predicted circulating levels of copper and zinc are associated with osteoarthritis but not with rheumatoid arthritis, Osteoarthritis and Cartilage, https://doi.org/10.1016/j.joca.2021.02.564