Causal association of genetically determined caffeine intake from tea or coffee with bone health: a two-sample Mendelian randomization study

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Short running tile: Caffeine and bone: a Mendelian-randomization study

Abstract

Background: Relationship of caffeine intake and consumption of caffeinated beverages, such as tea and coffee, with bone health remains controversial. This study aimed to evaluate whether genetically determined caffeine intake from tea or coffee has causal effects on overall total body bone mineral density (TB-BMD) and fracture. We also assessed the association with TB-BMD in five age strata.

Methods: Using two-sample Mendelian Randomization approach, summary statistics were retrieved from genome-wide association studies (GWAS)/GWAS meta-analyses of caffeine intake from tea (n=395,866)/coffee (n=373,522), TB-BMD (n=66,628) and fracture (n=426,795). Inverse variance weighted method was adopted as the main univariable analysis. Multivariable analysis was conducted to evaluate whether the causal effect is independent. *Results:* In univariable analysis, genetically determined caffeine intake from tea had positive association with overall TB-BMD (per SD increase in genetically determined caffeine intake, beta of TB-BMD [in SD]: 0.166; 95% CI: 0.006-0.326) and inverse association with fracture (OR=0.79; 95% CI: 0.654-0.954). Genetically determined caffeine intake from coffee was also positively associated with overall TB-BMD (beta=0.231; 95% CI: 0.093-0.369). The association remained significant after adjustment for smoking in multivariable analysis. Genetically determined caffeine intake from tea or coffee were both positively associated with TB-BMD in the age strata of 45-60 years, but we lacked evidence of association in other strata.

Conclusions: Genetically, caffeine intake from tea or coffee may be beneficial to bone health. Due to the ascertainment method of caffeine intake from tea, our study also implied genetically higher tea consumption may improve TB-BMD and lower fracture risk.

Key words: Caffeine, tea, coffee, bone mineral density, fracture, Mendelian randomization

Key messages

What is already known on this topic

Published studies demonstrated controversial findings on the relationship of caffeine intake and consumption of caffeinated beverages, such as tea and coffee, with bone health.

What this study adds

This two-sample Mendelian Randomization study demonstrated that genetically determined caffeine intake from tea or tea consumption was causally associated with improved overall TB-BMD and reduced fracture risk. Genetically determined caffeine intake from coffee was also positively associated with overall TB-BMD.

How this study might affect research, practice or policy

Caffeine and other active ingredients in tea may improve bone health. Tea may be included in the diet as a strategy to improve BMD and prevent osteoporosis-associated fracture.

Introduction

Osteoporosis is a chronic bone disease characterized by low bone mineral density (BMD) and deterioration of bone microarchitecture, leading to increased fracture risk. BMD measured by dual-energy X-ray absorptiometry (DXA) is the gold standard for diagnosis of osteoporosis. Osteoporosis-related fracture is estimated to incur significant economic burden, particularly amid the aging population [1]. Moreover, the median one-year all-cause mortality rate post hip fracture remained high at 22.8%, even a decreasing or stabilized trend was observed [2]. Therefore, identifying causal and modifiable risk factors (such as diet), is urgently required for timely prevention of osteoporosis and its associated fracture.

Tea and coffee are the most frequently consumed beverages [3], and also the major dietary sources of caffeine [4]. Yet, whether caffeine intake has any effect on bone health remains controversial [5-9]. Notably, individuals' habitual caffeine intake [10], consumption of tea and coffee [11,12] were linked to genes involved in the pharmacokinetic and pharmacodynamic response to caffeine, such as aryl hydrocarbon receptor and cytochrome P450 family 1 subfamily A member. Instead of solely habitual preference, the role of genetics enables the use of genetic variants as instrumental variables for caffeine intake or consumption of tea/coffee in Mendelian randomization (MR) studies. Since genetic variants are randomly allocated at conception, MR approach is less susceptible to residual confounding and reverse causality, providing stronger evidence of causal inference compared to conventional observational studies [13]. Aiming to clarify the controversial role of caffeine intake and explore the optimal dietary requirement in improving bone health, we assessed the causal effect of genetically determined caffeine intake from tea or coffee separately on DXA-derived total body bone mineral density (TB-BMD) and fracture using MR approach.

Materials and methods

Study design

The univariable two-sample MR approach was adopted, primarily aimed to examine the total causal effect of genetically determined caffeine intake from tea or coffee (exposure) on overall TB-BMD in the total sample and fracture (outcome). TB-BMD and fracture were the primary outcome as the genome-wide association study (GWAS) meta-analysis of TB-BMD [14] was the largest GWAS of DXA-derived BMD to-date while fracture was the clinical outcome of osteoporosis. The GWAS of combined caffeine intake from tea and coffee was also publicly available, but this phenotype was specifically related to caffeine intake from both tea and coffee, not either of them [10]. Notably, an individual with the habit of regular coffee intake is less likely having the concurrent habit of regular tea consumption, which was also implied by the weak phenotypic correlation between caffeine intake from tea and coffee respectively (Spearman's rank correlation coefficient r=-0.33) [10]. As the combined caffeine intake phenotype may not provide much clinical implication, its relationship with bone health was evaluated as an additional analysis. Due to the age-dependent features of both the genetic loci and their effect size for TB-BMD [14], if causal association was identified for overall TB-BMD, we also evaluated whether genetically determined caffeine intake has differential causal effects on TB-BMD in five age strata (below 15 years, 15-30 years, 30-45 years, 45-60 years, and 60 years or above) as secondary analysis. As a supplementary analysis for comparison, the causal association of genetically determined caffeine intake with DXA-derived BMD at lumbar spine (LS-BMD), femoral neck (FN-BMD) and forearm (FA-BMD) was assessed despite the relatively low statistical power.

If significant causal relationship was observed in the above univariable analyses, multivariable MR (MVMR) analysis was conducted to confirm whether caffeine intake was independently affecting bone health. Given the close correlation between smoking and caffeine intake demonstrated by both conventional observational [15] and genetic correlation [16] studies, and genetic instruments may be associated with several correlated exposures [17], MVMR analysis adjusted for beta estimates of cigarette smoking was performed. The study design and assumptions of MR analyses are illustrated in Figure 1.

Data sources

Summary statistics of caffeine intake from tea, coffee, combined caffeine intake from both beverages, TB-BMD, fracture, LS-BMD, FN-BMD, FA-BMD, and cigarette smoking were retrieved from the largest publicly available GWAS. Summary statistics of caffeine intake from tea, coffee, and combined caffeine intake were obtained from a GWAS by Said *et al* conducted in the UK Biobank participants, who were asked the average number of cups of tea and coffee they drank each day, and the type of coffee they usually drank [10]. The descriptions of data sources for all exposures and outcomes are detailed in Table 1. The selection of genetic instruments was described in Supplementary Methods 1.

Univariable MR analysis

The inverse variance weighted (IVW) method was adopted as the main analysis in the current MR study [18]. Several MR methods with different assumptions were applied as sensitivity analyses, including weighted median [19], MR-Egger regression [20], contamination mixture [21], and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) [22] methods, which are described in Supplementary Methods 2. Upon exclusion of pleiotropic outliers identified by

MR-PRESSO [22], the main and sensitivity MR analyses were repeated. Cochran's Q test was also conducted to evaluate the heterogeneity across the final genetic instruments. For analyses with TB-BMD as the outcome, results were presented as change in TB-BMD in standard deviation (SD) per SD increase in genetically determined caffeine intake. For analyses with fracture as outcome, the results were presented as the odds ratio (OR) of fracture per SD increase in genetically determined caffeine intake. Statistical power was calculated online (https://sb452.shinyapps.io/power/) [23]. If there was participant overlap between exposure and outcome datasets, the bias and Type I error were estimated by a web-based tool (https://sb452.shinyapps.io/overlap/) [24]. All analyses were conducted in R (version 4.1.3) using the "MendelianRandomization", "TwoSampleMR" and "MRPRESSO" packages.

MVMR analysis

Several MVMR methods were developed as the extended versions of univariable MR methods, including MVMR-IVW [25], MVMR-Egger [26] and MVMR-PRESSO. "MVMR" and "MRPRESSO" packages in R were utilized to provide the causal estimates. The MVMR-Egger intercept test and MVMR-PRESSO global test were employed to detect horizontal pleiotropy in MVMR analyses.

Results

Genetic instruments adopted in the MR analyses

The final lists of genetic instruments applied in each univariable and multivariable MR analysis were included in Supplementary Tables S1-S25, and Supplementary Tables S26-30, respectively. Caffeine intake from tea or coffee shared two common genetic instruments, while

the combined caffeine intake shared up to five and six genetic instruments with caffeine intake from tea or coffee, respectively (Supplementary Tables 1-6). Information of the genetic instruments adopted in each analysis are summarized in Table 2. The F-statistic were relatively high in all analyses (≥90.87). The Cochran's Q tests were all statistically insignificant, indicating the absence of heterogeneity among the instruments. The Type I error and bias incurred due to sample overlap between the exposure and outcome datasets were estimated to be 0.002-0.004 and 0.05 respectively (Table 2), which is minimal. Power calculation for MR analysis of genetically determined caffeine intake from tea (Supplementary Figures S1-S3), coffee (Supplementary Figures S4-S6), and combined caffeine intake (Supplementary Figures S7-S9) with TB-BMD, fracture, LS-BMD, FN-BMD and FA-BMD are presented in Supplementary Results 1.

Primary analysis – Causal association of genetically determined caffeine intake from tea or coffee with overall TB-BMD and fracture

Univariable IVW analysis suggested genetically determined caffeine intake from tea had a positive causal association with overall TB-BMD (per SD increase in genetically determined caffeine intake from tea, beta of overall TB-BMD in SD: 0.166; 95% Confidence Interval [CI]: 0.006-0.326; p=0.042; Figure 2a and Supplementary Figure S10) and an inverse association with reduced risk of fracture (OR=0.79; 95% CI: 0.654-0.954; p=0.014; Figure 2a and Supplementary Figure S11). The significant associations were also observed in the sensitivity analyses of contamination mixture and/or MR-PRESSO method, with insignificant MR-Egger intercept and MR-PRESSO global tests (Figure 2a). Leave-one-out analysis showed that the significant associations with TB-BMD and fracture were not driven by any single instrument (Supplementary Figures S12-S13). Upon adjustment for the beta estimates of smoking in

MVMR analysis, the association with overall TB-BMD (beta=0.172; 95% CI: 0.007-0.336; p=0.041) and fracture (OR=0.811; 95% CI: 0.671-0.978; p=0.029) remained significant in MVMR-IVW method. Similar estimates were observed from MVMR-PRESSO method. Both MVMR-Egger intercept and MVMR-PRESSO global tests were insignificant (Figure 2b), suggesting horizontal pleiotropy was unlikely.

Similarly, genetically determined caffeine intake from coffee had a positive association with overall TB-BMD in univariable IVW (beta=0.231; 95% CI: 0.093-0.369; p=0.001; Figure 2c and Supplementary Figure S14), contamination mixture and MR-PRESSO analyses. As MR-PRESSO global test was insignificant but not the MR-Egger intercept test (Figure 2c), we cannot rule out the possibility of horizontal pleiotropy. No sufficient evidence could support the association of genetically determined caffeine intake from coffee with fracture (Figure 2c and Supplementary Figure S15). Based on the leave-one-out analysis, no single instrument drove the association (Supplementary Figures S16-S17). Upon adjustment for smoking, the causal association with overall TB-BMD remained significant in MVMR-IVW (beta=0.222; 95% CI: 0.075-0.37; p=0.003) and MVMR-PRESSO (beta=0.222; 95% CI: 0.062-0.383; p=0.01) methods. Insignificant MVMR-Egger intercept and MVMR-PRESSO global tests suggested that horizontal pleiotropy was less likely (Figure 2d).

In the additional analysis, we had insufficient evidence to support any causal association of genetically determined combined caffeine intake from both tea and coffee with overall TB-BMD and fracture (Supplementary Results 2 and Supplementary Figures S18-S22).

Secondary analysis – Causal association of genetically determined caffeine intake from tea or coffee with TB-BMD in five age strata

We further assessed whether the association of genetically determined caffeine intake from tea or coffee with TB-BMD was age-specific. Insufficient evidence could support any association of genetically determined caffeine intake from tea with TB-BMD in the age strata below 15 years, 15-30 years, 30-45 years, and 60 years or above (Table 3 and Supplementary Figure S23). Positive causal association was observed for genetically determined caffeine intake from tea with TB-BMD in the age strata of 45-60 years in univariable IVW (beta=0.313; 95% CI: 0.06-0.566; p=0.015), weighted median, contamination mixture and MR-PRESSO methods (Table 3 and Supplementary Figure S23d). Leave-one-out analysis did not show any single instrument was driving the association (Supplementary Figure S24). After adjustment for smoking, the association with TB-BMD in the age strata of 45-60 years remained significant in MVMR-IVW (beta=0.326; 95% CI: 0.067-0.585; p=0.014) and MVMR-PRESSO (beta=0.326; 95% CI: 0.064-0.588; p=0.019) methods (Supplementary Figure S25).

Positive association was observed for genetically determined caffeine intake from coffee with TB-BMD in the age strata of 45-60 years in univariable IVW (beta=0.24; 95% CI: 0.008-0.472; p=0.043), contamination mixture and MR-PRESSO methods, but not in the other four age strata (Table 3 and Supplementary Figure S26). Leave-one-out analysis showed that no single instrument drove the association (Supplementary Figure S27). After adjustment for smoking, the positive association in the age strata of 45-60 years remained significant in both MVMR-IVW (beta=0.251; 95% CI: 0.015-0.488; p=0.037) and MVMR-PRESSO (beta=0.251; 95% CI: 0.029-0.474; p=0.03) analyses (Supplementary Table S28).

In the secondary analysis, univariable and multivariable MR-Egger intercept and MR-PRESSO global tests were all statistically insignificant (Tables 3 and 4, Supplementary Figures S25 and S28), suggesting the absence of horizontal pleiotropy.

In the supplementary analysis (Supplementary Results 3), no sufficient evidence could support the causal association of genetically determined caffeine intake from tea (Supplementary Table S31 and Supplementary Figure S29), coffee (Supplementary Table S32 and Supplementary Figure S30), and combined caffeine intake (Supplementary Table S33 and Supplementary Figure S31) with LS-BMD, FN-BMD and FA-BMD, respectively.

Discussion

To the best of our knowledge, this is the first study demonstrating the causal association of genetically determined caffeine intake from tea or coffee with bone health. We showed that genetically higher caffeine intake from tea or coffee had an independent causal effect on improving overall TB-BMD. The positive association was also observed for TB-BMD in the age strata of 45-60 years. Notably, genetically higher caffeine intake from tea was additionally associated with a reduced risk of fracture independently, but such association was not observed for genetically higher caffeine intake from coffee.

The positive causal association of genetically determined caffeine intake from tea or coffee with overall TB-BMD and TB-BMD in the age strata of 45-60 years were robustly supported by the univariable IVW analysis, and the sensitivity analyses of contamination mixture and/or MR-PRESSO methods. Although similar causal estimates were yielded by weighted median

method, statistical significance was not achieved in all analyses. This may be attributed to the relative low power of weighted median method when all instruments are valid, or there exists invalid instruments that have balanced pleiotropic effects on the outcome [21]. Notably, the positive causal association of caffeine intake from tea or coffee with TB-BMD remained significant in MVMR analysis after adjustment for smoking, indicating the beneficial effect of caffeine intake from either beverage on TB-BMD is independent. A hypothesized mechanism linking caffeine with bone metabolism is that caffeine is an antagonist of adenosine receptors. Caffeine inhibits the binding of adenosine to its receptors and thus limiting the functions modulated by the adenosine receptor signalling, including osteoclast formation, bone resorption [27], osteoblast proliferation, osteoblast differentiation and bone formation [28]. While several observational studies were conducted to examine the relationship of caffeine intake with BMD, the results were contradictory. A few cross-sectional studies did not identify any significant association between caffeine intake and BMD [5-8], but one study suggested that caffeine consumption was positively associated with lumbar spine BMD in female aged 30-39 while inversely associated with BMD in male aged 40-49 [6]. A meta-analysis revealed an elevated risk of fracture among individuals with the highest caffeine consumption with reference to those with the lowest caffeine consumption [9], while a population-based cohort study detected a J-shaped relationship between caffeine consumption and hip fracture risk [29]. These conflicting findings can be explained by their different study designs, limitation of conventional observational studies such as reverse causality, the heterogenous assessment of caffeine intake, and definition of high versus low caffeine consumption in the constituting studies of meta-analysis. Another possible explanation of the discrepancy is the residual confounding in conventional observational studies. For instance, cigarette smoking was highly correlated with caffeine intake [15,16], while cigarette smoking was also associated with higher rate of bone loss and increased risk of fracture [30,31]. Observational studies without

adjustment for cigarette smoking as a confounder likely underestimated the genuine association. In the present MR study, genetic instruments associated with known bone-related factors like cigarette smoking and BMI were excluded. The independence and exclusion restriction assumptions were likely valid. Our MVMR analysis further suggested caffeine intake from tea or coffee could improve TB-BMD independent of smoking.

Although genetically determined caffeine intake from tea or coffee had an independent protective effect on bone health, our additional MR analysis did not support any relationship of combined caffeine intake from both tea and coffee with TB-BMD and fracture. One likely explanation is that GWAS of combined caffeine intake identified a substantial proportion of genetic variants that are specifically related to caffeine intake from both beverages, but not either of them [10]. This aligns with our MR study that only up to one-third of genetic instruments for combined caffeine intake was common with those for caffeine intake from tea or coffee. Another possibility may be the weak phenotypic correlation among the three caffeine intake phenotypes. Caffeine intake from tea was inversely and weakly correlated with caffeine intake from coffee (r=-0.33) [10]. While combined caffeine intake had a weak phenotypic correlation with caffeine intake from tea (r=0.307), its correlation with caffeine intake from coffee was stronger (r=0.715) [10].

Findings related to caffeine intake from tea in this study is also indicative of daily tea consumption, as the caffeine intake from tea was estimated by multiplying the number of cups of tea daily by 30mg, the standardized caffeine content per cup of tea [10]. Our current study implies that genetically determined caffeine intake from tea, or genetically predicted tea consumption, is an independent protective factor of bone health in respect of TB-BMD and

fracture. Comparison of our study finding with the conflicting results from published studies on the relationship of tea consumption with bone health was presented in Supplementary Discussion 1. Several studies also investigated the association between coffee consumption and bone health. A comparison of the current study with these published studies was made in Supplementary Discussion 2.

This study has potential clinical implication. Our finding suggests the lifelong effect of genetically higher caffeine intake from tea or tea consumption on improvement of the overall TB-BMD and reduction of fracture risk, leading to the hypothesis that appropriate tea consumption may benefit bone health. While BMD increases steadily at childhood and rises sharply during adolescence until peak bone mass is reached at approximately the age of thirties, BMD remains stable till the age of fifties. The age-specific effect of genetically determined caffeine intake on TB-BMD in the age strata of 45-60 years is particularly essential, as bone loss accelerates after the age of 50, while the prevalence of osteoporosis was estimated to be 6.6% and 22.1% respectively in male and female aged ≥50 in Europe in 2019 [32]. Tea may be included in the diet as a strategy to improve BMD and prevent osteoporosis-associated fracture, especially due to its benefit on TB-BMD during the age of 45-60 years at which individuals would shortly experience rapid bone loss. Although we observed similar causal effect of genetically higher caffeine intake from coffee on overall TB-BMD in the total sample and the age strata of 45-60 years, our study did not support its effect on fracture reduction. Similar null association was observed for genetically predicted coffee consumption and fracture [33]. It is likely that other active ingredients in tea like fluoride [34] and polyphenols [35], particularly flavonoids [36-39], act together with caffeine to improve bone health. As a caffeinated beverage, tea may serve as a better protector of bone than coffee. Despite the long speculation that caffeine was linked to the development of osteoporosis via altering of calcium

metabolism and suppression of vitamin D receptor [40], our study has added new evidence to the current literature of contradictory findings. This would likely facilitate future review of the optimal dietary requirement in improving bone health, as the current MR study is unable to evaluate the previously reported J-shaped relationship between caffeine intake and fracture risk.

This study has several strengths. The MR approach utilizes genetic variants as instrumental variables representing lifelong effect of genetically determined caffeine intake from tea, coffee, or the combined caffeine intake, enabling the investigation of long-term effect on bone health when compared to cohort studies and RCT. By MR-Steiger filtering, we only included genetic instruments which explained the variance of exposure more than the outcome. The instruments are unlikely affected by reverse causality, strengthening the evidence of causal inference. The high F-statistic of the instruments (Table 2) indicated that weak instrument bias is unlikely. Moreover, the finding is robustly supported by various univariable and multivariable MR analyses with different assumptions. There are also limitations. First, two-sample MR approach assumes a linear relationship between the exposure and outcome. If the genuine relationship between caffeine intake with fracture is J-shaped as reported in a cohort study [29], we may have underestimated the causal effect. Second, the study has limited power in the secondary analysis evaluating the association of genetically determined caffeine intake with TB-BMD in the five age strata, and supplementary analyses assessing the causal effect on LS-BMD, FN-BMD and FA-BMD. The absence of association may be due to the low power. Future MR studies are warranted when larger GWAS of DXA-derived BMD, preferably age- and sex-specific GWAS, become available. Nevertheless, we had sufficient power in the primary analysis (Supplementary Results 1, Supplementary Figures S1-S6). Third, the GWAS of caffeine intake [10], TB-BMD [14] and fracture [41] comprised UK Biobank participants. Participant overlap between exposure and outcome datasets may cause bias towards the

346 confounded association [24]. Yet, we estimated the bias and Type I error rate to be 0.002-0.004
347 and 0.05 respectively (Table 2), which is minimal. This aligns with the findings of a simulation
348 study that two-sample MR methods could be applied safely in large single sample like UK
349 Biobank, except for MR-Egger [42]. In addition to the low statistical power of MR-Egger
350 method, this might explain why insignificant associations were observed from our MR-Egger
351 regression analysis. Nonetheless, due to the robust causal estimates obtained from other
352 sensitivity analyses, this does not affect the conclusion of our study. Fourth, as we retrieved
353 summary statistics of caffeine intake from the GWAS conducted by Said *et al*, this study also
354 shared the same limitations of the GWAS [10]. For instance, tea and coffee drinking habit was
355 collected from self-reported questionnaire at one time-point that did not capture change in the
356 drinking behaviour. The different preparation methods of coffee were not considered. The
357 standardization of caffeine content as 30mg per cup of tea is considered inaccurate since
358 different types of tea have variable caffeine content. The estimated caffeine intake may deviate
359 from the actual intake [10]. Lastly, the summary statistics were retrieved from GWAS/GWAS
360 meta-analysis of predominantly Europeans, limiting the generalizability of findings to other
361 ethnicities.

362

363 To conclude, the current study provides robust evidence that genetically higher caffeine intake
364 from tea and tea consumption may causally improve TB-BMD and reduce fracture risk.
365 Meanwhile, genetically higher caffeine intake from coffee is associated with TB-BMD but not
366 fracture. In addition to caffeine, other active ingredients in tea may play a role in protecting the
367 bone. This may provide insights on the optimal dietary requirements amid the contradictory
368 findings reported by conventional observational studies on the relationship between caffeinated
369 beverages and bone health.

Table 1. Description of data sources from which summary statistics were retrieved for the study.

Phenotype	Sample size	Population	Assessment method	Genetic analysis
Exposures				
Caffeine intake from tea [10] (Primary analysis)	395,866	European	The participants were asked the average number of cups of tea they drank each day. Daily caffeine intake from tea was estimated as the product of the number of cups of tea consumed daily and the caffeine content per cup of tea [standardized to 30mg].	A GWAS conducted among the UK Biobank participants. The estimated daily caffeine intake from tea, coffee, and the combined caffeine intake from both beverages of the UK Biobank participants were inverse rank normalized. Linear mixed model was applied in the genetic analysis of the three phenotypes separately, with adjustment for age, sex, genotyping array, and the first 30 principal components.
Caffeine intake from coffee [10] (Primary analysis)	373,522		The participants were asked the average number of cups of coffee they drank each day, and the type of coffee they usually drank. Daily caffeine intake from coffee was estimated as the product of the number of cups of coffee consumed daily and the caffeine content per cup of coffee, with different types of coffee having different caffeine content.	
Combined caffeine intake from both tea and coffee [10] (Additional analysis)	362,316		Daily combined caffeine intake was estimated as the sum of the daily caffeine intake from both tea and coffee, which was applicable only to participants who provided data on both beverages.	
Related exposure adjusted for in MVMR analysis				
Smoking initiation [43]	1,232,091 (557,337 cases and 674,754 controls)	European	In each individual study, the participants were asked to report whether they had ever been regular tobacco smokers. Thus, this is a binary phenotype.	A GWAS meta-analysis of 35 individual studies. Each study generated the GWAS summary statistics based on a standard analytic plan. For studies with related individuals, they adjusted

Phenotype	Sample size	Population	Assessment method	Genetic analysis
				for the covariates (age, age squared, sex, and principal components), inverse-normalized the residuals, and estimated the additive genetic effect of the variants using a linear mixed model with genetic kinship matrix. For studies comprising unrelated individuals, the additive genetic effects were estimated using the logistic model upon adjustment for the same covariates. After correction for residual stratification using study-specific genomic controls, meta-analysis was eventually conducted.
Outcomes				
Total body BMD [14] (Primary analysis)	66,628	Predominantly European ancestry (86%)	DXA was used to measure the total body BMD in individual cohorts based on standard protocols. For paediatric cohorts comprising individuals aged below 15 years, BMD of the total body less head was applied following the recommendation of the International Society for Clinical Densitometry.	A GWAS meta-analysis performed by the GEnetic Factors for OSteoporosis Consortium (GEFOS). In each individual study, DXA-derived TB-BMD was firstly adjusted for covariates (including age, weight, height and principal components) using the linear regression model, followed by inverse normal transformation of the resulting residuals, and association tests of single nucleotide polymorphisms (SNPs) with the normalized TB-BMD. GWAS meta-analysis was conducted for the overall TB-BMD in the total sample, and TB-BMD across five age strata, including the age-groups below 15 years

Phenotype	Sample size	Population	Assessment method	Genetic analysis
				(n=11,807), 15-30 years (n=4,180), 30-45 years (n=10,062), 45-60 years (n=18,805) and 60 years or above (n=22,504).
Fracture [41] (Primary analysis)	426,795 (53,184 cases and 373,611 controls)	European	The fracture status of the UK Biobank participants was ascertained through self-reported questionnaire, and diagnosis records at the Hospital Episodes Statistics database that captured medical records at hospitals of the National Health Service.	Summary statistics were retrieved from the discovery cohort comprising UK Biobank participants of a GWAS meta-analysis. Linear mixed non-infinitesimal model was applied in the genetic analysis, with age, sex, genotyping array, assessment centre, and the first 20 principal components as covariates.
BMD at lumbar spine [44] (Supplementary analysis)	28,498	European	BMD at lumbar spine (L1-L4) was measured by DXA and standardized within each cohort.	Summary statistics were retrieved from the discovery stage of a GWAS meta-analysis of DXA-derived BMD at lumbar spine, femoral neck and forearm. In each cohort, the standardized BMD was adjusted for covariates, including age, age squared, sex and weight, and the additive genetic model was applied in genetic analysis for each skeletal site. Fixed-effect meta-analysis of the cohort-level data was eventually conducted.
BMD at femoral neck [44] (Supplementary analysis)	32,735	European	BMD at femoral neck was measured by DXA and standardized within each cohort.	
BMD at forearm [44] (Supplementary analysis)	8,143	European	BMD at distal 1/3 of radius was measured by DXA and standardized within each cohort.	

Table 2. Summary of the genetic instruments included in each univariable MR analysis.

Outcome	Number of genetic instruments included in the analysis[#]	Variance explained by genetic instruments on exposure (%)	F-statistic	Cochran's Q test		Participant overlap between exposure and outcome	
				Q-statistic	Heterogeneity p value	Bias	Type I error rate
Exposure: Genetically determined caffeine intake from tea							
TB-BMD[~]							
Overall TB-BMD in total sample	24-4-2-2[a]-0=16	0.38	94.24	20.434	0.156	0	0.05
Below 15 years	24-4-4-2[a]-0=14	0.352	99.63	4. 6	0.983	NA	
15–30 years	24-5-9-1[b]-0=9	0.236	105.13	1.598	0.991	NA	
30–45 years	24-4-6-2[a]-0=12	0.333	111.11	5.848	0.883	NA	
45-60 years	24-4-3-1[b]-0=16	0.38	94.24	11.556	0.712	0	0.05
60 years or above	24-4-5-2[a]-0=13	0.334	102.35	4.191	0.98	0	0.05
Fracture^	24-6-0-1[a]-1=16	0.371	92.68	24.056	0.064	0.004	0.05
Lumbar spine BMD	24-4-3-2[a]-0=15	0.367	97.32	14.229	0.582	NA	
Femoral neck BMD	24-4-1-2[a]-0=17	0.39	90.87	17.396	0.36	NA	
Forearm BMD	24-4-7-1[b]-0=12	0.343	113.63	1.702	0.999	NA	
Exposure: Genetically determined caffeine intake from coffee							
TB-BMD[~]							
Overall TB-BMD in total sample	24-2-2-2[c]-1=17	0.485	108.36	21.053	0.177	0	0.05
Below 15 years	24-2-8-1[d]-0=13	0.458	133.64	7.74	0.805	NA	
15–30 years	24-2-8-2[c]-0=12	0.42	133.05	5.851	0.883	NA	
30–45 years	24-2-4-3[e]-0=15	0.461	116.71	6.333	0.957	NA	
45-60 years	24-2-8-1[d]-0=13	0.445	129.99	8.65	0.799	0	0.05
60 years or above	24-2-5-2[c]-0=15	0.453	114.52	11.055	0.682	0	0.05

Outcome	Number of genetic instruments included in the analysis[#]	Variance explained by genetic instruments on exposure (%)	F-statistic	Cochran's Q test		Participant overlap between exposure and outcome	
				Q-statistic	Heterogeneity p value	Bias	Type I error rate
*Fracture^*	24-2-0-3[f]-2=17	0.498	111.18	20.8	0.186	0.003	0.05
Lumbar spine BMD	24-2-4-2[g]-0=16	0.476	113.09	10.141	0.811	NA	
Femoral neck BMD	24-2-1-3[h]-0=18	0.51	107.63	7.906	0.969	NA	
Forearm BMD	24-2-8-2[c]-0=12	0.42	132.92	1.738	0.999	NA	
Exposure: Genetically determined combined caffeine intake from both tea and coffee							
Overall TB-BMD in total sample~	37-7-3-6[i]-0=21	1.095	192.96	31.711	0.047	0	0.05
*Fracture^*	37-7-0-8[j]-3=19	1.061	206.82	20.086	0.328	0.002	0.05
Lumbar spine BMD	37-7-13-4[k]-0=13	0.988	281.49	27.558	0.006	NA	
Femoral neck BMD	37-7-4-6[m]-0=20	1.085	200.84	18.654	0.479	NA	
Forearm BMD	37-7-10-5[n]-0=15	0.992	245.25	5.261	0.982	NA	

Number of genetic instruments included in the analysis = Number of independent genome-wide significant instruments identified from GWAS of caffeine intake from tea - Genetic instruments excluded due to lack of proxies - Genetic instruments excluded by MR-Steiger filtering – Genetic instruments that may affect the outcome via alternative pathways – Pleiotropic outliers identified by MR-PRESSO.

~ A small proportion of the study participants in the GWAS of TB-BMD was UK Biobank participants (n=1,559), which was in overlap with the GWAS of caffeine intake from tea and/or coffee. The bias and Type I error incurred were estimated assuming that the bias of the observational estimate is 0.4 per standard deviation increase in the exposure. The percentage of sample overlap between the exposure and outcome data sets is taken with respect to the larger data set [24] (i.e. caffeine intake), with overlap of 2.3%, 3%, and 4.4% for overall TB-BMD in the total sample, TB-BMD in the age strata of 45-60 years, and 60 years or above, respectively.

^ As both the GWAS of caffeine intake from tea/coffee and fracture comprised UK Biobank participants only, the bias and Type I error incurred were estimated assuming that the bias of the observational estimate (in log odds ratio) is 0.4 [equivalent to a bias of observational estimate (in odds ratio) of approximately 1.5] per standard deviation increase

in the exposure. The percentage of sample overlap between the exposure and outcome data sets is taken with respect to the larger data set [24] (i.e. fracture),with overlap of 92.8%, 87.5%, and 84.9% for caffeine intake from tea, coffee and combined intake respectively.

[a] rs1481012 and rs28429148; [b] rs1481012. They were instruments for caffeine intake from tea. It was significantly associated with Type II diabetes and/or serum urate level, with genome-wide significance. To avoid violation of the independence and exclusion assumptions, this instrument was excluded from the MR analysis.

[c] rs1327259 and rs2726513; [d] rs1327259; [e] rs1327259, rs2726513 and rs9941349; [f] rs1327259, rs780094 and rs13397165; [g] rs2726513 and rs9941349; [h] rs11127048, rs1327259 and rs2726513. These SNPs were instruments for caffeine intake from coffee. They were significantly associated with Type II diabetes, obesity, hip circumference, waist circumference, waist hip ratio, body mass index (BMI), weight and/or height, with genome-wide significance. To avoid violation of the independence and exclusion assumptions, these instruments were excluded from the MR analysis.

[i] rs2231142, rs215601, rs4418728, rs6265, rs489693 and rs7398196; [j] rs1260326, rs2231142, rs1490384, rs215601, rs6265 and rs489693; [k] rs2231142, rs1490384, rs215601 and rs6265; [m] rs1260326, rs2231142, rs4418728, rs6525, rs489693 and rs7398196; [n] rs2231142, rs4418728; rs4265, rs489693 and rs7398196. These SNPs were instruments for the combined caffeine intake from tea and coffee. They were significantly associated with coronary artery disease, Type II diabetes, gout, serum urate level, alcohol consumption, smoking, BMI, hip circumference, waist circumference, waist hip ratio, weight, height, obesity and/or overweight, with genome-wide significance. To avoid violation of the independence and exclusion assumptions, these instruments were excluded from the MR analysis.

Table 3. Secondary Mendelian randomization analyses examining the causal association of genetically determined caffeine intake from tea or coffee with total body bone mineral density (TB-BMD) in five age-strata.

	Beta estimate* (95% CI)	p-value	MR-Egger intercept test (p-value)	MR-PRESSO global test (p-value)
Exposure: Genetically determined caffeine intake from tea				
Below 15 years				
IVW	0.118 (-0.208 to 0.444)	0.479		
Weighted median	0.142 (-0.271 to 0.555)	0.500		
Contamination mixture	0.12 (-0.2 to 0.44)	0.479		
MR-Egger	0 (-0.72 to 0.72)	1.000	0.719	
MR-PRESSO	0.118 (-0.096 to 0.332)	0.256		0.988
15-30 years				
IVW	0.26 (-0.451 to 0.97)	0.474		
Weighted median	0.394 (-0.483 to 1.27)	0.379		
Contamination mixture	0.26 (-0.46 to 0.97)	0.474		
MR-Egger	0.976 (-0.674 to 2.626)	0.246	0.346	
MR-PRESSO	0.26 (-0.114 to 0.633)	0.148		0.986
30-45 years				
IVW	0.311 (-0.07 to 0.692)	0.109		
Weighted median	0.354 (-0.17 to 0.878)	0.185		
Contamination mixture	0.31 (-0.06 to 0.69)	0.109		
MR-Egger	0.438 (-0.428 to 1.304)	0.321	0.749	
MR-PRESSO	0.311 (-0.001 to 0.623)	0.050		0.819
45-60 years				
IVW	0.313 (0.06 to 0.566)	0.015		
Weighted median	0.415 (0.069 to 0.762)	0.019		
Contamination mixture	0.37 (0.08 to 0.65)	0.017		
MR-Egger	0.184 (-0.388 to 0.756)	0.528	0.622	
MR-PRESSO	0.313 (0.072 to 0.554)	0.014		0.722

	Beta estimate* (95% CI)	p-value	MR-Egger intercept test (p-value)	MR-PRESSO global test (p-value)
60 years or above				
IVW	-0.028 (-0.273 to 0.217)	0.824		
Weighted median	-0.017 (-0.334 to 0.299)	0.914		
Contamination mixture	-0.03 (-0.27 to 0.21)	0.824		
MR-Egger	0.071 (-0.474 to 0.616)	0.798	0.69	
MR-PRESSO	-0.028 (-0.189 to 0.133)	0.713		0.979
Exposure: Genetically determined caffeine intake from tea				
Below 15 years				
IVW	0.199 (-0.083 to 0.48)	0.167		
Weighted median	0.125 (-0.233 to 0.483)	0.494		
Contamination mixture	0.2 (-0.08 to 0.48)	0.167		
MR-Egger	0.114 (-0.454 to 0.682)	0.695	0.736	
MR-PRESSO	0.199 (-0.053 to 0.45)	0.111		0.836
15-30 years				
IVW	-0.157 (-0.691 to 0.376)	0.563		
Weighted median	-0.354 (-1.137 to 0.429)	0.376		
Contamination mixture	-0.16 (-1.1 to 0.79)	0.563		
MR-Egger	-0.116 (-1.187 to 0.955)	0.832	0.93	
MR-PRESSO	-0.157 (-0.594 to 0.279)	0.445		0.693
30-45 years				
IVW	0.221 (-0.106 to 0.548)	0.186		
Weighted median	0.229 (-0.223 to 0.681)	0.321		
Contamination mixture	0.22 (-0.1 to 0.54)	0.186		
MR-Egger	0.261 (-0.359 to 0.88)	0.409	0.881	
MR-PRESSO	0.221 (-0.02 to 0.461)	0.069		0.902

	Beta estimate* (95% CI)	p-value	MR-Egger intercept test (p-value)	MR-PRESSO global test (p-value)
45-60 years				
IVW	0.24 (0.008 to 0.472)	0.043		
Weighted median	0.225 (-0.073 to 0.523)	0.139		
Contamination mixture	0.24 (0.01 to 0.47)	0.043		
MR-Egger	0.173 (-0.271 to 0.617)	0.444	0.731	
MR-PRESSO	0.24 (0.029 to 0.45)	0.029		0.813
60 years or above				
IVW	0.008 (-0.199 to 0.216)	0.936		
Weighted median	-0.015 (-0.288 to 0.257)	0.911		
Contamination mixture	0.01 (-0.2 to 0.21)	0.937		
MR-Egger	-0.137 (-0.533 to 0.258)	0.496	0.396	
MR-PRESSO	0.008 (-0.194 to 0.211)	0.930		0.718

* Beta estimate is presented as standard deviation (SD) change in TB-BMD per SD increase in genetically determined caffeine intake from tea or coffee.

Data availability: The summary statistics used in this study can be downloaded from the respective GWAS/ GWAS meta-analyses cited in Table 1.

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Figure legends

Figure 1. Study design of the Mendelian randomization (MR) study and the key assumptions of MR approach.

(a) Univariable MR analysis

(b) Multivariable MR (MVMR) analysis

Figure 2. Primary Mendelian randomization analysis evaluating the causal effects of genetically determined caffeine intake from tea or coffee on overall total body bone mineral density (TB-BMD) and fracture.

(a) Univariable analysis evaluating the causal effects of genetically determined caffeine intake from tea on overall TB-BMD and fracture.

(b) Multivariable analysis evaluating the causal effects of genetically determined caffeine intake from tea on overall TB-BMD and fracture, adjusted for smoking.

(c) Univariable analysis evaluating the causal effects of genetically determined caffeine intake from coffee on overall TB-BMD and fracture.

(d) Multivariable analysis evaluating the causal effects of genetically determined caffeine intake from coffee on overall TB-BMD, adjusted for smoking.

Supplementary Materials

Supplementary Methods 1. Selection of genetic instruments.

Independent SNPs were identified from the GWAS of caffeine intake from tea and/or coffee, which were obtained by clumping the genome-wide significant SNPs ($p<5x10^{-8}$) with thresholds of $r^2 \geq 0.005$ and physical distance ≤5Mb [1]. A total of 24, 24 and 37 independent SNPs meeting a more stringent threshold of $p<1.67x10^{-8}$ defined by the study [1] were initially selected as genetic instruments for caffeine intake from tea, coffee, and the combined caffeine intake, respectively. Summary statistics of the genetic instruments were retrieved from the outcome datasets and harmonized. For genetic instruments absent from the outcome datasets, they were replaced by proxies in high linkage disequilibrium (LD; $r^2 \geq 0.8$) yet still significantly associated with the respective exposure of caffeine intake ($p<1.67x10^{-8}$). Proxies were also identified for palindromic instruments with minor allele frequency (MAF) >0.3 to avoid strand ambiguity. If any genetic instrument explained the variance of the outcome more than the exposure, they were identified by MR-Steiger filtering [2] and excluded from further analysis to minimize bias due to reverse causality. To ensure the independence and exclusion restriction assumptions (Figure 1) hold, any genome-wide significant association of the instruments with confounders and/or alternative factors that affect the outcome (such as body mass index [BMI], height, weight, etc) were identified via PhenoScanner [3]. Instruments potentially violating any of the two assumptions were

removed from MR analysis. For each analysis, proportion of phenotypic variance explained by the final set of instruments on the exposure was calculated by summing the variance explained by the instruments using the formula: effect size^2 x 2 x MAF x (1-MAF).

Supplementary Methods 2. Univariable MR analysis.

The inverse variance weighted (IVW) method assumes all genetic instruments are valid and combines the ratio estimates of each instrument in random-effect meta-analysis [4]. It was adopted as the main analysis in the current MR study. As the presence of invalid instruments might bias the causal estimates generated by IVW method, several MR methods with different assumptions were applied as sensitivity analyses. The weighted median method gives consistent causal effect estimates when at least 50% of the instruments are valid [5]. For MR-Egger regression, its intercept assesses the average pleiotropic effect across the genetic instruments, while the slope robustly estimates the causal effect after correction for bias due to directional pleiotropy, given that the weaker Instrument Strength Independent of Direct Effect (InSIDE) assumption holds [6]. The contamination mixture method estimates the causal effect efficiently with little bias and the lowest mean squared error compared to other MR methods, even if <40% instruments are invalid under the plurality assumption [7]. For MR Pleiotropy Residual Sum and Outlier (MR-PRESSO), its global test detects the presence of horizontal pleiotropy, while the outlier test provides a precise outlier-corrected causal estimate provided that <50% instruments are horizontal pleotropic [8].

Supplementary Results 1. Power calculation for MR analysis.

We had adequate statistical power in the primary analysis, with ≥80% power in detecting approximately ≥0.18 and 0.16 SD change in TB-BMD per SD increase in genetically determined caffeine intake from tea or coffee separately (Supplementary Figures S1 and S4). We also had ≥80% power in detecting an association of genetically determined caffeine intake from tea or coffee with fracture, if the OR of fracture was ≤0.8 and 0.83, respectively (Supplementary Figures S2 and S5).

In the secondary analysis evaluating the association of genetically determined caffeine intake with TB-BMD in the five age strata, we had particularly low power in the age strata of 15-30 years, with ≥80% power in detecting approximately ≥0.9 and 0.67 SD change in TB-BMD per SD increase in genetically determined caffeine intake from tea or coffee respectively (Supplementary Figures S1 and S4).

In the supplementary analysis evaluating the causal association of genetically determined caffeine intake from tea or coffee with LS-BMD, FN-BMD and FA-BMD, we did not have adequate statistical power mainly due to the small sample size of the outcome GWAS, especially for FA-BMD. Our study had ≥80% statistical power to detect any genuine causal association if beta estimate ≥0.25, 0.28 and 0.53 SD for FN-

BMD, LS-BMD and FA-BMD respectively per SD increase in genetically determined caffeine intake from tea (Supplementary Figure S3). We had ≥80% power if the beta estimate ≥0.22, 0.24 and 0.48 SD for FN-BMD, LS-BMD and FA-BMD respectively per SD increase in genetically determined caffeine intake from coffee (Supplementary Figure S6). For the MR analyses for genetically determined combined caffeine intake, we had ≥80% power if the beta estimate ≥0.15, 0.17 and 0.32 SD for FN-BMD, LS-BMD and FA-BMD respectively (Supplementary Figure S9).

Supplementary Results 2. Additional analysis evaluating the causal association of genetically determined combined caffeine intake with overall TB-BMD and fracture.

We did not have sufficient evidence to support the causal association of genetically determined combined caffeine intake with overall TB-BMD and fracture in all the applied MR methods (Supplementary Figures S18-S20). Both MR-Egger intercept test and MR-PRESSO global test were statistically insignificant, implying that horizontal pleiotropy was not likely. Leave-one-out analyses showed that no single instrument was driving the null association (Supplementary Figures S21-S22).

Supplementary Results 3. Supplementary analysis examining the causal association of genetically determined caffeine intake from tea, coffee, and combined intake with BMD at lumbar spine, femoral neck and forearm.

The supplementary analysis was conducted for the purpose of comparison with other MR studies, despite the relative low power. We did not have sufficient evidence to support the causal association of genetically determined caffeine intake from tea (Supplementary Table S31 and Supplementary Figure S29), coffee (Supplementary Table S32 and Supplementary Figure S30), and combined caffeine intake (Supplementary Table S33 and Supplementary Figure S31) with LS-BMD, FN-BMD and FA-BMD, respectively. All MR-Egger intercept and MR-PRESSO global tests were insignificant, except for the MR-PRESSO global test in evaluating the causal association of genetically determined combined caffeine intake with LS-BMD ($p=0.039$; Supplementary Table S33) that the possibility of horizontal pleiotropy cannot be completely ruled out in this case. Leave-one-out analysis suggested that no single instrument was driving the association (Supplementary Figures S32-S34).

Supplementary Discussion 1. Comparison of the current study with published studies on the relationship of tea consumption with bone health.

Conflicting results on the relationship of tea consumption with bone health were seen among cohort studies [9-14] and randomized controlled trial (RCT) [15,16]. While some cohort studies supported that tea consumption had a protective effect on bone health by improving BMD, lowering the risk of osteoporosis or fracture [9-11], a few did not reveal any association with BMD [12] and hip fracture risk [13,14]. Possible reasons for the discrepancy include the variation in follow-up time, in addition to the different ethnicities and known limitations of cohort studies like residual confounding. Previously, a six-month RCT showed that green tea polyphenols increased the ratio of serum bone-specific alkaline phosphatase to tartrate-resistant acid phosphatase, and improved muscle strength among post-menopausal women with osteopenia [15]. Meanwhile, a twelve-month RCT suggested that decaffeinated green tea extract did not improve BMD among post-menopausal women who were obese or overweight [16]. Despite the strong evidence of causality provided by RCT, only short-term effect of green tea extract on bone health were examined by the published studies [15,16]. Conversely, genetic variants were utilized in MR approach, enabling us to investigate the life-long effects of genetically predicted tea intake on bone health. Although a MR

study found null association between genetically predicted tea consumption and osteoporosis, the lack of statistical power to confirm a causal relationship was acknowledged [17].

Supplementary Discussion 2. Comparison of the current study with published studies on the relationship of coffee consumption with bone health.

Several studies investigated the association between coffee consumption and bone health. A meta-analysis showed that individuals with high coffee consumption had a reduced risk of osteoporosis compared to those with low coffee consumption [18]. This is partially consistent with our finding, as caffeine is the major pharmacoactive ingredient in coffee. Nevertheless, our recent study demonstrated that some metabolites associated with coffee intake were not related to caffeine metabolism, but they still contribute to the positive association of coffee intake with BMD [19], implying the presence of other bone-protecting ingredients in coffee. Meanwhile, meta-analysing 13 cohort studies suggested a potential J-shaped relationship between coffee consumption and fracture [18]. It remains unknown if the relationship between caffeine intake from coffee and fracture is also J-shaped. A published MR study evaluated the causal effect of genetically predicted coffee consumption on estimated BMD (eBMD) and fracture, but no association was found [20]. Our study had discrepant finding in respect of TB-BMD, which could be due to the intrinsic difference between TB-BMD and ultrasound-based eBMD. First, they are measured at different skeletal sites. Total body DXA scans were made for TB-BMD measurement, except that the paediatric cohort measured the

BMD of total body minus head [21]. eBMD was estimated at heel by ultrasound. Second, eBMD-associated genetic loci were previously reported to have opposite directions with genetic loci associated with DXA-derived BMD [22]. Third, we previously demonstrated that TB-BMD and eBMD had a positive yet moderate genetic correlation (r=0.59) [23]. Even though eBMD can be measured quickly at a relatively lower cost, it could not replace DXA-derived BMD in diagnosis of osteoporosis. Thus, the reported null association of genetically predicted coffee consumption with eBMD [20] requires cautious interpretation. Both our analysis and the published study [20] retrieved summary statistics of fracture from the same GWAS [24]. There was insufficient evidence to support any causal effects of genetically determined caffeine intake from coffee or coffee consumption on fracture. Moreover, the same study observed null association for genetically predicted coffee intake with LS-BMD, FN-BMD and FA-BMD [20]. Null association was also observed in our supplementary analysis for these three BMD traits, probably due to the small sample size of the GWAS and limited power of the MR analysis (Supplementary Results 1, Supplementary Figure S3, S6 and S9).

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