Causal Effects of Tea Intake on Multiple Types of Fractures: A

Two-Sample Mendelian Randomization Study

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Abstract: Fracture is a global public health disease. Bone health and fracture risk have become the focus of public and scientific attention. Observational studies have reported that tea consumption is associated with fracture risk, but the results are inconsistent. The present study was conducted to evaluate whether tea consumption was causally associated with the risk of bone fracture through two-sample Mendelian Randomization (MR) analysis. We included a large genome-wide association study (GWAS) associated with tea consumption of 447,485 individuals and analyzed the effects of genetic instruments on fractures using fracture cases from the UK Biobank dataset (n=361,194). Inverse variance weighted (IVW) indicated no causal effects of tea consumption on fractures of the skull and face, shoulder and upper arm, hand and wrist, femur, calf, and ankle (odds ratio=1.000, P=0.881; OR=1.000, P=0.857; OR=1.002, P=0.339; OR=0.997, P=0.054; OR=0.998, P=0.569, respectively). Consistent results were also found in MR-Egger, weighted median, and weighted mode. Our research provided evidence that tea consumption is unlikely to affect the incidence of fractures.

Keywords: tea intake; fracture; Mendelian randomization; genome-wide association studies

1. Introduction

Fracture is usually defined as the interruption of bone integrity and continuity, which can be divided into the traumatic fracture and pathological fracture. Fractures can occur in any part of the body, among which the femur (hip), vertebra (spine), and distal radius (wrist) are the most common[1]. With the increasing aging trend of the global population and the improvement of living and medical conditions, bone health and fracture risk have become the focus of public and scientific attention [2].

Numerous studies have shown that fracture is closely related to race, region, age, occupation, lifestyle, diet, and disease[3]. Among them, the influence of dietary factors cannot be ignored, including coffee intake [4], alcohol consumption [5], dietary protein intake [6], etc. We are extraordinarily interested in the causality between tea consumption and fracture risk among dietary factors. Tea is the second-most consumed beverage in the world. It is reported that tea drinking has particular benefits in reducing the risk of osteoporosis [7], primary prevention of cardiovascular diseases [8], and blood glucose control [9]. Previous studies have investigated the relationship between tea consumption and fracture risk [10-13], but the results are not inconsistent.

Mendelian Randomization (MR) is considered a method comparable to randomized controlled studies (RCT) [14]. Genetic variation is used as an instrumental variable (IV) to derive the causal relationship between outcome and exposure by using large-scale genome-wide association study (GWAS) data[15], which can effectively avoid the confounding bias and reverse causal bias of traditional epidemiological studies. Therefore, this study uses an MR study to study the causal relationship between tea intake and fracture from the level of genetic variation, which is significant for preventing fracture, improving bone health, and formulating appropriate intervention measures.

2. Materials and Methods

2.1.Data Source

We conducted a two-sample MR study using aggregate data on tea intake and multiple types of fractures from different GWAS. To reduce the potential confounding bias caused by ethnic stratification, we limited the sample data to the European population.

The tea intake (phenotype code: 1488 RAW) data set was obtained from the diet data of the British Biobank. The tea intake in the dietary data began to recruit participants in 2006 and was measured in cups per day. Those with answers of < 0 or > 99 were excluded, and participants with > 20 were asked to reconfirm, including 447,485 samples of European descent. For more details regarding tea intake, please visit https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=1488. We extracted aggregate statistics on the skull and facial, shoulder and upper arm, hand and wrist, femur, calf, and ankle fractures from Chapter XIX Injury, poisoning, and certain other consequences of external causes of UK Biobank (GWAS Round 2). This data includes 361,194 cases of fracture. Details of the fracture were obtained at https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=41202.

2.2.MR Analysis

MR refers to the use of genetic variation to estimate the causal relationship between exposure and outcome. Exposure can be anthropometric indicators, laboratory testing indicators, or other risk factors that may affect the outcome. The outcome is usually a certain disease, but not limited to disease. The genetic variation must satisfy the hypothesis of IV: (1) closely associated with exposure; (2) not associated with confounding factors associated with exposure-outcome; (3) no direct relationship between genetic variation and outcome.

The present study obtained 41 unique SNP by selecting significant exposure of single nucleotide polymorphism (SNP) ($p < 5 \times 10^{-8}$) and checking for linkage disequilibrium (LD)

effect size outliers. Before performing the two-sample MR analysis, we unify the exposure and outcome data into one data set by removing palindromes SNP[16].SNPs with A / T or G / C allele are defined as palindrome SNP[17]. We chose fixed effect IVW for causal analysis because the causal relationship between SNPs was under-dispersed, while the random effect IVW model will exaggerate the variance[16]. IVW was insensitive to horizontal pleiotropy [18], so we used MR-Egger, weighted median and weighted model to evaluate the causality between tea intake and fracture[19]. Secondly, Cochran's Q test was used to evaluate the heterogeneity among different SNP. If P < 0.05, it was defined as significant heterogeneity[20]. Then the intercept term tested by the MR-Egger model was away from 0, indicating the existence of horizontal pleiotropy[21]. The leave-one-out method was used for sensitivity analysis[22]. Judge whether a bias was caused by weak IV on the results according to R² and F value test (see Table1 for calculation formula)[23]. F statistics > 10 indicated that weak IV deviations were unlikely[24].

All data were analyzed using R software v.4.0.3, Empower Stats, and MR-base. All images were generated by GraphPad Prism 9.0.0 and Adobe Illustrator 2021. P<0.05 indicated statistical significance. (unless stated separately)

3. Results

3.1.IV Selection

41 SNPs related to tea intake were selected in the MR analysis ($P < 5 \times 10^{-8}$, through LD analysis, r ²=0.0001, kb=10000). Among them, 8 SNPs were removed because of being palindromes. Finally,33 SNPs related to tea intake were selected to perform the following MR analysis (skull and facial fractures:32 SNPs, missing rs149805207) (Table 1). The F statistics of SNPs were all greater than 10 (mean value = 67, F _{min}= 30, F_{max} = 494), so it was unlikely to cause a result bias by instrumental variable.

3.2. Two-sample MR analysis

The Fixed effect IVW analysis indicated that the genetic prediction of drinking an extra cup of tea a day did not affect fracture risk. The OR value of increased tea intake for skull and facial fractures was 1.000 (95% CI, 0.997-1.003), shoulder and upper arm fractures were1.000 (95% CI, 0.997-1.004), hand and wrist fractures were 1.002 (95% CI, 0.998-1.005), femur fractures were 0.997 (95% CI, 0.993-1.000), calf and ankle fractures were 0.998 (95% CI, 0.993-1.004) (Figure 1). Similar results were observed in MR-Egger, weighted median, and weighted mode (Figure 1). The scatter plot of these results is shown in Figure 2. Cochran's Q test indicated no significant heterogeneity (Table 2), and the visualization results were shown in the funnel diagram (Supplementary Figure 1). The MR-Egger test did not detect horizontal pleiotropy (Table 2). The left-out method showed that the comprehensive effect of SNP was not changed or reversed one by one, which indicated that the result was credible (Supplementary Figure 2). The forest diagram of tea intake of each SNP and different fracture estimates was shown in Supplementary Figure 3.

SNP	FA	Position	FAF	BFTA	SF	Р	N	R^2	F
rs10741694	<u>с, с</u>	11 16286183	0.63	0.015	0.0022	7 90F-12	447485	0.0001045	47
rs10752269	Δ	10 12692902	0.05	0.0129	0.0022	1 30E-09	447485	0.0001045	37
rs10764990	A	10.129152608	0.61	0.0122	0.0021	1.90E-08	447485	0.0000706	32
rs11164870#	G	1.93552187	0.6	-0.012	0.0022	4.20E-08	447485	0.0000671	30
rs1156588	G	2.58515375	0.21	0.0155	0.0026	2.90E-09	447485	0.0000787	35
rs11587444	G	1.150722844	0.39	0.014	0.0022	1.00E-10	447485	0.0000934	42
rs12591786	Т	15.60902512	0.16	0.0184	0.0029	3.70E-10	447485	0.0000878	39
rs13282783	Т	8.22088975	0.29	0.0136	0.0024	7.90E-09	447485	0.0000744	33
rs132904#	С	22.41798896	0.78	0.0166	0.0026	7.80E-11	447485	0.0000945	42
rs141071726	А	7.17558580	0.03	0.0407	0.0068	2.20E-09	447485	0.0000799	36
rs1453548#	А	11.59192089	0.66	0.0133	0.0022	3.00E-09	447485	0.0000786	35
rs1481012	G	4.89039082	0.11	0.0262	0.0034	5.30E-15	447485	0.0001366	61
rs149805207*	G	6.137095269	0.01	0.0719	0.0126	1.10E-08	447485	0.0000730	33
rs17245213	А	11.1679769	0.21	0.0146	0.0026	2.00E-08	447485	0.0000704	32
rs17576658	А	13.100272019	0.25	0.0135	0.0025	4.10E-08	447485	0.0000673	30
rs17685	А	7.75616105	0.28	0.0231	0.0024	1.60E-22	447485	0.0002131	95
rs2117137	G	3.89525505	0.41	0.013	0.0022	1.70E-09	447485	0.0000812	36
rs2273447#	Т	20.62900120	0.2	0.0175	0.0026	3.30E-11	447485	0.0000983	44
rs2279844	А	17.40819809	0.38	-0.012	0.0022	4.00E-08	447485	0.0000674	30
rs2351187	А	10.86850616	0.32	0.0129	0.0023	1.60E-08	447485	0.0000714	32
rs2472297	Т	15.75027880	0.26	0.0533	0.0024	2.30E-109	447485	0.0011019	494
rs2478875	G	6.51283110	0.21	0.0219	0.0026	5.10E-17	447485	0.0001571	70
rs2645929	G	13.56444529	0.81	-0.015	0.0027	3.50E-08	447485	0.0000680	30
rs2783129#	G	13.80168720	0.48	0.0117	0.0021	3.80E-08	447485	0.0000676	30
rs34619	А	5.60465365	0.43	0.0117	0.0021	4.30E-08	447485	0.0000671	30
rs4410790	С	7.17284577	0.63	0.0406	0.0022	3.40E-76	447485	0.0007621	341
rs4808193	С	19.19410622	0.34	0.0151	0.0022	1.70E-11	447485	0.0001011	45
rs4817505	С	21.34343828	0.39	0.0151	0.0022	4.20E-12	447485	0.0001073	48
rs56188862	С	1.174189269	0.39	0.0158	0.0022	4.30E-13	447485	0.0001173	52
rs56348300#	G	9.7054124	0.18	0.0159	0.0027	6.10E-09	447485	0.0000755	34
rs57462170	А	3.50239803	0.11	0.0192	0.0034	1.90E-08	447485	0.0000707	32
rs57631352	G	19.4338173	0.3	0.0131	0.0023	1.70E-08	447485	0.0000712	32
rs6829	Т	13.111531264	0.6	0.0119	0.0022	3.70E-08	447485	0.0000677	30
rs713598#	G	7.141673345	0.4	0.0134	0.0022	5.20E-10	447485	0.0000862	39
rs72797284	G	5.152031650	0.27	0.0171	0.0024	7.00E-13	447485	0.0001152	52
rs7757102	G	6.137222671	0.56	0.0118	0.0021	3.10E-08	447485	0.0000684	31
rs9302428#	G	16.24717600	0.64	0.0122	0.0022	2.60E-08	447485	0.0000692	31
rs9624470	А	22.24820268	0.58	0.0252	0.0022	1.30E-31	447485	0.0003057	137
rs9648476	А	7.39293033	0.62	0.0125	0.0022	1.10E-08	447485	0.0000731	33
rs977474	Т	12.11284772	0.83	0.0218	0.0029	2.40E-14	447485	0.0001300	58
rs9937354	Α	16.53799847	0.42	0.0141	0.0021	4.90E-11	447485	0.0000966	43

Table1. Characteristics of single-nucleotide polymorphisms(SNPs) associated with tea consumption associated with tea consumption.

* Missing of the skull and facial fractures; #Palindromes were excluded. SNP, single-nucleotide polymorphism; EAF, effect allele frequency; EA, effect allele; BETA, beta. exposure; SE, standard error; P, Significance level of tea; R² was calculated as follows: $2*beta^2*EAF*(1-EAF)/(2*beta^2*EAF*(1-EAF) + se^2*2*N*EAF(1-EAF))$. The F-statistic for each SNP was calculated as follows: $F = (N-2) *R^2/(1-R^2)$.

Outcome	Coch	ran	MR-Egger		
Outcome	Q	Р	Intercept	Р	
Skull and Face	26.91	0.63	-0.0000034	0.96	
Shoulder and Upper Arm	18.53	0.96	0.000055	0.45	
Hands and Wrists	21.06	0.91	0.000039	0.58	
Femur	36.59	0.23	-0.000014	0.86	
Calves and Ankles	21.59	0.90	0.000026	0.82	

Table2. Results of horizontal pleiotropy and heterogeneity statistics.

Figure1. Forest plots of Mendelian Randomization(MR) study using genetically predicted tea intake with multiple types of fractures. Inverse variance weighted(IVW), MR-Egger, weighted median, and weighted mode were used in this study.

Fracture site	Method	OR (95%CI)		Р
Skull and Face	MR Egger	1.000 (0.994,1.007)		0.908
	Weighted median	1.001 (0.996,1.005)		0.802
	Fixed effects IVW	1.000 (0.997,1.003)		0.881
	Weighted mode	1.000 (0.996,1.004)		0.993
Shoulder and Upper Arm	MR Egger	0.998 (0.991,1.005)	•	0.553
	Weighted median	1.000 (0.996,1.005)		0.908
	Fixed effects IVW	1.000 (0.997,1.004)	e	0.857
	Weighted mode	1.001 (0.995,1.006)		0.786
Hands and Wrists	MR Egger	1.000 (0.993,1.007)		0.960
	Weighted median	1.000 (0.995,1.005)		0.940
	Fixed effects IVW	1.002 (0.998,1.005)		0.339
	Weighted mode	1.000 (0.995,1.005)		0.960
Femur	MR Egger	0.997 (0.990,1.005)		0.511
	Weighted median	0.999 (0.994,1.004)		0.633
	Fixed effects IVW	0.997 (0.993,1.000)	e	0.054
	Weighted mode	0.999 (0.993,1.004)		0.691
Calves and Ankles	MR Egger	0.997 (0.986,1.009)		0.642
	Weighted median	0.997 (0.990,1.005)		0.437
	Fixed effects IVW	0.998 (0.993,1.004)		0.569
	Weighted mode	0.997 (0.989,1.006)		0.514
		0.990	0.995 1.000 1.005	1.010
			OR(95%CI)	

Figure2. The scatter plot for MR analyses of causal associations between each tea intake SNP and multiple types of fractures.



4. Discussion

In this study, we used two samples of MR to investigate the causal relationship between tea intake and fracture. It revealed no causal relationship between genetically predicted tea intake and fracture risk among 361,194 fracture cases and 447,485 tea intake participants in the sample, supporting the results of most but not all observational studies.

Numerous studies have reported the relationship between tea intake and fracture risk. Veiga et al. studied the fracture risk of yerba mate intake in 95 postmenopausal women in southern Brazil. They found that yerba mate intake had no effect on fracture but affected bone metabolism[10]. Dai et al. found no relationship between tea intake and hip fractures in a prospective cohort of 63,257 Singaporean-based people aged 45 to 74 [25]. Hallström et al. conducted a cohort study of 31,527 Swedish women aged 40 to 76 years and found that tea consumption was not related to fracture risk [26]. In a case-control study on the effect of diet on the risk of postmenopausal hip and wrist fractures, Kreiger et al. revealed that tea intake was not associated with fracture risk (hip and wrist). These findings are in accord with our MR analysis [27]. However, Myers et al. found that higher black tea and specific classes of flavonoids intake were associated with a lower risk of fractures in older women[12]. A prospective study of 1027 Western Australian women by Devine et al. found that tea drinking was associated with hip bone protection in older women[28]. Shen et al. studied tea consumption and fracture risk among 453,625 Chinese adults and found that

daily tea drinkers had a lower risk of fracture hospitalization than non-tea drinkers over the previous 12 months[29].

The inconsistent results of studies may be related to the influence of tea components on bone metabolism, which explains the microscopic mechanism of the two effects from the perspective of bone mineral density and bone strength. Tea is rich in caffeine, polyphenols, flavonoids, alkaloids, etc. Their effects on bones can be divided into two categories. (1) Protective effect of tea components on bone. Polyphenols can enhance the ability of antioxidation and/or reduce the damage of oxidative stress, which has a beneficial effect on bone metabolism[30-32]. Flavonoids can affect bone health by increasing osteogenic gene expression, stimulating osteogenesis, and improving bone marker activity[33]. Similar results appeared in Chen et al. 's study on the effect of (-) -epigallocatechin-3-gallate (EGCG) on the osteogenic function of mouse bone marrow mesenchymal stem cells[12]. In addition, a study of postmenopausal women found that serum NOx levels increased significantly in women who drank yerba mate[10], and high levels of NO seemed to stimulate osteoprotegerin and inhibit RANK-RANKL binding from reducing bone resorption, acting as an estrogen mediator[34]. Other studies also found that drinking tea may increase bone density by affecting fluoride levels in the body[26, 35]. (2) The damaging effect of tea components on bone. The caffeine in tea hurts bones. Experimental studies have proved that high dose caffeine intake can affect the bone development of growing rats [36, 37]. The mechanism may be related to promoting calcium excretion in urine and feces[38, 39], resulting in a negative calcium balance, which reduces bone density and bone strength. This possible mechanism has also been verified in several studies on caffeine intake and human metabolism[40, 41]. Another interesting mechanism is that tea and flavonoids affect bone health by affecting cardiovascular health[12], a conjecture that needs to be confirmed by more studies. In addition, tea is a stimulant beverage containing many kinds of alkaloids such as caffeine, theobromine, and theophylline. It may promote the excitement of the motor nervous system, which increases the risk of falls and leads to fractures.

RCT is recognized as the gold standard for studying whether interventions affect health outcomes. However, RCT is often expensive, impractical, or has a high failure rate[42]. MR is a statistical method that uses a genetic variation to simulate RCT, infer the causal relationship between phenotypes, and understand the etiology of the disease process[16]. Compared with traditional epidemiological studies, the MR studies take advantage of the essentially unmodifiable nature of the germline genome, which is not susceptible to reverse causal bias and confounding factors[43]. Finally, 33 SNP were selected (32 SNP for skull and facial fractures), and IVW, MR-Egger, weighted median, and weighted model were used to analyze the causality between the two samples. The results still showed that tea intake had no causal relationship or effect on fracture risk. Thus, we speculate that the interaction of these two mechanisms may offset the impact of tea intake on bones. In addition, we believe that the human body, as an organic whole, has a robust homeostasis regulation system, and the protective and damaging effects of tea intake on the body are not enough to cause tissue and organ damage. As a result, a causal relationship between tea intake and fracture risk is unlikely.

A major strength of our study is that it is the first time to use the two-sample MR to analyze the causal relationship between tea intake and fracture risk. The method overcomes the inherent effects of residual confusion, reverse causality bias, and measurement errors in traditional epidemiology. Another advantage is that we use multiple types of fractures as outcome variables to avoid the partial generalization caused by a single site. However, this study has some inevitable limitations. Firstly, the results did not apply to other populations due to deviations from the data limited to European populations. Secondly, the tea varieties and tea production methods are multifarious, which is extraordinarily significant in exploring the causal relationship between tea intake and exposure genes. Further analysis of tea intake and human health should consider the overall impact of these factors.

Supplementary Materials: Please refer to the attached file for the following support information.

Table S1: For supplementary tables, see the CSV file of supplementary materials.

Figure S1: The funnel plot for MR analyses of causal associations between each tea intake SNP and multiple types of fractures.

Figure S2: Leave-one-out sensitivity analysis for multiple types of fractures using SNP-associated tea intake.

Figure S3: The forest plot for MR analyses of causal associations between each tea intake SNP and multiple types of fractures.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript: MR Mendelian randomization

IVW	Inverse variance weighted
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IV Instrumental variable

GWAS Genome-wide association study

OR Odds ratio

LD Linkage disequilibrium

- CI Confidence interval
- RCT Randomized controlled studies
- EAF Effect allele frequency
- EA Effect allele
- SE Standard error

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