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Genetic Association of eGFR with BMD: A Mendelian Randomization Study

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Epidemiology

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Genetic Association of eGFR with BMD: A Mendelian Randomization Study

By

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Abstract

Genetic Association of eGFR with BMD: A Mendelian Randomization Study
By Pu Wang

Background: Both chronic kidney disease (CKD) and osteoporosis are important public health issues worldwide with high prevalence especially among the older population. These two diseases have high comorbidity and share multiple risk factors. The estimated glomerular filtration rate (eGFR) is the primary measurement of the filtration function of kidney. A low bone mineral density (BMD) is often used to diagnose osteoporosis and predict future osteoporotic fractures. Several observational epidemiologic studies have reported strong association between eGFR and BMD among older populations. Understanding whether the associations reflect a causal relationship or mere correlation may inform whether targeting renal treatment could reduce risk for osteoporosis, or vice versa.

Methods: We performed a bidirectional two-sample Mendelian randomization (MR) analysis using publicly available summary-level data from a genome-wide association study (GWAS) of eGFR (n= 567,460), and a GWAS of BMD (n = 395,929) among participants of European ancestry to test the hypothesis that the association between eGFR and BMD is potentially causal. The two-sample MR analyses included inverse-variance-weighted (IVW) regression for estimating the causal effects, and MR-Egger regression for the sensitivity analyses.

Results: We found no evidence of causal effect of eGFR on BMD (225 SNPs; causal effect estimate per 1-unit increase in log(eGFR)=-0.0950, p-value=0.68). The intercept obtained from MR-Egger regression was 0.001 (p-value=0.53), suggesting no evidence of horizontal pleiotropy. Evaluation of the inverse direction of causality showed that there is no evidence of causal effect of BMD on eGFR either (878 SNPs; causal effect estimate per 1-SD increase in BMD=-2.99×10^{-5}, p-value=0.98). Results obtained from MR-Egger regression (intercept=0.000, p-value=0.054) suggested no evidence of horizontal pleiotropy.

Conclusion: In summary, our bidirectional MR analyses did not support the causal relationship between eGFR and BMD in either direction among the European ancestry. However, MR analysis does not exclusively address the causal relationship by itself, and further work would be needed to investigate such causal relationship among specific subgroups, or common risk factors underlying the observed association between eGFR and BMD.

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Introduction

Nowadays, we are facing a sizeable increase in the older population [1], and consequently, the prevalence of many age-related diseases will increase, including osteoporosis, and chronic kidney disease (CKD) and so on [2][3].

CKD is an important public health problem among older adults. In 2002, the US National Kidney Foundation Kidney Disease Outcomes Quality Initiative clinical practice guidelines defined CKD as kidney damage or glomerular filtration rate (GFR) lower than 60 mL/min per 1.73 m^2 for 3 months or longer [4]. The estimated glomerular filtration rate (eGFR) using either serum creatinine or cystatin C as well as patient characteristics is the primary tool for the assessment of kidney function [5]. According to data from the National Health and Nutrition Examination Surveys (NHANES) 1999-2004, around 40% of adults above age 60 meet the definition for CKD using the Modification of Diet in Renal Disease (MDRD) equation to estimate eGFR [6]. According to the 2010 Global Burden of Disease study [7], CKD was ranked 27th in the list of causes of total number of global deaths in 1990 (age-standardized annual death rate of 15.7 per 100,000), but rose to 18th in 2010 (annual death rate 16.3 per 100,000). This degree of movement up the list was second only to that for HIV and AIDS. Besides its high prevalence and death rate, many of other age-related diseases and conditions could be magnified in the CDK population, including osteoporosis fragility, cognitive impairment and so on [8].

Osteoporosis is defined as a condition of reduced bone strength leading to an increase risk of fracture [9]. Since bone quality cannot be measured directly in clinical practice, bone mineral density (BMD) is often used to diagnose osteoporosis and predict future osteoporotic fractures [10]. Osteoporosis could be defined by a T-score, the number of standard deviations (SDs) a person's BMD is below the mean BMD for the young normal health population, and the cut-point for the diagnosis of osteoporosis was set by the World Health Organization (WHO) at a T-score of -2.5 or lower [11]. Osteoporosis is considered as another serious public health issue due to its

prevalence worldwide, especially in many Western countries [12]. Currently it is estimated that over 200 million people worldwide suffer from osteoporosis [12]. Approximately 30% of all postmenopausal women have osteoporosis in the United States and in Europe. At least 40% of these women [13] and 15-30% of men [14] will sustain one or more fragility fractures in their remaining lifetime [15].

CKD and osteoporosis are two great public health concerns that may share multiple risk factors. CKD can lead to disturbed mineral homeostasis, including increasing or decreasing the circulating levels of calcium, phosphorus, vitamin D and parathyroid hormone [16]. And this effect on mineral homeostasis may be associated with increase bone fragility [17].

Several observational epidemiologic studies have reported that reduced kidney function is associated with increased risk of osteoporotic fractures among older populations [18][19][20][21]. The strong associations between eGFR and BMD suggest that shared physiological and genetic factors may underlie these conditions. Understanding whether the associations reflect a causal relationship or mere correlation may inform whether targeting renal treatment could reduce risk for lower BMD, or vice versa.

Genetic epidemiology can be used to evaluate the causality of risk factors with respect to potential health-related outcomes. Mendelian randomization (MR) is an instrumental variable (IV)-based analytical method that uses genetic variants, most commonly single nucleotide polymorphisms (SNPs), as IVs to infer causality in observational studies (see Figure 1) [22]. Due to the random assortment of gene variants during gametogenesis, MR method could overcome the major limitation of evidence from observational studies – unmeasured confounding [23][24].

However, MR relies on specific IV assumptions [25]:

1. The instrument must be reliably associated with the exposure.
2. The instrument must be independent of confounders.
3. The instrument must associate with the outcome only through the exposure.

Most MR studies have been focused on testing hypotheses that arose from associations between traits found in observational studies. Horizontal pleiotropy, the effect of a genetic variant on multiple biological pathways [25], is widely seen as a threat to the validity of MR studies because it violates the 3rd IV assumption. Several approaches have been developed to detect and correct for horizontal pleiotropy. MR-Egger regression [26] is one of such approaches, which is a weighted regression allowing one or more genetic variants to have pleiotropic effects, as long as the size of these pleiotropic effects is independent of the size of the genetic variants' effects on the exposure variable [26]. The slope of the MR-Egger regression is an estimate of the causal effect of the exposure on the outcome. The intercept in this regression is free to vary, and the degree to which it departs from zero could reflect the degree of pleiotropy present in the data [27].

There are a variety of MR approaches other than the standard MR design, including two-sample MR, bidirectional MR, two-step MR, and so on (see Figure 2). The standard MR (also called one-sample MR or single-sample MR) is conducted using genetic instruments, exposure and outcome of interest from individuals measured in the same sample (Figure 2a), while the two-sample MR is conducted based on data on the exposure and outcome measured in two different (or only partially overlapping) samples [28] (Figure 2b). Two-sample MR could be conducted based on publicly available genome-wide association study (GWAS) summary data. So, compared with standard MR, two-sample MR has many advantages especially when it is difficult and/or expensive to measure the exposure and outcome in the same set of individuals. In bidirectional MR, instruments for both exposure and outcome are used to evaluate whether the exposure causes the outcome or whether the outcome causes the exposure [29] (Figure 2c). This approach assumes that the causal association works through an underlying mechanism where it is possible to determine a single causal temporal direction. However, the complexity of biological systems, such as the feedback loops between the exposure and outcome variables, may lead to difficulty in

interpretation of results of such analyses [30]. Two-step MR could be used to assess whether an intermediate trait acts as a causal mediator between an exposure and an outcome [31]. As shown in Figure 2d, in the first step, genetic instruments for the exposure are used to estimate the causal effect of the exposure variable on the potential mediator, and in the second step, genetic instruments for the potential mediator are used to assess the causal effect of the mediator on the outcome. The evidence of association in both steps could help estimate the degree of mediation of association between the exposure and the outcome by the intermediate variable. Thus, the magnitude of both direct effect (the effect of exposure on the outcome independent of the mediator) and indirect effect (the effect of the exposure on the outcome via the mediator) can be estimated using this method [32]. However, additional assumptions are needed when using this approach. It requires linearity and homogeneity for both the exposure-mediator and exposure-outcome relationships and no statistical interaction between exposure and mediator [32].

To date, MR has been successfully applied to a wide range of observational associations, including applications to the causal effects of biomarkers on disease [33][34][35][36], understanding correlation between physiological measures [37][38] and estimating the causal effects of various behaviors [39][40]. Results of some MR studies supported previous observational findings. For example, by conducting MR analyses, Pichler et al. identified that higher serum iron levels lower the risk of Parkinson's disease [34] and Interleukin-6 Receptor Mendelian Randomization Analysis (IL6R MR) Consortium et al. validated that interleukin 6 (IL6) increases the risk of coronary heart disease [36]. At the meantime, results of some MR studies did not support previous observed associations. Palmer et al. concluded that the previously observed association between uric acid and coronary heart disease is in part due to confounding by BMI [35] and both studies conducted by Lewis et al. and Bjørngaard et al. showed that the anxiety and depression among smokers does not appear to be a consequence of smoking. [39][40]

Here, we conducted a bidirectional two-sample MR analysis to assess the potential causal association between eGFR and BMD in both directions. Two-sample inverse variance weighted MR (IVW-MR) as well as MR-Egger regression were performed with SNP-eGFR association from the CKDGen Consortium [41] and the SNP-BMD association from the UK Biobank [42].

Methods

We performed a two-sample Mendelian randomization analysis using GWAS data to test the hypothesis that the association between eGFR and BMD is causal.

Genetic variant instruments for eGFR

We retrieved GWAS summary statistics for eGFR from a published meta-analysis conducted by the Chronic Kidney Disease Genetics Consortium (CKDGen) (https://ckdgen.imbi.uni-freiburg.de/) [41].

The variable of interest, eGFR, was estimated from serum creatinine. Creatinine values obtained with a Jaffe assay before 2009 were calibrated by multiplying by 0.95 [43]. Studies on adults (>18 years of age) estimated GFR with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [44], by using the R package nephron [45]. Studies on individuals who were 18 years old or younger used the Schwartz formula [46]. eGFR was winsorized at 15 and 200 ml/min per 1.73m^2.

Variants were assigned to loci by selecting the SNP with the lowest P value across the genome as the index SNP, defining the corresponding locus as the 1 Mb segment centered on the index SNP, and repeating the procedure until no further genome-wide-significant SNPs remained. The extended major histocompatibility complex (MHC) region was considered as a single locus.

Among individuals having European ancestry, 225 independent SNPs were associated with eGFR at the genome-wide significance level ($p<5 \times 10^{-8}$).

Data on major and minor alleles for each SNP (after imputation and filtering, 8,834,748 variants in 567,460 individuals of European ancestry), along with allele frequencies, beta coefficients for allele dose and 1-unit change in log(eGFR), p-values and standard errors were extracted. Effect estimates were adjusted for age and sex.

Genetic variant instruments for BMD

The GWAS summary statistics for BMD was retrieved from a published GWAS based on UK Biobank data [42]. Heel bone mineral density (eBMD) was estimated based on an ultrasound measurement of the calcaneus by UK Biobank. The T-score is the number of standard deviations for bone mineral density relative to the mean. Individuals were excluded that exceeded the following thresholds for eBMD: males, ≤ 0.18 or ≥ 1.06 g/cm^2; females, ≤ 0.12 or ≥ 1.025 g/cm^2 [47].

The study assessed genetic associations between 20,259,828 imputed genetic variants and heel bone mineral density (eBMD) in 394,929 individuals of European ancestry. LMM-BOLT was used to perform a linear mixed model, controlling for sex, array batch, age, height, weight and the leading 10 genomic principal components as computed by the UK Biobank study. There were 1,362 independent SNPs that were associated with BMD ($p < 6.6 \times 10^{-9}$). The threshold of 6.6×10^{-9} was used for genome-wide significance to account for the large number of SNPs in the UK Biobank release, as established by Kemp et al. [47]. We extracted the beta coefficients of BMD T-score together with its standard errors, major and minor alleles, allele frequencies, and p-values.

Bidirectional Two-sample Mendelian Randomization

Two large samples used here do not overlap and both were individuals of European ancestry, which meets the two-sample MR assumption that both samples should come from the same population, but with no overlap.

Two-sample MR was conducted using the MendelianRandomization package in R [48] and using previously described methods and as summarized below [26]. The MR analyses were performed bidirectionally. In the first run, BMD T-score was considered as the outcome variable whereas log(eGFR) was considered as the exposure variable. In the second run, log(eGFR) was considered as the outcome variable and BMD T-score was considered as the exposure variable.

Wald ratios (β_{IV}) were calculated for each SNP by dividing the per-allele change in outcome variable (β_{ZY}) by the per-allele change in the exposure variable (β_{ZX}):

$$\beta_{IV} = \beta_{ZY}/\beta_{ZX}$$

95% confidence intervals (95% CI) were calculated from the standard error (SE) of each Wald ratio, which was derived from the SE of the variant-outcome association divided by the variant-exposure association. Individual Wald ratios and 95% CIs were compiled in a forest plot. Heterogeneity in Wald ratios was tested using Cochran's Q test. Funnel plots were plotted to display the MR estimate of individual genetic variants against their precision.

The effect of variant in BMD was reported as change in T-score of BMD per allele dosage [42]. CKDGen regressed sex- and age-adjusted residuals of the natural logarithm of eGFR on SNP dosage levels [41]. Thus, we present the number of standard deviation change in BMD per unit change in log(eGFR) and the change in log(eGFR) per unit change in standard deviation change in BMD.

Conventional linear regression analysis of the variant-exposure association and variant-outcome association for each instrument was undertaken and weighted by inverse variance, known as the inverse-variance-weighted (IVW) regression, to obtain the overall causal estimate.

To establish that the violation of the third assumption of IV analysis were not biasing the estimate of the causal association, MR-Egger regression was used to detect the horizontal pleiotropy. MR-Egger regression involves a weighted linear regression of $\hat{\beta}_{ZY}$ on $\hat{\beta}_{ZX}$ where the intercept is not constrained to pass through the origin [26]. The slope coefficient obtained from the MR-Egger

regression provides an estimate of the causal effect. And a non-zero intercept from the MR-Egger regression suggests that pleiotropic effects tend to be in the direction of the intercept term, which will bias IVW estimates.

Results

Causal association of eGFR on BMD

Table 1 shows the top 50 SNPs among the 225 SNPs associated with eGFR and selected as IVs for the analysis, with the effect alleles and frequencies, the effect size on log(eGFR) and strength of the association with BMD T-score.

The two-sample MR IVW analysis pooled results from individual SNPs, and showed no evidence of causal effect of eGFR on BMD (SD change in BMD per 1 unit increase in log(eGFR) = -0.0950; p=0.68; Figure 3). There was strong evidence of heterogeneity between variants (Q statistic = 5240.71, p=0.000; see Figure 4), suggesting that effect estimates are not consistent across these independent instruments.

The causal estimate obtained using MR-Egger regression was -0.4359 (p=0.458), with an intercept of 0.001 (p-value = 0.53; Figure 3). A funnel plot (Figure 5) showed that individual variants were symmetrically distributed around the point estimate. Together, these findings suggested no evidence of horizontal pleiotropy.

Causal association of BMD on eGFR

Among the 1,362 independent SNPs that were associated with BMD ($p < 6.6 \times 10^{-9}$) [42], 878 SNPs were also present in the summary statistic obtained from the eGFR GWAS meta-analysis. Table 2 shows the top 50 SNPs among the 878 SNPs associated with BMD T-score and selected as IVs for this analysis, with the effect alleles and frequencies, the effect size on BMD T-score and strength of the association with log(eGFR).

Using the IVW method for analyzing the causal effect BMD T-score on the log-transformed eGFR resulted in a statistically non-significant causal estimate of $\beta_{MR-IVW} = -2.99 \times 10^{-5}$ (p-value=0.98; Figure 6), suggesting that there was little evidence for a causal effect of BMD on eGFR. There was strong evidence of heterogeneity between variants (Q statistic = 3343.40, p=0.000; see Figure 7).

No evidence of horizontal pleiotropy at any of the IVs for BMD was found; the estimates of the intercept of MR-Egger regression was not significantly different from zero (0.000, p=0.054), and there was a lack of asymmetry of the funnel plots (Figure 8).

By utilizing the bidirectional two-sample MR design, we assessed whether eGFR had a causal effect on BMD and whether BMD had a causal effect on eGFR. Overall, we saw no evidence of a causal effect of eGFR on BMD as well as the inverse causal effect of BMD on eGFR among the European-ancestry individuals.

Discussion

We implemented a bidirectional two-sample MR approach to examine the potential causal relationship between eGFR and BMD. Although most observational studies suggested strong association between reduced kidney function and higher risk of osteoporotic fracture [18][19][20][21], our results didn't support a causal relationship between eGFR and BMD in either direction. To our knowledge, this is the first study to investigate the causal relationship between eGFR and BMD using the MR approach.

The mechanistic understanding of the association between eGFR and BMD is at the metabolic level, due to abnormalities in the parathyroid-calcium-phosphate axis as a result of reduced kidney function [49]. Cohort studies may be biased by confounders or reverse causality.

However, by using an IV approach, MR could be used to assess causal relationships between environmental exposures or intermediate phenotypes and disease outcome while minimizing or eliminating the possibility that reverse causation or confounding is responsible for the association. Though it is assumed that the instrument affects the disease outcome through and only through its effect on a specific phenotype/exposure, we tested this assumption by using MR-Egger regression and found no evidence of violation of this assumption.

There are some strengths of the study. First, multiple variants were used in this MR analysis to increase power and test assumptions [50]. Second, the sample size is large by using reported meta-analysis data by CKDGen Consortium and GWAS data based on UK Biobank, which also increases statistical power. Third, the two-sample MR design could help avoid the "winners' curse", which might underestimate true causal effects in one-sample MR and usual GWAS [50].Thus, the failure to detect the expected epidemiological association of eGFR and BMD by MR approach is therefore not due to lack of power.

The present study has several limitations. First, Bias could be generated from a weak genetic instrument in the direction of the null hypothesis [28]. Because all SNPs are genome-wide significantly associated with eGFR ($p<5 \times 10^{-8}$) and very large sample sizes were used here, the probability for weak instruments is low. Second, aggregate data was used in two-sample MR and therefore we are unable to test differences of effect in subgroups. A longitudinal study among European suggested that the association between decreased eGFR and femoral neck BMD is gender-specific [51]. Besides, UK Biobank is an older cohort of European ancestry, so the results may differ in younger populations or in other ethnic backgrounds. Using GWAS results of both sexes combined under a two-sample MR design, we cannot test whether effects of eGFR on BMD differ by sex. Third, some observational studies have shown increased risk of fracture among those with advanced CKD with lower eGFR compared to those with early CKD [18][19]. In this

MR analysis, eGFR was treated as a continuous variable, which may have reduced power if the causal effect is restricted to subjects with lower eGFR.

Overall, our bidirectional MR analyses didn't support the causal relationship between eGFR and BMD in either direction. Therefore, there is unlikely clinical benefit for osteoporosis by treating eGFR, or vice versa. However, MR analysis doesn't exclusively address the causal relationship by itself. Although many studies have investigated the association between kidney function and BMD, most are cross-sectional, ethnically diverse and few studies include older women [21]. Subsequently, there is a gap in knowledge regarding kidney function in the old and bone health. Further work would be needed to investigate such causal relationship among specific subgroups (e.g., women and elderly), or common risk factors underlying the observed association between eGFR and BMD.

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Tables and Figures

Table 1. Top 50 variants and effect alleles with frequencies and magnitude of effect on eGFR and strength of association with BMD.

Table 1. Variants and effect allels with frequencies and magnitude of effect on eGFR and strength of association with BMD.

SNP	chr	pos	EA	NEA	EA freq*	EA log(eGFR) beta	SE log(eGFR) beta	p-Value eGFR association	EA BMD (T-score) beta	SE BMD (T-score) beta	p-*Value* BMD association
rs1145077	15	45683795	T	G	0.38	-0.0086	0.0004	2.30E-132	0.0040	0.0022	2.80E-02
rs28817415	4	77401452	T	C	0.44	-0.0074	0.0003	9.70E-104	-0.0008	0.0021	9.30E-01
rs77924615	16	20392332	A	G	0.20	0.0096	0.0005	1.20E-99	-0.0059	0.0026	2.80E-02
rs10254101	7	151415536	T	C	0.28	-0.0068	0.0004	6.90E-66	-0.0024	0.0023	2.90E-01
rs3812036	5	176813404	T	C	0.26	-0.0069	0.0004	3.20E-64	-0.0011	0.0024	6.50E-01
rs1047891	2	211540507	A	C	0.31	-0.0065	0.0004	3.60E-64	-0.0040	0.0022	7.90E-02
rs9895661	17	59456589	T	C	0.82	0.0074	0.0005	1.40E-57	-0.0102	0.0028	1.20E-04
rs12207180	6	160633107	A	T	0.12	-0.0085	0.0005	1.20E-56	-0.0031	0.0031	1.80E-01
rs963837	11	30749090	T	C	0.55	-0.0055	0.0004	2.40E-54	0.0029	0.0021	9.20E-02
rs1362800	5	39378115	T	C	0.42	-0.0053	0.0003	8.50E-53	0.0249	0.0021	6.80E-35
rs881858	6	43806609	A	G	0.70	-0.0056	0.0004	1.10E-49	0.0017	0.0022	5.70E-01
rs4794813	17	37670994	A	T	0.25	0.0058	0.0004	1.20E-48	0.0079	0.0024	1.80E-03
rs6546869	2	73895765	A	G	0.22	0.0061	0.0004	1.70E-48	0.0102	0.0025	1.20E-04
rs8101667	19	33402419	T	C	0.33	0.0050	0.0004	2.20E-43	-0.0004	0.0022	9.00E-01
rs2039424	9	71432174	A	G	0.62	0.0048	0.0004	9.70E-41	0.0015	0.0022	5.70E-01
rs780093	2	27742603	T	C	0.39	0.0046	0.0004	4.50E-38	-0.0044	0.0021	7.90E-03
rs80282103	10	899071	A	T	0.92	0.0081	0.0006	2.60E-37	0.0073	0.0038	3.60E-02
rs62435145	7	1286567	T	G	0.69	-0.0055	0.0004	1.10E-36	0.0058	0.0023	1.10E-02
rs34861762	8	23748420	T	C	0.42	-0.0043	0.0003	2.60E-35	-0.0008	0.0021	9.20E-01
rs11062167	12	364739	A	G	0.53	-0.0042	0.0003	7.10E-34	0.0031	0.0021	2.20E-01
rs1397764	3	141750810	A	G	0.28	0.0047	0.0004	7.80E-34	-0.0020	0.0024	4.20E-01
rs4886755	15	76298132	A	G	0.50	0.0041	0.0003	2.50E-33	-0.0055	0.0021	8.50E-03
rs267738	1	150940625	T	G	0.79	-0.0050	0.0004	1.30E-32	0.0161	0.0025	2.30E-11
rs17216707	20	52732362	T	C	0.80	-0.0052	0.0005	3.70E-31	-0.0075	0.0027	2.70E-03
rs8096658	18	77156537	C	G	0.51	0.0046	0.0004	1.80E-29	0.0142	0.0021	5.50E-13
rs2440165	17	19428719	T	C	0.61	0.0041	0.0004	5.60E-29	0.0030	0.0021	3.50E-01
rs12736457	1	113258293	C	G	0.87	0.0056	0.0005	8.80E-27	-0.0078	0.0031	1.40E-02
rs113445505	19	38157969	T	C	0.37	0.0038	0.0004	2.00E-26	-0.0020	0.0021	2.30E-01
rs72759880	5	67750213	T	G	0.11	-0.0057	0.0005	3.30E-26	-0.0010	0.0034	5.00E-01
rs1548945	2	217665788	T	C	0.41	0.0037	0.0004	1.30E-24	-0.0009	0.0021	5.10E-01
rs79760705	5	53298716	T	G	0.11	0.0056	0.0006	2.60E-24	0.0047	0.0033	1.40E-01
rs9903801	17	58915261	C	G	0.15	0.0049	0.0005	1.00E-23	0.0570	0.0030	9.00E-88
rs10159261	1	15912987	T	G	0.31	-0.0038	0.0004	3.00E-23	0.0053	0.0022	3.60E-03
rs6973656	7	77422583	A	G	0.61	0.0035	0.0003	3.10E-23	-0.0004	0.0021	9.20E-01
rs112880707	22	40884662	T	C	0.11	0.0056	0.0006	6.70E-23	0.0083	0.0036	2.50E-02
rs2273684	20	33529766	T	G	0.54	0.0033	0.0003	2.60E-21	-0.0164	0.0021	3.60E-16
rs63934	11	2789062	A	G	0.82	0.0042	0.0004	2.60E-21	-0.0045	0.0027	4.90E-02
rs632887	12	3392351	A	G	0.59	0.0033	0.0004	1.10E-20	-0.0008	0.0021	3.20E-01
rs807624	2	15782471	T	G	0.34	0.0034	0.0004	1.50E-20	-0.0113	0.0021	5.70E-08
rs700753	7	46753684	C	G	0.34	0.0033	0.0004	7.50E-20	0.0032	0.0022	1.10E-01
rs2472297	15	75027880	T	C	0.26	0.0039	0.0004	8.20E-20	-0.0034	0.0024	2.00E-01
rs35472707	2	169995581	T	C	0.05	-0.0075	0.0008	9.50E-20	-0.0089	0.0048	1.30E-01
rs6492982	15	41399951	T	C	0.55	-0.0032	0.0004	5.80E-19	0.0010	0.0021	5.30E-01
rs187355703	2	176993583	C	G	0.97	0.0101	0.0011	9.50E-19	-0.0136	0.0066	3.10E-02
rs948493	11	65552154	T	C	0.36	-0.0032	0.0004	1.30E-18	0.0240	0.0022	9.70E-31
rs154656	16	89708003	A	T	0.44	-0.0031	0.0003	1.30E-18	-0.0016	0.0021	2.70E-01
rs11564722	11	2178330	T	C	0.24	0.0038	0.0004	1.40E-18	0.0031	0.0025	1.80E-01
rs11914389	3	38527215	T	C	0.43	0.0030	0.0003	2.10E-18	-0.0058	0.0021	5.20E-03
rs6087579	20	32985155	A	G	0.51	-0.0030	0.0003	2.50E-18	0.0184	0.0021	1.20E-19
rs3850625	1	201016296	A	G	0.12	0.0048	0.0006	3.60E-18	0.0034	0.0032	3.00E-01

*Frequency of effect allele in Wuttke et al. [41]

eGFR, estimated glomerular filtration rate; BMD, bone mineral density; Chr, chromosome; EA, effect allele; SE, standard error

Table 2. Top 50 Variants and effect alleles with frequencies and magnitude of effect on BMD and strength of association with eGFR.

SNP	chr	pos	EA	NEA	EA freq*	EA BMD (T-score) beta	SE BMD (T-score) beta	p-Value BMD association	EA log(eGFR) beta	SE log(eGFR) beta	p-*Value* eGFR association
rs11002954	10	54418743	T	G	0.87	0.1116	0.0031	1.00E-200	0.0003	0.0006	7.00E-01
rs115242848	2	119507607	T	C	0.99	-0.4213	0.0121	1.00E-200	-0.0008	0.0019	6.75E-01
rs11881367	19	33551428	A	G	0.91	-0.1127	0.0036	1.00E-200	0.0000	0.0006	9.79E-01
rs11898505	2	54684557	G	A	0.36	0.0787	0.0022	1.00E-200	-0.0012	0.0004	1.85E-03
rs1414660	1	240586695	T	C	0.81	-0.0904	0.0026	1.00E-200	0.0005	0.0005	2.96E-01
rs1871859	6	151898506	T	C	0.88	0.1059	0.0031	1.00E-200	-0.0006	0.0005	2.11E-01
rs1891002	6	151900047	A	T	0.71	0.0884	0.0023	1.00E-200	0.0000	0.0004	9.92E-01
rs2707518	7	120954908	T	G	0.61	-0.1798	0.0021	1.00E-200	0.0001	0.0004	8.78E-01
rs2982573	6	152010534	C	T	0.58	-0.0811	0.0021	1.00E-200	-0.0001	0.0003	7.62E-01
rs6978070	7	38152923	A	G	0.67	-0.0651	0.0022	1.00E-200	-0.0002	0.0004	5.92E-01
rs7070913	10	54420223	A	G	0.89	0.1385	0.0034	1.00E-200	0.0001	0.0006	9.12E-01
rs7121746	11	112437007	G	A	0.41	0.0640	0.0021	1.00E-200	0.0004	0.0004	3.02E-01
rs798914	7	120907715	G	C	0.43	-0.0688	0.0021	1.00E-200	0.0002	0.0004	5.45E-01
rs9482773	6	127459552	C	G	0.52	-0.0825	0.0021	1.00E-200	0.0003	0.0003	4.46E-01
rs9606139	22	19679303	A	G	0.89	0.1222	0.0034	1.00E-200	-0.0016	0.0007	1.36E-02
rs35107139	14	54419106	C	A	0.60	-0.0636	0.0022	9.30E-196	0.0018	0.0004	1.41E-06
rs10130587	14	54419110	C	G	0.60	-0.0634	0.0022	7.30E-194	0.0021	0.0005	7.49E-06
rs4895959	6	133575875	A	C	0.46	0.0588	0.0021	8.30E-190	-0.0011	0.0003	1.66E-03
rs144832051	2	119610406	T	C	0.98	-0.2027	0.0072	9.60E-187	-0.0002	0.0014	8.92E-01
rs4073566	2	119161638	A	C	0.23	0.0693	0.0025	2.60E-182	-0.0015	0.0004	3.96E-04
rs1159798	10	54412493	C	A	0.22	0.0701	0.0025	4.10E-182	-0.0007	0.0004	1.21E-01
rs4505759	4	1003022	T	C	0.69	-0.0618	0.0023	1.20E-179	-0.0004	0.0004	3.68E-01
rs138090420	7	121036166	A	T	0.96	-0.1592	0.0060	5.40E-164	0.0000	0.0011	9.78E-01
rs55849728	12	90533915	T	C	0.60	-0.0547	0.0021	6.70E-157	-0.0004	0.0004	3.17E-01
rs9594738	13	42952145	T	C	0.51	0.0519	0.0021	6.60E-145	-0.0002	0.0003	5.41E-01
rs4635400	18	13719510	A	G	0.64	0.0527	0.0022	2.80E-140	0.0001	0.0004	7.15E-01
rs28626308	19	33517515	T	C	0.96	-0.1261	0.0051	7.30E-140	-0.0026	0.0009	2.39E-03
rs7123749	11	27303855	G	A	0.37	0.0504	0.0022	1.90E-134	-0.0007	0.0004	5.32E-02
rs10931982	2	202832130	C	T	0.23	-0.0585	0.0025	1.80E-129	-0.0006	0.0005	2.47E-01
rs4144782	2	119601872	G	A	0.61	-0.0500	0.0021	7.40E-127	0.0003	0.0004	3.90E-01
rs6684375	1	22706434	T	C	0.82	-0.0631	0.0027	1.40E-126	0.0003	0.0005	5.02E-01
rs370387	3	41123984	A	G	0.45	-0.0476	0.0021	1.80E-126	-0.0003	0.0003	3.15E-01
rs2929308	8	9084121	A	T	0.49	0.0488	0.0021	2.00E-126	-0.0022	0.0004	1.95E-10
rs75230517	6	45106438	C	G	0.95	0.1077	0.0047	2.70E-125	-0.0022	0.0008	4.20E-03
rs10180713	2	54804693	G	A	0.27	-0.0528	0.0023	2.80E-121	-0.0020	0.0004	6.57E-07
rs4455680	6	151863505	A	G	0.78	-0.0590	0.0026	2.40E-119	0.0001	0.0005	8.78E-01
rs11840862	13	42956463	G	A	0.29	0.0514	0.0023	6.10E-118	0.0000	0.0004	9.93E-01
rs6905582	6	152000454	A	G	0.83	0.0619	0.0028	2.50E-116	0.0002	0.0005	6.12E-01
rs118115924	12	49379537	T	G	0.99	0.2116	0.0097	2.70E-111	-0.0036	0.0019	5.34E-02
rs117481343	12	13328208	T	C	0.97	-0.1371	0.0064	2.80E-110	0.0006	0.0011	5.81E-01
rs11887431	2	42267462	T	C	0.77	-0.0522	0.0024	5.00E-107	0.0005	0.0004	2.28E-01
rs2741856	17	41826839	C	G	0.92	-0.0794	0.0038	1.70E-104	0.0005	0.0006	3.98E-01
rs1890010	6	152085275	T	C	0.29	-0.0477	0.0023	1.10E-103	-0.0006	0.0004	1.13E-01
rs114124763	2	119012019	G	C	0.95	-0.1029	0.0049	4.90E-101	0.0010	0.0009	2.49E-01
rs947091	10	31054186	A	G	0.52	-0.0433	0.0021	1.30E-99	-0.0007	0.0003	3.23E-02
rs9898390	17	2177484	G	C	0.28	-0.0472	0.0023	3.50E-98	-0.0014	0.0004	1.12E-03
rs7217502	17	41801246	C	A	0.62	-0.0427	0.0022	1.60E-96	-0.0001	0.0004	7.17E-01
rs62007686	14	103899344	G	A	0.66	0.0436	0.0022	2.10E-91	0.0012	0.0004	5.53E-04
rs9982895	21	40343087	C	T	0.27	0.0466	0.0024	9.40E-91	0.0002	0.0004	6.25E-01
rs4655059	1	22735906	C	A	0.51	0.0416	0.0022	1.10E-88	0.0004	0.0003	2.15E-01

*Frequency of effect allele in Kim et al. [42]

eGFR, estimated glomerular filtration rate; BMD, bone mineral density; Chr, chromosome; EA, effect allele; SE, standard error

Figure 1. Directed acyclic graph of instrumental variable (IV) analysis using genetic variants as proxies for exposure of interest. Genetic variants (Z) associated with an exposure (X) such as eGFR can be used as proxies to determine the effect of the exposure (X) on the outcome (Y) such as BMD. The three assumptions: (1) the IV is robustly associated with the exposure; (2) the IV is not associated with confounding factors (C); and (3) there is no alternative way that the IV affects the outcome other than via the exposure.

* SNP, single nucleotide polymorphisms; eGFR, estimated glomerular filtration rate; BMD, bone mineral density; IV, instrumental variable.

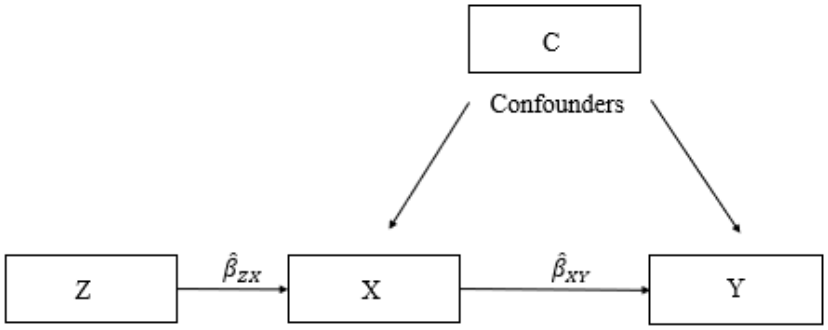

Figure 2. Different Mendelian randomization approaches. a. Standard MR. The causal relationship between the exposure (X) and the outcome (Y) is estimated by using genetic variants (Z) as an instrument. And the genetic instruments, exposure and outcome of interest are from individuals measured in the same sample. **b. Two-sample MR.** Two-sample MR is conducted based on data on exposure and outcome measured in two different samples. **c Bidirectional MR.** Instruments for both exposure and outcome are used to evaluate whether the exposure causes the outcome or whether the outcome variable causes the exposure variable. **d. Two-step MR.** In the first step, genetic instruments for the exposure are used to estimate the causal effect of the exposure variable on the potential mediator, and in the second step, genetic instruments for the potential mediator are used to assess the causal effect of the mediator on the outcome. The evidence of association in both steps could help estimate the degree of mediation of association between the exposure and the outcome by the intermediate variable.

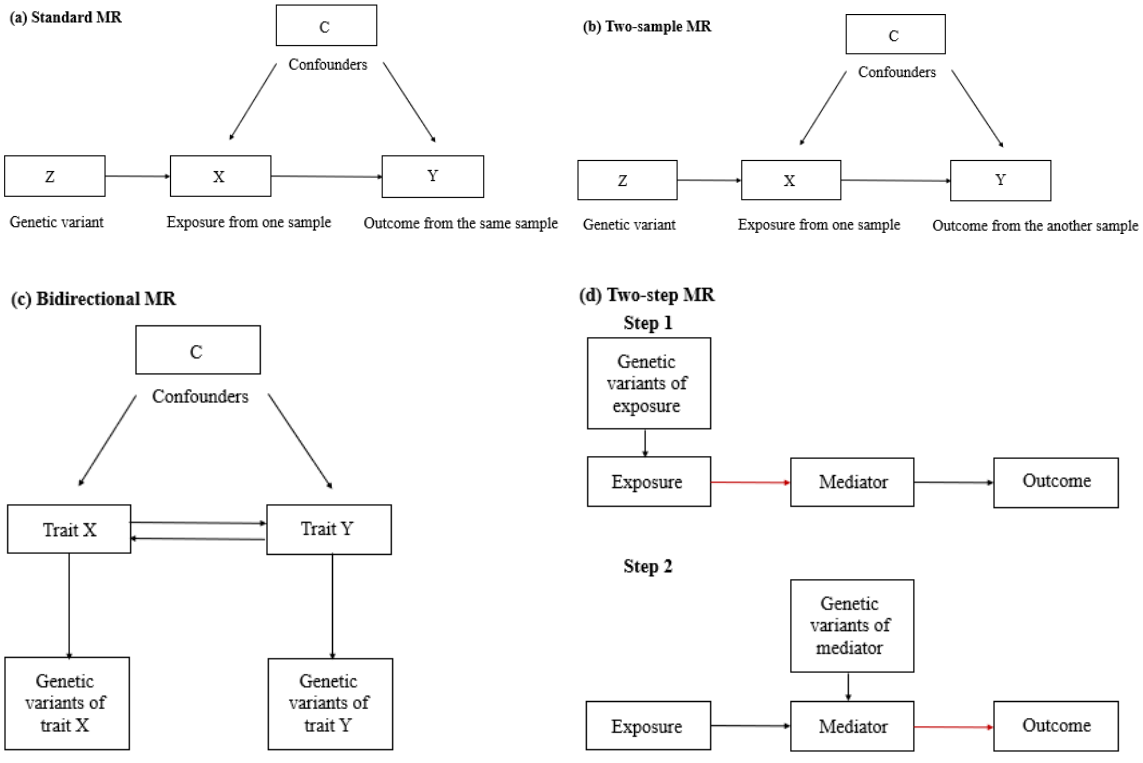

Figure 3. Scatterplot of the estimated effects of individual SNPs on log(eGFR) plotted against the estimated SNPs effects on BMD T-score using 225 SNP associated with eGFR. Effects of individual SNPs on log(eGFR) (x-axis), and BMD T-score (y-axis), as estimated by the respective genome-wide association studies. Error bars indicate 95% confidence intervals. The slopes of the lines are the estimated causal effects of log(eGFR) on BMD T-score, estimated using MR IVW method (light blue line) and MR-Egger method (dark blue line), respectively.

* SNP, single nucleotide polymorphisms; eGFR, estimated glomerular filtration rate; BMD, bone mineral density; SD, standard deviation.

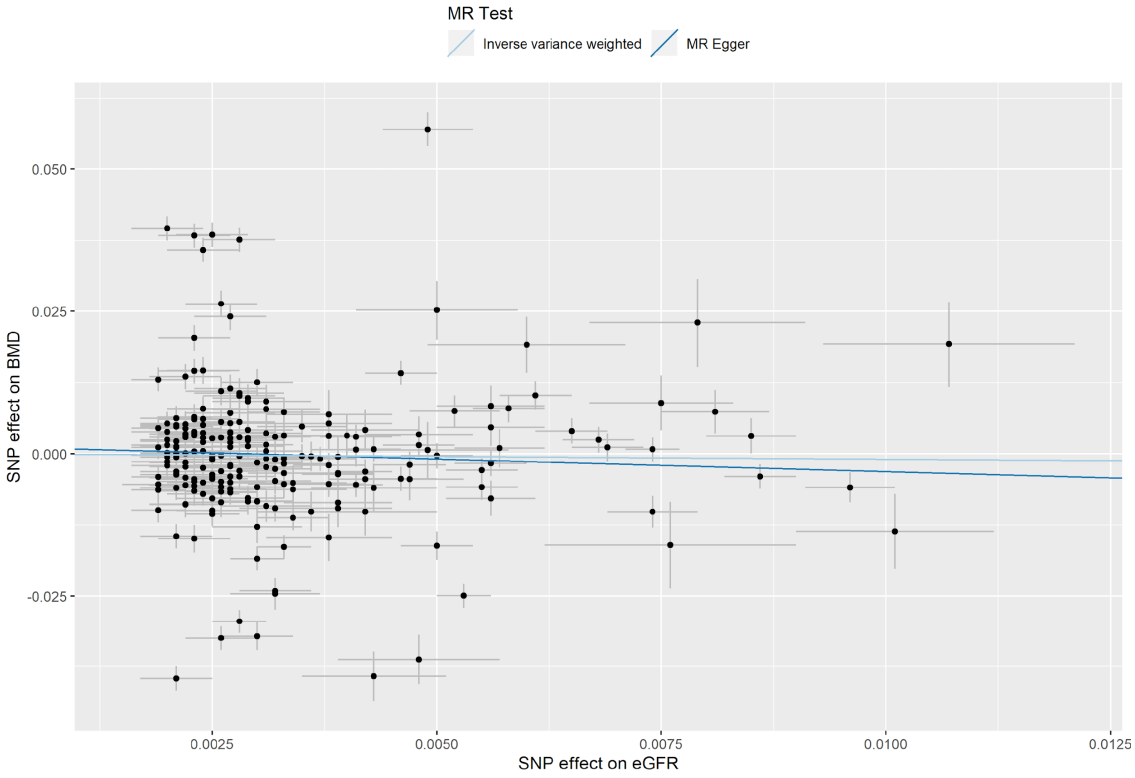

Figure 4. Forest plot of Wald ratio and 95% CIs generated from 225 SNPs (y-axis) associated with eGFR. Wald ratios for individual SNPs are listed according to magnitude of effect in the instrumental variable analysis and are presented with pooled effects using the IVW method and MR-Egger regression.

* IVW, inverse-variance weighted; MR, Mendelian randomization.

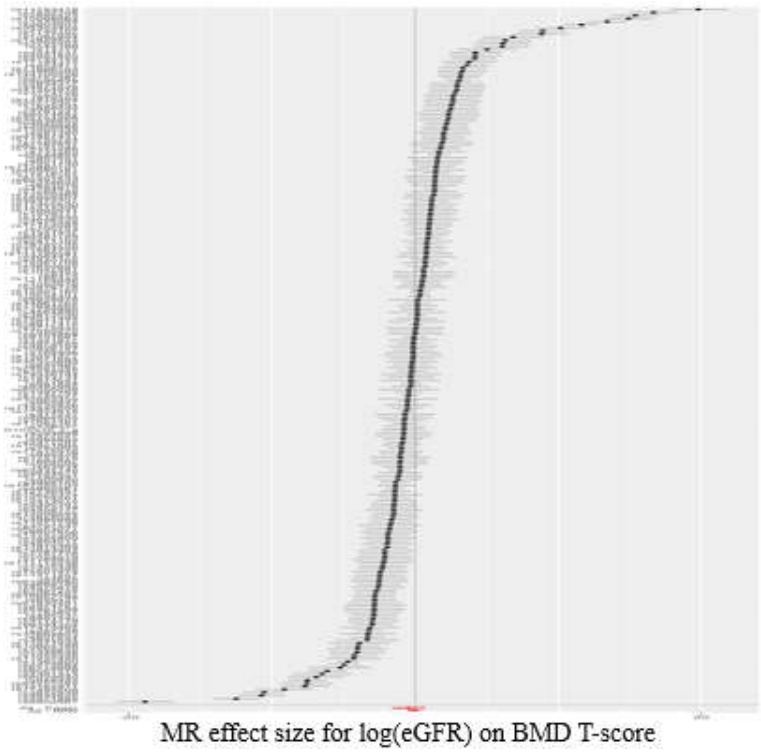

Figure 5. Funnel plot of instrument strength (y-axis) plotted against effect size of the causal estimates between log(eGFR) and BMD T-score (x-axis). Lack of asymmetry in the funnel plot suggests no overall horizontal pleiotropy on BMD. Vertical lines show the causal estimates using SNPs associated with eGFR combined into a single instrument for MR IVW (light blue) and MR-Egger (dark blue) regression analysis.

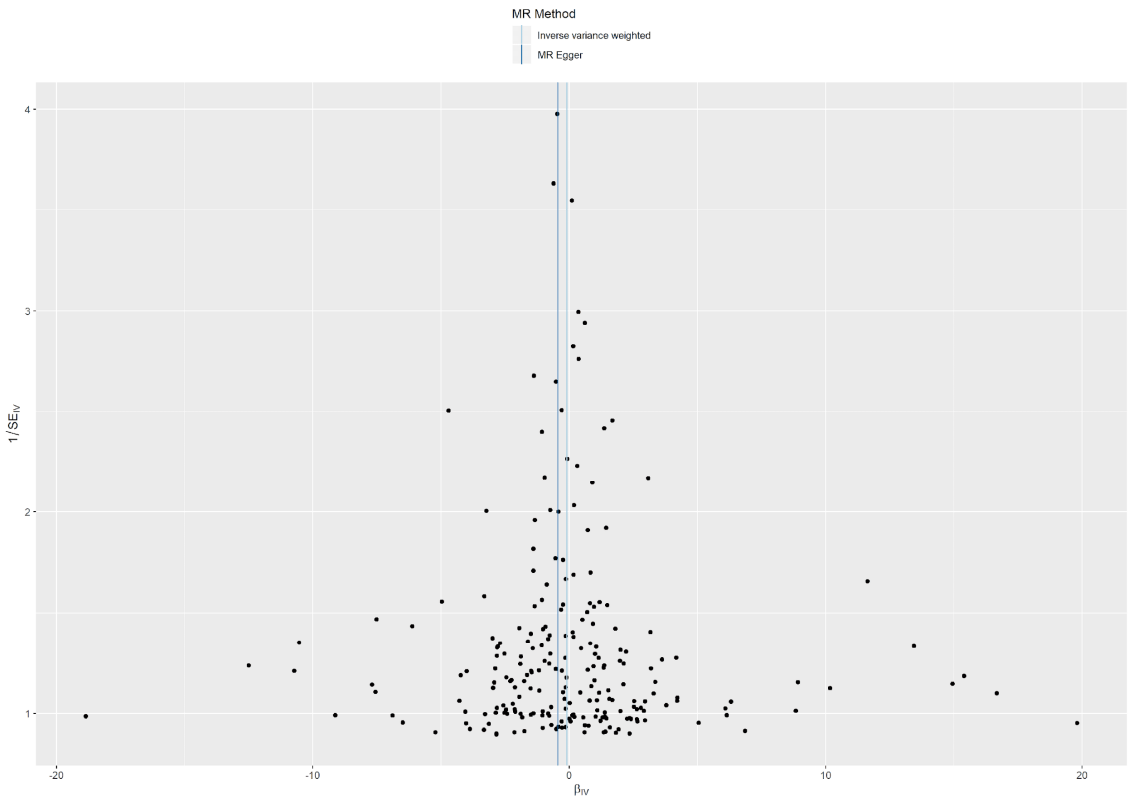

Figure 6. Scatterplot of the estimated effects of individual SNPs on BMD T-score plotted against the estimated SNPs effects on log(eGFR) using 878 SNP associated with BMD.

Effects of individual SNPs on BMD T-score (x-axis), and log(eGFR) (y-axis), as estimated by the respective genome-wide association studies. Error bars indicate 95% confidence intervals. The slopes of the lines are the estimated causal effects of BMD T-score on log(eGFR), estimated using MR IVW method (light blue line) and MR-Egger method (dark blue line), respectively.

* SNP, single nucleotide polymorphisms; BMD, bone mineral density; eGFR, estimated glomerular filtration rate; SD, standard deviation.

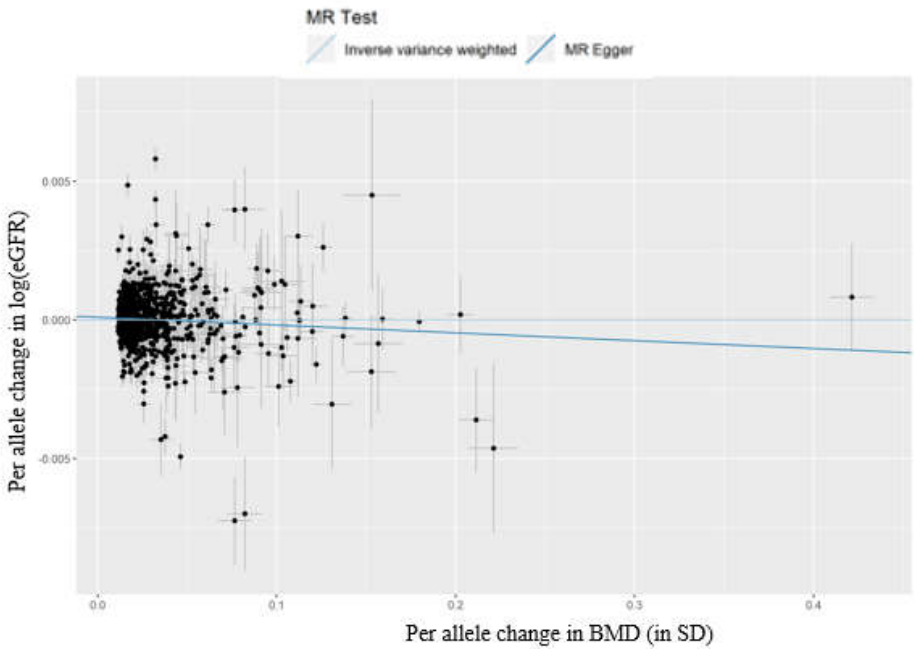

Figure 7. Forest plot of Wald ratio and 95% CIs generated from 878 SNPs associated with BMD. Wald ratios for individual SNPs are listed according to magnitude of effect in the instrumental variable analysis and are presented with pooled effects using the IVW method and MR-Egger regression.

* IVW, inverse-variance weighted; MR, Mendelian randomization.

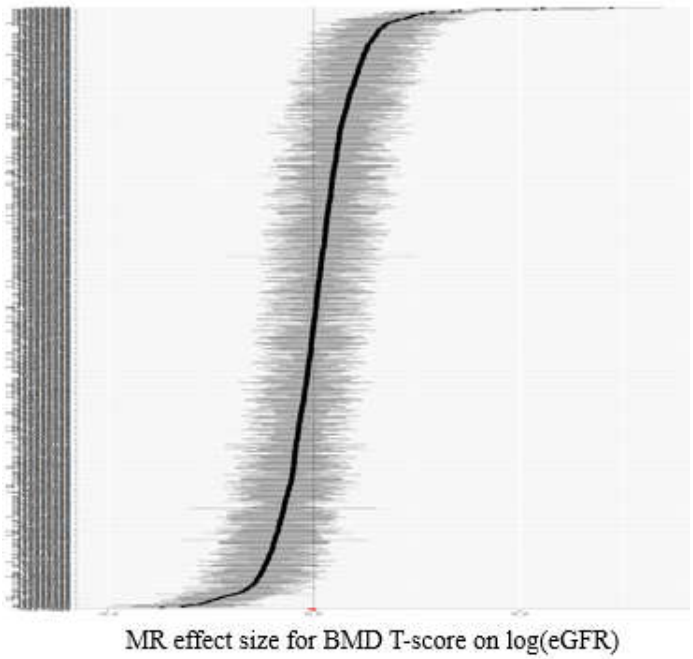

Figure 7. Funnel plot of instrument strength (y-axis) plotted against effect size of the causal estimates between BMD T-score and log(eGFR) (x-axis). Lack of asymmetry in the funnel plot suggests no overall horizontal pleiotropy on eGFR. Vertical lines show the causal estimates using SNPs associated with BMD combined into a single instrument for MR-IVW (light blue) and MR-Egger (dark blue) regression analysis.