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Original Article

Association between Chronic Hepatitis B and Osteoporosis: a two-sample mendelian randomization study

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Abstract

Introduction: Evidence suggests that metabolic abnormalities caused by chronic hepatitis B (CHB) are associated with osteoporosis (OP). However, whether there are causal relationships between CHB and OP remains undetermined. The present study assessed the causal effect of CHB on OP via two-sample Mendelian randomization (MR).

Methodology: We performed a two-sample MR study using summary-level data from genome-wide association studies (GWASs) of the CHB and OP patients derived from the BioBank Japan Project (BBJ). The single-nucleotide polymorphisms (SNPs) associated with CHB were selected as instrumental variables (IVs). The inverse variance-weighted (IVW) method was used as the primary statistical method to identify the consequence between CHB and OP. We additionally applied the other methods (weighted median method, simple mode method, and weighted mode method) to examine the consistency of the results. Cohran's Q test and Pleiotropy test were used to determine the horizontal pleiotropy and heterogeneities of these IVs on OP. The leave-one-out sensitivity test was used to evaluate the effect of a single IV on the ME results.

Results: Inverse-variance weighted analyses suggested that CHB was significantly associated with OP (OR = 1.011, 95% CI = 1.011 - 1.063, $p = 0.005$) without pleiotropy. The results of the heterogeneity test and the pleiotropy test revealed free heterogeneity and no pleiotropy in our IVW analysis ($p > 0.05$). Similar associations were detected with the weighted median and weighted mode methods (OR = 1.042, 95% CI = 1.007-1.079, $p = 0.017$; OR = 1.011, 95% CI = 1.011-1.063, $p = 0.032$).

Conclusions: This MR study revealed the causal effect of chronic hepatitis B on osteoporosis in East Asians.

Key words: chronic hepatitis B; osteoporosis; Mendelian randomization; hepatic osteodystrophy.

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Introduction

Hepatitis B (HBV) infection is a severe public health problem, especially in East Asia. In 2015, 1.34 million patients died of viral hepatitis (HBV accounted for 66%) [1]. The prevalence of chronic hepatitis B (CHB) is approximately 3.5% worldwide, and more than 200 million people peoples are positive for hepatitis B surface antigen (HBsAg), which the WHO reported in 2019 [2]. The HBV genome is classified into ten genotypes according to the genetic differences (A to J). Genotypes B and C account for most CHBs in Asian countries [3]. China has the highest infection rate of HBV in the world. The WHO indicated that approximately 10 million patients with CHB will die by 2030 [4]. Thus, more attention should be given to the CHB epidemic in China [5].

Osteoporosis (OP) is a systemic skeletal disease characterized by low bone mass and increased fracture risk [6]. In most countries, periodic bone mineral density (BMD) measurements are the current management approach to prevent the occurrence of fragile fractures in high-risk people (e.g. old adults or menopausal female) [7]. Although approximately 70% of chronic liver diseases can eventually lead to osteoporosis and a high fracture risk, few studies have reported on the liver bone axis [8]. Hepatic osteodystrophy (HOD) is a metabolic bone disease caused by chronic liver disease (CLD) and is usually characterized by decreased BMD and deterioration of bone structure. Numerous studies have indicated that the molecular pathways of bone loss differ between types of CLD [9]. In CHB patients, the serum level of

TNF-α, which is correlated with the severity of liver disease, is inversely correlated with disease severity [10]. However, no study has confirmed that HBV infection directly contributes to OP. Metabolic abnormalities caused by CLD could confound the relationship between CHB and OP.

Mendelian randomization (MR) is an impactful method that uses genetic variants as instrumental variables (IVs) to investigate the potential causal inference between the exposure and outcome. In observational epidemiological studies, MR is widely applied to overcome confounding, reverse causation, and various biases [11]. In this study, we used the genome-wide association study (GWAS) summary data of CHB as IVs and performed a two-sample MR to test for an association between CHB and OP.

Methodology

This two-sample Mendelian randomization (MR) method uses publicly available genome-wide association study (GWAS) summary data to assess the association between chronic hepatitis B (CHB) and osteoporosis (OP). A brief description of this MR design is shown in Figure 1. The Declaration of Helsinki statement was described in the original publications of these cohorts.

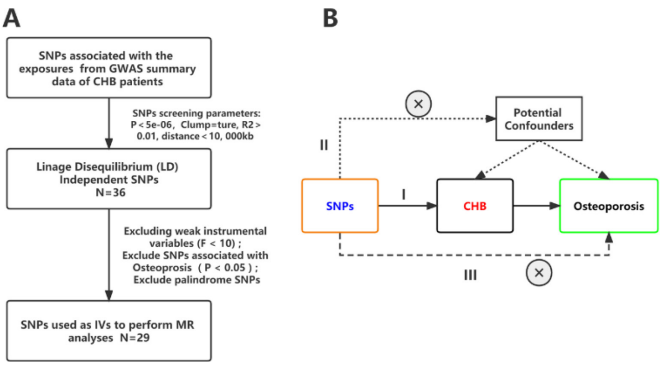
Data Sources for Exposures and Outcome

The GWAS summary statistics of this study were derived from the IEU platform (https://gwas.mrcieu.ac.uk/), derived from the BioBank Japan Project (BBJ). The gene-exposure data (CHB: bbj-a-99) included chronic hepatitis B, with 2234 cases, and 169588 controls. The gene-outcome data (OP: bbj-a-137) included 7,788 points, and 204,665 [12]. All the subjects included in the GWAS study were East Asian. The sequence of the SNP locus was 8,885,805. Detailed information is included in Table 1.

Selection of instrumental variables

The single-nucleotide polymorphisms (SNPs) associated with CHB were selected as instrumental variables (IVs). We used the threshold ($p < 5 \times 10^{-6}$) to choose the optimal IVs. To control linkage disequilibrium (LD) among the included IVs, the clumping process ($R^2 < 0.01$ and clumping distance = 10,000 kb) was conducted to screen the included SNPs. According to the above parameters, 36 SNPs were selected from the gene-exposure data. To avoid the weak IVs from being included in the MR analysis, the F statistics were calculated to assess the strength between IVs and CHB. All 36 SNPs with an F statistic > 10 were considered reliable IVs for CHB (Supplementary Table 1). The instrumental SNPs were all available in the gene outcome data. However, seven SNPs (rs75658393, rs9277947, rs2523490, rs62397979, rs154972, rs113258800, and rs4713602) with a $p < 0.05$ in the Gene-outcome data were excluded from IVs. Finally, we obtained 29 instrument SNPs to conduct the MR analysis. All SNPs were confirmed unrelated to confounding factors after being queried in the phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/). Detailed information on those IVs for CHB and osteoporosis is provided in (Supplementary Table 2).

Figure 1. The design of the two-sample MR analysis and the process of SNPs selection.



(A) The process of instrument selection from GWAS summary data of CHB patients; (B) the schematic diagram showing the design of the two-sample MR analysis.

Statistical analyses

Two-sample Mendelian randomization was performed via the Two-Sample MR packages in R software (v4.3.0). The inverse variance-weighted (IVW) method was used as the primary statistical method to identify the relationship between chronic hepatitis B (CHB) and osteoporosis (OP). We additionally applied the other methods (Weighted median method, Simple mode method, and Weighted

Table 1. The GWAS summary data is included in this study.

GWAS	Year	Population	Sex	Case	Control	Number of SNPs
Exposure						
Chronic hepatitis B	2019	East Asian	Males and Females	1,394	211,059	8,885,805
Outcome						
Osteoporosis	2019	East Asian	Males and Females	7,788	204,665	8,885,805

GWAS: genome-wide association study; SNP: single-nucleotide polymorphism.

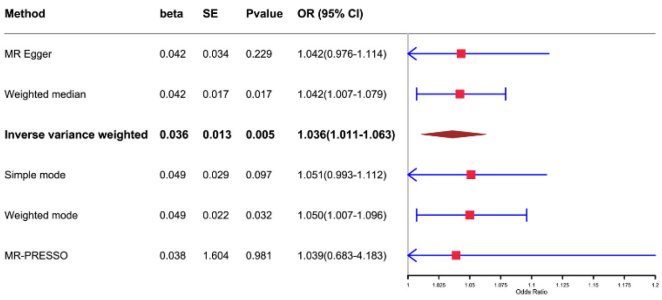
mode method) were used to examine the consistency of results. The weighted median method was applied to identify the weight of causality from more than half of the excellent IV. We additionally evaluated the sensitivity of this MR analysis through the following three aspects: the Heterogeneity test was used to assess the difference between each IV (Cohran's Q), the pleiotropy test was used to test horizontal pleiotropy between multiple IVs (MR-Egger intercept test and MR-PRESSO test analysed by MR-PRESSO packages in R software), and the Leave-one-out sensitivity test was used to evaluate the effect of a single IV on ME results. If the MR results estimated by the remaining IVs after removing a specific IV are very different from the total results, the MR results are sensitive to the IV. The formula of the F statistics is $F = \frac{(n-k-1)}{k}(\frac{R^2}{1-R^2})$, where N is the sample size of the CHB, K is the number of IVs associated with CHB, and R^2 is the proportion of the variability of the CHB explained by IVs. Finally, the statistical power of this two-sample MR analysis was estimated via an online tool (https://shiny.cnsgenomics.com/mRnd/).

Results

We investigated the causality of Chronic hepatitis B (CHB) to Osteoporosis (OP) by five models. The results of the two-sample MR analysis are shown in Figure 2. 28 LD-independent and appropriate IVs were enrolled from GWAS for CHB. The inverse variance-weighted (IVW) method indicated that CHB significantly affected the outcome of OP (OR = 1.011, 95% CI = 1.011-1.063, p = 0.005). Similar associations were conducted with the weighted median and weighted mode methods (OR = 1.042, 95% CI = 1.007-1.079, p = 0.017; OR = 1.011, 95% CI = 1.011-1.063, p = 0.032). The other MR methods did not yield significantly different results (Figure 3). The effect of each SNP on the outcome of the two-sample Mendelian randomization analysis is shown in Figure 4A.

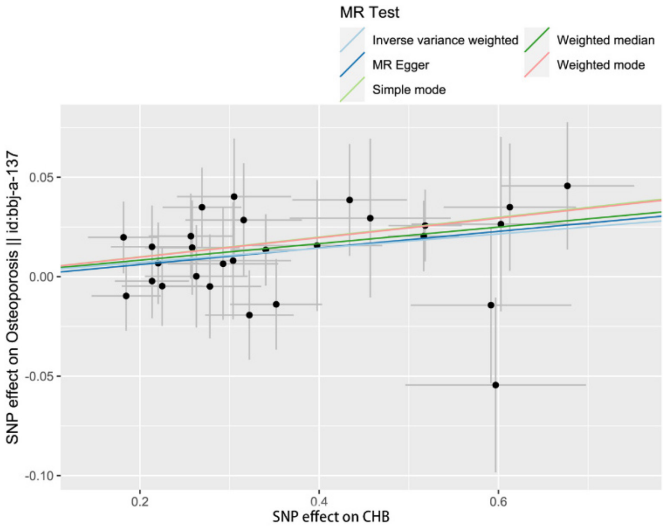
The F-statistics of the 28 LD-independent IVs were greater than 10 and without weak instrumental variable bias. Cochran's IVW Q test showed no significant heterogeneity in the independent IVs (Q = 14.631, p = 0.974). The funnel plot also showed no significant statistical bias in the study (Figure 5). The MR-PRESSO method and leave-one-out properties (Figure 4B) did not identify outliers. Furthermore, horizontal pleiotropy was not detected by the MR-Egger

Figure 2. Association of genetically predicted Chronic hepatitis B with risk of Osteoporosis.



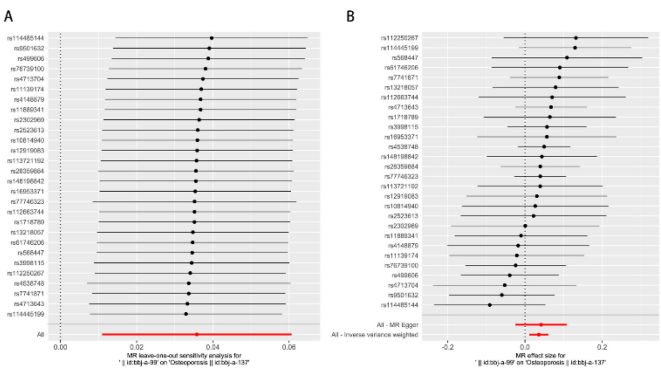
The inverse variance-weighted (IVW) method, MR-Egger regression, weighted median method, Simple mode method, and Weighted mode method are used for this MR analysis. The range of the abscissa of the forest plot is set as 1: 1.2.

Figure 3. Scatter plots for the relationship between Chronic hepatitis B and Osteoporosis.



Using inverse variance–weighted method (light blue), MR Egger (blue); simple median method (light green), weighted median method (green), and weighted method (orange).

Figure 4. Forest plot and Leave-one-out plot for each SNPs.



(A) The effect of each SNPs on the outcome of the two-sample Mendelian randomization analysis. (B) The Leave-one-out plot for the causal association between CHB and OP.

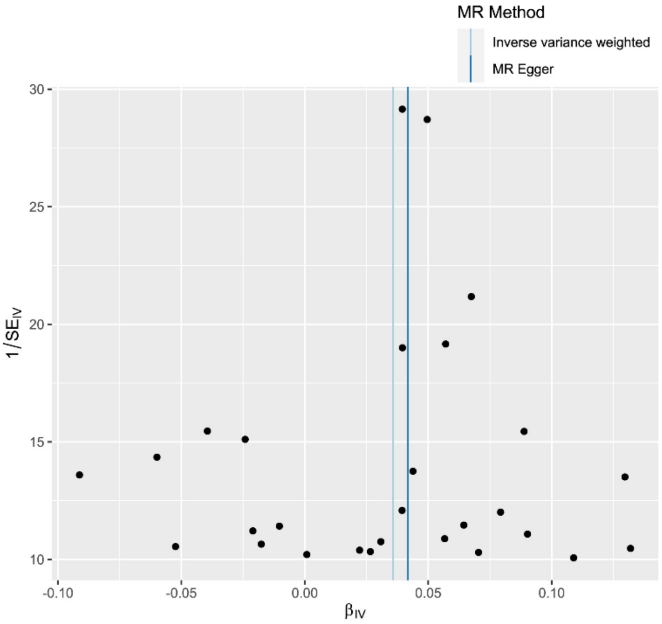
regression intercept (p = 0.851) and MR-PRESSO global test (p = 0.981).

Discussion

Although all CLD patients suffer from different degrees of osteoporosis, the mechanisms of altered bone metabolism differ between different types of CLD. We demonstrated a definite causal relationship between CHB and osteoporosis in the Asian population by a two-sample Mendelian randomization study. Previous studies have confirmed that 37.9% of patients with chronic viral hepatitis develop different degrees of osteoporosis [13,14]. Vitamin D is critical for bone mineralization and the absorption of calcium. Duarte MP's study finds that vitamin D does not contribute to high bone turnover in chronic viral hepatitis patients secondary to the hepatitis C virus [15]. Another study included 69 chronic hepatitis C (CHC) patients who underwent dual-energy X-ray absorptiometry. These results indicate that reduced BMD is associated with the severity of CHC [16]. Like CHC, chronic HBV infection is confirmed to increase the risk of developing osteoporosis, according to a case-control study [17]. However, the mechanism by which osteoporosis occurs during HBV infection remains unclear.

The altered bone metabolism induced by chronic liver disease (CLD) is hepatic osteodystrophy (HOD). Therefore, metabolic abnormalities caused by CLD are generally considered risk factors for HOD. A previous study has confirmed that a low body mass index (BMI) is inversely associated with BMD status [18]. A study from India that enrolled 72 patients (37 with alcoholism, 25 with hepatitis B, and 10 with hepatitis C) with cirrhosis indicated that reduced physical activity and low BMI are contributing factors to HOD [19]. Metabolic risk factors, such as BMI, hinder fibrosis regression in CHB patients [20]. Vitamin D3 is hydroxylated in the liver (the active metabolite $1,25(OH)_2$ vitamin D3 is formed in the kidney) and is critical for the homeostasis of bone metabolism. The $1,25(OH)_2$ vitamin D3 serum level plays an essential immune modulator in HBV patients. Necroinflammatory activity and fibrosis scores are associated with low vitamin D levels [21]. Another vitamin that plays a central role in synthesizing functionally active forms of several coagulation factors in the liver is vitamin K, which is required to form osteocalcin and osteonectin [22]. Serum insulin-like growth factor-1 (IGF-1) is reduced by the impaired somatotropic axis in CHB patients. The serum IGF-1 level is significantly decreased in children with end-stage liver disease, and the levels of BMD are entirely restored after transplantation [23]. Other metabolic indicators of CLD, such as iron, copper, and increased bilirubin, are also associated with a decreased BMD that IGF-1 regulates [24–26]. However, bone loss in CHB patients induced by metabolic abnormalities has not demonstrated a definite causal relationship between CHB and osteoporosis in Asia.

Figure 5. The funnel plot of appropriate IVs for this MR analysis.



Inverse variance–weighted method (light blue); MR Egger (blue).

Schieffer *et al.*'s study confirmed that viral hepatitis patients without cirrhosis already have significant bone loss. The BMD in the femoral neck (FN) and the lumbar spine (LS) was measured in 43 viral hepatitis patients (HCV n = 30 and HBV n = 13); 58% of patients had lower BMD, and 32% of patients were diagnosed with osteoporosis [27]. The transforming growth factor-β superfamily (TGF-β) is the most abundant cytokine in bone tissue. It can be activated as a chemotactic and growth factor for mesenchymal stem cells (MSCs) and bone progenitor cells [28]. TGF-β activity induces the hepatic stellate cells and enhances extracellular matrix (ECM) production. Therefore, TGF-β plays a crucial role in the fibrotic alterations associated with CHB. The persistent elevation of TGF-β in patients infected with HBV induces the expression of the O-glycosylated form of fibronectin, contributing to the development of osteoporosis [29]. The consistency of the serum TGF-β level and fibrosis progression after HBV infection is the probable mechanism by which HBV directly leads to OP.

Several GWASs in Asian populations have identified human leukocyte antigen (HLA) loci, such as

HLA-DP (rs9277535 and rs3077) and HLA-DQ (rs2856718 and rs7453920), that are associated with genetic susceptibility to CHB [30–32]. A previous study indicated that HLA-DQ is involved in the development of low BMD in nonceliac gluten sensitivity (NCGS) patients [33]. The protein levels of calcitriol, 25-dihydroxy vitamin D3, and IFN-gamma in human peripheral blood monocytes and U937 cells are correlated with the amount of mRNA specific for the HLA-DR alpha-chain [34]. A gene polymorphism may reflect the potential correlation between CHB and OP. However, further studies are needed to explore the solid evidence and detailed mechanism of the occurrence of osteoporosis induced by CHB.

This study has several limitations. First, only 29 instrument SNPs were included in the MR analysis. Larger scale GWAS data of CHB patients from East Asia should be used as exposure data for MR analysis. Second, this study focused mainly on East Asia, potentially reducing the generalizability of our findings. Third, we analysed the relationship between CHB and OP, and further analysis is needed on the relationship between early HBV infection and OP.

In conclusion, this MR study revealed causal relationships between chronic hepatitis B and osteoporosis, indicating that chronic hepatitis B may be directly involved in the pathogenesis of osteoporosis.

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Ethics approval and consent to participate

The data used in this study were obtained from a public database (the BioBank Japan Project). Ethics approval is not applicable to this study.

Availability of data and materials

The F-statistic for SNPs which are considered reliable IVs for CHB are available in Supplementary Table 1. Detailed information on those IVs for CHB and Osteoporosis are given in Supplementary Table 2.

Authors' contributions

Study concept and research elaboration: YYF, and DZX. Manuscript draft: DZX and WL. Data analysis: CMY and HYF. Manuscript revisions: ZYD, LY and XQF. Manuscript review: YYF. All authors gave consent to the publication of this study.

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Conflict of interests

No conflict of interests is declared.

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Annex – Supplementary items

Supplementary Table 1. The 36 single-nucleotide polymorphisms (SNPs) associated with CHB were selected as instrumental variables (IVs).

ID	se.exposure	beta.exposure	chr.exposure	pval.exposure	pos.exposure	SNP	effect_allele.exposure	other_allele.exposure	eaf.exposure
1	0.0413158	-0.213492	2	2.37E-07	191943742	rs11889341	T	C	0.306142
2	0.0898452	0.456981	4	3.65E-07	171148437	rs1718789	G	T	0.0499333
3	0.0384847	0.250841	6	7.13E-11	31178084	rs3130513	G	C	0.569239
4	0.057183	0.278046	6	1.16E-06	32819478	rs4148879	A	G	0.127178
5	0.0996843	-0.602753	6	1.48E-09	30690169	rs148198842	A	G	0.0450783
6	0.038588	-0.184621	6	1.71E-06	33836691	rs4713704	G	A	0.467687
7	0.0737906	0.612762	6	1.01E-16	33784833	rs3998115	C	G	0.917505
8	0.0638857	-0.433991	6	1.10E-11	33864354	rs7741871	A	G	0.108106
9	0.0614983	-0.458453	6	9.01E-14	32900651	rs154972	G	A	0.118573
10	0.0635089	-0.305051	6	1.56E-06	26175832	rs112250267	T	C	0.10544
11	0.0397601	0.516645	6	1.32E-38	33036401	rs77746323	T	C	0.598984
12	0.0614388	0.292817	6	1.88E-06	31320687	rs2523613	A	C	0.114118
13	0.0691837	-0.609692	6	1.22E-18	32965889	rs4713602	A	C	0.117758
14	0.0607406	0.540298	6	5.83E-19	31348779	rs2523490	T	A	0.874438
15	0.10073	-0.596902	6	3.11E-09	33376436	rs114485144	C	T	0.0420556
16	0.045763	-0.213435	6	3.10E-06	31286863	rs112663744	C	G	0.237144
17	0.0648016	0.315727	6	1.10E-06	32065113	rs61746206	T	C	0.0996474
18	0.0512678	0.352013	6	6.59E-12	32194308	rs499606	T	C	0.178778
19	0.0896192	-0.591708	6	4.04E-11	32171538	rs76739100	A	G	0.0546299
20	0.0726885	0.397639	6	4.49E-08	32682590	rs113721192	A	G	0.0940558
21	0.0567637	0.285867	6	4.75E-07	32395517	rs75658393	C	T	0.140572
22	0.0785411	-0.485219	6	6.50E-10	31483206	rs62397979	G	T	0.0714467
23	0.0861555	-0.757708	6	1.43E-18	32369605	rs113258800	G	A	0.0620008
24	0.0900457	-0.62967	6	2.69E-12	33197571	rs9277947	T	C	0.0585097
25	0.0745181	0.676957	6	1.04E-19	33594437	rs4713643	A	C	0.916226
26	0.0471514	0.256838	6	5.12E-08	26866144	rs13218057	G	T	0.680736
27	0.0493421	0.322148	6	6.63E-11	32420599	rs9501632	T	C	0.190956
28	0.0408114	0.518223	6	6.07E-37	32657505	rs4538748	C	T	0.648051
29	0.0394983	-0.340367	6	6.86E-18	32584330	rs28359884	A	C	0.500766
30	0.043984	0.269229	6	9.29E-10	32343715	rs114445199	A	G	0.258816
31	0.0396245	0.181669	9	4.54E-06	22021615	rs568447	A	G	0.398342
32	0.0646476	0.303951	9	2.58E-06	4328194	rs10814940	G	C	0.104695
33	0.0450695	0.224809	9	6.10E-07	83985424	rs11139174	G	A	0.240355
34	0.0451168	0.220315	16	1.04E-06	11188930	rs12919083	C	A	0.23608
35	0.0528411	0.258305	16	1.02E-06	54357670	rs16953371	T	C	0.162354
36	0.057438	-0.262897	19	4.72E-06	33113295	rs2302969	A	G	0.128256

Supplementary Table 2. The 29 instrument SNPs to conduct the MR analysis.

	se.exposure	beta.exposure	pval.exposure	pos.exposure	SNP	effect_allele.exposure	other_allele.exposure	eaf.exposure	R2	F
32	0.0646476	0.303951	2.58E-06	4328194	rs10814940	G	C	0.104695	0.453263717	39.82685916
33	0.0450695	0.224809	6.10E-07	83985424	rs11139174	G	A	0.240355	0.615528523	76.91075156
10	0.0635089	-0.305051	1.56E-06	26175832	rs112250267	T	C	0.10544	0.448075358	39.0008682
16	0.045763	-0.213435	3.10E-06	31286863	rs112663744	C	G	0.237144	0.619256811	78.13428607
20	0.0726885	0.397639	4.49E-08	32682590	rs113721192	A	G	0.0940558	0.463291503	41.46855127
30	0.043984	0.269229	9.29E-10	32343715	rs114445199	A	G	0.258816	0.631122229	82.19284071
15	0.10073	-0.596902	3.11E-09	33376436	rs114485144	C	T	0.0420556	0.303546038	20.93799797
1	0.0413158	-0.213492	2.37E-07	191943742	rs11889341	T	C	0.306142	0.656464551	91.80000826
34	0.0451168	0.220315	1.04E-06	11188930	rs12919083	C	A	0.23608	0.608621042	74.70548508
26	0.0471514	0.256838	5.12E-08	26866144	rs13218057	G	T	0.680736	0.766522415	157.7185096
5	0.0996843	-0.602753	1.48E-09	30690169	rs148198842	A	G	0.0450783	0.320969417	22.70791799
35	0.0528411	0.258305	1.02E-06	54357670	rs16953371	T	C	0.162354	0.537522886	55.83541031
2	0.0898452	0.456981	3.65E-07	171148437	rs1718789	G	T	0.0499333	0.318816539	22.48431942
36	0.057438	-0.262897	4.72E-06	33113295	rs2302969	A	G	0.128256	0.480360806	44.40876175
12	0.0614388	0.292817	1.88E-06	31320687	rs2523613	A	C	0.114118	0.464594808	41.68643649
29	0.0394983	-0.340367	6.86E-18	32584330	rs28359884	A	C	0.500766	0.738616476	135.7512327
3	0.0384847	0.250841	7.13E-11	31178084	rs3130513	G	C	0.569239	0.705863503	115.2855323
7	0.0737906	0.612762	1.01E-16	33784833	rs3998115	C	G	0.917505	0.417771401	34.47054662
4	0.057183	0.278046	1.16E-06	32819478	rs4148879	A	G	0.127178	0.474795079	43.42905922
28	0.0408114	0.518223	6.07E-37	32657505	rs4538748	C	T	0.648051	0.696260907	110.122058
25	0.0745181	0.676957	1.04E-19	33594437	rs4713643	A	C	0.916226	0.427833941	35.92163883
6	0.038588	-0.184621	1.71E-06	33836691	rs4713704	G	A	0.467687	0.718581842	122.6668239
18	0.0512678	0.352013	6.59E-12	32194308	rs499606	T	C	0.178778	0.563016249	61.89543787
31	0.0396245	0.181669	4.54E-06	22021615	rs568447	A	G	0.398342	0.710348043	117.814222
17	0.0648016	0.315727	1.10E-06	32065113	rs61746206	T	C	0.0996474	0.434876482	36.96796454
19	0.0896192	-0.591708	4.04E-11	32171538	rs76739100	A	G	0.0546299	0.34620627	25.43883251
8	0.0638857	-0.433991	1.10E-11	33864354	rs7741871	A	G	0.108106	0.460753132	41.04721196
11	0.0397601	0.516645	1.32E-38	33036401	rs77746323	T	C	0.598984	0.714374573	120.1523089
27	0.0493421	0.322148	6.63E-11	32420599	rs9501632	T	C	0.190956	0.570196671	63.73205185