Association of Serum 25-Hydroxyvitamin D with Metabolic Syndrome and Type 2 Diabetes: A Mendelian Randomization Study

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Abstract

Background: Vitamin D deficiency is common around the world, but the association between vitamin D deficiency with metabolic syndrome and its associated diseases is unclear.

Methods: A subset of 2393 participants from the Nantong Chronic Diseases Study (NCDS) of 2017-2018 were included in this study.The risk of MS and its associated diseases from low vitamin D levels were assessed by genetic scores using two 25(OH)D synthesis single nucleotide polymorphisms (SNPs) (DHCR7-rs12785878 and CYP2R1-rs10741657), one transport SNP (GC-rs2282679) and one catabolism SNP (CYP24A1-rs6013897).

Results: Odds Ratios (ORs) for decreased risk of MS and type 2 diabetes (T2D) was 0.73 and 0.79 in the deficient, 0.53 and 0.67 in the insufficient, and 0.54 and 0.60 in the sufficient categories of serum vitamin D levels, respectively. Mendelian randomization analysis showed per 25nmol/L higher genetically instrumented serum 25(OH)D concentration using the two synthesis SNPs: DHCR7+CYP2R1 genes, associated with a 7% lower risk of T2D. The highest tertile vs the lowest tertile of genetic scores using the three SNPs of DHCR7+CYP2R1+GC genes showed a 10% lower risk of T2D. Also, the group with higher genetic scores among these two and three SNPs were both associated with lower risk of abnormal diastolic blood pressure (DBP) (P=0.0162 and 0.0045 respectively).

Conclusions: Our Mendelian randomization analysis showed no genetic evidence for a causal role of lower vitamin D level in the development of MS, but showed a causal role in the development of T2D and DBP in middle-aged and elderly participants from rural China.

Background

Metabolic syndrome (MS) is a cluster of conditions, including abdominal obesity, hypertension, dyslipidemia, and hyperglycemia [1], contributing to increased risk of diabetes, heart disease and death [2]. It causes serious burden on public health and management is difficult [3]. China and many other Asian countries, have been experiencing a dramatic increase in MS and its associated disease incidence, especially in the middle-aged and elderly Chinese population [4-7]. The prevalence of MS, type 2 diabetes (T2D) and hypertension were about 18.4%, 8.5% and 36.6% respectively in the middle-aged Chinese population and 22.8%, 15.3% and 55.7% respectively in the elderly Chinese population during 2014–2015 [4, 6, 7]. The etiology of MS and its associated diseases is a complex interaction of multiple genetic and environmental factors, and the suggested heritability estimates range from 13–30% [8, 9].

Vitamin D deficiency is common in European, Indian, South American and Chinese populations, especially the middle-aged and elderly Chinese population[10, 11]. Vitamin D deficiency is associated with MS [10], hypertension [12], cardiovascular disease (CVD) [13], glucose homeostasis and type 2 diabetes (T2D) [14] as well as obesity and abdominal obesity [15]. Serum 25-hydroxyvitamin D [25(OH)D], a generally accepted clinical indicator of circulating vitamin D levels was found to be inversely associated with MS and T2D, among middle-aged and elderly individuals from China [10, 16]. However,

the rationale for low levels of vitamin D contributing to MS and its associated diseases are unclear. Studies of genetic variants that specifically affect 25(OH)D concentration can provide a causal association inference.

Advances in methodology of large-scale genetic association studies and international collaboration have identified four single nucleotide polymorphisms (SNPs) from four genes that influence 25(OH)D concentration, which represent circulating vitamin D levels [17, 18]. Genetic variants of synthesis genes *DHCR7/NADSYN1* (7-dehydrocholesterol reductase) and *CYP2R1* (25-hydroxylase) affects the synthesis of 25(OH)D, transport gene *GC* (group-specific component) encodes the vitamin D binding protein, and catabolism gene *CYP24A* (24-hydroxylase) is involved in the clearance of 25(OH)D [19].

We calculated genetic scores as an instrumental variable to estimate the causal effect of circulating vitamin D on MS and T2D by Mendelian randomization (MR). MR refers to the random allocation of alleles during meiosis [20]. The allocation is expected to be independent of behavioral and environmental factors allowing estimation of non-confounded risk associations that are not due to reverse causality [20, 21]. Mendelian randomization uses genetic variants as instrumental variables to estimate the causal effect of phenotypes, such as vitamin D status on MS or its outcomes, and is believed to overcome unmeasured confounding [21]. Likewise, the causal association of vitamin D with metabolic diseases remains unclear. Previous studies have not provided consistent results [22-28]. It has been reported that every 10% increase in genetically instrumented 25(OH)D was associated with decreased diastolic blood pressure (DBP) and 8.1% decreased risk of hypertension [25]. A 25-nmol/L higher genetically instrumented 25(OH)D concentration was associated with a 14% lower risk of T2D using two synthesis SNPs while no association was found between 25(OH)D and T2D using the four vitamin D related SNPs [24]. However, some other studies from China have reported of no association between genetically determined 25(OH)D with MS and its metabolic traits [23] and T2D [26]. Nevertheless, these studies were not specifically targeted at middle-aged and elderly population and few included genetic scores of SNPs. Thus, we aimed to evaluate the association between serum 25(OH)D concentrate and its genetic scores with MS and its associated diseases, like T2D, in the middle-aged and elderly participants from east rural China.

Methods

Participants and study design

In the present study 2,393 participants aged above 45 years were a subset of the 16,320 participants from Haian County among the 70,458 participants of the Nantong Chronic Diseases Study (NCDS); a cohort study of people (aged 18-90 years) living in Nantong China. Baseline recruitment for the NCDS was conducted between 2017–2018. The eligible residents of six communities in Haian were invited to participate and 12,533 people who had no prior history of cancer were enrolled in the study (response rate: 76.8%), and 2,393 middle-aged and elderly people were selected, with a response rate of 96.7%. Information on socio-demographic characteristics, lifestyle factors, personal medical history and the

family history of chronic diseases were collected by trained interviewers at the in-person interview. Participants were asked to provide a fasting blood sample. The study protocol was approved by the Institutional Review Boards of Nantong University and the Nantong Centers for Disease Control. All participants provided written informed consent.

Anthropometric and biochemical measurements

Anthropometric measurements of weight, height, waist and hip circumferences (WC and HC) were taken twice according to a standard protocol. If the difference between the first two measurements was greater than 1 cm for circumference or 1 kg for weight, a third measurement was taken. The average of the two closest measurements were applied in the present study. From these measurements, the waist-hip ratio (WHR) and the body mass index (BMI) were calculated, BMI was calculated as weight in kg divided by the square of height in meters.

A 10 ml blood sample was drawn into an EDTA vacutainer tube, stored in a portable Styrofoam box with ice packs (0–4 ^{o}C) and were processed within 6 hours. Serum 25(OH)D concentration was assayed by enzyme linked immunosorbent assay (ELISA).

We defined 25(OH)D < 25 nmol/L as severe deficiency, 25 to < 50 nmol/L as deficiency, 50 to < 75 nmol/L as insufficiency and ≥ 75 nmol/L as sufficient [29]. Furthermore, fasting blood glucose (FBG) and blood lipids (triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c)) were measured. Insulin level was measured by chemiluminescent immunoassay (CLIA). Homeostasis model assessment of insulin resistance (HOMR-IR) was calculated based on the formula: HOMR-IR= (FBG(mmol/L)×Insulin(μIU/mL))/22.5. Blood pressure comprising systolic blood pressure (SBP) and DBP were taken twice with the interval time of more than 3 minutes. If the difference between the first two measurements was larger than 10 mmHg, a third measurement was taken; the average of the two closest measurements was applied in this study. Other demographic information, such as education, income, lifestyles factors (such as physical activity, smoking and drinking status), personal medical history, family history of chronic diseases, and vitamin D and calcium supplements were collected using a standard questionnaire.

Diagnostic criteria for MS and T2D

MS was defined based on joint interim statement of the International Diabetes Federation criteria [30] by adopting the Asian criteria for WC as having ≥ 3 of the following metabolic abnormalities: Central obesity: WC ≥ 85 cm for Chinese men and ≥ 80 cm for Chinese women; abnormal fasting serum TG ≥ 1.7 mmol/L or taking TG lowering medication; abnormal fasting serum HDL-c < 1.3 mmol/L for Chinese women and < 1.0 mmol/L for Chinese men or under treatment to raise HDL-c levels; abnormal blood pressure (hypertension): SBP ≥ 130 mmHg, DBP ≥ 85 mmHg or on antihypertensive medication; abnormal fasting serum glucose (diabetes) ≥ 5.6 mmol/L or on anti-diabetic medication.

T2D was defined as FBG ≥ 7.0 mmol/L and/or 2 hours oral glucose tolerance test (2h-OGTT) ≥ 11.1 mmol/L and/or treatment with anti-diabetic medication and/or previously diagnosed diabetes by physicians [31].

SNPs selection and genotyping

Four vitamin D-related SNPs: two synthesis SNPs (*DHCR7/NADSYN1*-rs12785878 and *CYP2R1*-rs10741657), one transport SNP (*GC*-rs2282679) and one metabolism SNP (*CYP24A1*-rs6013897) were selected on the basis of a recent MR study of Asian population [32]. These SNPs were significantly associated with plasma 25(OH)D concentration in previous genome-wide studies [18] and also used in Mendelian analyses in studies from China [23, 24]. All four SNPs were on the Hardy-Weinberg equilibrium HWE (P > 0.05), and frequency of the alleles was > 0.05.

Genotyping was performed on the iPLEX™ Sequenom MassARRAY® platform. Polymerase chain reaction (PCR) and extension primers were designed by using the MassARRAY Assay Design 3.0 software (Sequenom, Inc). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. On each 96-well plate, two negative controls (water), two blinded duplicates, and two samples were included.

Genetic Scores

We assumed an additive genetic model for the SNPs with scores of 0, 1 or 2 for genotypes containing 0, 1 or 2 alleles, respectively based on the relationship between SNPs and circulating vitamin D levels. We calculated genetic scores for two synthesis SNPs (*DHCR7*-rs12785878*+CYP2R1*-rs10741657), three SNPs (*DHCR7*-rs12785878*+CYP2R1*-rs10741657+*GC*-rs2282679) and all four SNPs.

Statistical analysis

Normally distributed continuous variables were expressed as mean ± standard deviation (SD) and compared using ANOVA test, non-normally distributed continuous variables were expressed as median (interquartile range (IQR)) and analyzed using Wilcoxon rank sum test, and categorical variables were expressed as percentage and analyzed by Pearson chi-square test between diabetes cases and diabetes non-cases. Odds Ratios (ORs) and 95% confidence intervals (CI) were estimated using logistic regression models to analyze the association between serum 25(OH)D concentration and its determined genetic scores and MS and T2D adjusted for confounders. All analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC) and P < 0.05 was considered statistically significant and were based on two-sided probability.

Results

Among the 2393 participants, the prevalence of MS and T2D were 31.2% and 15.1%. Table 1 presents the differences in select demographic characteristics, anthropometric measurements and lifestyle factors

between MS/T2D cases and MS/T2D non-cases. MS and T2D cases were both older, had higher weight, WC, BMI, WHR, and income, more likely to be 'ever drinker' and having family history of MS/T2D, and less proportion of exercising compared with MS and T2D non-cases. Moreover, MS cases were more likely to be 'current smoker' than MS non-cases.

We found significant differences in demographic and clinical characteristics in the quintile groups of serum 25(OH)D concentration (Table 2). Compared with the lowest quintile of serum 25(OH)D, fasting glucose, insulin level, HOMA-IR, TG and WC decreased gradually, and reached the minimum, while HDL-c increased gradually and came to the maximum in the highest quintile of serum 25(OH)D.

Table 3 presents the association of serum 25(OH)D concentration with MS and T2D in our study population. We have found direct significant association between serum 25(OH)D with both MS and T2D. Compared with the lowest quintile of serum 25(OH)D (< 28.4 nmol/L), ORs for decreased risk of MS and T2D was 0.67 and 0.71 in the third quintile of serum 25(OH)D (36.8-45.9 nmol/L), 0.62 and 0.64 in the fourth quintile of 25(OH)D (46.0-57.4 nmol/L), and 0.46 and 0.53 in the highest quintile of serum 25(OH)D (≥ 57.5 nmol/L) among middle-aged and elderly Chinese participants. Similarly, compared to the severe deficient category of vitamin D (< 25 nmol/L), there was decreased prevalence of MS and T2D with ORs of 0.73 and 0.79 in the deficient category of vitamin D (25(OH)D: 25 to < 50 nmol/L), 0.53 and 0.67 in the insufficient category of vitamin D (25(OH)D: 50 to < 75 nmol/L) and, 0.54 and 0.60 in the sufficient category of vitamin D (25(OH)D: ≥75 nmol/L) respectively. Overall, every 25 nmol/L increase in serum 25(OH)D concentration was associated with 22% and 14% lower risk of MS and T2D respectively.

Mendelian randomization analysis showed no significant association between serum 25(OH)D determining genetic variants with MS risk (Table 4). However, we found per 25 nmol/L higher genetically instrumented serum 25(OH)D concentration using two synthesis SNPs (*DHCR7*-rs12785878+*CYP2R1*-rs10741657) to be associated with a 7% lower risk of T2D. But between tertiles of genetic scores, these two SNPs did not show any significant association for lower risk of developing T2D. However, the highest tertile of genetic scores using three SNPs (*DHCR7*-rs12785878+*CYP2R1*-rs10741657+ *GC*-rs2282679) was associated with a 10% lower risk of T2D, compared with the lowest tertile group. Furthermore, we did not find any association between genetic scores of all four SNPs with T2D. Similarly, we found that the higher group of genetic scores in the two synthesis SNPs in *DHCR7*+*CYP2R1* genes and three SNPs in genes *DHCR7*+*CYP2R1*+*GC* were both associated with lower risk of abnormal DBP (P = 0.0162 and 0.0045, respectively). Moreover, no associations were found between genetically instrumented 25(OH)D concentration with lower risk of T2D. Also, null results were shown between any single SNPs with MS and T2D in the middle-aged and elderly participants from east rural China (data not shown).

Discussion

Vitamin D levels were known to influence MS and associated diseases but the causal or resulting direction of the association were uncertain. This study revealed that there was no genetic evidence indicating the causal role of lower vitamin D level in the development of MS, but we found higher serum

25(OH)D concentrates play a genetic causal role in lowering the risk of T2D and abnormal DBP in the middle-aged and elderly rural participants from east rural China .

Many epidemiological studies have found inverse associations between serum 25(OH)D level with MS and its associated diseases [10, 33, 34]. Previous studies have reported a positive correlation between Vitamin D levels and HDL-c, whereas an inverse association with TG, SBP and DBP,[10] T2D,[34] BMI and WC [35]. We found higher serum 25(OH)D concentration to be significantly associated with lower glucose concentrations, insulin level, HOMA-IR, WC and higher HDL-c. Also, fully adjusted ORs (95% CI) for decreased risk of MS and T2D were 0.46 (0.32-0.63) and 0.51 (0.40–0.63) in the highest quintile of serum 25(OH)D, compared with the lowest quintile of serum concentration. It was consistent with Bea's [33] and Afzal's [36] studies which found that serum 25(OH)D in the highest quartile decreased the risk for MS with OR= 0.52 (0.36–0.75) compared with the lowest quartile of 25(OH)D, multivariable adjusted hazard ratios of T2D were 1.35 (1.09–1.66) for lowest vs highest quartile of serum 25(OH)D. However, a number of randomized controlled trials (RCTs) have shown no association between vitamin D level and the incidence of MS and its associated diseases including T2D in elderly people [37-39]. Furthermore, a cohort study reported that after a year of vitamin D supplementation, those who improved their serum 25(OH)D concentrations with < 25 nmol/L, 25 to < 50 nmol/L, 50 to < 75 nmol/L, and ≥ 75 nmol/L had respectively 0.76, 0.64, 0.59, 0.56 times the risk for MS at follow up.[40]

Mendelian randomization studies showed no evidence that genetically increased serum 25(OH)D is associated with lower risk of MS, T2D or hypertension.[23, 27, 41] We did not find any association between vitamin D associated SNPs and genetic scores with risk of MS, while we found that genetically instrumented 25 nmol/L higher serum 25(OH)D using two synthesis SNPs were associated with only a 7% lower risk of T2D. It is consistent with Lu et al's study that 9% and 14% lower risk of diabetes in Chinese participants and in a meta-analysis, respectively [24]. Furthermore, Yuan's study found genetic variants associated with low plasma concentrations were associated with T2D (P =0.0290) [28]. We further found the highest serum 25(OH)D tertile vs the lowest serum 25(OH)D tertile of genetic scores using three SNPs (two synthesis and one transport) was associated with a 10% lower risk of T2D.

A previous study reported modest association between the two SNPs genetic scores of plasma 25(OH)D concentrations with hypertension (P = 0.0003) [25], but another study demonstrated no effect on blood pressure in the Chinese population [24]. We found that higher group of genetic scores for two synthesis SNPs, two synthesis plus one transport SNPs were both associated with lower risk of abnormal DBP (P = 0.0162 and 0.0045 respectively). As we know, vitamin D 'synthesis' genes *DHCR7*/ *NADSYN1* and *CYP2R1*, 'transport' gene *CYP24A1* and 'metabolism' gene *GC,* contribute to variability in the circulating vitamin D levels [42, 43]. Interestingly, genetic scores combined four SNPs in these four vitamin D associated genes had no association with T2D and SBP/DBP. This possibility from our findings could have arisen by chance and should be verified with further MR studies.

Using a genetic variant as proxy for vitamin D levels is supposed to give better causal inferences for several reasons. Firstly, unlike vitamin D levels, genetic variants are generally not associated with the

behavioral, social and physiological factors that confound the association between vitamin D and MS and its associated diseases. Second, genetic variants associated with vitamin D levels will not be influenced by other diseases, and the estimates will therefore be less biased. Third, often a genetic variant will reflect exposures throughout the life course and do not change with disease status [44-46]. Finally, using multiple SNPs in different gene loci to index vitamin D level, we were able to minimize the risk of pleiotropic effects, as the effects of alternative pathways reflected by individual SNPs could be strongly diluted when combined in a multi marker score [47].

A limitation of this study was the single measurement of vitamin D levels. Although mendelian randomization is a potentially powerful technique for strengthening causal inference, several issues could disturb the instrumental variable assumptions: developmental changes compensating for genetic variation; linkage disequilibrium between genotype and other causal variables; pleiotropy which refers to a single gene having multiple biological function [48] and epigenetic effects i.e. non-Mendelian, heritable changes in gene expression not accompanied by changes in DNA sequence [21, 49]. Our analysis is based on the assumption that genotype only affects MS and its associated diseases through vitamin D levels.

Conclusions

Serum 25(OH)D concentration was inversely associated with MS and T2D risk in our rural elderly participants. However, Mendelian randomization analysis showed concordance with genetic studies of 25(OH)D developing alleles using two synthesis SNPs and risk of T2D and abnormal DBP in the middle-aged and elderly participants from east rural China indicating a protective effect of higher serum 25(OH)D concentrations on the risk of developing T2D and abnormal DBP. Conversely, genetically determined vitamin D was not significantly associated with MS and T2D, and lower vitamin D level is unlikely to have a causal role in the development of MS and T2D. Therefore, further trials of vitamin D supplementation are required before advocating use of vitamin D supplements or food fortification for the prevention of MS and T2D.

Abbreviations

MS: Metabolic syndrome; MR: Mendelian randomization; T2D: Type 2 diabetes; BMI: Body mass index; CVD: cardiovascular disease; CI: Confidence intervals; CLIA: Chemilumineseent immunoassay; DBP: Diastolic blood pressure; ELISA: Enzyme linked immunosorbent assay; FBG: Fasting blood glucose; GS: Genetic Scores; HDL-c: High density lipoprotein cholesterol; HOMR-IR: Homeostasis model assessment of insulin resistance; IQR: Interquartile range; NCDS: Nantong Chronic Diseases Study; NHGRI: National Human Genome Resource Institute; OGTT: Oral glucose tolerance test; ORs: Odds Ratios; PCR: Polymerase chain reaction; SBP: Systolic blood pressure; SNP: single nucleotide polymorphism; SD: standard deviation; TG: Triglyceride; WC: Waist circumference; WHR: Waist-hip ratio.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki. The study protocols were approved by the Institutional Review Boards of Nantong University and the Nantong Centers for Disease Control. All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

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Authors' contributions

JX, JYL, YJG and QYL conceived and designed the experiments. SYW, YZ, LWC, JYL, XYZ and XJW contributed to data collection. JX, YJG, QYL analysed the data. JX, JYL, SYW, YJG and QYL drafted the manuscript and approved the final version for submission. All authors read and approved the final manuscript.

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Tables

Table 1 Characteristics of study participants with MS/T2D cases and non-cases (n=2393)

	MS cases (n=746)	MS non-cases (n=1647)	P	T2D cases (n=361)	T2D non-cases (n=2032)	P
Age (year,)	61.24±6.41	56.68±6.19	<0.0001	60.30±6.54	57.71±6.41	0.0004
Weight (kg,) *	64.35±18.41	58.85±18.20	<0.0001	66.26±18.92	54.87±18.63	<0.0001
WC (cm,) *	87.76±10.32	78.75±10.16	<0.0001	89.86±11.26	76.10±10.97	<0.0001
BMI (kg/m^2,) *	26.66±4.02	22.47±3.87	<0.0001	27.62±4.12	21.38±4.06	<0.0001
WHR ()*	0.94±0.14	0.88±0.12	<0.0001	0.95±0.13	0.87±0.11	<0.0001
Education (%)*			0.2590			0.6440
Illiterate	0.00	0.62		0.00	0.47	
Primary school	2.75	3.15		2.40	3.11	
Middle school	36.32	38.00		36.92	37.57	
High school	41.11	39.98		40.77	40.26	
Colleges and above	19.02	18.72		19.91	18.59	
Income (yuan/month, %)*			0.0001			0.0397
<2000	16.28	20.42		16.52	19.62	
2000-	40.69	42.01		39.49	41.94	
3500-	30.35	30.15		31.31	30.01	
>=3500	12.67	7.42		12.69	8.43	
Smoking status (%)*			<0.0001			0.4876
Never-smokers	50.45	62.09		57.03	58.71	
Ever smokers	12.03	11.22		10.42	11.66	
Current smokers	37.52	26.70		32.55	29.63	
Drinking status (%)*			0.0003			0.0032
Never-	54.67	61.02		56.15	59.56	

drinkers						
Ever drinkers	5.78	2.91		6.96	3.23	
Current drinkers	39.55	36.07		36.90	37.21	
Physical activity (%)*			0.0003			0.0418
No	82.13	75.53		81.74	76.89	
Yes	17.87	24.47		18.26	23.11	
CHD (%)*			0.3523			0.7289
No	96.99	97.72		97.10	97.56	
Yes	3.01	2.28		2.90	2.44	
Familial history of MS/T2D (%)			<0.0001			0.0403
No	74.62	85.68		78.50	82.89	
Yes	25.38	14.31		21.50	17.11	
Vitamin D supplement (%)			0.8296			0.8487
No	97.21	97.00		97.14	97.07	
Yes	2.79	3.00		2.86	2.93	
Calcium supplement (%)			0.5847			0.7391
No	96.45	96.04		95.87	96.19	
Yes	3.55	3.96		4.13	3.81	
Abbreviations: WC waist circumference, BMI body mass index, WHR waist-hip ratio, CHD coronary heart disease, *adjusted for age at interview.						

Table 2 Comparison of clinical characteristics in different groups of serum 25(OH)D concentration

	Vitamin D Concentration (nmol/L)					P
	Q1(<28.4)	Q2(28.5-36.7)	Q3(36.8-45.9)	Q4(46.0-57.4)	Q5(>57.5)	
n	317	322	330	312	314	
Age (year)	59.8±7.0	59.6±7.0	60.0±6.9	59.4±7.2	58.8±7.1	0.0700
Female n(%)	194(61.2)	190(59.0)	201(60.9)	188(60.3)	186(59.2)	0.9732
FBG (mmol/L M(IQU))	5.80(5.26-6.35)	5.69(5.14-6.33)	5.68(5.12-6.31)	5.60(5.13-6.21)	5.49(5.05-5.95)	0.0020
Insulin (pmol/L M(IQU))	88.2(62.3-124.0)	88.0(61.1-121.5)	83.2(62.2-114.4)	80.8(59.5-106.9)	73.2(55.1-98.9)	0.0001
HOMA-IR	1.69(1.20-2.32)	1.68(1.19-2.28)	1.60(1.17-2.11)	1.50(1.12-2.00)	1.41(1.08-1.90)	0.0002
TG (mmol/L M(IQU))	1.24(0.93-1.90)	1.23(0.92-1.90)	1.20(0.89-1.87)	1.09(0.77-1.65)	0.97(0.69-1.43)	<0.0001
HDL-c (mmol/L M(IQU))	1.21(0.88-1.87)	1.24(0.92-1.90)	1.24(0.93-1.91)	1.28(0.99-1.99)	1.35(1.06-2.02)	0.0001
BMI (Kg/m^2)	25.1±4.0	24.9±4.0	25.2±3.9	24.6±3.8	24.4±4.0	0.0642
WC (cm)	86.3±11.1	85.8±10.5	84.9±10.8	81.4±11.0	75.3±10.4	<0.0001
Hypertension n(%)	179(56.5)	181(56.2)	185(56.1)	167(53.5)	158(50.3)	0.4753
Familial history of diabetes n(%)	46(14.5)	49(15.2)	48(14.5)	40(12.8)	39(12.4)	0.8175
Familial history of CHD n(%)	80(25.2)	80(24.8)	83(25.2)	78(25.0)	72(22.9)	0.9597

Abbreviations: BMI body mass index, CHD coronary heart disease, FBG fasting blood glucose, HDL-c high density lipoprotein cholesterol, HOMA-IR homeostasis model assessment of insulin resistance, TG triglyceride, WC waist circumference, : mean ± standard deviation; M(IQU): median (interquartile range).

Table 3 Association of T2D and MS with serum 25 (OH)D concentration

	MS cases	OR(95%CI) *	T2D cases	OR(95%CI) **
Every increasing 5nmol/L 25(OH)D	746(31.2)	0.90(0.88-0.93)	361(15.1)	0.93(0.91-0.95)
Every increasing 25nmol/L 25(OH)D		0.78(0.59-0.87)		0.86(0.65-0.92)
Quintiles of 25(OH)D (nmol/L)				
Q1(<28.4)	154(35.2)	1.0	87(18.3)	1.0
Q2(28.5-36.7)	167(34.0)	0.90(0.80-0.95)	82(17.1)	0.88(0.73-0.96)
Q3((36.8-45.9)	161(31.6)	0.67(0.53-0.82)	75(15.1)	0.71(0.58-0.84)
Q4(46.0-57.4)	129(28.4)	0.62(0.47-0.80)	64(13.8)	0.64(0.49-0.87)
Q5(≥57.5)	135(26.6)	0.46(0.32-0.63)	53(11.1)	0.53(0.42-0.60)
P for trend		0.0001		0.0009
Clinical categories of 25(OH)D (nmol/L)				
<25	139(35.9)	1.0	71(18.3)	1.0
25-50	390(31.9)	0.73(0.58-0.88)	188(15.4)	0.79(0.63-0.93)
50-75	196(27.9)	0.53(0.38-0.82)	92(13.1)	0.67(0.52-0.86)
≥75	21(26.1)	0.54(0.37-0.77)	10(12.4)	0.60(0.49-0.85)
P for trend		0.0001		0.0001

* Adjusted for Age at interview, BMI, WHR, Income, Smoking status, Drinking status, Physical activity and Familial history of MS; ** adjust for Age, BMI, WHR, Income, Drinking status, Physical activity and Familial history of diabetes.

Table 4 Association of T2D, MS, abnormal SBP and DBP with vitamin D-determined Genetic Scores

Genetic Scores	OR(95%CI) for MS	OR(95%CI) for T2D	OR(95%CI) for abnormal SBP	OR(95%CI) for abnormal DBP
DHCR7+CYP2R1				
<2	1.0	1.0	1.0	1.0
=2	1.05(0.77-1.42)	0.91(0.82-1.02)	1.06(0.79-1.24)	1.20(0.89-1.61)
≥3	0.90(0.68-1.20)	0.88(0.68-1.03)	0.92(0.72-1.13)	0.72(0.56-0.89)
P for trend	0.3663	0.0516	0.0651	0.0162
per 25nmol/L higher 25(OH)D concentration	0.86(0.69-1.08)	0.93(0.75-0.99)	0.95(0.69-1.17)	0.80(0.59-1.08)
DHCR7+CYP2R1+GC				
<3	1.0	1.0	1.0	1.0
=3	0.78(0.60-1.01)	1.35(0.85-1.04)	1.21(0.88-1.06)	0.72(0.58-0.89)
≥4	0.87(0.67-1.14)	0.90(0.82-0.98)	0.91(0.86-1.08)	1.16(0.91-1.48)
P for trend	0.2792	0.0515	0.0978	0.0045
per 25nmol/L higher 25(OH)D concentration	0.92(0.73-1.15)	0.94(0.85-1.01)	0.97(0.69-1.00)	0.84(0.66-1.07)
DHCR7+CYP2R1+GC+CYP24A1				
<3	1.0	1.0	1.0	1.0
3-5	1.12(0.85-1.46)	0.85(0.88-1.04)	0.89(0.68-0.94)	0.79(0.60-1.02)
≥5	0.82(0.61-1.09)	1.04(0.71-1.02)	1.16(0.91-1.48)	0.88(0.67-1.05)
P for trend	0.1347	0.0589	0.2193	0.0638
per 25nmol/L higher 25(OH)D concentration	0.99(0.78-1.26)	0.88(0.75-1.05)	0.95(0.76-1.00)	0.92(0.69-1.00)

Adjusted for age at interview, BMI, WHR, income, smoking status, drinking status, physical activity and familial history of diabetes.