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Relationship between tobacco smoking and metabolic syndrome: a Mendelian randomization analysis

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Abstract

Background Numerous epidemiologic observational studies have demonstrated that smokers have an increased risk of developing cardiovascular-related diseases. However, less is known about the causal relationship between tobacco smoking and the metabolic syndrome. This study aimed to determine whether genetically predicted smoking is associated with metabolic syndrome using the Mendelian randomization (MR) approach.

Methods This paper used individual-level genetic and personal data from the Taiwan Biobank dataset, including 80,072 Han Chinese individuals (15,773 cases of metabolic and 64,299 controls; 21,399 smokers and 58,673 non-smokers). The literature was searched for smoking-associated single nucleotide polymorphisms (SNPs), and 14 SNPs satisfying MR assumptions were identified and used as instrumental variables. Weighted and unweighted genetic risk scores (GRSs) based on these significant SNPs were derived. MR analyses were performed using the two-stage approach of regression models.

Results Genetically predicted smoking is associated with a higher risk of metabolic syndrome (odds ratio [OR]: 1.49, 95% CI: 1.47–1.52 per 1 standard deviation increase) for weighted and unweighted GRSs. When Q1 was used as the reference group, the adjusted ORs of metabolic syndrome for Q2, Q3, and Q4 were 1.15 (1.08, 1.22), 2.17 (2.05, 2.30), and 4.23 (3.98, 4.49), respectively, for the weighted GRS. The corresponding ORs for Q2, Q3, and Q4 were 1.16 (1.09, 1.24), 2.17 (2.05, 2.30), and 4.26 (4.02, 4.53), respectively, for the unweighted GRS.

Conclusions Genetic predisposition toward tobacco smoking is strongly associated with a higher likelihood of metabolic syndrome. Further work is warranted to clarify the underlying mechanism of smoking in the development of metabolic syndrome.

Keywords Metabolic syndrome, Mendelian randomization, Smoking, Single nucleotide polymorphism

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Background

The prevalence and incidence of metabolic syndrome, which is considered a great public health problem, are rapidly growing worldwide in recent years. The prevalence of the metabolic syndrome is 13.6%–25.5% in the Taiwanese population, 11.9%–37.1% in the Asian population, 11.6%–26.3% in the European population, and 20%–25% in the world's population [1, 2]. Metabolic syndrome causes inflammatory response and endocrine and neurobiological pathology related with increased risks of diseases, such as type 2 diabetes, cardiovascular disease, kidney disease, atherosclerosis, cancer, and premature death [3, 4]. Furthermore, the high morbidity and mortality of these diseases result in increased burden on caregivers and healthcare systems.

Metabolic syndrome has been considered a multifactorial disorder and significantly associated with lifestyle factors, including tobacco smoking, diet, alcohol intake, physical inactivity, and poor sleep hygiene [5, 6]. Epidemiologic study has shown that tobacco smokers had a two or more times greater risk of metabolic syndrome, hypertriglyceridemia, and low high-density lipoprotein cholesterol (HDL-C) than nontobacco smokers [7, 8]. Given the observational nature of prior studies, associations between smoking and metabolic syndrome may be biased by unknown or residual confounding and reverse causation. The current evidence for the causal role of smoking on metabolic syndrome lacks experimental evidence for causality. Therefore, the causal effect of smoking on metabolic syndrome needs to be established, which will be crucial for the prevention and treatment of these diseases.

Mendelian randomization (MR) analysis has been proposed as an alternative statistical method when randomized controlled trials are not feasible to prevent the bias arising from potential unknown or residual confounding and reverse causality [9]. MR analysis uses exposure-related genetic variants not influenced by the onset of disease or confounding factors as instrumental variables to explore the potential causal association between exposure and disease [10]. Prior MR studies investigated the effects of smoking on ischemic stroke [11, 12], type 2 diabetes [13], heart failure [14], and blood pressure [15], not on metabolic syndrome. In addition, these MR studies regarding smoking were conducted in Western populations. The causal association between smoking and metabolic syndrome has not been examined yet. Therefore, this paper explored the potential causal associations between smoking and metabolic syndrome by adopting an MR method using genetic variants selected from candidate gene and genome-wide association study (GWAS) approach. Potential causality was tested by exploring whether the genetic predisposition toward tobacco smoking is associated with increased likelihood of metabolic syndrome.

Methods

Study subjects and data source

The inclusion criteria of study subjects were participants of the Taiwan Biobank, a community population of Taiwan, comprising Han Chinese who were 30–70 years old without cancer history and enrolled during 2008–2020. In early 2005, the "Taiwan Biobank" was created as a part of Taiwan's strategic development in promoting the country as an island of biomedicine [16]. The Taiwan Biobank project plans to conduct a large-scale community-based cohort, then track these participants' health-related status and lifestyle behaviors for at least ten years. This community-based cohort study plans to recruit 200,000 volunteers aged 30–70 years with no history of cancer. Currently, the total number of individuals in the Taiwan Biobank is approximately 126,000. The exclusion criteria are those who did not have information regarding lifestyle factors, physical examination, blood test, or whole-genome genotyping data. The number of individuals who fulfill the above criteria is about 116,066 with 9,809,486 variants (Fig. 1). Study subjects were excluded if GWAS data did not pass the quality control criteria, leaving 90,381 study subjects with 2,581,477 variants. Additional 2,580,949 single nucleotide polymorphisms (SNPs) were excluded because they were not reported to be associated with smoking. Then, 528 smoking-related variants identified in the literature were extracted from the dataset. Furthermore, 10,309 persons were excluded because of missing data, and 514 variants were deleted because of violation of MR assumptions 1 or 3, resulting in 80,027 persons with 14 variants.

Measurements

Sociodemographic factors, lifestyle behaviors, laboratory examination, and disease history

Sociodemographic factors comprised age, gender, educational attainment, married status, individual and household income, residential area, job occupation, and status for living alone. Lifestyle behaviors consisted of tobacco smoking, coffee intake, alcohol drinking, and physical activity. Questions about lifestyle behaviors asked respondents about their usual or typical behaviors. Smoking status was categorized as current, past, and never users. Participants were considered nonsmokers if they self-reported to have never smoked or have not continuously smoked for at least six months. Past smokers were those who self-reported to have continuously smoked for at least six months but were current nonsmokers. Current smokers were those who self-reported to have continuously smoked for at least six months

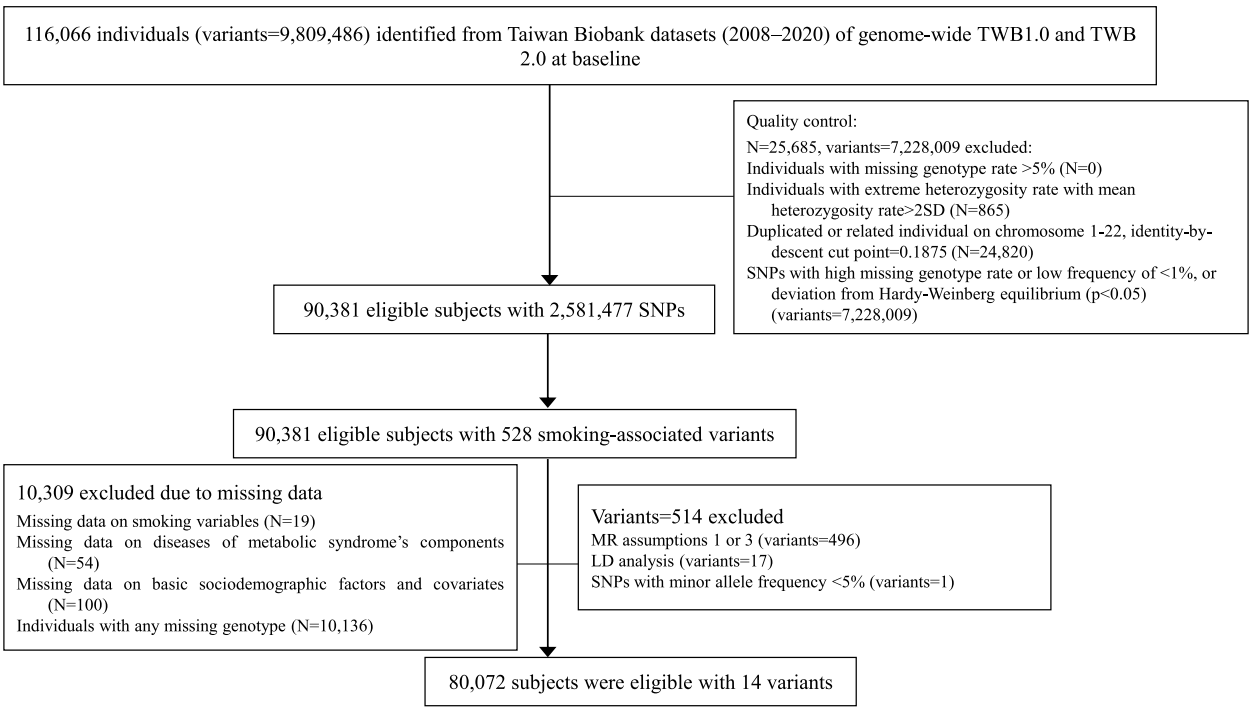

Fig. 1 Study flowchart for study subject and SNP selection

and were current smokers. The present study defined smokers as those who were past and current smokers. Past and current smoking were classified as smokers because former and current smokers are associated with an increased incidence of metabolic syndrome [17]. Coffee intake was categorized as "yes" if participants self-reported they had coffee habit and "no" otherwise. Alcohol drinking was categorized as current, never, and past users. Participants were considered nondrinkers if they self-reported they did not drink or drank less than 150 cc of alcohol per week continuously for six months. Past drinkers were those who abstained from alcohol for more than six months. Current drinkers were those who drank at least 150 cc of alcohol per week continuously for six months. Physical activity was categorized as "yes" if participants self-reported a habit of exercising at least three times per week (each exercise time > 30 min) and "no" otherwise.

Total cholesterol (TC), HDL-C, low density lipoprotein cholesterol (LDL-C), triglycerides (TG), fasting plasma glucose (FPG), blood urea nitrogen (BUN), creatinine, and uric acid were examined at the Department of Clinical Laboratory, Linkou Chang Gung Memorial Hospital. A checklist of disease history for respondents, respondents' father, mother, and siblings on valve heart disease, coronary heart disease, arrhythmia, cardiomyopathy, congenital heart disease, hyperlipidemia, hypertension, stroke, and diabetes was taken by self-reported questionnaires.

Definition of metabolic syndrome

The modified definition of metabolic syndrome as described in the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATP III) was be used [18]. According to the ATP III, the metabolic syndrome components are as follows: hyperglycemia (FPG ≥ 100 mg/dl or those who were taking antidiabetic drugs), hypertriglyceridemia (serum triglycerides ≥ 150 mg/dl or those who were taking cholesterol-lowering drugs), hypertension (blood pressure > 130/85 mmHg or those who were taking antihypertensive drugs), abdominal obesity (waist circumference > 90 cm in men and waist circumference > 80 cm in women), and low HDL-C (serum HDL-C < 40 mg/dl in men and HDL-C < 50 mg/dl in women).

SNPs genotyping for genetic instruments in MR analysis

DNA samples from the Taiwan Biobank were genotyped using the TWB array and run on the Axiom genome-wide array plate system (Affymetrix, Santa Clara, CA, USA). In the present study, each SNP was assessed to learn whether the SNPs (in the founders) are in Hardy–Weinberg Equilibrium (HWE) by using PLINK (v1.9)

[19]. Pairwise linkage disequilibrium among SNPs was quantified by correlation coefficient r^2 in Haploview (v4.2) [20]. Imputation of the database was carried out using IMPUTE2 [21] with a reference derived from the 1000 Genomes Project. The genetic variants were selected based on studies in literature using candidate gene and GWAS approach. SNPs not found in the Taiwan biobank dataset or with minor allele frequencies < 5% were removed. Genetic variants (i.e., SNPs) from *CHRNA5-A3-B4* gene (60 SNPs) and other SNPs from GWAS for smoking (528 SNPs) that can be found in Taiwan biobank dataset were selected. The SNPs satisfying MR assumptions 1 and 3 were trimmed for linkage disequilibrium at a threshold of r^2 at 0.2.

Statistical analysis for MR analysis

Hardy–Weinberg equilibrium was tested in participants using the Chi-square test for goodness of fit. Sociodemographic factors, lifestyle behaviors, laboratory data, and medication were evaluated between persons with and without smoking or metabolic syndrome using two-sample t test and Chi-square test as well as among persons with subgroups of unweighted and weighted genetic risk using analysis of variance and Chi-square test.

First, the relationship between smoking and metabolic syndrome was analyzed using unconditional logistic regression analysis. Second, the relationship of smoking with smoking-related SNPs was investigated. To verify whether selected SNPs can be utilized as instrumental variables for MR analysis, the associations between selected SNPs and smoking were quantified using logistic regression models with each SNP coded as 0, 1, or 2 according to the number of minor alleles, that is, additive model for MR assumption 1. Then, MR assumption 3 was assessed using the same approach. An unweighted smoking genetic risk score (GRS) was created by counting their alleles of SNPs individually associated with smoking. In addition, a weighted allele score was created by summing each genotype multiplied by its estimated coefficient from the logistic regression models, divided by the sum of weights [22]. The weighted and unweighted GRSs were divided into quartiles for categorical analyses.

Finally, a formal MR analysis was performed. The causal effect of smoking on metabolic syndrome was quantified by instrumental variable analysis using two-stage regression with multivariate adjustment. The first stage comprised the ordinary logistic regression of tobacco smoking, resulting in predicted likelihood of tobacco smoking, that is, genetic variants–tobacco smoking associations. The second stage comprised a logistic regression of metabolic syndrome on the predicted likelihood of tobacco smoking estimated in the first stage. Different definitions of instrumental variables including unweight and weighted GRSs were used to examine the robustness of these associations. The data analysis was based on complete case analysis, i.e., participants with missing data on the variables of interest were excluded. SAS version 9.4 (SAS Institute Inc., Cary, NC) was used. All reported p values are two sided, and the level of significance is 0.05.

Results

A total of 80,072 study subjects were eligible for analysis with a mean age of 49.7 years with a standard deviation (SD): 10.7 years, of whom 34.8% were men. Table 1 compares the basic sociodemographic factors, lifestyle habits, anthropometric and biochemical markers, and comorbidities based on status of smoking and metabolic syndrome. The prevalence of metabolic syndrome was statistically higher in persons with smoking habit than those without smoking habit ($p < 0.001$), and the crude odds ratio (OR) was 1.90 (95% CI: 1.83, 1.98). After multivariate adjustment, metabolic syndrome was statistically associated with smoking (1.16 [95% CI: 1.10, 1.22]).

MR assumptions 1 and 3 were assessed for all SNPs, and 32 SNPs satisfied SNP-level MR assumptions 1 and 3 by using an additive model. After performing LD analysis, 14 SNPs were left (Supplementary Fig. 1). Supplementary Fig. 2 presents the forest plot of ORs for significant SNPs associated with smoking, satisfying MR assumption 1 with an additive model (14 SNPs with $p < 0.05$). The coding of SNPs with negative associations (OR < 1) was reversed as 2, 1, or 0 based on the number of minor alleles (four SNPs). Supplementary Fig. 3 presents the forest plots of ORs for nonsignificant associations of these 14 SNPs with metabolic syndrome.

Next, weighted and unweighted GRSs were derived using these fourteen smoking-associated SNPs. GRS-level MR assumptions 1 and 3 were assessed, that is, the associations between weighted and unweighted GRSs and smoking and metabolic syndrome. The results revealed weighted and unweighted GRSs satisfied GRS-level MR assumptions 1 and 3, that is, weighted and unweighted GRSs were significantly positively associated with smoking status, and weighted and unweighted GRSs were not associated with metabolic syndrome either in continuous or categorical forms with and without adjustment (Table 2).

Then, the GRS-level MR assumption 2 was explored, that is, the associations between weighted and unweighted GRSs and covariates, including sociodemographic factors, lifestyle behaviors, clinical and biochemical markers, and comorbidities (Supplementary Table 1). All covariates satisfied MR assumption 2 except for gender. Thus, gender would not be considered in the

Table 1 Comparisons of sociodemographic factors, lifestyle behaviors, clinical and biochemical markers, and comorbidities according to smoking status and metabolic syndrome

		Smoking			Metabolic syndrome		
	All	**No**	**Yes**	***P* value**	**No**	**Yes**	***P* value**
Variables	**(*N* = 80,072)**	**(*N* = 58,673)**	**(N = 21,399)**		**(*N* = 64,299)**	**(*N* = 15,773)**	
Sociodemographic factors, n (%)							
Gender				< 0.001			< 0.001
Men	27,898 (34.84)	11,876 (20.24)	16,022 (74.87)		19,885 (30.93)	8013 (50.80)	
Women	52,174 (65.16)	46,797 (79.76)	5377 (25.13)		44,414 (69.07)	7760 (49.20)	
Age, years, mean ± SD	49.65 ± 10.65	49.84 ± 10.69	49.14 ± 10.51	< 0.001	48.63 ± 10.58	53.81 ± 9.87	< 0.001
Education level (year)				< 0.001			< 0.001
≤ 6	4132 (5.16)	3416 (5.82)	716 (3.35)		2724 (4.24)	1408 (8.93)	
7–12	29,548 (36.9)	21,047 (35.87)	8501 (39.73)		22,829 (35.5)	6719 (42.60)	
≥ 13	46,392 (57.94)	34,210 (58.31)	12,182 (56.93)		38,746 (60.26)	7646 (48.48)	
Marriage status				< 0.001			< 0.001
Unmarried	10,492 (13.10)	7688 (13.10)	2804 (13.10)		9102 (14.16)	1390 (8.81)	
Married	59,271 (74.02)	43,449 (74.05)	15,822 (73.94)		47,202 (73.41)	12,069 (76.52)	
Divorce or separation	6835 (8.54)	4488 (7.65)	2347 (10.97)		5440 (8.46)	1395 (8.84)	
Widowed	3474 (4.34)	3048 (5.19)	426 (1.99)		2555 (3.97)	919 (5.83)	
Living alone				0.14			0.80
No	73,558 (91.86)	53,951 (91.95)	19,607 (91.63)		59,060 (91.85)	14,498 (91.92)	
Yes	6514 (8.14)	4722 (8.05)	1792 (8.37)		5239 (8.15)	1275 (8.08)	
Lifestyle behaviors, n (%)							
Alcohol drinking				< 0.001			< 0.001
No	73,394 (91.66)	56,937 (97.04)	16,457 (76.91)		59,760 (92.94)	13,634 (86.44)	
Ever	2040 (2.55)	467 (0.8)	1573 (7.35)		1342 (2.09)	698 (4.43)	
Yes	4638 (5.79)	1269 (2.16)	3369 (15.74)		3197 (4.97)	1441 (9.14)	
Leisure time physical activity				< 0.001			< 0.001
No	48,101 (60.07)	34,821 (59.35)	13,280 (62.06)		38,836 (60.40)	9265 (58.74)	
Yes	31,971 (39.93)	23,852 (40.65)	8119 (37.94)		25,463 (39.60)	6508 (41.26)	
Clinical and biochemical markers, mean ± SD							
Waistline, cm	83.17 ± 10.17	81.84 ± 9.81	86.83 ± 10.25	< 0.001	80.9 ± 9.1	92.43 ± 8.97	< 0.001
BMI, kg/m^2	24.22 ± 3.77	23.87 ± 3.69	25.18 ± 3.84	< 0.001	23.43 ± 3.34	27.41 ± 3.75	< 0.001
SBP, mmHg	119.41 ± 17.87	118.29 ± 17.85	122.49 ± 17.58	< 0.001	115.97 ± 16.37	133.44 ± 16.86	< 0.001
DBP, mmHg	73.37 ± 11.03	72.28 ± 10.74	76.35 ± 11.25	< 0.001	71.48 ± 10.22	81.08 ± 10.86	< 0.001
FPG, mg/dL	95.76 ± 20.48	94.74 ± 18.94	98.57 ± 23.98	< 0.001	92.09 ± 13.43	110.73 ± 33.39	< 0.001
HbA1c, %	5.76 ± 0.8	5.74 ± 0.75	5.83 ± 0.91	< 0.001	5.62 ± 0.56	6.35 ± 1.24	< 0.001
TG, mg/dL	116.22 ± 97.32	108.19 ± 80.32	138.25 ± 130.71	< 0.001	95.78 ± 57.68	199.54 ± 160.84	< 0.001
TC, mg/dL	195.91 ± 35.95	196.71 ± 35.91	193.7 ± 35.97	0.024	195.1 ± 34.77	199.19 ± 40.25	< 0.001
HDL-C, mg/dL	54.61 ± 13.45	56.3 ± 13.36	50 ± 12.6	< 0.001	57.07 ± 12.93	44.59 ± 10.59	< 0.001
LDL-C, mg/dL	121.11 ± 31.84	121.01 ± 31.74	121.37 ± 32.13	0.16	120.71 ± 31.08	122.73 ± 34.73	< 0.001
Albumin, g/dL	4.51 ± 0.23	4.5 ± 0.23	4.55 ± 0.24	< 0.001	4.51 ± 0.23	4.55 ± 0.23	< 0.001
SCr, mg/dL	0.72 ± 0.31	0.67 ± 0.26	0.84 ± 0.4	< 0.001	0.7 ± 0.26	0.8 ± 0.47	< 0.001
eGFR, mL/min/1.73 m^2	102.54 ± 14.74	103.62 ± 14.14	99.59 ± 15.92	< 0.001	104.06 ± 13.92	96.37 ± 16.31	< 0.001
Comorbidities, n (%)							
Hypertension				< 0.001			< 0.001
No	70,545 (88.1)	52,463 (89.42)	18,082 (84.5)		60,322 (93.81)	10,223 (64.81)	
Yes	9527 (11.9)	6210 (10.58)	3317 (15.5)		3977 (6.19)	5550 (35.19)	
Hyperlipidemia				< 0.001			< 0.001
No	74,253 (92.73)	54,808 (93.41)	19,445 (90.87)		62,075 (96.54)	12,178 (77.21)	
Yes	5819 (7.27)	3865 (6.59)	1954 (9.13)		2224 (3.46)	3595 (22.79)	

Table 1 (continued)

		Smoking			Metabolic syndrome		
	All	**No**	**Yes**	***P* value**	**No**	**Yes**	***P* value**
Variables	**(*N* = 80,072)**	**(*N* = 58,673)**	**(N = 21,399)**		**(*N* = 64,299)**	**(*N* = 15,773)**	
Diabetes				< 0.001			< 0.001
No	76,109 (95.05)	56,092 (95.60)	20,017 (93.54)		63,044 (98.05)	13,065 (82.83)	
Yes	3963 (4.95)	2581 (4.40)	1382 (6.46)		1255 (1.95)	2708 (17.17)	
Stroke				< 0.001			< 0.001
No	79,588 (99.4)	58,408 (99.55)	21,180 (98.98)		64,059 (99.63)	15,529 (98.45)	
Yes	484 (0.6)	265 (0.45)	219 (1.02)		240 (0.37)	244 (1.55)	
Valvular heart disease				< 0.001			< 0.001
No	76,666 (95.75)	56,015 (95.47)	20,651 (96.50)		61,404 (95.50)	15,262 (96.76)	
Yes	3406 (4.25)	2658 (4.53)	748 (3.50)		2895 (4.50)	511 (3.24)	
Coronary artery disease				< 0.001			< 0.001
No	79,169 (98.87)	58,197 (99.19)	20,972 (98.00)		63,863 (99.32)	15,306 (97.04)	
Yes	903 (1.13)	476 (0.81)	427 (2.00)		436 (0.68)	467 (2.96)	
Arrhythmia				0.001			< 0.001
No	76,500 (95.54)	56,141 (95.68)	20,359 (95.14)		61,646 (95.87)	14,854 (94.17)	
Yes	3572 (4.46)	2532 (4.32)	1040 (4.86)		2653 (4.13)	919 (5.83)	
Cardiomyopathy				< 0.001			< 0.001
No	79,465 (99.24)	58,299 (99.36)	21,166 (98.91)		63,984 (99.51)	15,481 (98.15)	
Yes	607 (0.76)	374 (0.64)	233 (1.09)		315 (0.49)	292 (1.85)	
Congenital heart disease				0.80			0.06
No	79,919 (99.81)	58,559 (99.81)	21,360 (99.82)		64,186 (99.82)	15,733 (99.75)	
Yes	153 (0.19)	114 (0.19)	39 (0.18)		113 (0.18)	40 (0.25)	
Other heart disease				0.39			0.24
No	79,944 (99.84)	58,584 (99.85)	21,360 (99.82)		64,202 (99.85)	15,742 (99.80)	
Yes	128 (0.16)	89 (0.15)	39 (0.18)		97 (0.15)	31 (0.20)	

Differences in continue variables were tested using the Student's t test. Differences in categorical variables were tested using the chi-square test

BMI Body mass index, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *FPG* Fasting plasma glucose, *HbA1c* hemoglobin A1c, *TG* Triglyceride, *TC* Total cholesterol, *HDL-C* High-density lipoprotein-cholesterol, *LDL-C* Low-density lipoprotein-cholesterol, *SCr* Serum creatinine, *eGFR* estimated glomerular filtration rate

first stage of model for deriving the likelihood of smoking using GRSs.

Table 3 shows the ORs of metabolic syndrome for genetic-related smoking likelihood derived from the unweighted and weighted GRS without and with adjustment. Genetic-related likelihood of smoking was the p hat derived from the logistic regression model by regressing smoking status on unweighted GRS. The crude OR of metabolic syndrome per 1 SD increase in the genetic-related smoking likelihood without adjustment was 1.62 (1.60, 1.65). After multivariate adjustment of residuals from the stage 1 model, principal components analysis (PCA), and gender that did not satisfy MR assumption 2, OR of metabolic syndrome per 1 SD increase in the genetic-related smoking likelihood was 1.49 (1.47, 1.52). After grouping the genetic-related smoking likelihood derived from unweighted GRS with adjustment according to the quartiles, the highest metabolic syndrome prevalence rate was observed in Q4 (36.08%), and the lowest was in Q1 (9.98%). Using Q1 as the reference group, the adjusted ORs of metabolic syndrome for Q2, Q3, and Q4 of genetic-related smoking likelihood derived from unweighted GRS were 1.16 (1.09, 1.24), 2.17 (2.05, 2.30), and 4.26 (4.02, 4.53), respectively. The results for genetic-related smoking likelihood derived from weighted GRS were similar. After full adjustment, the OR of metabolic syndrome per 1 SD increase in the genetic-related smoking likelihood was 1.49 (1.47, 1.52). After grouping the genetic-related smoking likelihood from weighted GRS with adjustment, the highest metabolic syndrome prevalence rate was observed in Q4 (36.03%), and the lowest was in Q1 (10.02%). The adjusted ORs of metabolic syndrome for Q2, Q3, and Q4 of genetic-related smoking likelihood derived were 1.15 (1.08, 1.22), 2.17 (2.05, 2.30), and 4.23 (3.98, 4.49), respectively.

To evaluate whether current smoking and past smoking status impact the results, we categorized smokers into current and past smokers (see Supplementary Tables 2

Table 2 Odds ratios of smoking-related unweighted and weighted and GRSs derived from SNPs satisfying MR assumptions 1 and 3 for association between smoking and metabolic syndrome

Variables	n	Smoking			Metabolic syndrome		
		Yes, n (%)	Crude OR (95% CI)	Adjusted OR (95%CI)	Yes, n (%)	Crude OR (95% CI)	Adjusted OR (95%CI)
Unweighted genetic risk score							
Per standard deviation	80,072	21,399 (26.72)	1.08 (1.06, 1.09)***	1.08 (1.06, 1.11)***	15,773 (19.70)	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)
Q1: < 10	21,569	5421 (25.13)	1.00	1.00	4240 (19.66)	1.00	1.00
Q2: 10	13,928	3643 (26.16)	1.06 (1.01, 1.11)*	1.06 (1.00, 1.12)	2700 (19.39)	0.98 (0.93, 1.04)	0.99 (0.93, 1.05)
Q3: 11–12	27,323	7299 (26.71)	1.09 (1.04, 1.13)***	1.12 (1.06, 1.17)***	5461 (19.99)	1.02 (0.98, 1.07)	1.01 (0.96, 1.06)
Q4: ≥ 13	17,252	5036 (29.19)	1.23 (1.17, 1.29)***	1.26 (1.19, 1.33)***	3372 (19.55)	0.99 (0.94, 1.04)	0.98 (0.92, 1.04)
Weighted genetic risk score							
Per standard deviation	80,072	21,399 (26.72)	1.08 (1.06, 1.09)***	1.08 (1.06, 1.11)***	15,773 (19.70)	1.01 (0.99, 1.03)	1.00 (0.98, 1.03)
Q1: < 0.30	20,016	5019 (25.07)	1.00	1.00	3966 (19.81)	1.00	1.00
Q2: 0.30–0.33	20,020	5259 (26.27)	1.07 (1.02, 1.11)**	1.05 (1.00, 1.11)	3877 (19.37)	0.97 (0.93, 1.02)	0.96 (0.91, 1.02)
Q3: 0.34–0.39	20,020	5304 (26.49)	1.08 (1.03, 1.13)**	1.09 (1.03, 1.15)**	3999 (19.98)	1.01 (0.96, 1.06)	1.00 (0.95, 1.06)
Q4: ≥ 0.40	20,016	5817 (29.06)	1.22 (1.17, 1.28)***	1.25 (1.18, 1.32)***	3931 (19.64)	0.99 (0.94, 1.04)	0.98 (0.93, 1.04)

Multivariate model was adjusted for covariates that satisfied MR assumption 2

OR Odds ratio, *CI* Confidence interval

*: $P < 0.05$

**: $P < 0.01$

***: $P < 0.001$

Table 3 Odds ratios of metabolic syndrome for predictive smoking derived from unweighted and weighted GRS

Variables	n	Metabolic syndrome			
		Yes, n (%)	Crude OR (95% CI)	Adjusted OR (95%CI)[1]	Adjusted OR (95%CI)[2]
Phat of smoking derived from unweighted genetic risk score					
Per 1 standard deviation	80,072	15,773 (19.70)	1.62 (1.60, 1.65)***	1.63 (1.60, 1.65)***	1.49 (1.47, 1.52)***
Q1: < 0.16323	20,018	1997 (9.98)	1.00	1.00	1.00
Q2: 0.16323–0.21447	20,018	2364 (11.81)	1.21 (1.13, 1.29)***	1.21 (1.13, 1.29)***	1.16 (1.09, 1.24)***
Q3: 0.21448–0.29760	20,018	4189 (20.93)	2.39 (2.26, 2.53)***	2.38 (2.25, 2.52)***	2.17 (2.05, 2.30)***
Q4: ≥ 0.29761	20,018	7223 (36.08)	5.09 (4.82, 5.38)***	5.12 (4.85, 5.41)***	4.26 (4.02, 4.53)***
Phat of smoking derived from weighted genetic risk score					
Per 1 standard deviation	80,072	15,773 (19.70)	1.62 (1.60, 1.65)***	1.63 (1.60, 1.65)***	1.49 (1.47, 1.52)***
Q1: < 0.16323	20,018	2006 (10.02)	1.00	1.00	1.00
Q2: 0.16323–0.21447	20,018	2351 (11.74)	1.20 (1.12, 1.27)***	1.20 (1.12, 1.27)***	1.15 (1.08, 1.22)***
Q3: 0.21448–0.29793	20,018	4204 (21.00)	2.39 (2.25, 2.53)***	2.38 (2.25, 2.52)***	2.17 (2.05, 2.30)***
Q4: ≥ 0.29794	20,018	7212 (36.03)	5.06 (4.79, 5.34)***	5.08 (4.81, 5.37)***	4.23 (3.98, 4.49)***

Adjusted odds ratio[1]: adjusted for residual and PCA

Adjusted odds ratio[2]: adjusted for residual, PCA, and covariates that did not satisfy MR assumption 2

OR Odds ratio, *CI* Confidence interval, *Q1* the first quartile, *Q2* the second quartile, *Q3* the third quartile, *Q4* the fourth quartile

*: $P < 0.05$

**: $P < 0.01$

***: $P < 0.001$

and 3). The multivariate-adjusted OR for metabolic syndrome per 1 standard deviation (SD) increase in the genetic-related likelihood of current smoking, derived from both the unweighted and weighted GRS, was 1.31 (95% CI: 1.28, 1.33). In contrast, the multivariate-adjusted OR for metabolic syndrome per 1 SD increase

in the genetic-related likelihood of past smoking, also derived from both the unweighted and weighted GRS, was 1.54 (95% CI: 1.51, 1.57).

Discussion

The causal association between smoking and metabolic syndrome was explored using one-sample MR analysis with instrument variables of SNPs in *CHRNA5-A3-B4* and other genes identified from prior GWAS studies in adult persons who participated in the Taiwan Biobank study. After multivariate adjustment for residuals in the first stage, gender and ten principal components from PCA, the genetic-related smoking likelihood was positively linearly linked with metabolic syndrome, that is, persons with a higher likelihood of genetic predisposition to smoke were more likely to have metabolic syndrome. This study is the first to assess the causal link with experimental evidence between smoking and metabolic syndrome using the MR approach.

Randomized controlled trial is the closed approximation in design to an experiment, and a well-run trial may provide experimental evidence for confirming a causal association between an exposure and an outcome. For lifestyle behaviors, the exposure is generally a treatment, drug, or cessation program, and the outcome is the reduction of disease or mortality. For example, if a randomized trial demonstrated that a smoking cessation program reduction in smoking led to lower risks of metabolic syndrome, it provided experimental evidence. After thoroughly reviewing literature, a published protocol was found for an international randomized controlled trial evaluating the effect of combustion-free nicotine delivery system versus smoking cessation program on metabolic syndrome in persons with type 2 diabetes [23], but no findings for this international study were found. Using the MR approach, our study used an alternative approach to provide experimental evidence of causal association between smoking status and metabolic syndrome.

After searching the literature regarding MR studies of smoking in depth, the gene that has been used as genetic instrumental variables using candidate gene approach for smoking was *CHRNA5/A3/B4*, which was a candidate region for smoking behaviors and smoking-related diseases [24–28]. *CHRNA5/A3/B4* is an important nicotinic acetylcholine receptor, or *nAChRs*, gene cluster, which is located on chromosome 15 at region 15q24–25 and comprises the gene encoding for the α5, α3, and β4 subunits. Prior genetic studies identified SNPs in these three cluster *nAChR* genes as risk factors linked to multiple smoking-related phenotypes, including nicotine dependence [24], smoking cessation [25], smoking quantity [26], peripheral arterial disease [24], and lung cancer [24, 28]. A candidate gene study first reported the association between the SNP rs16969968 in *CHRNA5* and nicotine dependence [29]. The risk variant SNP rs16969968 in *CHRNA5* was associated with a twofold greater response in smoking quantity and nicotine dependence [30].

Numerous prior MR studies explored the associations between smoking and cardiovascular-related risk factors or disease such as ischemic stroke [11, 12], type 2 diabetes [13], heart failure [14], and cardiovascular risk factors [15, 31]. Two studies focused on the outcomes of cardiovascular risk factors similar to ours [15, 31]. A prior study investigated the associations between tobacco smoking and cardiovascular risk factors among adults aged 20 years or older in Norway using a single SNP rs1051730 as an instrument variable and found smoking may be causally linked with lower BMI, and waist and hip circumferences, but was not associated with higher levels of blood pressure, serum lipid, or glucose levels [31]. The other MR study examining the associations between ever smoking regularly and blood pressure was conducted in individuals of self-reported European ancestry from twenty-three studies using the genetic variants rs1051730 and rs16969968 as an instrumental variable [15, 31], but the association of ever smoking regularly with blood pressure was not found. In the present study, genetic predisposition to smoking was associated with a 1.49-fold higher risk of metabolic syndrome for every 1 SD increase in likelihood of genetic predisposition to smoking, which was consistent with previous observational epidemiologic studies [32, 33].

Many plausible underlying mechanisms may support the associations between smoking and metabolic syndrome and its components (Supplementary Table 4). One plausible mechanism is that smoking may reduce insulin sensitivity and development of insulin resistance [34], and increased insulin resistance may be the underlying cause that results in hemodynamic abnormal conditions contributing to metabolic syndrome. The other plausible mechanism is that smoking is linked with increased levels of inflammatory markers of fibrinogen and C reactive protein through triggering an immunologic response that results in vascular injury [35, 36]. In addition, smoking alters coagulation–fibrinolysis process [37], contributing to thrombosis through its action on platelets, endothelium, and fibrinogen [38].

The strength of the present study was the use of a sample with large size, standardized approach to collect data, and the MR approach for ruling out the potential impact of confounding and reverse causation on the associations between smoking and metabolic syndrome. However, several limitations should be noted. First, the present study considered a binary variable of smoking status because of high proportions of missing data for number of cigarettes per

day. Furthermore, persons who have quitted smoking were classified as alcohol drinkers. This classification would result in an underestimated risk of smoking on metabolic syndrome, which is a lesser threat to validity. Second, the findings were obtained from the Han Chinese population, and the external generalization of the study's findings to other populations might be limited because the present study's population may differ from other population in race, genes, and smoking behavior. The worldwide smoking prevalence ranged from 54.5% in Indonesia, 43.2% in Russia, and 41.5% in China to less than 10% in Costa Rica, Norway, and Iceland in men, whereas rates ranged from over 20% in Chile, Hungary, and France to less than 5% in Costa Rica, India, Mexico, Indonesia, China, and Korea in women [39]. In Taiwan, the smoking prevalence was 14.0% in 2017, decreasing dramatically from 20.9% in 2005 due to population-level intervention for tobacco control [40]. Third, this study adopted a cross-sectional design that determines smoking status and metabolic syndrome at the same time point; thus, it lacks a time sequence, that is, smoking was determined before the occurrence of metabolic syndrome. However, reverse causality can be preliminarily ruled out because genes are innately determined. Fourth, the study design is cross-sectional. The potential error arising from the impact of comorbidity or diagnosis of metabolic syndrome on smoking status cannot be controlled. It requires future research using a longitudinal study design to address this issue. Fifth, we searched extensively but were unable to find an external population. Therefore, no external population was used to derive SNP weights for constructing the GRS. Since the study lacked an external population for obtaining weighted SNP values, we employed both weighted and unweighted methods to determine the impact of weighting on the results. The findings from these methods were similar, suggesting that the results are not sensitive to the weighting. Finally, our study sample may not be representative of general population in Taiwan, so potential selection bias might exist. However, the present paper had an analytic objective, whether the study had enough number of study subjects with smoking or with metabolic syndrome is more important consideration, that is, sufficient power to assess the potential relationship between smoking and metabolic syndrome. On the contrary, the definition of smoking status used in the present study may decrease the effect size because past smokers were categorized as smokers. This assumption means the magnitude of true association may be greater than that observed in the present study.

Conclusion

The present paper presented experimental evidence for the causal association between tobacco smoking and metabolic syndrome in Han Chinese, which may provide knowledge for policy makers and public health professionals who design health education intervention.

Abbreviations

HDL-C	High density lipoprotein-cholesterol
MR	Mendelian randomization
GWAS	Genome-wide association study
SNPs	Single nucleotide polymorphisms
TC	Total cholesterol
LDL-C	Low density lipoprotein-cholesterol
TG	Triglycerides
FPG	Fasting plasma glucose
BUN	Blood urea nitrogen
ATP III	Third Report of the National Cholesterol Education Program's Adult Treatment Panel
HWE	Hardy–Weinberg Equilibrium
GRS	Genetic risk score
SD	Standard deviation
OR	Odds ratio
PCA	Principal components analysis

Supplementary Information

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Supplementary Material 1.

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Not applicable.

Authors' contributions

TCL and CCL were responsible for drafting the article, the conception and design of the study. TCL, CIL and SYY acquired data and analysed data. CSL and CHL interpreted data. All authors revised the manuscript and approved the final version. TCL and CCL are responsible for the integrity of the work as a whole.

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Data availability

The datasets generated during and analyzed during the current study are not publicly available due to the policy declared by Taiwan Biobank but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This present study was approved by the Ethics and Governance Council of Taiwan Biobank (approval number: TWBR10811-06) and the Ethical Review Board of China Medical University Hospital (CMUH109-REC3-187). All participants provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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