ARTICLE

Genetically predicted high body mass index is associated with increased gastric cancer risk

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Epidemiological studies have linked body mass index (BMI) with risk of gastrointestinal cancers. However, for gastric cancer, the relationship is more controversial. In particular, it is unclear whether the observed association is due to confounding or bias inherent in conventional observational studies. To investigate whether BMI is causally associated with gastric cancer risk, we applied Mendelian randomization using individual-level data from 2631 gastric cancer cases and 4373 cancer-free controls. We derived a weighted genetic risk score (wGRS) using 37 BMI-associated genetic variants as an instrumental variable. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for associations between genetically predicted BMI and gastric cancer risk. We observed that higher genetically determined BMI was associated with increased gastric cancer risk (per standard deviation (SD) increase in the wGRS: OR = 1.07, 95% CI: 1.02–1.13, $P = 4.94 \times 10^{-3}$). Compared with individuals in the bottom tertile of the BMI wGRS, those in the top tertile had 1.14-fold (95% CI: 1.01–1.29) increased risk of developing gastric cancer. Sensitivity analyses using alternative causal inference measures demonstrated consistent association. Our study indicated that genetically high BMI was associated with increased gastric cancer risk, suggesting that high BMI may have a causal role in the etiology of gastric cancer.

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INTRODUCTION

Gastric cancer is one of the leading causes of cancer morbidity and mortality worldwide, with around 42.5% of all cases occurring in China.[1,2] Gastric cancer is a multifactorial disease involving both genetic and environmental factors. *Helicobacter pylori* infection, cigarette smoking, alcohol drinking, and salted food intake are the established common risk factors for gastric cancer.[3] Over the past two decades, epidemiological studies have linked body mass index (BMI) with risk of gastrointestinal cancers including esophageal,[4] liver,[5] pancreatic,[6] and colorectal cancers.[7] However, for gastric cancer, the relationship is more controversial. Several large-scale cohort studies indicated a positive association between high BMI and increased risk of gastric cancer,[8–13] whereas others found no statistically significant relationship.[14–17] A recent meta-analysis of prospective studies reported that compared with individuals with normal BMI, those with BMI ≥ 30 kg/m^2 had a 13% (95% confidence interval (CI): 3–24%) increased risk of developing gastric cancer.[18] However, it remains unclear whether the observed association reflects a causal effect of BMI on gastric cancer, or is due to confounding or biases inherent in conventional epidemiological studies. For instance, the observational association between BMI and gastric cancer risk may be biased by reverse causality, or confounded by early life exposures, patterns of diet and other factors, which are difficult to measure or control for in traditional observational studies.

Mendelian randomization (MR) offers a way to investigate the nature of the association between BMI and gastric cancer risk. It utilizes instrumental variables such as genetic variants that act as proxies for environmental, social, or behavioral factors to make a causal inference of a given exposure and an outcome (such as BMI and gastric cancer).[19,20] Because genotype is presumed to be randomly allocated at conception, confounding factors are anticipated to be equally distributed among different genotypes. Those who inherited more BMI-increasing alleles from their parents are in effect being randomly assigned a 'higher BMI dosage'. As illustrated in Supplementary Figure 1, using BMI-related genetic variants to predict BMI, the MR approach shares many similarities with the 'intention-to-treat' analysis in a randomized clinical trial, and is often robust to the issues of confounding and reverse causality inherent in observational epidemiological studies.

In this study, we applied MR analysis to evaluate the association between BMI and risk of gastric cancer using individual-level data from 7004 subjects of Chinese Han descent. We derived a weighted genetic risk score (wGRS) comprising 37 BMI-related single nucleotide polymorphisms (SNP) identified by a meta-analysis of genome-wide association studies (GWAS) in East Asian-ancestry populations,[19] and assessed whether there is a causal relationship between BMI and gastric cancer risk.

MATERIALS AND METHODS

Study subjects

We used genetic data from the Nanjing/Beijing and the United States National Cancer Institute (NCI) gastric cancer studies. Full details of the study

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population have been described elsewhere.[20–22] For the Nanjing/Beijing study, individuals were derived from separate case–control studies conducted in Nanjing and Beijing. Cases were patients with histopathologically confirmed non-cardia gastric cancer. Controls were cancer-free individuals selected from local residents. Raw data related to the present study were deposited in GWAS Central database (http://www.gwascentral.org/) with the study accession number of HGVST1849. For the NCI study, data of gastric cancer cases and cancer-free controls were obtained from the public database of Genotype and Phenotypes (dbGaP, http://www.ncbi.nlm.nih.gov/gap) with the study accession number of phs000361.v1.p1. Study subjects were from two separate studies, the Shanxi Upper Gastrointestinal Cancer Genetics Project and the Linxian Nutritional Intervention Trials, which were conducted in the area of the Taihang Mountains in China. Data on height and weight of the control population were only available in the Nanjing Study. Height was measured to the nearest 0.1 cm and weight was measured to the nearest 100 g. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. All study subjects were unrelated individuals of Chinese Han descent. There was no overlap of participants between the Nanjing/Beijing study and the NCI study. All study subjects provided written informed consent, and the study protocols were approved by the relevant Institutional Review Boards.

Genotyping and imputation

Full details of the genotyping, quality control and imputation have been previously reported.[20] Briefly, individuals in the Nanjing/Beijing study were genotyped using the Affymetrix Genome-Wide Human SNP Array V.6.0 chips, and subjects in the NCI study were genotyped using the Illumina 660W-Quad chips. We used PLINK (v1.07) to carry out standard quality-control measures, which included removal of samples with low call rates, excessive heterozygosity, incorrect sex assignments, or ambiguous familial relationship. Genotype imputation was performed with IMPUTE2 software (V.2.2.2) using the 1000 Genomes Project Phase 3 as the reference.[23] Population structure was evaluated by principal components analysis using the software package EIGENSTRAT 3.0 for the Nanjing/Beijing study and the NCI study separately. Genotyped SNPs with call rate $<95\%$, minor allele frequency (MAF) $<1\%$, or P-value from an exact test of Hardy–Weinberg equilibrium in the control samples $<1\times10^{-5}$ were excluded. Imputed SNPs were restricted based on MAF $>1\%$ and overall IMPUTE2 INFO scores >0.8, which indicates a high degree of imputation accuracy.

Selection of BMI-associated SNPs

A recent meta-analysis of GWAS conducted by the Asian Genetic Epidemiology Network (AGEN) BMI-Consortium identified 16 SNPs associated with BMI at genome-wide significance level ($P<5\times10^{-8}$) in populations of East Asian-ancestry.[19] Moreover, the study also confirmed associations of 22 additional SNPs previously reported to be associated with BMI in populations of European ancestry ($P<0.05$).[19]

One variant, rs7701094, was excluded due to failure of imputation and was replaced by rs6890814 ($r^2=0.97$, INFO >0.90) (Supplementary Table 1). rs12229654 and rs671 were in moderate linkage disequilibrium (LD) ($r^2=0.58$, D' $=0.77$), and rs671 with the lower P-value for association with BMI was selected. The remaining 37 genetic variants were all from independent loci (defined as $r^2<0.01$) based on 1000 Genomes Project Phase 3 data.

Statistical analysis

Because the BMI value of the controls did not follow normal distribution (Shapiro–Wilk normality test $P=1.96\times10^{-6}$), sex-specific rank-based inverse normal transformation (INT) was applied to improve normality and keep consistent with the previous GWAS meta-analysis.[19] We created a wGRS predicting BMI by summing the dosages for BMI-increasing alleles in each individual across the 37 variants using the following formula:[24] wGRS $=\sum_{i=1}^{37}\beta_i\,\mathrm{SNP}_i$, where β_i is the effect estimate of the ith SNP for BMI reported in the GWAS meta-analysis and SNPi is the dosage of the effect allele (0, 1, or 2 for wild-type homozygous, heterozygous, or homozygous for the effect allele associated with higher BMI). Using the controls of the Nanjing study, we examined associations of the wGRS and each of the 37 genetic variants with BMI using generalized linear regression models. Pooled analyses using individual-level data from the Nanjing/Beijing and the NCI studies were

conducted to examine the associations of the BMI wGRS and each variant with gastric cancer risk. The restricted cubic spline function was applied using the logistic regression model to evaluate the shape of the association between the BMI wGRS and gastric cancer risk, and Wald test was used for non-linearity estimation. The BMI wGRS was also categorized into three groups based on its tertile distribution in controls, and was evaluated both as a continuous variable (scaled per SD) and a categorical variable (the bottom tertile as the reference group) using logistic regression models adjusted for potential confounders. Stratified analyses were also performed based on age, sex, and study site.

In addition to the wGRS approach, we estimated the causal effect of BMI on gastric cancer using the MR inverse-variance weighted (IVW) method with summary association of each genetic variant.[25] The Cochran's Q test was used to assess the heterogeneity for the genetic variants. IVW regression implemented in the gtx package (v0.0.8) in R software was applied to evaluate the combined association of the 37 BMI-related variants with gastric cancer risk. The potential causal association between BMI (X) and gastric cancer risk (Y) was modeled using BMI-related SNPs as the instrumental variables. The causal effect (β_{YX}) was calculated using the Wald estimator,[26] and the standard error (SE) for the causal effect was calculated using the delta method.[27] MR Egger regression, which is analogous to the casual effect approach using IVW regression, was also used to evaluate the potential pleiotropic effect of the genetic variants.[28] It estimates an intercept term in the regression, and a significant, non-zero intercept implies directional bias among the genetic instruments. Sensitivity analyses were also performed to assess the robustness of the association using an unweighted BMI genetic risk score and a core wGRS derived exclusively from SNPs exhibiting genome-wide significant associations with BMI in East Asian populations. All statistical analyses were performed using PLINK(v 1.07), R (v.3.3.0), and SAS (9.3). Two-sided P-values <0.05 were considered statistically significant.

RESULTS

Genetically predicted high BMI associates with gastric cancer risk

A total of 2631 gastric cancer cases and 4373 cancer-free controls were used in the current analyses. Selected characteristics of cases and controls are summarized in Supplementary Table 2. When we regressed the observed BMI on the wGRS among controls, individuals carrying greater numbers of BMI-increasing alleles had higher attained BMI ($r^2=0.01$, $P=2.17\times10^{-3}$). As shown in Figure 1, the mean BMI value of subjects in the highest wGRS decile was 1.72 kg/m² greater than that of individuals in the lowest decile (trend test $P=1.48\times10^{-3}$).

The mean BMI wGRS was greater in gastric cancer cases (1.11 ± 0.12) than in controls (1.10 ± 0.12) (Student's t-test $P=3.58\times10^{-3}$). Using the restricted cubic spine function in the
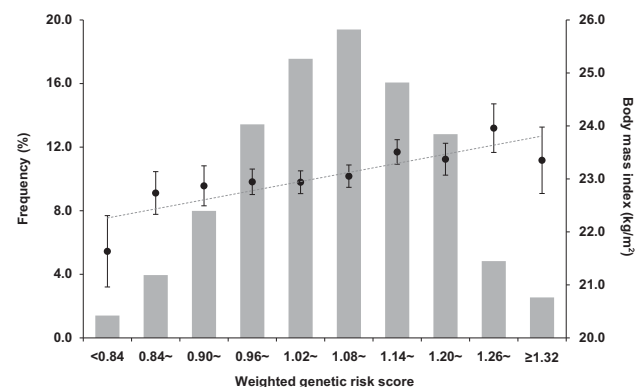

Figure 1 Distributions of the wGRS and mean BMI by wGRS groups in the control population. The mean values of BMI were greater in higher wGRS categories (trend test $P=1.48\times10^{-3}$), for example, the mean BMI value of subjects in the highest wGRS decile was 1.72 kg/m² greater than individuals in the lowest decile.

Table 1 Effect estimates for associations of genetic instruments with BMI and gastric cancer risk

SNP	Description	Nearby gene	Alleles (1/2) [c]	EAF	P_{HWE}	SNP-BMI association[a]			EAF in cases	EAF in controls	SNP-gastric cancer association[b]		
						β	SE	P			OR	95% CI	P
rs1514175	chr1:g.74991644A>G	TNNI3K	A/G	0.75	0.641	0.081	0.048	0.093	0.77	0.77	1.01	0.93-1.09	0.883
rs2815752	chr1:g.72812440A>G	NEGR1	A/G	0.92	0.964	−0.006	0.077	0.935	0.92	0.91	1.07	0.94-1.21	0.295
rs574367	chr1:g.177873210G>T	SEC16B	T/G	0.20	0.890	0.047	0.053	0.368	0.22	0.20	1.11	1.02-1.21	0.017
rs12463617	chr2:g.629244C>A	TMEM18	C/A	0.91	0.359	0.126	0.075	0.096	0.90	0.90	1.05	0.93-1.18	0.418
rs1861411	chr2:g.58904177G>A	FANCL	A/G	0.40	0.258	0.032	0.043	0.466	0.41	0.41	1.02	0.95-1.09	0.623
rs6545814	chr2:g.25131316A>G	ADCY3/RBJ	G/A	0.43	0.585	0.054	0.043	0.206	0.43	0.42	1.05	0.98-1.12	0.194
rs10513801	chr3:g.185822353T>G	SFRS10/ETV5	T/G	0.97	0.940	0.125	0.121	0.301	0.97	0.97	1.17	0.95-1.43	0.141
rs2535633	chr3:g.52859630C>G	ITH4	G/C	0.39	0.321	−0.009	0.042	0.832	0.50	0.52	0.98	0.91-1.05	0.573
rs16858082	chr4:g.45175804C>T	GNPDA2	T/C	0.36	0.706	0.052	0.043	0.231	0.34	0.35	0.96	0.89-1.03	0.268
rs261967	chr5:g.95850250A>C	PCSK1	C/A	0.43	0.223	0.053	0.043	0.219	0.43	0.43	1.02	0.95-1.10	0.537
rs888789	chr5:g.124317011G>A	FLJ35779	A/G	0.48	0.078	0.075	0.041	0.064	0.46	0.46	0.99	0.92-1.06	0.722
rs6890814	chr5:g.124310278G>C	ZNF608	C/G	0.48	0.889	−0.015	0.042	0.718	0.48	0.48	0.99	0.93-1.07	0.876
rs4713766	chr6:g.34244245C>A	NUDT3	A/C	0.09	0.044	−0.040	0.070	0.564	0.10	0.09	1.05	0.93-1.18	0.431
rs9356744	chr6:g.20685486T>C	CDKAL1	T/C	0.58	0.339	−0.051	0.043	0.235	0.59	0.59	1.03	0.95-1.10	0.485
rs9473924	chr6:g.50834157G>T	TFAP2B	T/G	0.27	0.022	0.032	0.049	0.518	0.28	0.28	0.98	0.91-1.06	0.610
rs17150703	chr8:g.9745798G>A	MSRA	G/A	0.84	0.930	0.107	0.057	0.061	0.86	0.85	1.00	0.90-1.10	0.944
rs4735692	chr8:g.76615663G>A	HNF4G	A/G	0.38	0.424	0.043	0.044	0.323	0.41	0.40	1.02	0.95-1.10	0.518
rs11142387	chr9:g.72998332A>C	KLF9	C/A	0.30	0.454	0.061	0.045	0.177	0.32	0.31	1.06	0.98-1.14	0.127
rs1211166	chr9:g.87285992A>G	NTRK2	A/G	0.78	0.879	−0.037	0.050	0.46	0.78	0.78	1.02	0.94-1.11	0.636
rs11191580	chr10:g.104906211T>C	NT5C2	C/T	0.32	0.994	0.027	0.045	0.554	0.27	0.29	0.92	0.85-1.00	0.043
rs10160804	chr11:g.8600240C>A	RPL27A	A/C	0.47	0.127	0.028	0.041	0.493	0.46	0.46	0.99	0.92-1.06	0.716
rs11030104	chr11:g.27684517A>G	BDNF	A/G	0.49	0.808	0.098	0.042	0.019	0.53	0.52	1.02	0.95-1.09	0.613
rs11604680	chr11:g.47457539A>G	MTCH2	G/A	0.33	0.670	−0.008	0.044	0.858	0.32	0.32	0.97	0.90-1.04	0.423
rs2237892	chr11:g.2839751C>T	KCNQ1	T/C	0.34	0.392	−0.032	0.045	0.478	0.32	0.32	1.00	0.93-1.08	0.950
rs671	chr12:g.112241766G>A	ALDH2	G/A	0.73	0.386	0.032	0.046	0.486	0.82	0.80	1.12	1.02-1.22	0.013
rs897057	chr12:g.50266279C>T	FAIM2	C/T	0.75	0.623	−0.063	0.059	0.285	0.80	0.80	1.04	0.95-1.13	0.407
rs7989336	chr13:g.97017548G>A	HS6ST3	A/G	0.49	0.415	−0.036	0.042	0.397	0.50	0.49	1.03	0.96-1.11	0.346
rs9568867	chr13:g.54107352G>A	OLFM4	A/G	0.23	0.353	0.081	0.049	0.102	0.25	0.24	1.03	0.95-1.11	0.457
rs4776970	chr15:g.68080886T>A	MAP2K5	A/T	0.22	0.867	0.021	0.051	0.676	0.23	0.23	1.05	0.97-1.14	0.257
rs1558902	chr16:g.53803574T>A	FTO	A/T	0.12	0.803	0.083	0.064	0.195	0.12	0.12	1.04	0.93-1.16	0.474
rs2531995	chr16:g.40134670C>T	ADCY9	T/C	0.32	0.480	0.007	0.045	0.886	0.35	0.33	1.05	0.98-1.13	0.180
rs4788102	chr16:g.28873398G>A	SH2B1	A/G	0.11	0.464	0.110	0.070	0.115	0.16	0.15	1.08	0.98-1.18	0.129
rs7503807	chr17:g.78591111A>C	RPTOR	A/C	0.70	0.239	−0.084	0.046	0.072	0.69	0.69	1.00	0.93-1.08	0.939
rs9299	chr17:g.46669430C>T	HOXB5	T/C	0.54	0.206	0.101	0.043	0.019	0.52	0.54	0.90	0.84-0.97	0.004
rs591166	chr18:g.57841589T>A	MC4R	A/T	0.20	0.918	−0.055	0.052	0.299	0.24	0.22	1.08	0.99-1.17	0.071
rs11671664	chr19:g.46172278G>A	GIPR/QPCTL	G/A	0.54	0.388	−0.012	0.042	0.767	0.54	0.55	0.97	0.91-1.04	0.431
rs3810291	chr19:g.47569003G>A	TMEM160	A/G	0.28	0.742	−0.069	0.046	0.134	0.30	0.29	1.02	0.94-1.10	0.627
Unweighted GRS, per SD						0.084	0.029	4.37×10^{-3}			1.05	1.00-1.11	0.037
Core wGRS, per SD						0.077	0.029	9.43×10^{-3}			1.06	1.01-1.12	0.013
wGRS, per SD						0.091	0.029	2.17×10^{-3}			1.07	1.02-1.13	4.94×10^{-3}
MR (IVW)[d]						—	—	—			1.07	1.02-1.12	5.50×10^{-3}

Abbreviations: BMI, body mass index; CI, confidence interval; EAF, effect allele frequency; GRS, genetic risk score; HWE, Hardy–Weinberg equilibrium; IVW, inverse-variance weighted; MR, Mendelian randomization; OR, odds ratio; SD, standard deviation; SNP, single nucleotide polymorphism; SE, standard error; wGRS, weighted genetic risk score.
[a]Results (EAF, β, SE and P) were derived from population-based controls in our study and were adjusted for age, sex and the first principal component.
[b]Results (EAF, β, SE and P) were derived from pooled-analysis of the Nanjing/Beijing study and the NCI study and were adjusted for age, sex, study site and the first principle component.
[c]Alleles (1/2), Allele1 (effect allele) associated with increased BMI in the initial study; Allele2, reference allele.
[d]The effect estimate was scaled to per SD to make it comparable to the wGRS method.

logistic regression model, we did not observe a non-linear association between the BMI wGRS and risk of gastric cancer (P for non-linearity $= 0.709$), suggesting a potential linear relationship. As shown in Table 1, logistic regression analysis indicated that higher genetically predicted BMI was associated with increased risk of gastric cancer (per SD increase in the wGRS: OR $= 1.07$, 95% CI: 1.02–1.13, $P = 4.94 \times 10^{-3}$). Compared with individuals in the bottom tertile of the BMI wGRS, those in the top tertile had a 1.14-fold (95% CI: 1.01–1.29) increased risk of developing gastric cancer (trend test $P = 0.031$) (Table 2). Subgroup analyses by sex showed similar effect size for men (OR $= 1.07$, 95% CI: 1.01–1.14, $P = 0.016$) and for women (OR $= 1.08$, 95% CI: 0.98–1.19, $P = 0.113$), though the later was not statistically significant (P for heterogeneity $= 0.889$). Stratification analyses by age group and study site did not reveal significant heterogeneity between different strata (Supplementary Table 3).

Gastric cancer risk estimates using individual variants and genetic risk score heterogeneity

To determine if an individual variant used in our wGRS drove this result, we calculated effect estimates for all the SNPs on gastric cancer risk. As listed in Table 1, 27 out of 37 variants showed a positive and

Table 2 Associations of categories of genetic risk score (wGRS) predicting between BMI and gastric cancer risk

wGRS categories	Cases, n (%)	Controls, n (%)	OR	95% CI	P^a
T1	810 (30.79)	1456 (33.30)		Ref.	
T2	887 (33.71)	1459 (33.36)	1.09	0.97–1.23	0.153
T3	934 (35.50)	1458 (33.34)	1.14	1.01–1.29	0.031
P for trend					0.031

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; Ref, reference; wGRS, weighted genetic risk score.
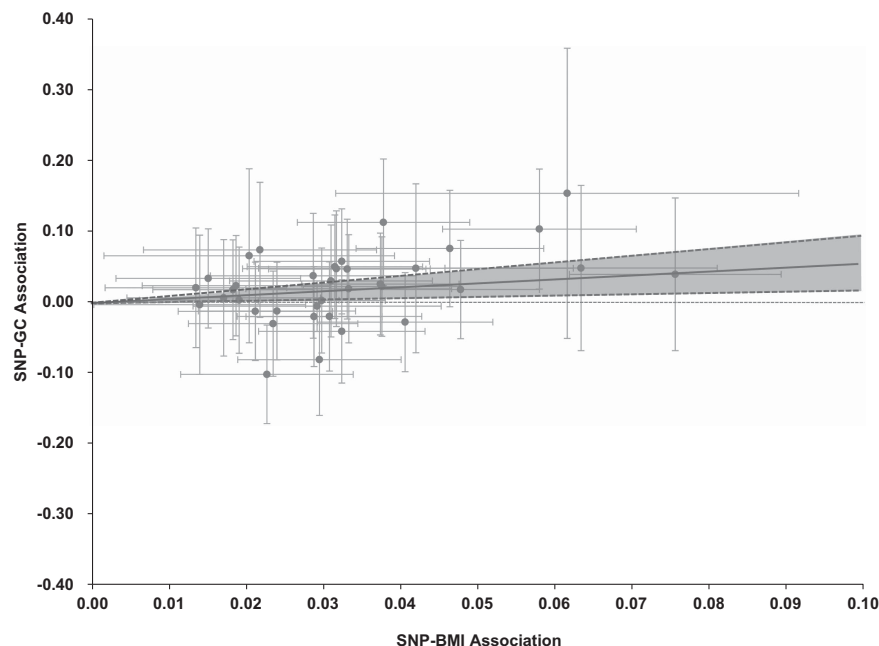[a]Adjusted for age, sex, study site, and the first principle component.

consistent association between the allele-increasing BMI and gastric cancer, and only two were statistically significant. Cochran's Q test did not detect evidence for heterogeneity ($P = 0.232$), suggesting the effect estimated from the wGRS was not driven by outlier genetic variants that have strong effects on gastric cancer risk.

In addition to the wGRS approach, we also estimated the potential causal effect of BMI on gastric cancer using the MR IVW method with summary statistics of each genetic variant. The potential causal effect of each variant was evaluated using the Wald estimator, and the combined effect estimate was calculated using the IVW method (Supplementary Table 4). As shown in Figure 2, a similar positive association of BMI with gastric cancer risk was observed compared with that using the wGRS (OR $= 1.07$, 95% CI: 1.01–1.13, $P = 5.50 \times 10^{-3}$).

Sensitivity analysis for BMI genetic instruments using alternative causal inference methods

We then performed MR Egger regression analyses to evaluate the potential pleiotropic effect of the genetic instruments. We did not detect bias for the BMI wGRS ($P = 0.181$) (Supplementary Figure 2). Notably, the direction of estimated bias was negative, suggesting that putative bias present across the selected genetic variants resulted in an underestimation of the causal effect on gastric cancer risk.

To determine if the analyses was robust to the choice of weights used in the genetic risk score analysis, we evaluated the causal association of BMI and gastric cancer risk using an unweighted genetic risk score. As expected, the association attenuated but remained statistically significant (per SD: OR $= 1.05$, 95% CI: 1.00–1.11, $P = 0.037$). The OR per $5 kg/m^2$ increase in BMI was 1.03 (95% CI: 1.00–1.06). Moreover, we constructed a core wGRS based exclusively on 15 SNPs exhibiting genome-wide significant associations with BMI in East Asian populations, and found that the core

Figure 2 Plots of the effect size and 95% CI of each SNP on BMI and gastric cancer risk. The X axis plots the previously published β-estimate for the association of each SNP with BMI. The Y axis plots the β-estimate from the multivariable logistic regression model for the association of each SNP with gastric cancer risk in our study population. Error bars indicate 95% CIs of the effect estimate. A regression line with 95% CIs is plotted using the MR IVW method.

wGRS for BMI was also significantly associated with gastric cancer risk (per SD: OR = 1.06, 95% CI: 1.01–1.12, P = 0.013).

DISCUSSION

In the current study, we took advantage of previously identified genetic variants that are associated with BMI in East Asian populations and applied the MR approach to examine the relationship between genetically predicted BMI and gastric cancer risk. We found that per SD increase in the BMI wGRS was associated with a 7% (95% CI: 2–12%) increase in gastric cancer risk. Sensitivity analyses using alternative causal inference measures demonstrated consistent association, suggesting the robustness of our primary findings. Our study provides the first evidence of a potential causal effect of high BMI on gastric cancer risk, which is in line with the evidence obtained from a recent population-based cohort study of 5.24 million UK adults that found a 5 kg/m^2 increase in BMI was associated with a 8% increased risk of gastric cancer among non-smokers.[8]

Although our MR analyses indicate a potential causal relationship between BMI and gastric cancer risk, the underlying mechanism by which elevated BMI increases gastric cancer risk remains unclear. Several possible explanations have been proposed. First, obesity is recognized as a low-grade chronic inflammatory state that leads to elevated levels of pro-inflammatory factors, such as tumor necrosis factor-alpha, interleukin-6 and C-reactive protein, which may promote cancer development.[29] Moreover, accumulation of adipose tissue also leads to the deregulation of insulin and insulin growth factor-1 signaling pathways, increased production of sex hormones and upregulated levels of adipokines, such as leptin and adiponectin, which may further promote cancer cell growth and impair apoptosis.[29] Finally, obesity also promotes gastroesophageal reflux, which may be caused by elevated intra-abdominal pressure, and its transition to the potentially precancerous state of Barrett's esophagus.[30] Though these explanations are biologically plausible, further work will be required to elucidate the exact mechanisms by which adiposity confers the increased risk of gastric cancer.

A recent meta-analysis of prospective observational studies of BMI and gastric cancer risk reported a statistically significant association for men (OR = 1.27, 95% CI: 1.09–1.48), but not for women (OR = 1.04, 95% CI: 0.79–1.39).[18] In the current study, we also observed a significant association of genetically predicted BMI with gastric cancer risk in men, but not in women. Previous studies have suggested that estrogen may be responsible for the gender differences in morbidity and mortality observed in gastric cancer. For example, women with delayed menopause and increased fertility have a lower risk of gastric cancer, and drugs which block estrogen activity, such as Tamoxifen, are linked to higher rates of gastric cancer in women.[31] *In vivo* studies also demonstrated that treating mice with estrogen dramatically lowers their rates of gastric cancer caused by chronic *Helicobacter pylori* infection.[32] It is possible that estrogen may protect women from the low-grade chronic inflammation state associated with obesity. Nevertheless, because the point estimates for women were of similar order and in the same direction as that seen for men, we cannot rule out a causal relationship between BMI and gastric cancer risk for women. Because the proportion of women in our study subjects was relatively small, it is possible that we did not observe evidence of a causal effect for women due to lack of statistical power. Studies with a larger sample size of women are warranted to elucidate the effect of BMI on gastric cancer risk in women.

In addition, findings from the observational studies have consistently reported positive association of BMI with gastric cardia cancer, while evidence remains inadequate for non-cardia cancer.[4,18,33,34]

Unfortunately, in the current study, we did not perform stratification analysis by gastric cancer subsite, because we did not obtain subsite information from the NCI study. However, we found evidence of association between genetically predicted BMI and non-cardia cancer in the Nanjing/Beijing study where all the cases were non-cardia gastric cancer patients.

MR analyses rely on three key assumptions. The first one is that the instrumental variable is associated with exposure of interest. Because each SNP only explained a small amount of variance in BMI, we used multiple genetic variants and created a wGRS to better proxy our exposure of interest, which reduced the chance of weak instrument bias. As the current genetic loci identified by GWASs only explain a small amount of variance in BMI,[19] we were not able to test the full spectrum of genetically influenced BMI. Further insight may be available from whole-genome sequence analysis, though they are unlikely to substantially increase the variance explained at present. The second assumption is that the instrumental variable is not associated with potential confounders of the exposure-outcome association. Because genotype is presumed to be allocated randomly at conception, covariates are anticipated to be randomly distributed with respect to genotype. Accordingly, we did not observe associations of wGRS with age, gender, or study site. The third assumption is that no other pathway exists between the instrumental variable and the outcome. It is possible that some SNPs used to derive the wGRS are associated with BMI as well as other factors if BMI is causally associated with these secondary traits (vertical pleiotropy). It is also possible that a genetic variant is associated with multiple pathways, including those not involving BMI (horizontal pleiotropy). In the current study, we performed MR Egger regression, which did not detect bias for the BMI wGRS. Sensitivity analyses using alternative causal inference measures demonstrated consistent association. In addition, because the analyses were restricted to participants of Chinese ancestry, our results may not necessarily apply to other races. However, this fact also minimizes the risk of population stratification affecting the results of our instrumental variable analyses. Because the coefficients used to construct the BMI wGRS were derived from rank-based INT-transformed BMI data, we could only calculate OR per 5 kg/m^2 change in BMI using the unweighted GRS as the genetic instrument. The predicted association, a 3% increase in gastric cancer risk per 5 kg/m^2 increase, in BMI was smaller than that estimated in the observational study using measured BMI.[8] However, this point estimate may not be appropriate or correct, since the sample size of our control population with BMI data available was small, and the BMI values did not follow normal distribution. Moreover, the unweighted GRS could only explain very small amount of variance in BMI. Nevertheless, this did not reduce the significance of the current study, which provided genetic evidence for the causal relationship between high BMI and gastric cancer risk.

In summary, our analyses provide evidence for a possible causal association between BMI and gastric cancer risk, suggesting that high BMI plays a role in the etiology of gastric cancer. However, sex- and subsite-specific effects of BMI on gastric cancer risk require further investigation.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)