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Gene-environment interactions and the risk of Barrett's Esophagus in three US cohorts

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Abstract

Background: Several single nucleotide polymorphisms (SNPs) have been associated with Barrett's esophagus (BE) risk. Additionally, environmental factors including smoking, alcohol consumption and heartburn increase BE risk. However, data on potential interactions between these genetic and environmental factors on BE risk are scant. Understanding how genes and environmental risk factors interact may provide key insight into the pathophysiology of BE, and potentially identify opportunities for targeted prevention and treatment.

Objectives: To examine the main effects and the potential effect-modification between known genetic loci (SNPs) and established environmental risk factors for BE.

Patients and Methods: We performed a nested case-control study using data on 401 incident BE cases and 436 age-matched controls from the Nurses' Health Study, Nurses' Health Study II, and Health Professionals Follow-up Study cohorts, who gave blood and completed biennial questionnaires. Overall, we genotyped 46 SNPs identified in previous BE GWAS as well as SNPs in candidate genes related to BE susceptibility (i.e., related to excess body fat, fat distribution, factors associated with insulin resistance, and inflammatory mediators). A genetic risk score

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(GRS) was constructed to evaluate the combined effect of the selected SNPs on BE risk. Interactions between SNPs and BE risk factors were also assessed.

Results and Conclusions: We observed a suggestive, but not statistically significant, association between our GRS and BE risk: a one-allele increase in the unweighted GRS increased the risk of Barrett's esophagus by a factor of 1.20 (95%CI=1.00–1.44; p=0.057). We did not observe any meaningful multiplicative interactions between smoking, alcohol consumption or heartburn duration and BE genotypes. When we assessed the joint effect of weighted GRS and BE risk factors, we did not observe any significant interaction with alcohol and heartburn duration, while smoking showed a significant multiplicative interaction (p=0.016).

Keywords

smoking; alcohol; heartburn; genetic susceptibility; gene-environment interactions; Barrett's esophagus

INTRODUCTION

Barrett's esophagus (BE), a condition marked by specialized intestinal metaplasia of the esophageal mucosa in response to gastroesophageal reflux, is associated with an increased risk of esophageal adenocarcinoma.[1] However, the relatively high prevalence of BE in industrialized nations (~1–2%)[2–4], coupled with the low absolute annual risk (~0.1%) of esophageal adenocarcinoma in the setting of BE[5], has caused a softening of guidelines that call for routine screening for, and surveillance of, this condition.[2] Moreover, a significant percentage of patients presenting with esophageal adenocarcinoma have no symptoms of gastroesophageal reflux, and thus would not necessarily trigger a screening endoscopy.[6] This has led many to seek risk scores or predictive models, either for BE itself or to identify cases more likely to progress to cancer, to better identify individuals who might benefit from screening in a more cost-effective manner.[7]

A genetic susceptibility to BE was first suggested by familial clustering of cases, including data supporting an autosomal dominant pattern of inheritance in some families.[8] More recently, genetic linkage studies and genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in several genes that may confer greater risk for both BE and esophageal adenocarcinoma,[9–13] and which can also be combined to build a polygenic risk score for BE.[14]

In addition to genetic risk factors, environmental and lifestyle risk factors have been shown to contribute to BE risk. Several common, modifiable factors have been associated with BE, such as body mass index (BMI),[15] smoking,[16] alcohol consumption,[17] and heartburn duration.[18]

However, data on potential interactions between these genetic and environmental factors on BE risk are scant.[19] Understanding how genes and environmental risk factors interact may provide key insight into the pathophysiology of BE and may identify opportunities for targeted prevention and treatment.[13]

We therefore examined the main effects and the potential effect-modification between known BE genetic loci (SNPs) and established environmental risk factors for BE in 401 incident BE cases and 436 age-matched controls from the Nurses' Health Study, Nurses' Health Study II and Health Professionals Follow-up Study cohorts. In addition, we also developed a genetic risk score (GRS) for BE based on previously-identified susceptibility loci. This polygenic risk score may in the future be incorporated in risk-assessment scores, or more realistically, may inform on biological mechanisms underlying BE development and progression to dysplasia/cancer.

METHODS

Study Populations

The Nurses' Health Study (NHS) is a prospective cohort study of 121,700 female registered nurses in 11 US states aged 30–55 years at cohort inception in 1976 (<http://www.channing.harvard.edu/nhs/>). Participants complete biennial questionnaires to update information on demographic characteristics, lifestyle factors, and newly diagnosed diseases. Between 1989 and 1990, overall 32,826 NHS cohort participants provided blood samples. Details of the blood collection and archival methods have been described previously.[20] The Nurses' Health Study II (NHS-II) cohort was established in 1989 to recruit a population younger than the original NHS cohort. This prospective cohort includes 116,686 female registered nurses from 15 US states, aged 25–44 years at cohort inception, who complete follow-up using methodology similar to the NHS. Between 1996–1999, overall 29,612 NHS-II cohort participants provided blood samples. The Health Professionals Follow-up Study (HPFS) is a prospective cohort study of 51,529 US men aged 40–75 years at cohort inception who enrolled in 1986 (<https://www.hsph.harvard.edu/hpfs>). Biennial follow up is similar to the NHS and NHS-II. Between 1993 and 1999, overall 18,159 HPFS cohort participants provided blood samples. The demographic and risk factor characteristics of the participants from all three cohorts who provided blood samples are very similar to those of the cohorts overall.[21]

The NHS and NHS-II study protocols were approved by the Human Research Committee of Partners Healthcare, and the HPFS study protocol was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health, with informed consent from all participants.

Barrett's esophagus ascertainment and case-control selection

The present study includes BE cases diagnosed between 1976–2012 in NHS; 1989–2012 in NHS2; 1986–2012 in HPFS. All potential BE cases were initially identified based on the nurse's or health professional's self-reported diagnosis, and then verified through review of medical records by study physicians (please see Supplementary Materials for details on BE ascertainment).

BE cases and matched controls were selected from NHS, NHS-II and HPFS participants who gave a blood sample. Regarding BE cases, we included all documented incident cases through 2012, and excluded BE cases with a prior history of malignancy (other than non-

melanoma skin cancer). Eligibility for selection as a control required an available blood sample, a prior upper gastrointestinal endoscopy and no cancer diagnosis at the time the matched case was diagnosed. We randomly selected one (NHS, HPFS) or two (NHS2) controls matched to each case on year of birth, gender, month/year of blood draw, and diabetes status.

Non-genetic (lifestyle) exposures

Body Mass Index (BMI), smoking, alcohol consumption and heartburn assessment were based on self-report questionnaires that were completed every two years. Self-reported lifestyle exposures in our cohorts have been shown to be highly accurate in our cohorts (please see Supplementary Materials for details on non-genetic lifestyle exposures ascertainment).

Genetic Exposures

SNP selection: We genotyped SNPs identified in previous BE GWAS as well as SNPs in candidate genes related to BE susceptibility. Based on the Catalog of Published Genome-Wide Association Studies[22] and previously published GWAS,[9–12] we identified SNPs that have been associated with an increased risk of both BE and esophageal adenocarcinoma on a genome-wide significance level (5×10^{-8}). Moreover, SNPs in candidate genes in pathways related to excess body fat, fat distribution, factors associated with insulin resistance, and inflammatory mediators have been genotyped. Specifically, SNPs in insulin-like growth factor 1 (IGF-1), IGF binding proteins IGFBP-1, -2 and -3, adiponectin gene (ADIPOQ) and its receptor (ADIPOR1, ADIPOR2), leptin, and ABO blood type were genotyped.[23–44] After reviewing the literature, a total of 56 SNPs were found to be associated with BE susceptibility either from GWAS or candidate-gene approach studies. After excluding ten SNPs (two monomorphic, eight not in H-W equilibrium), we included a total of 46 SNPs in our study. In Supplementary Table 1 we include a list of all the genotyped SNPs and their association with BE.

DNA extraction: DNA was extracted in 96-well plate format. 50ul of buffy coat were diluted with 150ul of PBS and processed via the QIAmp™ (QIAGEN Inc., Chatsworth, CA) 96-spin blood kit protocol. The average yield from 50ul of buffy coat was 5.5ug with a standard deviation of 2.2 (range 2.0–16.4).

Genotyping: SNP were genotyped by Taqman SNP allelic discrimination, based on 5' nuclease allelic discrimination using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) adapted to 384-well format. Overall, 98% of DNA samples were successfully genotyped after questionable calls were repeated; quality control (QC) analyses showed almost perfect concordance.

Genetic risk score (GRS): We first assessed the main effects of individual SNPs on BE risk in our data. Then, to maximize power to detect any potential interactions, and to test if there was an effect of the overall genetic burden in BE risk, we constructed a GWAS-based GRS. For the main analysis, the SNPs that reached genome-wide significance (10^{-8}) in the original studies,[9–12] and were successfully genotyped in our dataset, were considered for

inclusion in the GRS. Monomorphic SNPs (N=2), as well as SNPs that did not follow Hardy-Weinberg equilibrium in controls (N=8), were excluded, leaving a total of 3 SNPs in the main GRS. We defined this as GRS_B to denote that this GRS is composed of SNPs that have been significantly associated with BE in prior studies. For our primary analysis of the GWAS-based GRS_B, we used SNP-specific weights based on the beta coefficients reported in the original landmark studies by Su *et al.* [10] (for rs8257809 and rs9936833), and by Levine *et al.* [11] (for rs2687201). We also assessed an unweighted GRS_B where each SNP was assigned the same weight. Additionally, we assessed an unweighted GRS including all SNPs included in the study, both from candidate genes and GWAS studies, regardless their level of significance. After excluding those that were not in HW equilibrium in controls and monomorphic SNPs, a total of 46 SNPs were included. We defined this as GRS_C to denote that this GRS is composed of the complete SNPs considered for our study, including those from candidate genes. To reduce bias, only participants without any missing data in any of the SNPs were included in the all GRS analyses.

Statistical analysis:

We performed logistic regression to determine the associations between BMI, smoking, alcohol consumption, heartburn duration, individual SNPs, and BE risk. We calculated multivariable adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI). We assessed multiplicative interactions between SNPs and BE risk factors by including an interaction term for each SNP and BE risk factor. We also created models to assess the association between GRS and BE risk. All analyses were adjusted for cohort (NHS, NHS-II, HPFS). All reported P-values are two sided, and an α level of 0.05 was used to define statistical significance. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC) and R package (R Foundation, Vienna, Austria).

RESULTS

Through 2012 we identified a total of 401 incident BE cases and 436 controls from the NHS (196 BE cases and 194 controls; female nurses), NHS-II (35 cases and 72 controls; female nurses) and HPFS cohorts (170 cases and 170 controls; male health professionals). Participants had complete information on anthropometric, socio-demographic, and lifestyle characteristics, as well as high quality genetic data available.

Table 1 shows the main effects of environmental BE risk factors in the study population. After adjusting for cohort, we observed an increased risk of BE in current smokers compared to never smokers (OR=1.65, 95% CI=1.01–2.72). We did not observe an association between BMI and BE risk: compared to participants with healthy weight (BMI<25), the OR for overweight participants (BMI 25.0–29.9) was 1.09 (95%CI = 0.80–1.48), and OR=0.99 (95%CI=0.61–1.58) for obese participants (BMI ≥ 30.0) (Table 1). A non-significant increased risk of BE was seen in the subset of overweight and obese women from the NHS cohort only, but not in NHS-II nor HPFS participants (results not shown). Alcohol drinking was positively associated with BE risk, compared to non-drinkers, the OR for those who drink less than 20 grams of alcohol per day was 1.58 (95% CI=1.10–2.27), OR=1.91 (95% CI=1.09–3.35) for those drinking between 20 and 40 grams per day, and

OR=2.30 (95% CI=1.05–5.16) for those drinking over 40 grams per day (Table 1). We observed a strong positive association between heartburn duration and risk of BE: the longer the heartburn symptoms were present, the higher the risk of BE. Compared to participants who never had reflux, the OR for those with heartburn during 1 to 5 years was 1.69 (95% CI=1.18–2.43); OR=3.90 (95% CI=2.48–6.21) for those participants who had heartburn for 5 to 15 years, and OR=4.80 (95% CI=3.10–7.54) for participants with heartburn for more than 15 years (Table 1).

Table 2 presents the associations between each SNP included in the GRS_B and BE risk. These include the GWAS hits that reached significance for association with BE in the original study and were successfully genotyped in our dataset. Out of the three SNPs, only the SNP rs2687201 (an A/C single-nucleotide variation on human chromosome 3p14 near the transcription factor FOXP1, which regulates esophageal development), was associated with BE risk in our data ($p=0.019$). Supplementary Table 1 presents the main effect of all the 46 SNPs from either candidate genes or GWAS approaches, on BE risk. Only two SNPs, rs2687201 and rs732392 (a T/G single nucleotide variation on chromosome 11 close to GALNT18 that is involved in the pathway protein glycosylation), showed a nominally-significant association with BE risk in our data. However, these associations did not remain statistically significant when corrected for multiple testing.

Table 3 shows the association between the GWAS-based GRS_B and BE risk. In the multi-variable adjusted model, a one-allele increase in the unweighted GRS_B increased the risk of BE by a factor of 1.20 (95% CI=1.00–1.44; $p=0.057$). In the weighted GRS_B , the increased risk per unit of the score (which takes into account the number of alleles and the magnitude of its effects) was 3.13 (95% CI=0.95–10.45). The OR for each standard deviation of the weighted GRS_B was 1.20 (95% CI=0.99–1.45; $p=0.062$). We additionally built an unweighted GRS_C including a total of 46 SNPs from candidate genes and GWAS approaches, and assessed the association with BE risk. We did not observe an increased risk of BE per allele in the score: OR=0.99 (95% CI=0.84–1.16).

Interactions between each individual candidate SNP and BE risk factors were also assessed. We did not observe any significant multiplicative interactions between smoking, alcohol consumption or heartburn duration and the individual SNPs, although the effects of genetic and environmental risk factors were additive (results not shown). Only the interaction between smoking and the SNP rs2687201 was suggestive, but non-statistically significant ($p=0.061$). In order to maximize the power to detect any potential interactions, and to test if there was an effect of the overall genetic burden in BE risk, we additionally assessed the joint effects of the weighted GWAS-based GRS_B and BE risk factors (Table 4). Table 4a presents the joint effect of smoking and the weighted GRS_B on BE risk. Using never smokers with a low weighted GRS_B (defined as below the median) as a reference, we observed a significant multiplicative interaction between smoking and the GRS_B as related to BE risk ($p=0.016$). While we did not observe an increased risk of BE in participants with either high weighted GRS_B (defined as above the median) alone or ever smokers with low genetic risk, there was a nearly 50% increased risk of BE in ever smokers with a high weighted GRS_B , even though this did not reach statistical significance (Table 4a). However, we found no significant multiplicative interactions between alcohol consumption (Table 4b),

or heartburn duration (Table 4c) and the weighted GRS_B as related to BE risk. In addition, we also assessed the joint effects of the unweighted GRS_B and BE risk factors (Supplementary Table 2a-c), and unweighted GRS_C and BE risk factors (Supplementary Table 3a-c), and did not observe any significant interaction.

DISCUSSION

We present a nested case control study of 401 incident BE cases and 436 controls from the well-characterized NHS, NHS-II and HPFS cohorts, assessing the main effects, as well as the gene-environment interactions between environmental factors and genetic susceptibility on BE risk. We confirmed the previously described associations between smoking, alcohol consumption and heartburn duration and BE risk in the subset of our cohorts with genetic data available. Two SNPs showed a nominal association with BE in our data: rs2687201 and rs732392 (Supplementary Table 1). The rs2687201 variant is located at 3p14, near the transcription factor FOXP1. This family of transcription factors is involved in a wide range of biological functions, including lung and esophageal development, as well as cancer development or suppression, depending upon context.[45] Less is known about the rs732392 variant, located on chromosome 11 close to genes GALNT18, involved in protein glycosylation and modification, and present ubiquitously in all organs. Although, after correcting for multiple comparisons, we did not find significant associations between individual SNPs and BE risk in our data, we found a suggestive, though non-statistically significant, association between our GWAS-based GRS_B and BE risk: a one-allele increase in the unweighted GRS_B increased the risk of Barrett's esophagus by a factor of 1.20 (95% CI=1.00–1.44; $p=0.057$). Finally, we did not observe any meaningful multiplicative interactions between smoking, alcohol consumption or heartburn duration and BE genotypes. However, when we examined the joint effect of GWAS-based weighted GRS_B and smoking, we did observe an interaction ($p=0.016$), even though none of the joint categories were themselves significant, probably due to low power. We did not observe any association or interaction when we examined the joint effect of GWAS-based GRS_B and alcohol and heartburn, nor did we observe any significant interaction between BE risk factors and unweighted GRS_B and GRS_C . These results provide insight into the relative contribution of genetic and lifestyle risk factors for BE and add to our understanding of both the epidemiology and pathophysiology of the disease.

Our findings are consistent with other published works on smoking[16], alcohol[17] and heartburn[18]. However, we were not able to replicate our previous findings with BMI[15], probably because of limited power from a smaller sample size. Another reason could be that our previous findings were observed exclusively in women, whereas this study included both genders. Since, waist circumference or waist-to-hip ratio[46], rather than BMI, has been more strongly associated with Barrett's esophagus in men, this may have diluted the effect of BMI in this mixed group of participants.

To our knowledge, only one other study from the BEACON consortium has examined the interaction between genetic factors and reflux, smoking and BMI for the risk of Barrett's esophagus and esophageal adenocarcinoma.[19] This study had a large sample size, but included only seven SNPs, and data on participants' characteristics were obtained

retrospectively. The overall conclusions of this study are compatible with ours. While rs2687201 modified the association between gastroesophageal reflux disease and risk of BE ($p=0.0005$), similar to our study, no interaction between the SNPs and smoking or BMI was observed and there was a suggestive, but non-statistically significant, interaction of rs2687201 with smoking ($p=0.065$). Our results are also comparable to the findings of another recent study within the BEACON consortium that developed a risk prediction model by combining non-genetic and genetic risk factors. The authors found that while a polygenic risk score was itself significantly associated with BE risk, its addition to a risk prediction model based on non-genetic factors did not offer a substantial additional prediction value to justify its use in clinical practice.[14]

Our study has numerous strengths. It is nested within three large and well-characterized cohorts with detailed prospective assessment of anthropometric, socio-demographic, and lifestyle characteristics. To our knowledge this is the first study that suggests that there might be an association between a GRS built using SNPs associated with BE at genome-wide significant levels, as well as interactions between GRS and environmental risk factors for BE. While these results were not statistically significant, they offer “signals” on the potential existence of an association which can be explored by future studies.. Furthermore, this study represents the first detailed exploration of interactions between environmental risk factors for BE and SNPs associated with risk factors for BE, such as SNPs associated with excess body fat, fat distribution, factors associated with insulin resistance, and inflammatory mediators.

We also recognize several limitations. First, since, large sample sizes are required to detect either the main effect of SNPs or gene-environment interactions, especially when ORs for the main effects are small, as can be the case for genetic variants, this may have limited our power to identify gene-environment interactions[47]. Thus, we cannot exclude that the lack of observed associations in our study could reflect false negatives results. Even though we were not able to replicate the main effects of GWAS hits in our dataset, the direction of the associations were consistent with previous literature.[9–12,48] In addition, we genotyped specific candidate SNPs based on the best available studies at the time of our analysis,[9–12] and thus have not been able to include some of the more recently discovered SNPs that are associated with BE.[13,14] However, differently from other studies we also included SNPs from candidate genes related to BE susceptibility. Second, our study included more females (60% of the total sample) than males. Considering that Barrett’s esophagus has a male predominance (male/female ratio of 2:1),[2] this factor could limit the significance and generalizability of these findings. Finally, our populations of health professionals tend to be somewhat healthier than the general population, and additionally, all study subjects included in the present study are of European ancestry, so our results may not generalize to other racial or ethnic groups. However, the homogeneity among NHS, NHS-II, and HPFS participants strengthens the internal validity of these findings by maximizing the quality of reported data.

In summary, in this study using data from three large and well-characterized US cohorts we assessed the main effects, as well as the gene-environment interactions, between environmental factors and genetic susceptibility on BE risk. Our results further support the

strong association between smoking, alcohol consumption and heartburn, and BE risk. Although we did not find significant associations between individual SNPs and BE risk, we showed that SNPs associated with BE at genome-wide significant levels can be combined into a GRS with a potential positive association with BE risk. Even though we did not observe any meaningful multiplicative interactions between smoking, alcohol consumption or heartburn duration and BE genotypes, the suggestive interaction between smoking and BE genotypes, as well as the interaction between smoking and the weighted GRS_B, suggests that further study of gene-environment interactions are required to improve our understanding of BE epidemiology and pathophysiology, and to better target prevention and screening to the most appropriate patients. These pilot results suggest that larger studies in this area are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

BE	Barrett's Esophagus
BMI	body mass index
CI	confidence interval
GRS	genetic risk score
GWAS	genome-wide association studies
HPFS	Health Professionals Follow-up Study
NHS	Nurses' Health Study
OR	odds ratio
SIM	specialized intestinal metaplasia
SNP	single nucleotide polymorphism

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STUDY HIGHLIGHTS

What is KNOWN

- Initial data suggest that there may be interactions between genetic and environmental factors that predispose to Barrett's esophagus (BE).

What is new here

- We developed a genetic risk score using SNPs known to be associated with BE at genome-wide significant levels
- The genetic risk score was marginally associated with the risk of BE, and showed a significant interaction with smoking, but not with other environmental factors
- We also explored for the first time the interaction between SNPs in candidate genes related to BE susceptibility and environmental risk factors
- Understanding how genes and environmental risk factors interact may provide key insight into the pathophysiology of BE, and potentially identify opportunities for targeted prevention and treatment.

Table 1.

Main effect of non-genetic risk factors on Barrett's Esophagus risk

	Controls (N=436)	Cases (N=401)	OR ^I	95% CI	p-value
Smoking					
Never	208 (48%)	168 (42%)	1.00	Reference	
Former	193 (44%)	189 (47%)	1.14	0.87–1.56	0.30
Current	35 (8%)	44 (11%)	1.65	1.01–2.72	0.048
BMI (kg/m²)					
<25	243 (56%)	216 (54%)	1.00	Reference	
25–29.9	148 (34%)	146 (36%)	1.09	0.80–1.48	0.56
30	45 (10%)	39 (10%)	0.99	0.61–1.58	0.95
Alcohol (g/day)					
No drinkers	103 (24%)	61 (15%)	1.00	Reference	
<20	283 (65%)	278 (69%)	1.58	1.10–2.27	0.014
20–40	36 (8%)	43 (11%)	1.91	1.09–3.35	0.024
>40	14 (3%)	19 (5%)	2.30	1.05–5.16	0.039
Heartburn Duration (years)					
0	173 (43%)	83 (22%)	1.00	Reference	
1–5	147 (36%)	116 (30%)	1.69	1.18–2.43	4.58×10 ⁻³
>5–15	42 (10%)	80 (21%)	3.90	2.48–6.21	6.45×10 ⁻⁹
>15	43 (11%)	101 (27%)	4.80	3.10–7.54	4.59×10 ⁻¹²

^I adjusted by age and cohort

Table 2.

Main effect of SNPs included in the GWAS-based Genetic Risk Score on Barrett's Esophagus

SNP	Controls	Cases	OR ^I	95% CI	p-value
rs2687201					0.019
C/C	179 (43%)	204 (52%)	1.00	Reference	
A/C	190 (45%)	144 (37%)	0.65	(0.48–0.88)	
A/A	52 (12%)	45 (11%)	0.78	(0.50–1.23)	
rs9257809					0.70
A/A	375 (88%)	341 (85%)	1.00	Reference	
A/G	49 (11%)	55 (14%)	1.19	0.79–1.81)	
G/G	3 (1%)	3 (1%)	1.00	(0.20–4.98)	
rs9936833					0.70
T/T	156 (37%)	155 (39%)	1.00	Reference	
C/T	201 (47%)	176 (44%)	0.88	(0.65–1.19)	
C/C	70 (16%)	70 (17%)	0.96	(0.64–1.43)	

^I adjusted by age and cohort

Table 3.

Association between the GWAS-based Genetic Risk Score and Barrett's esophagus risk

GRS	Controls	Cases	OR ^I	95% CI	p-value
Unweighted GRS _B	410	392	1.20	1.00–1.44	0.057
Weighted GRS _B	410	392	3.13	0.95–10.45	0.062

^I multivariable adjusted OR: cohort, smoking, alcohol, energy intake, and heartburn duration

Table 4a.

Joint effect of smoking and weighted GRS_B on Barrett's esophagus risk.

	Never Smokers	Ever Smokers
Low GRS_B	95 CO, 74 CA OR = 1.00 (REF)	107 CO, 96 CA OR = 0.80 95% CI: 0.47–1.35
High GRS_B	86 CO, 81 CA OR = 0.78 95% CI: 0.45–1.36	88 CO, 113 CA OR = 1.49 95% CI: 0.86–2.60

ORs adjusted by cohort, alcohol, energy intake, and heartburn duration

P-interaction = 0.016

Table 4b.

Joint effect of alcohol and weighted GRS_B on Barrett's esophagus risk.

	Low Alcohol (0–2.8 g/day)	High Alcohol (>2.8–81.7 g/day)
Low GRS_B	103 CO, 78 CA OR = 1.00 (REF)	97 CO, 92 CA OR = 1.04 95% CI: 0.61–1.77
High GRS_B	93 CO, 84 CA OR = 1.25 95% CI: 0.74–2.14	80 CO, 111 CA OR = 1.35 95% CI: 0.80–2.29

ORs adjusted by cohort, smoking, energy intake, and heartburn duration

P-interaction = 0.26

Table 4c.

Joint effect of heartburn duration and weighted GRS_B on Barrett's esophagus risk.

	Heartburn duration ≤ 5 years	Heartburn duration > 5 years
Low GRS_B	64 CO, 50 CA OR = 1.00 (REF)	43 CO, 81 CA OR = 2.55 95% CI: 1.52–4.33
High GRS_B	61 CO, 54 CA OR = 1.43 95% CI: 0.86–2.40	36 CO, 88 CA OR = 2.86 95% CI: 1.69–4.90

ORs adjusted by cohort, smoking, alcohol, and energy intake

P-interaction = 0.74

Note: all stratified analysis have been done using the weighted GWAS-based weighted GRS_B dichotomized based on the median