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Original Article

The effects of the interaction of genetic predisposition with lifestyle factors on bladder cancer risk

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Objectives

To investigate the association of polygenic risk score (PRS) and bladder cancer (BC) risk and whether this PRS can be offset by a healthy lifestyle.

Methods

Individuals with BC (n = 563) and non-BC controls (n = 483 957) were identified in the UK Biobank, and adjusted Cox regression models were used. A PRS was constructed based on 34 genetic variants associated with BC development, while a healthy lifestyle score (HLS) was constructed based on three lifestyle factors (i.e., smoking, physical activity, and diet).

Results

Overall, a negative interaction was observed between the PRS and the HLS (P = 0.02). A 7% higher and 28% lower BC risk per 1-standard deviation (SD) increment in PRS and HLS were observed, respectively. A simultaneous increment of 1 SD in both HLS and PRS was associated with a 6% lower BC risk. In addition, individuals with a high genetic risk and an unfavourable lifestyle showed an increased BC risk compared to individuals with low genetic risk and a favourable lifestyle (hazard ratio 1.55, 95% confidence interval 1.16–1.91; P for trend <0.001). Furthermore, population-attributable fraction (PAF) analysis showed that 12%–15% of the BC cases might have been prevented if individuals had adhered to a healthy lifestyle.

Conclusion

This large-scale cohort study shows that a genetic predisposition combined with unhealthy behaviours have a joint negative effect on the risk of developing BC. Behavioural lifestyle changes should be encouraged for people through comprehensive, multifactorial approaches, although high-risk individuals may be selected based on genetic risk.

Keywords

bladder cancer, polygenic risk score, healthy lifestyle, cohort study, gene–environment interaction

Introduction

Bladder cancer (BC) is the most common malignancy of the urinary tract and the seventh leading cause of death from cancer, with nearly 550 000 new diagnoses and 200 000 deaths per year worldwide [1]. Given its high frequency of recurrence, BC is reported to be among the most expensive life-time treatments of all cancers, resulting in burden to the healthcare system [2]. Therefore, early access and adequate provision of healthcare services are crucial for BC detection and better oncological control in the long run. The role of genetic susceptibility in BC carcinogenesis has been well recognized as an essential component in cancer research. Several studies have reported an familial aggregation, and an estimated familial risk of 30% was revealed [3,4], indicating the importance of genetic factors in the development of BC.

Over the past decade, large genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) related to BC [5]. These SNPs have a low to moderate effect on BC risk, with odds ratios (ORs) ranging from 1.1 to 1.6. A polygenic risk score (PRS) based

on SNPs is considered to be a useful and generally applicable tool to evaluate the inherited risk for each individual. For individuals who carry more than one risk allele, the PRS could calculate the cumulative effect of all genetic predisposition and, thereby, increase the predictability of incident BC [6]. Apart from genetics, studies demonstrated that lifestyle factors also play an essential role in BC development and a possible complex interplay between genetic and lifestyle factors [7]. There is considerable evidence that individuals who avoid smoking, who are physically active and who have a healthy diet have a lower BC risk [8–10]. Studies have combined lifestyle factors to create a composite lifestyle score to investigate the relationship between lifestyle factors and other health conditions, such as cardiovascular disease and diabetes [11]; however, research on the effect of such a healthy lifestyle score (HLS) and BC risk is currently insufficient. In addition, it is possible that the genetic risk can be offset by certain lifestyle factors. Studies that examined whether the risk of BC reduction was associated with adherence to a healthy lifestyle have varied based on genetic predisposition and thereby have yielded inconsistent results.

In this study, we used individual as well as summary data from the UK Biobank to establish the effect of both PRS and HLS on BC risk. In addition, the interaction between PRS and HLS and BC risk was assessed.

Methods and Materials

Study Population

The UK Biobank is a large prospective cohort consisting of more than 0.5 million participants aged 40–69 years and recruited across the UK between 2006 and 2010 [12]. At baseline, participants were required to complete a series of touch-screen questionnaires, provide biological samples and undergo various physical assessments.

The studies involving human participants were reviewed and approved by NHS National Research Ethics Service North West (11/NW/0382). All participants gave informed consent at recruitment.

The UK Biobank dataset for this project included 502 505 participants. Exclusion criteria included the withdrawal of informed consent, lack of genetic data or incomplete data for assessment of dietary intakes, and diagnosed BC at baseline. Finally, 484 520 individuals were eligible for analysis (Figure S1).

Ascertainment of Bladder Cancer

The definitions for BC cases are presented in Table S2. Our analysis was restricted to BC patients (corresponding International Classification of Diseases [ICD] codes C67.0, C67.1, C67.2, C67.3, C67.4, C67.5, C67.6, C67.7, C67.8, C67.9, D09.0 (ICD10) and 1880, 1882, 1884, 1886, 1888, 1889, 2337 [ICD9] and self-report/doctor diagnosis [code 1035 in field 20 001]). Hospital admission data were available for participants until August 2020.

Genotyping and SNP Selection

A detailed description of the genotyping process, imputation, and quality control in the UK Biobank study has been published elsewhere [13]. Briefly, the SNPs were genotyped using the custom UK Biobank Lung Exome Variant Evaluation Axiom (807, 411 markers) or the UK Biobank Axiom array (825 927 markers) and then imputed using merged UK10K and 1000 Genomes Project Phase 3 panels as the reference panel. We selected 34 SNPs representative of loci associated with BC (Table S1) based on the GWAS-Catalogue (https://www.ebi.ac.uk/gwas/). All the included SNPs were identified in the UK Biobank, and no proxy SNPs were used.

Calculation of the Polygenic Risk Score for Bladder Cancer

A previously described weighted method was used to calculate the PRS for BC based on the 34 selected SNPs [14]. Each SNP was weighted by its relative effect size (β-coefficient). We used β-coefficients derived from GWAS-Catalogue to obtain more precise effect sizes of these SNPs on BC. The PRS was calculated using the following equation, where SNPi (i = 1, 2,..., 34) is the risk allele number of each SNP.

$$PRS = \beta 1 * SNP1 + \beta 2 * SNP2 + \ldots\ldots + \beta 34 * SNP34 * 34/sum\ of\ the\ \beta - coefficients.$$

The calculated PRS ranged from 3.65 to 30.50 in the UK Biobank. A higher PRS indicates a higher genetic predisposition to BC.

Assessment of Healthy Lifestyle Score and Covariates

An HLS was constructed based on three well-established BC risk factors (smoking, physical activity and diet) [8–10], assessed at baseline using a touch-screen questionnaire. Participants scored one point for each of three healthy behaviours defined based on national recommendations (full details in Table S3) [15]. Smoking was categorized as smoking or non-smoking; smoking was scored as 0 while non-smoking was scored as 1. Regular physical activity was defined as meeting the American Heart Association recommendations of at least 150 min of moderate activity per week or 75 min of vigorous activity per week (or an equivalent combination) or engaging in moderate physical activity at least 5 days a week or vigorous activity once a

week [16]. The individuals who met the criteria of regular physical activity were scored as 1, while those who failed to meet the criteria were scored as 0. Healthy diet was based on intakes of at least four of seven commonly eaten food groups according to recommendations on dietary priorities for human health [17], individuals who met the criteria of healthy diet consumption were scored as 1, while those who failed to meet the criteria were scored as 0. Taking the three components of healthy lifestyle together, the unweighted HLS ranged from 0 to 3, with higher scores indicating higher adherence to healthy lifestyle, and was categorized as unfavourable (no healthy lifestyle factors), moderate (one healthy lifestyle factor), intermediate (two healthy lifestyle factors), and favourable (three healthy lifestyle factors) lifestyles. A weighted HLS was then derived based on the β-coefficients of each lifestyle factor in the multivariate Cox regression model with all three lifestyle factors and adjustment for age, sex, and assessment centre (22 categories), according to the following equation. In this study, the unweighted lifestyle score was used in the main analysis, while weighted lifestyle score was used as a sensitivity analysis to validate the calculation method of healthy lifestyle score.

$$Weighted\ HLS = \beta 1 * smoking + \beta 2 * physical\ activity + \beta 3 * diet * 3/sum\ of\ the\ \beta - coefficients$$

Statistical Analysis

We used univariate and multivariate Cox regression models to calculate the hazard ratios (HRs) and 95% CIs for the association between PRS and/or HLS and BC risk. The proportional hazard assumption was examined for each analysis and no evidence of violation was found. In addition, the appropriateness of the use of the log-normal distribution was tested using a Wald test, and again no evidence of violation was found. The interaction effect of HLS and PRS on BC risk was tested by including a multiplicative interaction term in the Cox regression models. Continuous values of the PRS and weighted HLS were z-score-transformed and used in the further analyses for appropriate scaling for clinical interpretation, in which the score of each individual minus the mean of PRS/weighted HLS and then divided by the standard deviation (SD) of PRS/ weighted HLS. The transformed PRS was categorized by quartile as low (Quartile 1), moderate (Quartile 2), intermediate (Quartile 3), and high (Quartile 4). The standardized weighted HLS was also categorized by quartile as unfavourable (Quartile 1), moderate (Quartile 2), intermediate (Quartile 3), and unfavourable (Quartile 4). Several potential confounders were included in our multivariable-adjusted models: Model 1 was the crude model without adjustments; Model 2 was adjusted for age (in years, continuous) and sex (male or female); Model 3 was further adjusted for assessment centre (22 categories), body mass index (BMI [kg/m^2]; continuous), ethnicity (White or non-White), education (A' levels/AS levels or equivalent, college or university degree, O' levels/GCSEs or equivalent, CSEs or equivalent, NVQ or HND or HNC or equivalent, other professional qualifications, for example, nursing, teaching, none of the above, or missing), Townsend deprivation index (continuous), household income (< £18 000, £18 000–30 999, £31 000–£51 999, £52 000–£100 000, >£100 000, or missing), alcohol consumption (never or special occasions only, one to three times/month, one or two times/week, three or four times/week, or daily/almost daily), history of diabetes (yes or no), vitamin or mineral supplement use (yes or no). For evaluation of the association between PRS and BC risk, a smoking factor (smoking and non-smoking), physical activity factor (regular or non-regular), and diet factor (healthy or non-healthy) were included as additional confounder in the sensitivity analyses. Missing data were coded as a missing indicator category for categorical variables (i.e., education level, history of diabetes and household income) or replaced by the mean value for the continuous variables (i.e., Townsend deprivation index), if applicable.

In addition, the HRs for BC risk were estimated by combining the different categories of HLS and PRS. Here, 16 groups were created: low HLS and low PRS; low HLS and intermediate PRS; low HLS and moderate PRS; low HLS and high PRS; intermediate HLS and low PRS; intermediate HLS and intermediate PRS; intermediate HLS and moderate PRS; intermediate HLS and high PRS; moderate HLS and low PRS; moderate HLS and intermediate PRS; moderate HLS and moderate PRS; moderate HLS and high PRS; high HLS and low PRS; high HLS and intermediate PRS; high HLS and moderate PRS; high HLS and high PRS. The group with highest HLS and lowest PRS was used as reference. The population-attributable fraction, an estimate of the proportion of events that would have been prevented if all individuals had avoided favourable lifestyle (i.e., failed to meet any healthy lifestyle criteria), was calculated and stratified by PRS category. Furthermore, the following sensitivity analyses were conducted: (i) excluding individuals under the age of 60 years; (ii) excluding non-White individuals; (iii) evaluating the effect of genetic risk based on unrelated participants; (iv) excluding individuals whose BC diagnosis was solely derived from self-report; (v) weighting HLS score with BC risk; and (vi) excluding incident cases of BC occurring during the first year of follow-up.

Statistical analyses were performed with R software (version 4.0.5) and STATA 14 SE (Stata Corporation, College Station, TX, USA). Tests were two-sided, and P values < 0.05 were taken to indicate statistical significance.

Results

Population Characteristics

Table 1 shows the distribution of the characteristics of the participants by BC cases and non-BC cases. In total, 484 520 study participants contributed 7 260 837 person-years of follow-up (median for BC cases 3.40 years and for non-BC cases 15 years), with 563 incident BC cases (420 male, 143 female) diagnosed. The mean (SD) age at recruitment was 56.52 (8.09) years, and 263 500 of the participants (54.4%) were female. Most participants had either one (45.45%) or two (44.06%) of three healthy lifestyle factors. The mean (SD) PRS and HLS for BC cases were 25.50 (4.04) and 1.21 (0.62), respectively, and for non-BC cases they were 16.48 (2.71) and 1.43 (0.66), respectively (Table 1). The PRS was shown to be normally distributed (Figure S2). In addition, participants with BC were generally older (62 vs 57 years), were more often men (75% vs 46%), were heavier smokers (20 vs 11 pack-years) and had higher BMI (28 vs 27 kg/m^2; Table 1).

Association between the Polygenic Risk Score and Bladder Cancer Risk

Table 2 shows the results for the associations between PRS and BC risk. In multivariable-adjusted analyses (Model 3), higher PRS was significantly associated with higher BC risk (comparing the highest with the lowest PRS quartile: HR 1.26, 95% CI 1.10–1.46). Relative to the mean values, each SD increment in PRS was associated with a 7% (95% CI 3–12) higher BC risk (P = 0.004) of BC. Additional adjustment for lifestyle factors did not change these results, indicating that genetics has an independent role in BC development (Table S4). Similar results were observed in the sensitivity analyses based on multivariable-adjusted analyses (Model 3) by stratifying sexes (comparing the highest with the lowest PRS quartile: male: HR 1.28, 95% CI 1.12–1.45, HR per 1-SD increment 1.10, 95% CI 1.02–1.26, P for trend = 0.002; female: HR 1.26, 95% CI 1.13–1.40, HR per 1-SD increment 1.12, 95% CI 1.03–1.28, P for trend = 0.001) and excluding: (i) people aged <60 years (comparing the highest with the lowest PRS quartile: HR 1.37, 95% CI 1.19–1.48, HR per 1-SD increment 1.05, 95% CI 1.01–1.11, P for trend <0.001); (ii) non-White (comparing the highest with the lowest PRS quartile: HR 1.32, 95% CI 1.18–1.47, HR per 1-SD increment 1.09, 95% CI 1.05–1.12; P for trend <0.001); (iii) self-report BC (comparing the highest with the lowest PRS quartile: HR 1.32, 95% CI 1.19–1.46, HR per 1-SD increment 1.08, 95% CI 1.05–1.24; P for trend <0.001); (iv) related participants (comparing the highest with the lowest PRS quartile: HR 1.28, 95% CI 1.11–1.48, HR per 1-SD increment 1.07, 95% CI 1.03–1.12; P for trend = 0.002); (v) incident cases of BC occurring during the first year of follow-up (comparing the highest with the lowest PRS quartile: HR 1.30, 95% CI 1.11–1.51, HR per 1-SD increment 1.08, 95% CI 1.03–1.13; P for trend = 0.003 [Table S5]).

Association between the Healthy Lifestyle Score and Bladder Cancer Risk

Table 2 shows the results for the associations between the HLS and BC risk based on multivariable-adjusted analyses (Model 3). HLS was inversely associated with BC risk (comparing the highest with the lowest weighted HLS quartile: HR 0.57, 95% CI 0.32–0.74, HR per 1-SD increment 0.72, 95% CI 0.68–0.76; P for trend <0.001). A similar inverse association was observed using the weighted HLS (comparing the highest with the lowest HLS quartile: HR 0.78, 95% CI 0.70–0.86, HR per 1-SD increment 0.92, 95% CI 0.89–0.95; P for trend <0.001 [Table S6]). Also, the same pattern was shown based on multivariable-adjusted analyses (Model 3) by stratifying sexes (comparing the highest with the lowest HLS quartile: male: HR 0.57, 95% CI 0.42–0.84, HR per 1-SD increment 0.70, 95% CI 0.67–0.74, P for trend <0.001; female: HR 0.39, 95% CI 0.28–0.55, HR per 1-SD increment 0.62, 95% CI 0.58–0.65; P for trend <0.001), and excluding: (a) people aged <60 years (comparing the highest with the lowest weighted HLS quartile: HR 0.47, 95% CI 0.33–0.67, HR per 1-SD increment 0.68, 95% CI 0.62–0.75; P for trend <0.001); (b) non-White (comparing the highest with the lowest PRS quartile: HR 0.60, 95% CI 0.41–0.88, HR per 1-SD increment 0.72, 95% CI 0.68–0.77; P for trend <0.001); (c) self-report BC (comparing the highest with the lowest PRS quartile: HR 0.59, 95% CI 0.42–0.83, HR per 1-SD increment 0.71, 95% CI 0.67–0.75, P for trend <0.001); and (d) incident cases of BC occurring during the first year of follow-up (comparing the highest with the lowest PRS quartile: HR 0.28, 95% CI 0.15–0.50, HR per 1-SD increment 0.60, 95% CI 0.56–0.65; P for trend <0.001 [Table S7]).

Association between Interaction of PRS and HLS with Bladder Cancer Risk

In the multivariable-adjusted model (Model 3) in Table 2, each 1-SD increment in the PRS was associated with a 0.07 increment of BC risk (P = 0.004), while each 1-SD increment in the HLS was associated with a 0.33 reduction in BC risk (P < 0.001). However, when considering the joint effect of genetic and lifestyle factors, we observed an interaction effect between PRS and HLS on BC risk; each 1-SD increment of PRS*HLS was associated with a reduction of BC risk (β = −0.06, P = 0.002), indicating that the increased BC risk by PRS was offset by HLS (Table S8). In addition, through the assessment of interaction between the PRS and each HLS component (i.e., smoking, physical activity and diet), we observed that each 1-SD increment of PRS*each HLS component was associated with a reduction of BC risk

Table 1 Characteristics of included participants in the UK Biobank cohort.

Characteristics	Case *N* = 563	Non-case *N* = 483 957	*P**
Female, *n* (%)	143 (25.40)	263 357 (54.42)	<0.001
Age, years†	62.32 ± 5.67	56.50 ± 8.09	<0.001
BMI, kg/m^2	28.13 ± 4.51	27.42 ± 4.78	<0.001
Townsend deprivation index¶	−1.13 ± 3.11	−1.31 ± 3.09	0.03
Ethnicity			
White (%)	499 (88.63)	405 987 (83.89)	<0.001
Non-White (%)	64 (11.37)	77 970 (16.11)	
Smoking status, *n* (%)			
Never	185 (32.86)	264 274 (54.61)	<0.001
Former	287 (50.98)	166 524 (34.41)	
Current	91 (16.16)	50 678 (10.47)	
Prefer not to answer	0	2481 (0.51)	
Smoking pack-years§	20.07 ± 21.59	10.57 ± 15.49	<0.001
Education‡, ***n* (%)**			
A' levels/AS levels or equivalent	39 (6.93)	53 822 (11.12)	<0.001
College or University degree	149 (26.47)	156 701 (32.38)	
O' levels/GCSEs or equivalent	102 (18.12)	102 146 (21.11)	
CSEs or equivalent	9 (1.60)	26 141 (5.40)	
NVQ or HND or HNC or equivalent	53 (9.41)	31 680 (6.55)	
Other professional qualifications, e.g., nursing, teaching	40 (7.10)	24 990 (5.16)	
None of the above	159 (28.24)	82 263 (17.00)	
Unknown (%)	12 (2.13)	6214 (1.28)	
Household Income, *n* (%)			
<£18 000	154 (27.35)	93 755 (19.37)	<0.001
£18 000 to £30 999	136 (24.16)	104 966 (21.69)	
£31 000 to £51 999	106 (18.83)	107 796 (22.27)	
£52 000 to £100 000	67 (11.90)	84 126 (9.97)	
>£100 000	7 (1.24)	22 396 (4.63)	
Unknown	93 (16.52)	70 918 (14.65)	
Alcohol consumption, *n* (%)			
Never	41 (7.28)	38 901 (8.04)	<0.001
Special occasions only	59 (11.08)	55 602 (11.49)	
One to three times a month	43 (7.64)	53 836 (11.12)	
Once or twice a week	144 (25.58)	124 677 (25.76)	
Three or four times a week	131 (23.27)	111 667 (23.07)	
Daily or almost daily	143 (25.40)	98 192 (20.29)	
Unknown	2 (0.28)	1082 (0.22)	
History of diabetes, *n* (%)			
Yes	97 (17.23)	27 710 (5.73)	<0.001
No	466 (82.77)	456 247 (94.27)	
Vitamin and/or Mineral Supplements, *n* (%)			
Yes	30 (5.33)	27 990 (5.78)	0.01
No	533 (94.67)	455 967 (94.22)	
Follow-up, median years	3.40	15.00	<0.001
HLS	1.21 ± 0.62	1.43 ± 0.66	<0.001
0 (unfavourable), *n* (%)	51 (9.06)	34 711 (7.17)	
1 (moderate), *n* (%)	352 (65.52)	219 874 (45.51)	
2 (intermediate), *n* (%)	153 (27.18)	218 155 (45.08)	
3 (favourable), *n* (%)	7 (1.24)	11 217 (2.32)	
PRS‡	25.50 ± 4.04	16.48 ± 2.71	<0.001
Q1 (low)	123 (21.85)	122 166 (25.24)	
Q2 (moderate)	131 (23.27)	119 891 (24.77)	
Q3 (intermediate)	155 (27.53)	121 910 (25.19)	
Q4 (high)	154 (27.35)	119 990 (24.79)	

*Data are mean ± SD, unless otherwise indicated. Lifestyle risk categories were defined according to HLS as unfavourable (Q1), intermediate (Q2), moderate (Q3), and favourable (Q4). P value <0.05 was considered statistically significant. BMI, body mass index; HLS, healthy lifestyle score; PRS, polygenic risk score; Q, quartile; SD, standard deviation. *Calculated using chi-squared test for categorical variables and t-test for continuous variables between bladder cancer cases and non-cases. †Age at the time of recruitment. ‡Higher education was defined as A' levels/AS levels or equivalent, college or university degree, O' levels/GCSEs or equivalent, CSEs or equivalent, NVQ or HND or HNC or equivalent, other professional qualifications, e.g., nursing, teaching, none of the above. §Pack-years was defined as the number of cigarettes smoked per day multiplying the years of smoking. ¶Socioeconomic status assessed with the Townsend deprivation index, which combines information on social class, employment, car availability, and housing. Genetic risk categories defined according to a PRS as low (Q1), intermediate Q2), moderate (Q3), and high (Q4).*

Table 2 Association of polygenic risk score and healthy lifestyle score with bladder cancer risk.

	Quartile of PRS: HR (95% CI)				Continuous PRS: HR (95% CI)			
	Q1 n_{case} = 123	Q2 n_{case} = 131	Q3 n_{case} = 155	Q4 n_{case} = 154	β	SE	*P*	per 1-SD HR
Model 1	Ref.	1.15 (0.99–1.33)	1.19 (1.03–1.37)	1.28 (1.11–1.48)	0.07	0.02	0.002	1.07 (1.03–1.12)
Model 2	Ref.	1.13 (0.97–1.31)	1.17 (1.01–1.36)	1.26 (1.09–1.45)	0.07	0.02	0.005	1.07 (1.02–1.12)
Model 3	Ref.	1.13 (0.97–1.31)	1.18 (1.02–1.36)	1.26 (1.10–1.46)	0.07	0.02	0.004	1.07 (1.03–1.12)
	Four scores of HLS: HR (95% CI)				**Continuous HLS: HR (95% CI)**			
	0 n_{case} = 51	1 n_{case} = 352	2 n_{case} = 153	3 n_{case} = 7	β	SE	*P*	per 1-SD HR
Model 1	Ref.	1.01 (0.85–1.21)	0.46 (0.38–0.55)	0.31 (0.18–0.53)	−0.49	0.02	<0.001	0.61 (0.56–0.65)
Model 2	Ref.	0.98 (0.83–1.17)	0.55 (0.46–0.67)	0.43 (0.25–0.73)	−0.37	0.03	<0.001	0.69 (0.64–0.74)
Model 3	Ref.	1.01 (0.85–1.20)	0.66 (0.52–0.84)	0.57 (0.32–0.74)	−0.33	0.02	<0.001	0.72 (0.68–0.76)

Cox regression models were performed using z-score transformed values of the PRS and HLS. Model 1 was crude model without adjustment. Model 2 adjusted for age (in years, continuous) and sex (male or female). Model 3 was further adjusted for assessment centres (22 categories), BMI (in kg/m^2; continuous), ethnicity (White or non-White), education (A′ levels/AS levels or equivalent, college or university degree, O′ levels/GCSEs or equivalent, CSEs or equivalent, NVQ or HND or HNC or equivalent, other professional qualifications, e.g., nursing, teaching, none of the above, or missing), Townsend deprivation index (continuous), household income (<£18 000, £18 000–£30 999, £31 000–£51 999, £52 000–£100 000, >£100 000, or missing), alcohol consumption (never or special occasions only, one to three times/month, one or two times/week, three or four times/week, or daily/almost daily), history of diabetes (yes or no), vitamin or mineral supplement use (yes or no). Reference group was lowest PRS (Q1) or lowest HLS (0). P < 0.05 was considered statistically significant. PRS, polygenic risk score; HLS, healthy lifestyle score; Q, quartile; SE, standardized error; SD, standardized deviation; HR, hazard ratio; Ref, reference.

(PRS*smoking, β = −0.13, *P* < 0.001; PRS*physical activity, β = −0.11, *P* = 0.003; PRS*diet, β = −0.04, *P* < 0.001), which suggests smoking might have the most impact in averting the effect of PRS (Table S9).

Results from the HLS and PRS combined categories showed that a higher PRS was associated with a higher risk of BC, and the association was more pronounced among participants who had a lower HLS; similarly, a higher HLS was associated with a lower BC risk and the association was stronger among participants with a lower PRS (Table 3). Compared to individuals in the lowest quartile of PRS and the highest quartile of the HLS, those in the highest quartile of the PRS and the lowest quartile of the HLS had a 1.55 increase in BC risk (95% CI 1.16–1.91, *P* for trend <0.001 [Table 3]). The observed findings were maintained when stratifying the analysis by PRS category (Table S10): individuals with the highest adherence to HLS showed a consistent decreased risk of BC compared to those with the lowest HLS in the low PRS group (HR 0.58, 95% CI 0.44–0.77; *P* for trend <0.001), the intermediate PRS group (HR 0.49, 95% CI 0.38–0.64; *P* for trend <0.001), the moderate PRS group (HR 0.50, 95% CI 0.36–0.55; *P* for trend <0.001), and the high PRS group (HR 0.73, 95% CI 0.56–0.95; *P* for trend <0.001).

Table 3 Risk of bladder cancer according to healthy lifestyle category within each genetic risk category.

	PRS				*P* trend	*P* interaction
	Q1	Q2	Q3	Q4	<0.001	0.02
HLS						
3	Ref.	0.24 (0.17–0.36)	0.68 (0.48–0.84)	0.74 (0.51–0.88)		
2	0.75 (0.42–0.86)	0.84 (0.67–0.94)	0.85 (0.68–0.97)	0.91 (0.81–1.01)		
1	1.15 (0.84–1.37)	1.19 (0.88–1.38)	1.32 (0.90–1.46)	1.33 (0.92–1.50)		
0	1.34 (0.95–1.58)	1.35 (0.99–1.48)	1.54 (1.16–1.89)	1.55 (1.16–1.91)		

Multivariate logistic regression models were performed using z-score transformed values of the PRS. Model 1 was crude model without adjustment; Model 2 adjusted for age (in years, continuous) and sex (male or female); Model 3 was further adjusted for assessment centres (22 categories), BMI (in kg/m^2; continuous), ethnicity (Caucasian or no-Caucasian), education (A′ levels/AS levels or equivalent, college or university degree, O′ levels/GCSEs or equivalent, CSEs or equivalent, NVQ or HND or HNC or equivalent, other professional qualifications, e.g., nursing, teaching, none of the above, or missing), Townsend deprivation index (continuous), household income (<£18 000, £18 000–£30 999, £31 000–£51 999, £52 000–£100 000, >£100 000, or missing), alcohol consumption (never or special occasions only, 1 to 3 times/month, 1 or 2 times/week, 3 or 4 times/week, or daily/almost daily), history of diabetes (yes or no), vitamin or mineral supplement use (yes or no). Reference group was lowest PRS and highest HLS (Q1). P < 0.05 was considered statistically significant. PRS, polygenic risk score; HLS, healthy lifestyle score; Q, quartile; SE, standardized error; SD, standardized deviation; HR, hazard ratio; Ref, reference.

Population-Attributable Fractions

Results of the PAF analysis showed that 12%–15% of the BC cases might have been prevented if individuals had adhered to a healthy lifestyle. In addition, among individuals with a high PRS, the PAF remained approximately 10% (Table S11).

Discussion

The present study showed that an individual's genetic risk and lifestyle are independently and jointly associated with BC risk. Individuals with a high genetic risk and an unfavourable lifestyle had an increased BC risk compared to individuals with a low genetic risk and a favourable lifestyle. In addition, results indicate that the BC risk attributed to an individual's genetic predisposition could be offset by a healthy lifestyle (Figure S3).

The levels and differences in absolute BC risk across different genetic risk scores are in line with a Chinese case–control study, also showing that a higher PRS was associated with an increased BC risk [18]. Although several European studies showed an increased BC risk for individuals with a positive BC family history [3,4], so far, only one study reported the association between PRS and BC risk in a European population based on a pan-cancer design [19]. This study, therefore, with more details, investigated the combined effect of different lifestyle factors and genetic predisposition and BC risk, showing that adherence to a healthy lifestyle reduced the risk of BC and offset the effect of genetics. However, results are not surprising since previous research clearly showed that modifiable lifestyle factors and health behaviours including diet, physical activity, and smoking affect BC carcinogenesis [20]. Furthermore, the interaction between genetics and modifiable factors has been shown to influence BC risk, including; gene–smoking interaction [21], gene–diet interaction [22] and gene–energy-intake interaction [23].

A wide range of mechanisms have been proposed to explain how genetic and lifestyle factors, and the interaction between these two, might influence BC risk. Common genetic variants were found to affect: (i) the immune response [24]; (ii) the regulation of endocytosis [25]; and (iii) the inflammatory procedure [26]. Beyond the effect of SNPs, our PRS analysis strengthened the hypothesis that BC risk is associated with the occurrence of a wide spectrum of genetic alterations, suggesting that an entire complex network of genes might play an important role in the regulation of cell division, resulting in BC development. In addition to endogenous factors (i.e., genetic susceptibility), normal urothelium is also exposed to exogenous (i.e., lifestyle) factors that might cause DNA damage by inducing the overexpression of oncogenes. The most well-known lifestyle factor influencing BC risk is cigarette smoking, accounting for 50% of the male BC cases, and 25% of the female BC cases. Exposure of human normal urothelial cells to smoke-induced morphological change, and epithelial mesenchymal transition (EMT) and mitogen-activated protein kinase (MAPK) activation [27], by activation of the extracellular signal-regulated kinases (ERK1/2) pathway [28]. Another well-known modifiable factor effecting BC risk is physical activity. However, the exact biological mechanisms through which physical activity may influence BC risk have not yet been elucidated. It might be possible that physical activity: (i) increases the carcinogenic detoxification; (ii) promotes DNA repair processes; (iii) modifies cell proliferation; (iv) influences the differentiation and apoptosis mechanisms; (v) reduces chronic inflammation; and/or (vi) enhances the immune function [29]. Looking at the interaction between genetic and lifestyle factors, previous research reported a gene–diet interaction that could potentially influence BC risk, by showing that intake of vegetables was associated with a reduced BC risk among current smokers only. This association was shown to be stronger among individuals with the GSTM1 wild-type genotype compared to the GSTM1 non-mutated genotype [30]. In line with this result, the current study shows that the protective effect of a high HLS on BC risk is modified by an individual's PRS, indicating that individuals with a high PRS may benefit more from adherence to a healthy lifestyle in terms of BC prevention compared to individuals with a low PRS.

The major strength of this study is the large population size, providing the ability to perform detailed analyses with sufficient statistical power to detect small effect sizes. Another strength is the use of a comprehensive diet quality score and an accurate PRS, based on a comprehensive package of SNPs. Furthermore, data were available for multiple covariates, which offered the opportunity for a detailed model adjustment and performance of sensitivity analyses.

The study also has several limitations. First, unlike the genetic variants, adherence to a healthy lifestyle was not randomly assigned, therefore could be biased. Second, since the assessment of HLS (i.e., smoking, physical activity and diet) was conducted at baseline, bias derived from the fact that the lifestyle has changed over time could not be ruled out. Third, SNPs contributing to the PRS may also have a pleiotropic effect on lifestyle factors, which might inflate the associations of PRS and HLS with BC risk. Fourth, although analyses were adjusted for known potential sources of bias, the possibility of unmeasured confounding and reverse causation remains. Fifth, lifestyle factors were self-reported, therefore possible measurement and classification errors are probably biased toward the null and would underestimate the BC risk associated with poor health behaviours and lifestyle factors. Sixth, given that a minority of participants were non-White, the results obtained from this study should be interpreted with caution with regard to other ethnicities. Finally, this

sample was restricted to volunteers aged 60–73 years at baseline and, therefore, further research is warranted to investigate to what degree these findings can be generalized to other age groups.

In summary, this study shows that a healthy lifestyle and a low genetic risk are associated with a decreased BC risk. In addition, an interaction between these factors was observed, whereby a favourable healthy lifestyle is likely to offset a high genetic predisposition. These findings provide important evidence to support tailoring lifestyle recommendations to an individual's genetic makeup for BC prevention. However, more studies with a large sample size and precise measures of lifestyle exposures are needed to corroborate our results.

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Disclosure of Interests

All the authors declare no conflicts of interest.

Author Contributions

Conceived and designed the study: E.Y.W.Y, A.W; supervision: M.P.Z, A.W; conducted data analyses and interpretation and drafted the manuscript: E.Y.W.Y, A.W; data curation: E.Y.W.Y, Y.X.L, Y.T.C; Critical revision of the manuscript: Q.Y.T, S.M, S.Z.W, W.C.L, M.P.Z, A.W. All authors read and approved the final manuscript.

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A healthy lifestyle and a low genetic risk are associated with a decreased bladder cancer risk, in which a favourable healthy lifestyle is likely to offset a high genetic predisposition. These findings provide important evidence to support tailoring lifestyle recommendations to an individual's genetic makeup for bladder cancer prevention.

Data Availability Statement

This work was conducted using the UK Biobank Resource. The UK Biobank is an open access resource and *bona fide* researchers can apply to use the UK Biobank dataset by registering and applying at http://ukbiobank.ac.uk/register-apply/. Further information is available from the corresponding author upon request.

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Abbreviations: BC, bladder cancer; BMI, body mass index; GWAS, genome-wide association studies; HLS, healthy lifestyle score; HR, hazard ratio; ICD, International Classification of Diseases; OR, odds ratio; PAF, population-attributable fractions; PRS, polygenic risk score; SD, standard deviation; SNP, single nucleotide polymorphisms.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flowchart of participants included in the current UK Biobank study.

Figure S2. Distribution of the polygenic risk score for bladder cancer.

Figure S3. Summary of main findings and concepts of the current study.

Table S1. Characteristics of 34 BC-associated SNPs in the UK biobank.

Table S2. Definitions of disease diagnosis.

Table S3. Components and scaling methods of the healthy lifestyle score (HLS) used in the UK Biobank study.

Table S4. Association between polygenic risk score and bladder cancer risk based on Model 3 with additional adjustment of lifestyle factor.

Table S5. Association between polygenic risk score and bladder cancer risk based on Model 3.

Table S6. Association between weighted healthy lifestyle score and bladder cancer risk.

Table S7. Association between healthy lifestyle score and bladder cancer risk based on Model 3.

Table S8. Association of interaction of polygenic risk score and healthy lifestyle score with bladder cancer risk.

Table S9. Association of interaction between polygenic risk score and each healthy lifestyle component with bladder cancer risk based on Model 3.

Table S10. Association between healthy lifestyle score and bladder cancer risk stratified by polygenic risk score.

Table S11. Population-attributable fraction per behavioural lifestyle to unfavourable lifestyle.