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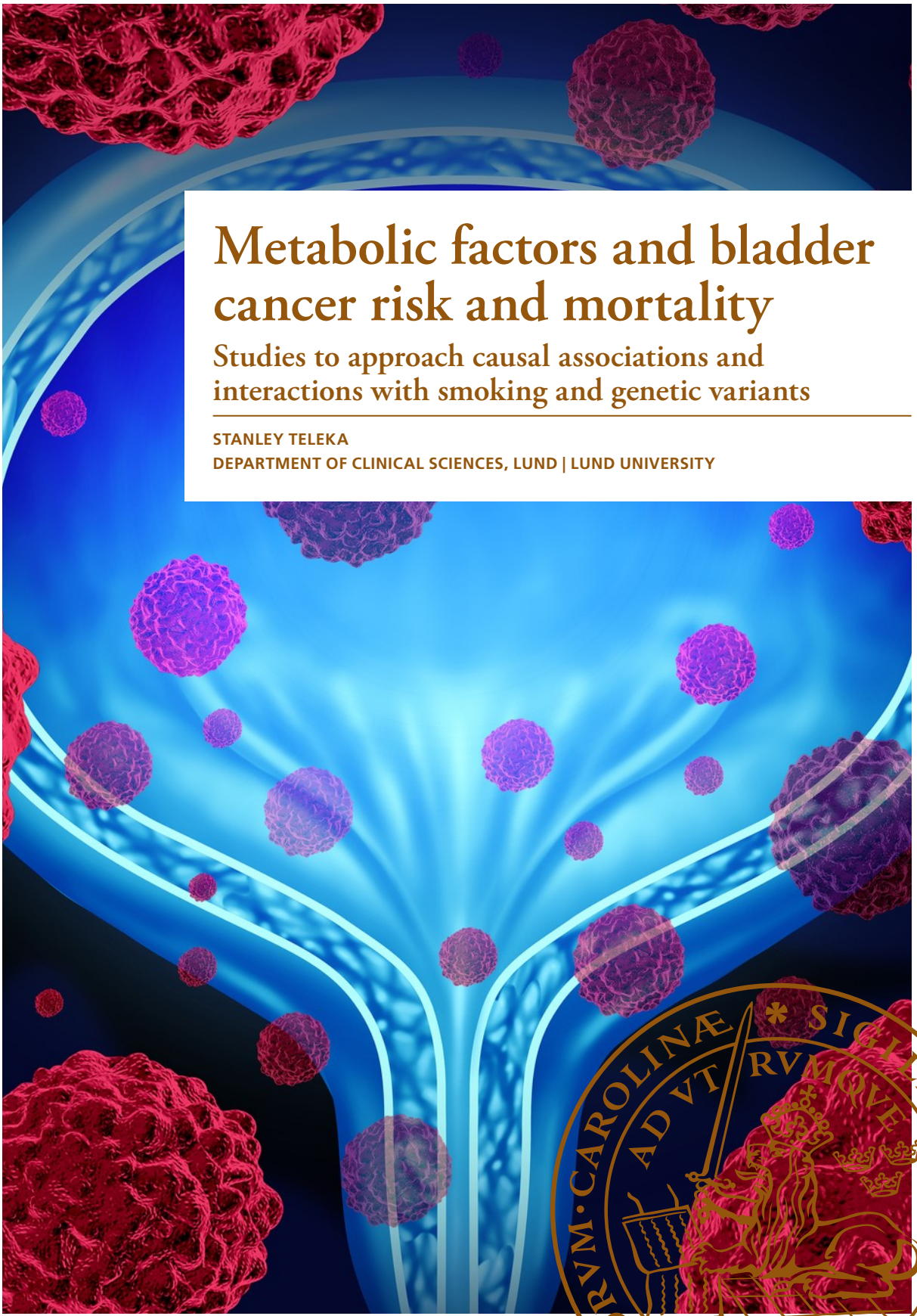

Metabolic factors and bladder cancer risk and mortality

Studies to approach causal associations and interactions with smoking and genetic variants

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Stanley Teleka

LUND
UNIVERSITY

DOCTORAL DISSERTATION
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Associate professor Jennifer Lyn Baker, Center for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Denmark

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Title and subtitle: Metabolic factors and bladder cancer risk and mortality: Studies to approach causal associations and interactions with smoking and genetic variants

Abstract:

Urothelial bladder cancer (BC) is one of the most common cancers in developed countries. It has one of the highest recurrence rates among solid tumours, resulting in regular treatment and follow-up, making it one of the most expensive cancers to treat, and a significant public health burden. Derangement of metabolic factors such as body mass index (BMI), blood pressure (BP), glucose, triglycerides (TG) and cholesterol (TC) has resulted in global epidemics such as obesity, hypertension, diabetes and dyslipidemia, which collectively impact cardiovascular disease and certain cancers. Previous studies investigating the link between metabolic factors and BC have shown inconsistent results, furthermore, additive and multiplicative interactions, which may inform biological mechanisms, are rarely investigated in such studies.

This thesis aimed to clarify the link between metabolic factors and BC, especially BP, by investigating the association between metabolic factors and BC risk (total, and separately into sub-groups based on tumour characterstics) and BC-specific mortality, using conventional survival analysis and additionally for BP, Mendelian Randomization (MR) analysis, and to assess additive and multiplicative interaction with smoking and BC genetic variants in such associations.

In studies that spanned several prospective cohorts, we found, as main findings among men: a positive association between systolic BP (SBP) and muscle-invasive BC (MIBC)/aggressive urothelial cancer risk (paper I-IV), and a stronger association among never-smokers, both in conventional survival analysis and MR analysis of a Swedish cohort, a positive association between BMI, TG and TC and non-muscle invasive BC (NMIBC) risk, and a positive association between SBP and TG and risk of BC-specific mortality. Among women (paper I), we found an inverse association between BMI and total BC risk, a positive association between glucose and MIBC risk, and a positive association between TG and risk of BC-specific mortality. In the interaction analysis among men, SBP and DBP did not interact with *NAT2* in relation to total BC risk; however, SBP and a genetic score for BC positively interacted on an additive scale, in relation to MIBC risk.

In conclusion, abberations in metabolic factors was associated with BC outcomes; the associations differed depending on the sub-group of population and the specific outcome being investigated. Among men, SBP consistently showed a positive association with MIBC risk, furthermore, SBP and the genetic risk of BC positively interacted on an additive scale. The thesis highlights the importance of investigating associations in specific sub-groups of the population and specific BC outcomes, and assessing interaction to explore potential biological mechanisms and inform public health.

Key words: bladder cancer, body mass index, blood pressure, systolic blood pressure, diastolic blood pressure, glucose, triglycerides, cholesterol, risk, specific mortality, Mendelian randomization analysis

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"O Lord, by these things men live, and in all these things is the life of my spirit…"

Isaiah 38:16, Bible (King James Version)

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List of papers

This doctoral thesis is based on the following four papers

I. **Teleka S**, Häggström C, Nagel G, Bjørge T, Manjer J, Ulmer H, Liedberg F, Ghaderi S, Lang A, Jonsson H, Jahnson S, Orho-Melander M, Tretli S, Stattin P, Stocks T. (2018), Risk of bladder cancer by disease severity in relation to metabolic factors and smoking: A prospective pooled cohort study of 800,000 men and women. **Int J Cancer** 2018; 143: 3071-3082. https://doi.org/10.1002/ijc.31597

II. **Teleka S**, Jochems S.H.J, Häggström C, Wood A.M, Järvholm B, Orho-Melander M, Liedberg F, Stocks T. (2021), Association between blood pressure and BMI with bladder cancer risk and mortality in 340,000 men in three Swedish cohorts. **Cancer Med** 2021; 00: 1–8. https://doi.org/10.1002/cam4.3721

III. **Teleka S**, Hindy G, Drake I, Poveda A, Melander O, Liedberg F, Orho-Melander M, Stocks T. Blood pressure and bladder cancer risk in men by use of survival analysis and in interaction with *NAT2* genotype, and by Mendelian randomization analysis. **PLoS One**. 2020 Nov 25;15(11):e0241711. doi: 10.1371/journal.pone.0241711.

IV. **Teleka S,** Melander O, Orho-Melander M, Liedberg F, Jirström K, Stocks T. Interaction between blood pressure and genetic score for bladder cancer, and risk of urothelial carcinoma. **Manuscript**

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Papers not included in the thesis

I. Nagel G, Bjørge T, Jaensch A, Peter RS, Häggström C, Lang A, Engeland A, **Teleka S**, Jirström K, Lindquist D, Stattin P, Ulmer H, Concin H, Stocks T. Metabolic factors and the risk of small intestine cancers: Pooled study of 800 000 individuals in the metabolic syndrome and cancer project. **Int J Cancer**. 2021; 1– 9. https://doi.org/10.1002/ijc.33530

II. Fritz J, Bjørge T, Nagel G, Manjer J, Engeland A, Häggström C, Concin H, **Teleka S**, Tretli S, Gylling B, Lang A, Stattin P, Stocks T, Ulmer H. The triglyceride-glucose index as a measure of insulin resistance and risk of obesity-related cancers. **Int J Epidemiol**. 2020 Feb 1;49(1):193-204. doi: 10.1093/ije/dyz053.

III. Drake I, Dias JA, **Teleka S**, Stocks T, Orho-Melander M. Lifestyle and cancer incidence and mortality risk depending on family history of cancer in two prospective cohorts. **Int J Cancer**. 2020 Mar 1;146(5):1198-1207. doi: 10.1002/ijc.32397. Epub 2019 May 21.

IV. Mutie PM, Drake I, Ericson U, **Teleka S**, Schulz CA, Stocks T, Sonestedt E. Different domains of self-reported physical activity and risk of type 2 diabetes in a population-based Swedish cohort: the Malmö diet and Cancer study. **BMC Public Health**. 2020 Feb 21;20(1):261. doi: 10.1186/s12889-020-8344-2.

V. **Teleka S**, Chijuwa A, Senga E, Chisi JE. Cytosine arabinoside reduces the numbers of granulocyte macrophage colony forming cells (GM-CFC) and high proliferative potential colony forming cells (HPP-CFC) in vivo in mice**. Malawi Med J**. 2011 Dec;23(4):118-21.

Abbreviations and symbols

2SLS- Two stage least square

ASR- Age standardised rates

BC- Bladder cancer

BP- Blood pressure

CHARGE- Cohorts for Heart and Aging Research in Genomic Epidemiology

CI- Confidence interval

CwC- Construction workers cohort

DNA- Deoxyribonucleic acid

EORTIC- European Organisation for Research and Treatment of Cancer

GSTM1- Glutathione-s-transferase-mu-1

GWAS- Genome-wide association studies

HR- Hazard ratio

IARC- International Agency for Research on Cancer

ICBP- International Consortium for Blood Pressure Genome - Wide Association Studies

ICD- International Classification of Diseases

InSIDE- Instrumental strength independent of direct effects

IV- Instrumental variable

IVW- Inverse variance weighted

LM- Lunn-McNeil

LR- Likelihood ratio

LUTS- Lower respiratory tract symptoms

MDCS- Malmö Diet and Cancer Study

Me-Can- Metabolic Syndrome and Cancer Project

MIBC- Muscle-invasive bladder cancer

MPP- Malmö Preventive Project

MR- Mendelian Randomization

NAT2- N-acetyl-transferase-2

NICE- National Institute for Health and Care Excellence

NMIBC- Non-muscle invasive bladder cancer

PH- Proportional hazards

pT- Pathological T stage

PUNLMP- Papillary urothelial neoplasm of low malignant potential

RCS- Restricted cubic splines

RDB- Regression dilution bias

RDR- Regression dilution ratio

RERI- Relative excess risk of interaction

RR- Relative risk

SBP- Systolic blood pressure

SD- Standard deviation

SNP- Single nucleotide polymorphism

SNRUBC- Swedish National Register for Urinary Bladder Cancer

TNM- Tumour-Nodes-Metastases

TURBT-Trans-urethral removal of bladder tumour

UC- Urothelial cancer/carcinoma

UK- United Kingdom

VHM&PP- Vorarlberg Health Monitoring and Prevention Programme

VIP- Västerbotten Intervention Programme

wGRS- Weighted genetic risk score

Populärvetenskaplig Sammanfattning

Cancer i urinblåsan är en av de vanligaste cancerformerna i höginkomstländer. Denna cancer har hög återfallsrisk, vilket gör den till en av de mest kostsamma att behandla. Dessutom drabbas patienter negativt både fysiskt och psykiskt av återkommande behandlingar och återbesök, vilket gör blåscancer till ett viktigt folkhälsoproblem. Förhöjda nivåer av blodtryck och kroppsmasseindex (BMI) liksom förhöjda nivåer av blodglukos, triglycerider och kolesterol, sammantaget kallat metabola faktorer, kan leda till sjukdomstillstånd som hypertoni (förhöjt blodtryck), fetma, diabetes och dyslipidemi (abnormala nivåer av blodfetter). Dessa tillstånd bidrar till kardiovaskulära sjukdomar som globalt utgör den vanligaste dödsorsaken. Tidigare studier av sambandet mellan metabola faktorer och blåscancer har varit bristfälliga och samspelet mellan dessa metabola faktorer och andra kända riskfaktorer för blåscancer, såsom rökning och genetisk risk, har knappast studerats. Detta är viktigt för att kunna bidra med vidare information om biologin bakom blåscancer och i förlängningen också applicera fynden på folkhälsonivå.

Syftet med avhandlingen var att klargöra sambanden mellan nämnda metabola faktorer, särskilt blodtryck, och risk för insjuknande och död i urinblåsecancer. Kliniskt kan blåscancer delas i två subgrupper beroende på om tumören växer in i urinblåsans muskel: den mindre aggressiva icke-muskelinvasiva blåscancern och den mer aggressiva muskelinvasiva blåscancern. Eftersom dessa kan skilja sig i hur de utvecklar sig och avseende riskfaktorer, undersökte vi blåscancerrisk separat i dessa två subgrupper. Vi använde statistiska metoder för konventionell analys, och blodtryck undersöktes även med så kallad Mendelsk randomiseringsanalys i samband med blåscancerrisk totalt. Denna metod använder sig av genetiska varianter som markör för riskfaktorn av intresse vilket har fördelen att inflytandet av störfaktorer som rökning minimeras. Vi undersökte också interaktionen mellan dessa metabola faktorer och rökning respektive genetiska varianter för blåscancer, i samband med blåscancerrisk.

I fyra delstudier fann vi att högre nivåer av systoliskt blodtryck bland män var förenat med en ökad risk av blåscancer totalt, vilket delvis tillstyrktes av den Mendelska randomiseringsanalysen, och med muskelinvasiv blåscancer där sambandet var något starkare bland män som aldrig hade rökt (studie 1-4). Vi fann också att högre nivåer av BMI, triglycerider och kolesterol var kopplade till högre

risk av icke-muskelinvasiv blåscancer, och högre nivåer av systoliskt blodtryck och triglycerider visade samband med en ökad risk att dö av blåscancer (studie 1 och 2). Bland kvinnor fann vi att högre nivåer av BMI var relaterat till en minskad blåscancerrisk. Vi fann även att högre nivåer av blodglukos var kopplat till en högre risk för icke-muskelinvasiv blåscancer och högre nivåer av triglycerider var relaterat till en högre risk att dö av blåscancer (studie 1). Undersökningen av interaktioner visade att högre nivåer av systoliskt blodtryck och högre genetiskt score för blåscancerrisk interagerade additivt och positivt i samband med risk för muskelinvasiv blåscancer (studie 4).

Sammanfattningsvis utgjorde förhöjda nivåer av metabola faktorer en ökad risk av olika blåscancerutfall. Sambanden skiljde sig beroende på vilken subgrupp av populationen, och vilket utfall av blåscancer, som studerades. Bland män utgjorde förhöjda nivåer av systoliskt blodtryck konsekvent en ökad risk för muskelinvasiv blåscancer, och systoliskt blodtryck interagerade också positivt och additivt med genetisk risk för blåscancer, i samband med muskelinvasiv blåscancer. Denna avhandling understryker betydelsen av att undersöka samband i specifika subgrupper av populationen och i relation till olika blåscancerutfall. Den markerar också betydelsen av att studera interaktioner för att undersöka biologiska mekanismer och formera folkhälsorekommendationer.

Popular science summary (Chechewa)

Khansa ya chikhodzodzo ndi imodzi mwa ma Khansa omwe anafalikila ku mayiko otukuka. Khansayi ili ndi kuthekera kwakukulu kobweleranso m'thupi mwa munthu akachira kotelo kuti kupeza chinthandizo cha Khansa imeneyi kumatenga ndalama zambiri. Kuonjezera pa chiphinjo cha zachuma chomwe chimabwera chifukwa cha nthendayi, odwala Khansayi amakhala okhudzika m'malingaliro mwawo ndi nkhawa chifukwa amakhala akupitapita ku chipatala kukasaka chithandizo; pa chifukwachi, Khansayi ndi yofunika kwambiri pa nkhani ya za umoyo. Zinthu zokhudzana ndi kagayidwe ka chakudya m'thupi monga kuthamanga msanga kwa magazi (BP), kukwela kwa shuga wa m'thupi ndi mafuta opezeka m'thupi komanso mulingo wa mafuta a mu thupi malingana ndi kutalika komanso kulemera kwa munthu (BMI) zimatha kuyambitsa nthenda monga kukwela kwa kuthamanga kwa magazi, kunenepa kwambiri, matenda a shuga ndi kusakhazikika kwa mlingo wa mafuta a m'thupi. Matenda onsewa amaonjezela ku mavuto a matenda a mtima, omwe ali otsogolera potenga miyoyo ya anthu dziko lonse lapansi. Kafukufuku wa m'buyomu amene ankawunikira pa ubale wa kagayidwe ka zakudya m'thupi ndi Khansa ya chikhodzodzo anali osadalilika. Komanso, m'mene zinthu zokhudzana ndi kagayidwe ka chakudya zimakhudzana ndi njira zina zoti zithanso kuyambitsa Khansa ya chikhodzodzo ndi nkhani yokuti siimaunikilidwa pafupi pafupi. Kotelo, kafukufuku ameneyu ndi ofunikira poperaka chidziwitso chokhudza Khansa ya chikhodzodzo, komanso atha kuthandizila popereka chidziwitso pa nkhani ya za umoyo wa anthu.

Cholinga cha pepalali, chinali kufotokoza za ubale omwe ulipo pakati pa zinthu zokhudzana ndi kagayidwe ka chakudya m'thupi (makamaka kuthamanga kwa magazi) ndi Khansa ya chikhodzodzo. Izi tipanga pophunzira za kuyanjana komwe kulipo pakati pa kagayidwe ka chakudya ndi chiopsyezo cha Khansa ya chikhodzodzo, komanso chiopsyezo cha imfa chomwe chimatha kukhalapo chifukwa cha Khansa imeneyi. Ku chipatala, Khansa ya chikhodzodzo inagawidwa mumagulu awili kutengela ndi m'mene chotupa chalowelera mu minyewa ya chikhodzodzo. Gulu loyamba ndi la Khansa ya chikhodzodzo yochepa ukali yosalowelera mu minyewa (NMIBC) ndipo gulu la chiwili ndi la Khansa ya chikhodzodzo yolusa kwambiri yolowelera mu minyewa (MIBC). Chifukwa choti magulu awiri amenewa atha kusiyana m'mene ziopsyezo zawo zimakhalila,

tinapanga kafukufuka wa gulu lililonse mwapadera. Tinagwiritsa ntchito njira ya kawelengera wa nthawi zonse osanthula kupulumuka, ndiponso pa za kuthamanga kwa magazi, tinagwiritsa njira yosanthula yotchedwa Mendelian Randomization (MR) kuti tifufuze mayanjano. Chachiwiri, tikuyembekezela kufufuza kugwirizana komwe kumakhalapo pakati pa zinthu zokhudzana ndi kagayidwe ka chakudya m'thupi komanso kusuta fodya ndi zotsatira za Khansa ya chikhodzodzo.

Mukufufuza komwe kudachitika pamaphunziro anayi, tinapeza kuti mwa abambo: kukwela kwa kuthamanga kwa magazi (SBP) kumagwirizana ndi kukwera kwa chiopsyezo cha MIBC, mukawelengera wa nthawi zonse osanthula kupulumuka komanso mu kusanthula kwa MR. Kuyanjana uku kumawoneka kwa mphamvu yambiri pochita kafukufuku pa anthu omwe sanasutepo (pepala 1–4). Tinapezanso kuti kukwela kwa BMI ndi mafuta osungidwa m'thupi osiyanasiyana kumagwirizana ndi kukwera kwa chiopsyezo cha NMIBC, komanso kukwela kwa SBP ndi mafuta osungidwa m'thupi kumagwirizana ndi kuwonjezereka kwa chiopsyezo chomwalira ndi Khansa ya chikhodzodzo (pepala 1 ndi 2). Mwa amayi, tinapeza kuti kukwera kwa BMI kumagwirizana ndi kutsika kwa chiopsyezo cha Khansa ya chikhodzodzo. Tinapezanso kuti kuwonjezeka kwa shuga wa mthupi kumagwirizana ndi kukwera kwa chiopsyezo cha MIBC komanso kukwela kwa mafuta osungidwa mu thupi kumagwirizana ndi kuwonjezeka kwa chiopsyezo chomwalira ndi Khansa ya chikhodzodzo (pepala 1). Pofufuza kuyanjana, tinapeza kuti kuonjezera SBP ndi kukwera kwa chiopsyezo cha majini omwe akhoza kutenga mosavuta Khansa ya chikhodzodzo zimalumikizana bwino pa mulingo wowonjezera poyerekeza ndi MIBC.

Pomaliza, pamakhala kugwirizana pakati pa milingo yachilendo ya zinthu zokhudza kagayidwe ka chakudya m'thupi ndi kuwonjezereka kwa chiopsyezo cha zotsatira za Khansa ya chikhodzodzo. Kugwirizanaku kumasiyana kutengela ndi gulu la anthu lomwe linagwiritsidwa ntchito komanso chotsatira chenicheni chomwe chimafufuzidwa pa nthawiyo. Pakati pa abambo, kuonjezereka kwa SBP kumapangitsa nthawi zonse kuti chiopsyezo cha MIBC chikwere, komanso kukweza mlingo wa SBP ndi chiopsyezo cha majini omwe ametenga mosavuta Khansa ya chikhodzodzo zimalumikizana bwino pa mulingo wowonjezera. Pepalali likuwonetsa kufunikira kofufuza kuyanjana m'magulu a anthu ndi zotsatira za Khansa ya chikhodzodzo. Likuwonetsanso kufunikira kofufuza kuyanjana kwa njira za chilengedwe zoti tigwiritse ntchito kudziwitsa za umoyo wa anthu.

Background

Bladder cancer

Epidemiology of bladder cancer

Bladder cancer (BC) is one of the most common forms of cancer, ranking as the 10th most common cancer (6th among men and 13th among women) worldwide. An estimated 550,000 new cases of BC (approximately 425,000 in males and 125,000 in females) were diagnosed in 2018[1-3]. The incidence of BC differs between geographical regions, this difference may be due to differences in risks factors, but may also be due to differences in the detection, diagnostic procedures and registration practices, especially the recording and reporting of non-muscle invasive BC (NMIBC)[4]. The incidence of BC is higher in the developed compared to the developing world (**Figure 1**), however, the incidence in the developing world is estimated to rise due to population growth and increase in life expectancy[5-7].

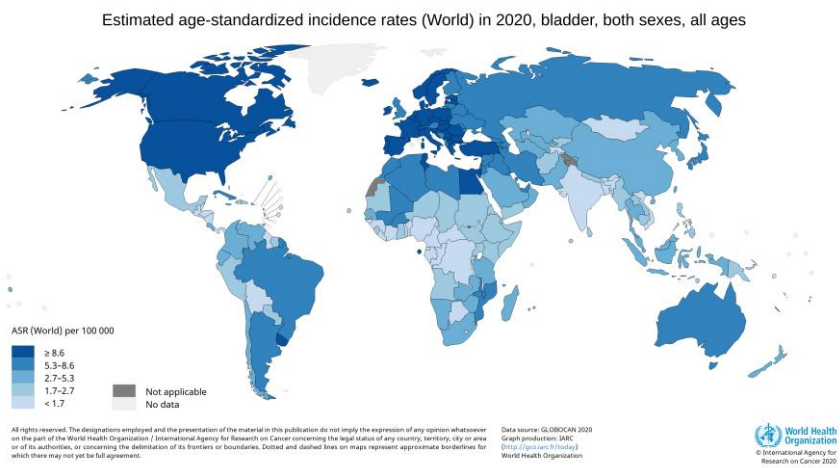

Figure 1. Age-standardized incidence rates for bladder cancer world-wide from GLOBOCAN © International Agency for Research on Cancer 2020.

Out of tumours that originate from cells of the "urothelium" which include the renal calyces, pelvis, ureters and urethra, BC is by far the most common, accounting for up to 95% of all urothelial cancer (UC) cases[8]. In developed countries, urothelial BC is the predominant histological sub-type accounting for approximately 95% of all incident cases, other histological subtypes (including pure squamous cell carcinoma, adenocarcinoma and small-cell carcinoma) account for the remaining 5% and usually entail a poor prognosis[9]. The incidence of BC across time varies by region, but typically reflect smoking patterns (with a 20-30 years lag-time) for both men and women[6, 10]. For men in the Nordic countries, the incidence of BC steadily increased from 1960, reaching its peak between 1990 and 1995, after which it began to slowly decline (**Figure 2**). With regards to women and in the same time period, it has been steadily increasing[11, 12].

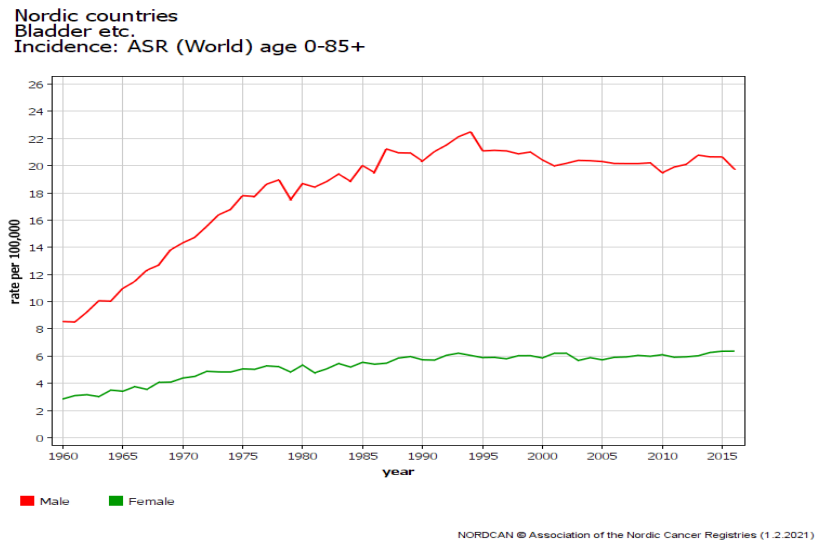

Figure 2. Age-standardized incidence rates for bladder cancer (between 1960-2016) in the Nordic countries NORDCAN © 2009 Association of the Nordic Cancer Registries.

With regards to mortality, BC ranks 13th among cancers. Like with incidence, mortality varies between regions (**Figure 3**). This is partly due to differences in quality and efficiency in healthcare systems. Consequentially, developing countries tend to have relatively higher mortality rates despite the relatively lower incidence rates[2]. There has been a decline in BC-specific mortality in developed countries, including the Nordic countries (**Figure 4**). The decline may be due to improvement in treatment, however, this decrease is still observable in the late 80s and early 90s,

suggesting that the decrease in mortality maybe due to reasons other than improvement in treatment[11, 12].

BC has, among solid tumours, one of the highest recurrence rates despite adequate treatment. This results in frequent follow-up and treatment, making BC one of the most expensive cancers to treat[13-15].

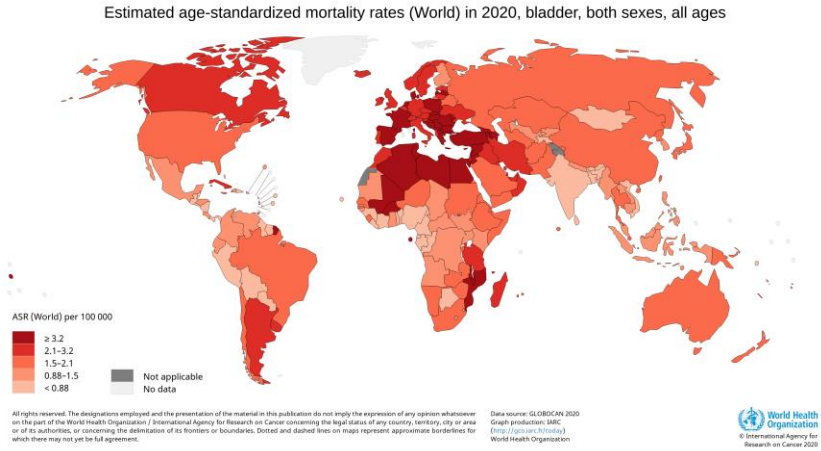

Figure 3. Age-standardized mortality rates for bladder cancer world-wide from GLOBOCAN © International Agency of Research on Cancer 2020.

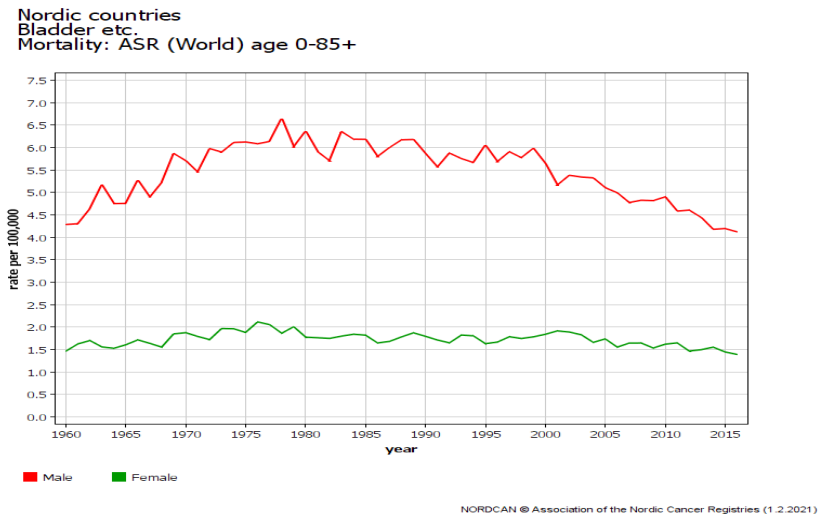

Figure 4. Age-standardized bladder cancer specific mortality rates (between 1960-2016) in the Nordic countries NORDCAN © 2009 Association of the Nordic Cancer Registries.

Risk factors for bladder cancer

Smoking

Smoking is recognized as the single most important modifiable risk factor for BC. In Europe, the proportion of BC attributed to smoking is 43% among men and 26% among women[16]. Current and historic patterns of BC incidence and mortality are reflected in the smoking patterns, with a lag-time of 20-30 years between exposure to cigarette smoking, and BC diagnosis. For example, the incidence of BC in the 2010s was highest (howbeit not uniformly) in geographic areas which had high smoking rates in the 1980s[6, 10]. Current smokers are at between 3 to 4 times increased risk of developing BC compared to never-smokers, while the corresponding risk among ex-smokers is 2-times increased risk. Smoking cessation reduces risk of BC, the reduction in risk increases with time but does not return to the baseline risk (risk among never-smokers). This suggests that the effect of smoke-related carcinogens linger on for the remaining life time[16, 17]. In addition to increasing the risk of BC, there is evidence that current smokers are more likely to present with more aggressive disease, and at diagnosis, are at higher risk of BC recurrence, progression and BC-specific death compared to never-smokers[15, 17].

Tobacco smoke is an abundant source of carcinogens, which include polycyclic aromatic hydrocarbons, nitrogen-based carcinogens such as aromatic amines, N-nitroso compounds, and heterocyclic amines[18]. These carcinogenic compounds cause DNA damage through base modification, double stranded breaks and formation of DNA adducts (portions of DNA attached to carcinogenic molecules). Carcinogens from tobacco smoke inhaled through the lungs are excreted through the renal system, where they concentrate and exert their carcinogenic effect in the urinary bladder[16]. The susceptibility to BC from tobacco smoking is modified by germ-line mutation in a gene that code for carcinogen-detoxifying enzymes, with the most consistent evidence being for the glutathione-s-transferase-mu-1 (*GSTM1*) and N-acetyltransferase-2 (*NAT2*) genes[1].

Age

Age is widely accepted as the strongest non-modifiable risk factor for BC. For individuals younger than 40 years, BC is rare, and the mean age at diagnosis is 73 years. The incidence of BC is 11-times higher among those older than 65 years compared to those younger than 65 years[19, 20]. Several explanations linking age to risk of BC have been proposed. Firstly, accumulation of environmental risk factors such as smoking and occupational carcinogens sufficient to trigger BC occur with the passage of time. Secondly, age allows the necessary accumulation of cellular changes required to initiate BC carcinogenesis[19]. The lag-time between environmental exposures (e.g. 20-30 years for smoking) and the first clinical signs and symptoms of BC might explain the first appearance of BC in the elderly[19, 21]. Thirdly, the diminished capacity to void the bladder that accompanies age, combined with reduced fluid intake due to the bothersome lower urinary tract symptoms (LUTS), may increase the duration of exposure and concentration of carcinogens present in

urine to the bladder. Lastly, activation (proto-oncogenes) or inactivation (tumour suppressor genes) of certain genes (that regulate the cell-cycle) that accompany advanced age may lead to the development of BC[22].

Similar to incidence, mortality due to BC is greater among the elderly[18]. Furthermore, studies have shown that elderly patients are at higher risk of being diagnosed with more advanced disease and are at higher risk of recurrence and progression compared to younger patients[10]. The higher risk of advanced disease at presentation and the relatively less aggressive treatment may partially explain the higher mortality among the elderly[23].

Sex

The incidence of BC is generally between 3 to 4 times higher among men[1, 6, 10]. In Sweden, the age standardized incidence (ASR, [per 100,000]) rates (2020) are 17.5 and 5.3 among men and women respectively[24]. The difference in incidence between men and women has been attributed to several factors, including differences in the distribution of environmental factors such as smoking and occupational carcinogens[10]. With regards to smoking, the prevalence of smoking is higher among men compared to women (31% and 6% for men and women above the age of 15 years respectively)[2]. However, it is believed that the sex differences to smoking exposure only partially explain the differences in incidence[25]. Likewise, differences in exposure to occupational carcinogens has been implicated, however, research in this area is lacking[1, 6, 25]. With regards to biological factors, the role of sex hormones in carcinogenesis, as well as sex differences in the ability to detoxify BC-related carcinogens, have been implicated to contribute to the difference in incidence between men and women[25].

Mortality due to BC (in absolute numbers) is higher among men compared to women[5]. In Sweden (2020) the ASR are 3.7 and 1.4 among men and women respectively. However, the mortality relative to incidence is higher among women compared to men[24]. Previous studies have indicated that women are more likely to be diagnosed with more advanced disease compared to men. Furthermore, prognostic studies indicate that the risk of recurrence and progression is higher among women compared to men[22, 25]. No clear explanation for the poorer prognosis among women is known; however, some studies have suggested that the presentation of more advanced disease is due to a delay in diagnosis among women. Furthermore, haematuria (presence of blood in urine) or LUTS are more likely to be attributed to a urinary tract infection than malignancy in women, and the poorer survival has been attributed to lower efficacy of treatment among women[3].

Occupational Exposures

Exposure to specific occupational carcinogens have been shown to increase the risk of BC and are estimated to account up to 10% of the attributable risk[1, 6, 26]. The agents implicated in relation to BC carcinogenesis are aromatic amines, polycyclic aromatic hydrocarbons and chlorinated hydrocarbons. Exposure to these chemicals most often

occurs in industrial plants which process paint, rubber, textile, dye, leather, metal and petroleum products[1, 4, 6, 26].

The biggest limitation in studies of occupational exposure is the heterogeneity in the classification of occupation. Chemical exposure occurs from the actual task being performed at the workplace, while some individuals in the occupational facility are exposed to the carcinogens, others within the same facility may have not. Current classification of occupational exposures such as the Nordisk Yrkesklassificering (Nordic Occupational Classification) may lump up together such individuals yet have different exposures to these agents[1]. It is well documented in studies that germ-line mutations in genetic variants involved in detoxification of BC carcinogens modify the relationship between exposure to these carcinogens and BC risk[1]. This is because mutations in such genes likely lead to longer exposure to occupational carcinogens[1, 6].

Genetic factors

Genetic factors account for about 31% of all BC cases[27]. Family history of BC is associated with at least a two-fold increase in risk among 1st degree relatives[28-31]. Before the advent of genome-wide association studies (GWAS), identification of germ-line mutations associated with BC were based on two targeted-approaches: 1) linkage studies of high risk families and 2) candidate gene studies[32]. With regards to linkage studies of high risk families, very few high-penetrance mutations have been identified to date[33], as such a polygenic basis for BC was assumed, whereby the risk of developing BC was accounted by a number of genetic polymorphisms, each conferring a small and additive risk of BC[27]. With regards to candidate gene studies, it had already been proposed from concurrent studies, that the association between tobacco smoking, industrial carcinogen and BC was related to genetic variation in enzymes responsible for detoxifying urothelial carcinogens. As such, most studies focused on genetic variants involved in chemical carcinogenesis[33]. With the exception of two of the most studied genetic variants, *GSTM1* and *NAT2*, candidate gene studies were largely unfruitful in discovering more genetic variants that were associated with BC[4]. Single nucleotide polymorphism (SNP) is the most common type of genetic variation in humans, and in the era of GWAS, at least 28 SNPs associated with BC have been discovered, other variants have been discovered through other types of studies/methodologies[33, 34]. Despite the discovery of these variants, a large part of BC heritability is still considered unknown (missing/hidden heritability). This may be due to that BC is largely environmentally driven or due to undiscovered gene-gene or gene-environmental interactions[27, 33, 35].

NAT2

NAT2 is a xenobiotic enzyme responsible for detoxification of carcinogenic chemicals[36]. The capacity for the *NAT2* enzyme to detoxify carcinogens depends on the polymorphism in the gene. Rapid acetylation status is given to individuals who carry the wild-type polymorphism and slow acetylation status to those who carry the mutated-types polymorphism. Those who carry the mutated polymorphism can

further be sub-divided into intermediate acetylation if only one of the two alleles is mutated and ultra-slow acetylation if both alleles are mutated[37]. While both candidate gene studies and GWAS have shown an association between *NAT2* and BC risk, it is believed that the impact of *NAT2* lies in its ability to modify the effect of environmental exposures[33, 38]. This example of gene-environment interaction has been validated in studies on interaction between *NAT2* and tobacco smoking in relation to BC and in studies on interaction between *NAT2* and occupational exposures in relation to BC[38, 39].

Other risk factors

Other environmental risk factors documented to increase the risk of BC include arsenic and nitrates found in drinking water[40]. Dietary factors such as low intake of fruits and vegetables, low intake of water and high intake of processed red meat have been implicated. Iatrogenic risk factors include exposure to external beam radiation, which usually occurs in the treatment of other urogenital cancers (such as cervical and prostate cancers). Treatment with drugs that include cyclophosphamide and pioglitazone have been shown to increase the risk of BC[1, 6]. Association between metabolic factors and BC is described in further detail later.

Review of the anatomy and physiology of the bladder

The bladder is a hollow and muscular organ that functions as a reservoir for urine. It is located medially in the pelvis immediately behind the pubis symphysis. Its shape and size depends on how much urine it has stored. When empty, it is pyramid-like in shape and confined entirely in the pelvis, as it fills up, it assumes an ovoid shape and its superior surface raises into the abdomen[41, 42]. The bladder wall is made up of 3 principal layers: the mucosa, submucosa and muscularis layers. The mucosal layer comprises the innermost layer of the bladder, it consists of epithelial cells called the "urothelium" and a basement membrane. The submucosa, also referred to as the lamina propria, is a layer of connective tissue immediately beneath the mucosa, unlike the mucosa, it contains blood vessels and nerves. The muscularis layer lies beneath the lamina propria and consists of 3 layers of muscles, an outer and inner layer longitudinal muscles, which sandwich a circular layer between them. On the superior surface, covering the muscles is a membrane called the serosa, which is a reflection of the peritoneum. The adventitia (a layer of connective tissue) covers the muscles on the rest of the bladder. Outside the serosa/adventitia, a layer of fat known as "perivesical fat" covers the bladder[42, 43].

Carcinogenesis

BC results from the process of 'carcinogenesis' which can be defined as a complex cascade of events that turns normal cells into cancer cells[44]. The process of cell

renewal and cell death is a tightly regulated process, however, the cellular and genetic changes that result from carcinogenesis lead to uncontrolled proliferation of cancer cells[45]. In urothelial BC, carcinogenesis occurs in the cells lining the urothelium and it may take many years from the triggering event to the diagnosis of disease[46].

Diagnosis, management and prognosis of bladder cancer

From a clinical point of view, BC can be separated into two disease entities, NMIBC and muscle-invasive BC (MIBC). NMIBC constitute 75% of patients at presentation and include tumours confined to the mucosa (stage Ta, Tis) and the lamina propria (stage T1, [**Figure 5**]). The remaining 25% of the patients will present with MIBC (stage T2-T4) and metastatic disease (distal [M1] and/or lymph node spread [from N1], not shown in figure)[10, 47-50].

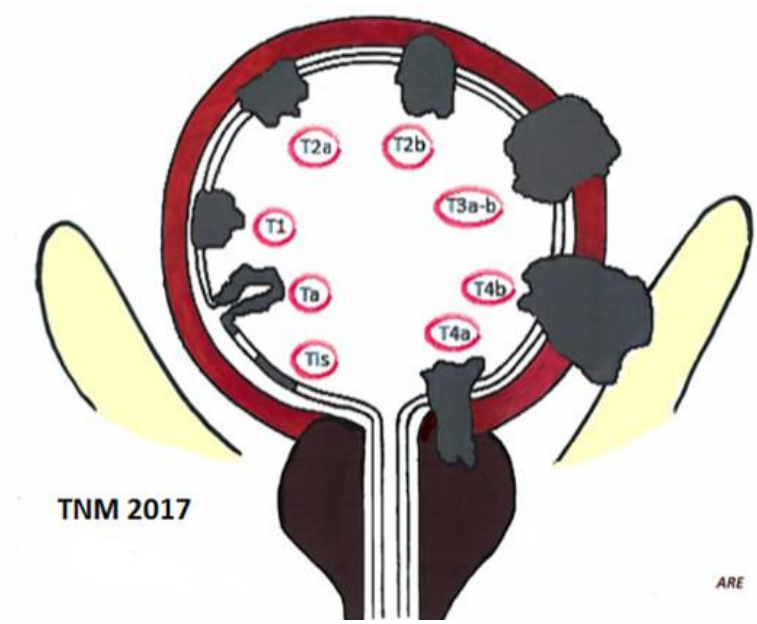

Figure 5. Tumour stages of bladder cancer according to the TNM classification (2017) COPYRIGHT © INTERNETMEDICIN AB

Classification into NMIBC and MIBC is based on the (Tumour-Nodes-Metastases) TNM classification system, which can be further sub-divided into clinical and pathological staging. Clinical staging is usually conducted at initial presentation and is based on physical examination, transurethral resection of bladder tumour (TURBT) and radiological imaging. Pathological staging of the primary tumour (pT) is more accurate, it is based on histopathological examination and requires the

full examination of all the layers of the bladder wall to appropriately evaluate the highest possible pT category[51].

While stage entails the extent to which the tumour has locally invaded the surrounding tissue, grade, the extent to which tumour cells differ in appearance and function to normal cells, is another way of describing how aggressive a tumour is. There have been several iterations of grading systems for BC by the world health organization (WHO), including the 1973, 1999, 2004 and 2016 versions (**Table 1**)[52-55].

Table 1. WHO grading systems for urothelial bladder cancer

Version				
1973	Grade 1		Grade 2	Grade 3
1999	PUNLMP	Grade 1	Grade 2	Grade 3
2004/2016	PUNLMP	Low grade	High grade	

Abbreviations: WHO, world health organization; PUNLMP, papillary urothelial neoplasm of low malignant potential.

Diagnosis

There is no universally accepted algorithm for BC screening. Haematuria is the most common symptoms in BC (3 year positive predictive value=7.5%, among men). If BC is suspected, a focused physical examination, accompanied by radiological imaging, urine cytology and cystoscopy (with TURBT) follow. The TURBT is a critical step, as biopsy specimen is collected for histopathological evaluation[10, 48].

Management

An important part in the management of NMIBC is to determine the likelihood that the tumour will recur or progress (risk stratification), which in turn will guide the type of treatment to be given. Several organizations have developed a risk stratification tool for NMIBC (**Table 2**). Depending on the classified risk, management of NMIBC includes TURBT to remove all visible tumours, and Intravesical instillation, with higher risk tumours, requiring more aggressive treatment (Intravesical installation- immunotherapy instead of chemotherapy) and follow-up (restaging TURBT, maintenance therapy). Radical cystectomy and bilateral lymphadenectomy is the definitive treatment for MIBC (localized), this procedure may also be performed on some high risk NMIBC. Bladder-sparing treatment is usually reserved for patients unfit for surgery or who want to preserve their bladder and includes a combination of TURBT, radio-sensitizing chemotherapy and radiotherapy. Patients with metastatic disease are treated with radical cystectomy followed by some form of systemic chemotherapy[10, 48, 50].

Table 2. Risk stratification in non-muscle invasive bladder cancer

Risk Category	Description
Low risk	Urothelial BC with any of the following: • Single tumour, stage (pTa), grade (G1), less than 3 cm in diameter. • Single tumour, stage (pTa), grade (G2 [low grade]), less than 3 cm in diameter. • Any PUNLMP.
Intermediate risk	Urothelial BC with any of the following: • Single tumour, stage (pTa), grade (G1), more than 3 cm in diameter. • multiple tumours, stage (pTa), grade (G1). • Single tumour, stage (pTa), grade (G2 [low grade]), more than 3 cm in diameter. • Multiple tumours, stage (pTa), grade (G2 [low grade]). • Stage (pTa), grade (G2 [high grade]). • Any tumour, stage (pTa), grade (G2) with no further specifications • Recurrence of any low risk tumour within 1 year of last recurrence.
High risk	Urothelial BC with any of the following: • Stage (pTa), grade (G3) • Stage (pT1), grade (G2/G3) • pTis (Cis) • Aggressive morphological variants of urothelial BC.

Abbreviations: BC, bladder cancer; pT, pathological T stage; G, grade; PUNLMP, papillary urothelial neoplasm of low malignant potential. The table is based on data from the National Institute for Health and Care Excellence (NICE) and European Organization for Research and Treatment of Cancer (EORTC).

Prognosis

In NMIBC, grade is the most important prognostic factor for disease recurrence and progression. Fifteen year progression free survival for low grade Ta tumours is 95%, which drops to 61% for high grade T1 tumours. Between 50-70% of NMIBC will recur and between 10-20% will progress to MIBC[49]. MIBC has a poor prognosis. Stage is the most important prognostic factor, and for organ-confined disease, five-year survival is between 35-50% despite treatment[56]. Approximately 50% progress to metastatic disease with a 5-year survival of less than 10%[10, 48, 50].

Metabolic factors

Blood pressure

Blood pressure (BP) is the force that blood exerts on the walls of arteries as it moves through the circulatory system. Systolic BP (SBP), the peak BP during systole (when the heart contracts and pushes blood from its chambers into the arteries) and diastolic BP (DBP), the lowest BP during diastole (when the heart chambers dilate and refill with blood) are two commonly used BP indices[57, 58]. Elevated BP, also

known as hypertension is one of the most important risk factors for cardiovascular and chronic kidney disease[59-61]. Globally, cardiovascular diseases (CVDs) are the leading cause of death accounting for approximately 17 million deaths out of which 9 million are attributed to complications of hypertension[62, 63]. Hypertension defined as SBP≥ 140 mmHg, DBP≥90 mmHg has a global prevalence of more than 1 billion, and this value is projected to increase by ≥50% by the year 2025[63]. This projected increase is mainly attributed to ageing populations, increased exposure to unhealthy lifestyles, including high salt intake, low potassium intake, unhealthy diet, smoking, low physical activity, alcohol intake and overweight and obesity[64, 65].

Despite the discovery of the aforementioned environmental risk factors for hypertension and a well-understood physiological basis, the exact biological mechanism behind approximately 90% of all cases of hypertension remains unclear (primary hypertension)[63, 66, 67]. While the role of environmental factors in determining BP is established, between 30 to 50% of the variability in BP is estimated to be inherited[63, 68, 69]. It is widely agreed upon that genetic component of BP at population level is mostly determined by the cumulative effects of many genetic variants, each with a small-effect (however, a few genetic variants display a Mendelian mode of inheritance), supporting a polygenic mode of inheritance (**Table 3**)[67, 70, 71].

Table 3 Comparison between polygenic and Mendelian modes of inheritance

Polygenic mode of inheritance	Mendelian mode of inheritance
Usually located in the non-coding (intron) part of the genome.	Usually located in the coding (exon) part of the genome.
Variation in several genes contribute to the same trait.	Variation in one (or few) gene(s) contributes to one trait.
Each contributing variant has a small effect and low penetrance.	The contributing variant usually has a large effect and high penetrance.
Mutation in multiple gene loci required achieve necessary threshold to develop the trait or disease	Mutation in one (or few) gene locus is enough to develop the trait or disease
Examples of disease or trait: SBP, BMI and T2D	Examples of disease or trait: Huntington disease, cystic fibrosis and MODY

Abbreviations: SBP, systolic blood pressure; BMI, body mass index; T2D, type 2 diabetes; MODY, maturity onset diabetes in young.

Since the variation of BP explained by most individual SNPs from GWAS is very small, individual studies are likely to be underpowered (to detect small effects) due to small sample sizes. To circumvent this challenge, several studies maybe pooled to form large consortium (e.g. the International Consortium of Blood Pressure Genome Wide Association Studies [ICBP])[72]. To date more than 900 BP SNPs have been discovered, most of them through the largest available consortia[62]. Despite the discovery of a large number of SNPs, the variation of BP explained by the SNPs is minimal, for SBP, approximately 5.7%. Some research investigators have postulated that the missing heritability is due to some yet-to-be discovered variants that are rare and have modest to large effects on BP. Due to a large number of traits exhibiting some form of missing heritability, the paradigm regarding genetic

mechanisms is shifting from "common disease-common variants" hypothesis to "common disease-rare variants hypothesis" and genetic sequencing is shifting from the use of standard GWAS to whole genome/exome sequencing[62, 63].
While the link between BP and CVD is well-established, the link between BP and cancer has only come into focus in recent times. This link may be due to shared common risk factors and pathophysiological pathways[73, 74]. To date, the most compelling evidence of an association between BP and site-specific cancers, is between BP and renal cell carcinoma (location, kidney). There is some evidence (howbeit inconsistent) that support associations with other specific sites including the colon and rectum, endometrium, breast and prostate[73, 75]. With BC, the few noteworthy conventional studies that have investigated associations were within the Metabolic Syndrome and Cancer (Me-Can) project (a pattern consistent in the other metabolic factors)[75, 76], other studies in the area have been inconsistent for the most part, typically due to small study size, lack of detailed adjustment of smoking, and combining sub-groups (e.g. men and women), who may have different risk[77].

Obesity

Obesity can be defined as abnormal or excessive accumulation of body fat resulting from imbalance between energy intake and expenditure[78]. Over the past few decades, there has been a marked increase in world-wide prevalence of obesity and overweight in a phenomenon known as the "obesity epidemic". It has been estimated that if prevailing trends continue, 20% of the world's adult population will be obese and 38% overweight by the year 2030[78, 79]. Not all individuals exposed to an obesogenic environment become obese, suggesting the role of genetic mechanisms. Twin, family and adoption studies estimate that the component of obesity accounted by inheritance ranges between 40-70%. While no fewer than 10 types of monogenic obesity have been discovered, the majority of the genetically determined obesity is polygenic in nature. This is supported by the approximately 750 gene loci associated with obesity[80, 81].

Body mass index (BMI), defined as weight in kilograms (Kg) divided by height in meter squared (m^2) is universally used as a proxy marker for obesity. It is a highly reproducible measure with little measurement error. The WHO classify BMI into 4 major categories: <18.5 Kg/m^2, underweight; 18.5-24.9 Kg/m^2, normal weight; 25-29.9 Kg/m^2, overweight; ≥ 30 Kg/m^2, obese[2] [78, 82, 83]. It is increasingly being recognized that distribution of fat (not captured by BMI) as opposed to the total amount fat is associated with increased metabolic risk and mortality[83, 84]. Subcutaneous fat accounts for 80-90% of total body fat, however, the remaining 5-20% of total body fat is accounted by visceral fat. Visceral fat surrounds organs and blood vessels, it releases fatty acids, pro-inflammatory agents and other chemical messengers collectively called adipokines that lead to the development of a myriad of metabolic aberrations[81, 84, 85]. Other measures of obesity such as waist

circumference (WC) and waist hip ratio are more strongly correlated with visceral fat compared to BMI[81, 86]. However, BMI is strongly correlated with WC (r> 0.8), furthermore, it is a highly reproducible measure with little measurement error[87-89].

While the health impact of obesity is mainly focused on its role in the development of CVDs, it has also been linked to cancers at several sites including the oesophagus (adenocarcinoma), gallbladder, colon, rectum, pancreas, breast (post-menopausal), liver, kidney, prostate, ovaries and endometrium[83, 90-92]. With regards to total BC risk, 3 out of 4 meta-analyses have shown a modest, but significant positive association[93-95], with one showing a null association[96]. Findings from individual studies have largely been inconsistent[97-100]. This may be due to residual confounding (as a result of lack/inadequate adjustment) by smoking (and other potential confounders), variation in the cut-off points for BMI, and combining sub-populations with potentially different aetiologies. Furthermore, the association between BMI and BC risk may differ according to aggressiveness of disease. While one study found no difference in risk by tumour aggressiveness, studies in this area are still lacking[97]. Studies investigating the association between BP and BC-specific death are inconsistent and difficult to interpret, due to differences in study settings, timing of BMI measure and definition of the time-scale in the analysis[101, 102]

Glucose

Glucose is a 6-carbon sugar and is the primary source of energy for cells in the human body. Once in the body, it travels through the bloodstream to energy-requiring cells. In healthy individuals the level of blood glucose is tightly regulated through homeostatic mechanisms[103, 104]. Hyperglycaemia occurs when the level of blood glucose exceeds 125mg/dL while fasting, or exceeds 180mg/dL, two hours post-prandial[105]. Hyperglycaemia is the hallmark feature of diabetes, a set of heterogeneous diseases characterized by persistent hyperglycaemia leading to macrovascular and microvascular complications[106-108]. Previous studies have reported associations between elevated blood glucose (hyperglycaemia) and cancer risk and mortality (for total cancer, and at specific sites)[107]. In relation to BC, evidence is limited. Two large studies have investigated the association in a European population. In one Me-Can study, they found no association between glucose and total BC risk for both men and women[76], furthermore, evidence of associations separately by aggressiveness of disease is lacking.

Triglycerides

Triglyceride is a lipid molecule composed of 3 fatty acids that are attached to a glycerol backbone. Triglycerides are the main constituent of the fat stores in the body[109]. Because triglycerides are not soluble in an aqueous solution, they are

transported in the bloodstream by large macromolecular structures in the form of very low density lipoproteins (VLDL), low density lipoproteins (LDL) and chylomicrons, and like glucose, it displays post-prandial variation[109, 110]. Epidemiologic and clinical studies demonstrate that elevated triglycerides are an independent risk factor for cardiovascular events[110]. However, epidemiological evidence for an association between triglycerides and total cancer risk and at specific sites is inconsistent[111]. In relation to BC risk and mortality, studies have generally shown a null association. However, most of the studies were small, combined men and women and only investigated total BC risk[9, 112, 113].

Total cholesterol

Cholesterol is a complex, 4-ringed lipid molecule that is a structural component of cellular membranes of eukaryotic cells, it is also the only precursor for steroid hormones[114]. Like all lipids, it mixes poorly with aqueous solutions and is thus transported via several carriers[114, 115]. Elevated blood cholesterol is established as an independent risk factor for cardiovascular events. Chronically elevated cholesterol leads to atherosclerosis (hardening and narrowing of arteries), which can lead to complete occlusion of the artery[114-116]. In relation total cancer risk, in general inverse or null associations have been observed[111, 117, 118]. Specifically with BC risk, studies have generally show a null association, but suffer from the same limitation as mentioned for Triglycerides[76, 117].

Rationale

BC is a common cancer form in developed countries, furthermore it is an expensive cancer to treat, making it a significant public health burden. While 50% of the risk can be accounted by smoking in the general population, other established risk factors occur in specific sub-populations but do not fully account for the remaining proportion in the general population.

Derangement of metabolic factors including BMI, BP and glucose, triglycerides and cholesterol catalyse health burdens of global proportions such as the obesity, hypertension and diabetes epidemics. While the majority of the health burden of these metabolic factors are in the form of cardiovascular diseases, epidemiological evidence is gathering linking them to cancer overall, and at specific anatomical sites. Due to shared risk factors and pathophysiological pathways, it is plausible that these metabolic factors may account for a proportion of the risk (not accounted by smoking) of BC in the general population. Furthermore, many of the previous studies that investigated metabolic factors in relation to BC risk, investigated BC as a single entity, without taking into account that the aetiology of BC may differ depending on tumour characteristics such as stage and grade. Investigating BC separately based on the aforementioned tumour characteristics may clarify the associations and potentially reveal new biological mechanisms.

Interaction between risk factors in relation to a specific disease is a known phenomenon and reflects complex biological mechanism. A proportion of the risk of BC within the population may be attributed to the joint effect of several exposures. Furthermore, assessing interaction between metabolic factors and other established risk factors for BC may provide insight into potential biological mechanisms linking metabolic factors to BC.

Aims

Overall aim

The overall aim of this doctoral thesis was to investigate the association between metabolic factors and BC risk and mortality and the interaction in such associations with smoking and BC genetic variants.

Specific Aims

Paper I: To investigate the sex-specific associations between metabolic factors and risk of total BC and separately for NMIBC and MIBC, and risk of BC-specific mortality, and to investigate the interaction between metabolic factors and smoking in such associations.

Paper II: To investigate the associations between BMI, SBP and DBP, and risk of total BC, and separately for NMIBC and MIBC risk and by tumour grade (for NMIBC risk) among men, and in an attempt to disentangle the confounding effect of smoking, among never-smokers. Furthermore, to investigate the association between BMI and BP and risk of BC-specific mortality from the time of baseline examination in the full population, and among cases from the time of diagnosis.

Paper III: To investigate the association between SBP and DBP, and risk of total BC, and separately for NMIBC risk and MIBC risk using conventional survival analysis, and in a Mendelian randomization analysis, and to study the interaction with *NAT2* in such associations among men.

Paper IV: To investigate a BC weighted genetic risk score (wGRS), SBP and DBP, and their interaction, in relation to UC risk overall and separately for aggressive and non-aggressive UC risk in men.

Theoretical framework

Previous studies investigating the association between these metabolic factors and BC have generally shown inconsistent findings due to several limitations (**Table 4**). Furthermore, additive interaction is rarely investigated in epidemiological studies, and with regards to the association between metabolic factors and BC, it is a research gap which has not been explored previously. The design of the PhD project is two-fold, firstly to address many of the major limitations in such associations in an attempt to clarify them, and for BP, to address causality. Secondly, the focus was to investigate interaction between the metabolic factors and other established environmental and genetic factors.

Table 4. Summary of weakenesses and research gaps and what measures were taken to address them according to each paper.

Paper	Exposure(s)	Outcome(s)	Limitation(s) and research gap(s) addressed	Measures taken
I.	Blood pressure, BMI, glucose, triglycerides and total cholesterol	Total BC, NMIBC, MIBC and BC-specific mortality	Low statistical power	Large sample size and number of cases (n, 811,633 [cases, 3,737])
			Confounding by smoking	Detailed smoking data
			Combining sub-groups with potentially different risk profile or aetiology	Investigate men and women separately and investigate BC separately for NMIBC and MIBC
			Potential interaction with smoking	Additive and multiplicative interaction
II.	Blood pressure and BMI	Total BC, NMIBC (grade 1-3), MIBC, BC-specific mortality and all-cause mortality	Confounding by smoking	Investigated associations only among "never-smokers"
			Combining sub-groups with potentially different aetiology	Further incorporate classification by grade in addition to classification by muscle-invasiveness
III.	Blood pressure	Total BC, NMIBC and MIBC	Known and unknown confounders, residual confounding	Mendelian randomization analysis
			Potential interaction with *NAT2*	Additive and multiplicative interaction
IV.	Blood pressure	Total UC, non-aggressive UC and aggressive UC	Potential interaction with a weighted BC genetic score composed of several genetic variants	Additive and multiplicative interaction for total UC and separately for non-aggressive and aggressive UC

Abbreviations: BMI, body mass index; BC, bladder cancer; NMIBC, non-muscle invasive BC; MIBC, muscle-invasive BC; UC, urothelial cancer; *NAT2*, n-acetyltransiferanse 2.

Methods and subjects

Study populations

To fulfil the specific aims of this thesis, we used several population-based prospective cohorts (**Figure 5**). In **paper I**, we used the Me-Can 2.0; in **paper II**, we pooled 3 Swedish cohorts (Västerbotten Intervention Programme [VIP], Malmö Preventive Project [MPP] and the Construction Workers Cohort [CWC]); in **paper III**, we pooled 2 Swedish cohorts (Malmö Diet and Cancer Study [MDCS] and MPP) and we also used the UK-biobank; in **paper IV**, we used the MDCS.

The Metabolic Syndrome and Cancer Project

The Me-Can was initiated in 2006 with the overall aim of generating a large, pooled cohort to study components of the metabolic syndrome in relation to cancer risk[119]. It is a pooling of 6 (initially 7) population-based cohorts from Sweden (VIP and MPP), Norway (Oslo study 1, Age 40-Programme, Norwegian Counties Study (NCS) and Austria (Vorarlberg Health Monitoring and Prevention Programme [VHM&PP])[120]. Initially, the Me-Can consisted of approximately 580,000 participants with 36,000 incident cancer cases, this first iteration of the project was called Me-Can 1.0. In 2015, cohort information and linkages were additionally extended to include additional participants and observations (VIP [2006-2014] and VHM&PP [2003-2005]), additional follow-up time and additional variables, including smoking duration and intensity, and BC tumour characteristics. This follow-up project (Me-Can 2.0) included approximately 814,000 participants with 84,000 incident cancer cases[119]. A description of each individual cohort included in the Me-Can will immediately follow. Ethical approval was obtained from ethical committees in Sweden, Norway and Austria[120].

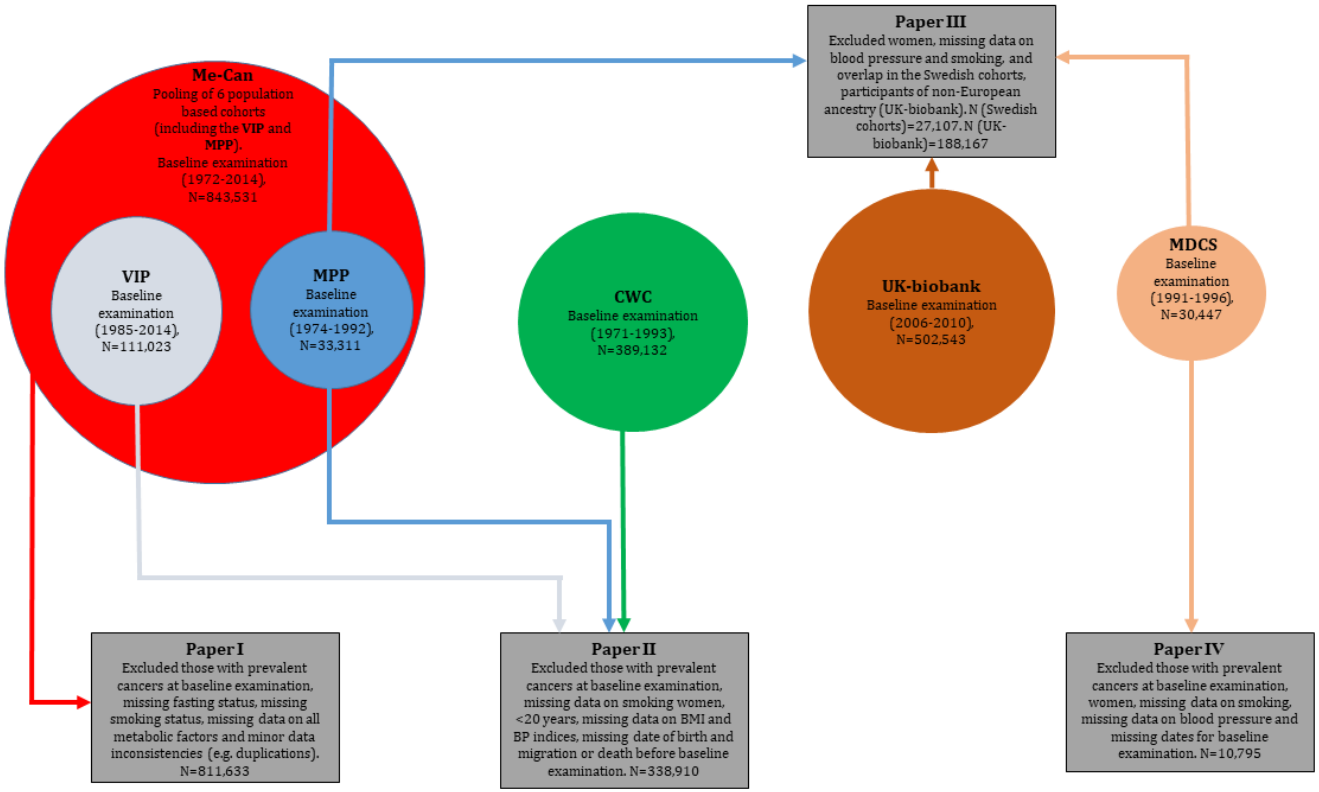

Figure 5. Study populations in **paper Ⅰ-Ⅳ**. **Abbreviations**: Me-Can, Metabolic Syndrome and Cancer Project; VIP, Västerbotten Intervention Programme; MPP, Malmö Preventive Project; CWC, Construction Workers Cohort; MDCS, Malmö Diet and Cancer Study; BMI, body mass index; BP, blood pressure

The Västerbotten Intervention Programme

The VIP was conceived with the aim of preventing CVD and diabetes in the residents of Västerbotten County, located in Northern Sweden. It initially started as the Norsjö Project in Norsjö County (1985). In an effort to prioritize preventive measures, the project was integrated into primary healthcare, where the primary health centre staff took on the responsibility of inviting all inhabitants to the partake in a health examination and a comprehensive questionnaire, by 1991 the entire Västerbotten county was covered[121]. In addition to the baseline assessment, participants were invited to a repeat health examination and questionnaire when they reached 30 (only before 1996), 40, 50 and 60 years of age. Participants were requested to fast for at least 4 hours before a health examination, from 1992 onwards, this was changed to at least 8 hours of fasting. The cohort is on-going and the participation rate over the years has been at least 60%[120].

The Malmö Preventive Project

The MPP was started in 1974 with the aim of inviting a pre-specified section of the adult middle-aged population living in the city of Malmö, South of Sweden, to find individuals at high risk of CVD, alcohol abuse, breast cancer and diabetes, so that they benefit from preventive intervention[122]. Invited participants underwent a comprehensive screening for risk factors that included a physical examination, radiological and laboratory investigations and concluded with a self-administered questionnaire. Participation rate over the baseline years (1974-1992) ranged from 64-78%. A majority of men were screened in the first half of the baseline years (1974-1982), while a majority of the women were screened in the latter half (1981-1992).

The Oslo study 1

The Oslo study 1, like all the other included Norwegian cohorts, was initiated by the Norwegian Institute of Public Health, as such, they are all uniform and conform to the same survey design. The Oslo study 1 began in 1972 with the aim of conducting epidemiological research to prevent CVD. It was the first of 2 rounds of surveys conducted exclusively among men aged between 40 and 49 years, living in Oslo, the capital city of Norway. In addition, a random selection of men (7%) aged 20-39 years were also invited. The survey had an average participation rate of about 60%[120].

The Age 40-Programme

The Age 40-Programme was started in 1985 with the aim of conducting epidemiological research in relation to CVD[123]. By 1993, the survey had covered all the counties in Norway. During the baseline years of 1985-1999, county residents aged 40-42 years were invited to undergo a detailed physical examination. The average participation rate over the baseline years was 69%[120].

The Norwegian Counties Study

The aim of the NCS was to screen individuals at high risk of CVD, who might benefit from prevention interventions. It was started in 1974 in 3 Norwegian counties (Oppland, Finnmark and Sogn og Fjordane) and was conducted over 3 specific time periods. In the first time period (1974-1978), all residents aged 35-49 years and a random sub-set of 20-34 year-olds from the 3 counties were invited to the screening. Those invited to the second and third time periods (1977-1983 and 1985-1993, respectively) were a mixture of previous and new participants. Over all three time periods, the participation rate ranged from 78-90%[120].

The Vorarlberg Health Monitoring and Prevention Programme

The VHM&PP is a population-based surveillance program located in the province of Vorarlberg in western Austria. The main aim of the surveillance was to screen for chronic diseases, with priority given to cancer and CVD. All adults (19 year of age or older) in the province were invited via various mediums of communication to participate in a health examination annually. The overall attendance rate between 1985-2003 was approximately 66%[120].

The Construction Workers Cohort

The CwC was a nation-wide initiative founded by the Swedish Foundation for Occupational Safety and Health with the object of coordinating all activities regarding Occupational health among construction workers living in Sweden. Every 2-5 years, construction workers were invited to undergo a health examination which additionally included a comprehensive questionnaire on occupation and smoking habits. Despite being a voluntary initiative, at least 80% of the construction workers completed one health examination. At the time, female construction workers comprised only 5.3% in the country, which contributed to the low proportion of women in the CWC[124, 125].

The Malmö Diet and Cancer Study

The MDCS began as joint initiative between the Medical Faculty at Lund University, the Swedish Medical Research Council, Swedish Cancer Society and International Agency for Research on Cancer (IARC) with the aim of investigating the link between diet and cancer[126]. The time period for baseline examination extended from 1991-1996, during which all Malmö residents born between 1926 and1945 were invited. In 1994, the inclusion criteria expanded to encompass those born 1923-1945. Furthermore, participants must have had a good command of the Swedish language and full mental capacity. Baseline examination required two visits to a MDCS assessment centre: the first visit included collection of

anthropometric measurements and blood samples, concluding with the distribution of a questionnaire, the second visit included a detailed diet history assessment by a qualified interviewer and an error check of the completed questionnaire[127]. The participation rate in the MDCS was approximately 40% over the baseline years[128]. The MDCS had ethical approval from the Lund University Ethics Committee, a written informed consent was obtained from every participant.

The UK-biobank

The UK-biobank was established by the Medical Research council and Wellcome trust to investigate genetic and non-genetic risk factors for major diseases of middle and old age[129]. With baseline years spanning from 2006-2010, eligible individuals were invited to 22 assessment centres across the United Kingdom (UK) to undergo a health examination, which included a physical examination, collection of blood, urine and saliva by a qualified nurse[130]. Additionally, participants had to complete touch-screen questionnaire. The attendance rate for the UK-biobank was approximately 5% [131]. The UK-biobank was approved by the Northwest Multi-Centre Research Ethics Committee, a written informed consent was obtained from every participant[132].

Assessment of main exposures

Blood pressure

Paper I: Since the Me-Can was a pooling of 6 different cohorts, the methods for assessing the exposures (including BP) differed between the individual cohorts. The number of BP readings ranged from 1-3, seated or supine, with an interval between readings ranging between 1 to 10 minutes. Aside from the Age 40-Programme, where they used an automated device, a standard mercury sphygmomanometer was used to read the BP, a more detailed description of BP assessment in the Me-Can has been previously published[120].

Paper II: BP was taken in a supine position (VIP, seated after 2009) with a standard mercury sphygmanometer in all the cohorts. In the MPP, BP was recorded twice with a 10 minute interval in-between readings, the average of the two readings was then recorded as the actual BP. In the VIP and CWC, BP was taken once after a rest of 5 minutes.

Paper III: In the MDCS, BP was taken twice with a 5 minute interval in-between readings using a standard mercury sphygmomanometer. The average of the two readings was then recorded as the actual BP. BP assessment in the MPP is described

above. In the UK-biobank, two BP readings were taken while seated, with 1 minute interval in-between readings using an automated device (OMRON Healthcare, Europe B.V. Kruisweg 577 2132 NA Hoofddorp).

Paper IV: BP assessment in the MDCS is described above.

From **paper I-IV**, we investigated BP in several ways. In **paper I**, BP, in the main analysis, was assessed as the quantity mid-BP (= [SBP+DBP]/2). This is because mid-BP had been shown more informative than SBP and DBP, at least with respect with CVD[133]. Secondly, it would allow us to compare our findings with those of the only other large prospective studies at the time[75, 76], one of which originated from the same cohort[76]. Lastly, we investigated 4 other metabolic factors and deduced that assessing BP as a single quantity would reduce the number of tests performed, in subsequent papers where we assessed fewer exposures, we investigated BP separately in the form of SBP and DBP. In **paper I, III and IV**, we transformed the BP variable(s) into z-scores using the following equation: $z = (x-u)/\sigma$, where x is the actual level, u is the mean, and σ is the standard deviation (SD), transforming variables into z-scores (standardised separately by cohort and sex) allowed us to directly compare the estimates of the variables since they are quantified on the same scale. This was especially useful and informative in paper I where we investigated 5 exposures. **In paper II and III**, we assessed SBP and DBP per 10mmHg. In **paper III and IV**, we additionally investigated SBP and DBP in specified categories. In **paper IV** and in relation to the categorical analysis, we investigated the p-value for trend across categories, which was achieved by incorporating the categories (each category was the value of the mean for that category) as a continuous variable in the Cox regression model and testing its coefficient using the Wald test.

Body mass index

Paper I and II: Height and weight were taken with light clothing and no shoes. BMI was calculated as weight (Kg)/[height (M)]^2.[131]As with BP, we investigated BMI throughout the papers in an identical manner. In all the papers, and when not investigating BMI as the main exposure, we adjusted for BMI in the form of quantiles.

Glucose, triglycerides and total cholesterol

Glucose, triglycerides and cholesterol were only investigated in **paper I**. The fasting status (time since the last meal), blood component, and method used to measure glucose, triglycerides and total cholesterol differed between cohorts. In the Norwegian cohorts, fasting was not required, thus most of the samples taken (96%) were non-fasting. In contrast, most of the samples taken in the Swedish and Austrian cohorts were in a fasting state (>8 h). In the Norwegian cohorts serum was used, in

the VHM&PP and VIP plasma, and in the MPP, whole-blood[120]. The whole-blood values in the MPP were converted to the equivalent of serum levels by dividing by 1.15. Aside from the Oslo study 1 and NCS, which used the non-enzymatic method, all the other cohorts used the enzymatic methods. Using special formulae, levels for the enzymatic method were transformed to make them comparable to those of the non-enzymatic method. We assessed these metabolic factors in the form of z-scores (separately by cohort, sex and fasting status). Glucose and triglycerides demonstrated a right skewed distribution, we therefore log-transformed the variables using the natural log before z-score transformation.

Selection of SNPs and genotyping

Paper III: In the MDCS we used a set of 29 pre-selected SNPs (**Appendix I**). The selection of the SNPs was based on two large BP consortia (SNPs were included if they achieved genome-wide significance), the ICBP and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium[72, 134]. The dataset containing the 29 SNPs was static, consequentially, SNPs from the most recent GWAS at the time of this study could not be added. However, two publications originating from the MDCS based their genetic risk score on these 29 SNPs[135, 136]. DNA samples were genotyped on the MALDI-TOF mass spectrometer (Sequenom MassArray, Sequenom, San Diego, CA, USA). SNAP v2.2.2 was used to identify proxy SNPs in the case that commercial primers were not available. TaqMan and KASPar allelic discrimination were used to genotype individual SNPs that failed genotyping by Sequenom. An internal quality control was carried out to remove SNPs that deviated from Hardy-Weinberg equilibrium (HWE) and had low genotype rate among other parameters.

In the UK-biobank, SNPs (**Appendix II**) were identified in the ICBP and 14 other consortia. We searched for SNPs that were discovered in populations of European ancestry, and were validated through replication at least once. In addition, they must have been discovered outside the UK-biobank. This is because in 2-sample Mendelian randomization (MR) analysis, complete or partial overlap in samples used to discover the SNPs and in the sample used in the MR analysis leads to weak instrumental bias, biased towards the confounded observational association. DNA samples were genotyped on Affymetrix (ThermoFisher Scientifics) on two similar, custom-designed axiom arrays (UK-BiLEVE and UK-biobank). In addition to the internal quality control, for the MR analysis, we removed SNPs in linkage disequilibrium, had a low genotype rate, low minor allele frequency and were out of HWE using PLINK software. LDlink, a web-based tool was used to identify suitable proxy SNPs for missing candidate SNPs.

Paper IV: SNPs related to BC were identified in GWAS that spanned from 2008 to 2017. Included SNPs were discovered in populations of European Ancestry. The

Illumina GSA version 1 genotyping array was used to genotype the DNA samples. Low quality samples were removed in the internal quality control.

In **Paper III and IV**, we generated weighted genetic risk scores (wGRS) by calculating the genotype dosage of each SNP in an additive fashion (0, 1 and 2 for each risk increasing allele), after-which each SNP was multiplied by its respective weight, i.e. the beta-coefficient from the association of each SNP with BP (**paper II**) or BC (**paper IV**). The beta-coefficients were obtained from the GWAS where the SNPs were selected from. This is followed by summing up across all the variants with the following equation (wGRS (per individual) = $[\beta_1 \times SNP_1 + \beta_2 \times SNP_2 + \ldots \beta_x \times SNP_x]$/number of SNPs). In some BC GWAS, the association between a SNP and BC was expressed as odds ratio (OR), in such a case, the OR was converted into its beta-coefficient using the natural log. Alternatively, we could have used an unweighted genetic score (without taking into account the beta-coefficients), however, this lacks biological specificity since it is not expected that each SNP has an equal effect on the trait, and inadvertently reduces statistical power[137] .

In **Paper II**, we investigated the interaction with *NAT2*, SNP rs1495741 (A/G), where "A/A" polymorphism reflected fast acetylation, "A/G" reflected intermediate acetylation and "G/G" reflected slow acetylation. In the analysis, we combined fast and intermediate acetylators due to small numbers.

Assessment of covariates

Information on date of birth, smoking, physical activity and education were obtained from questionnaires integrated into the baseline examination. With regards to smoking, we incorporated information on smoking status and smoking dosage (among current smokers) into one variable. Therefore, the smoking variable was categorical with the following classification: never-smokers, ex-smokers and current smokers (quantiles [based on pack years]). In **paper III**, due to limitations in the CWC, we used smoking status (never-smokers, ex-smokers and current smokers) as the only control for smoking. Ex-smokers represented a heterogeneous group, and specifying them may be challenging if information on e.g. "years since cessation" is lacking. We would have stratified the ex-smoker group by accumulated pack-years, but opted to keep them as a single category since it did not materially improve adjustment. We tested the best way to formulate the smoking variable by testing several of them against lung cancer a priori in **paper I**, where end-point data on lung cancer was also available.

Like with smoking, we incorporated all the other aforementioned covariates into the models in the form of categories. Components of covariates such as the Charlson comorbidity index and treatment from BC were obtained from medical records and

quality registers. Selection of co-variables was based on literature and data availability.

Follow-up and outcome assessment

Follow-up and assessment of outcome for all the papers are briefly summarized in **Table 5**.

Table 5. Follow-up and outcome assessment in paper I-IV

Criteria	Paper I	Paper II	Paper III	Paper IV
Cancer diagnosis, mortality and migration	Identified through linkages with the respective national cancer registers, cause of death registries and population registries. In the VHM&PP cohort, information on migration was not obtained. In the UK-biobank, information on migration was obtained from the National Health Services, among other sources. In the Nordic countries, linkage to these registries is made possible by a unique identification number possessed by every individual living in those countries.			
Follow-up of linkages	Until 31 December 2012 for the Norwegian cohorts and 31 December 2014 for the Swedish and Austrian cohorts	Until 31 December 2014	Until 31 December 2016 for the Swedish cohorts. Until 31 December 2015 in the UK-biobank	Until 31 December 2018
Definition of BC*	According to ICD-7 (181.[0-6]) and 10 (C67 [0-9]), including carcinoma in situ (D09.[0-1])	According to ICD-10 including carcinoma in situ	According to ICD-9 (188.[0-9]) and 10, including carcinoma in situ	According to ICD 10 (C64-68 [0-9]) including carcinoma in situ
Classification of tumour	NMIBC (Ta, T1, CIS), MIBC (T2-T4, including metastatic tumours)	NMIBC (by grade[WHO, 1999]) and MIBC	NMIBC and MIBC	Non-aggressive UC and aggressive UC (based on muscle invasiveness and UC-specific mortality
Evaluation of tumor stage	Clinical staging based on histology (from biopsy), palpation and radiological imaging			Tumours re-evaluated for pathological staging
Definition of bladder cancer death	BC (ICD 7-10) recorded as the underlying cause of death in the cause of death registry			UC (ICD10) recorded as the underlying cause of death in the cause of death registry

*In **paper IV**, we used all cancers of the urothelium (renal collecting tubules, calyces, pelvis, ureters, urethra and bladder). Abbreviations: VHM&PP, Vorarlberg Health Monitoring and Prevention Programme; BC, bladder cancer; ICD, International Classification of Disease; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer; WHO, world health organization; UC, urothelial cancer.

Selection

In **paper I-IV** the main reasons for exclusion at baseline are as follows:

Prevalent cancers- Not only did we exclude participants with prevalent BC/UC, we also excluded those with other prevalent cancers, this is because the prevalent cancer might have an impact on the level of the exposure, which is especially important for metabolic factors which may be altered due to constitutional symptoms (e.g. unintended weight loss and fever) caused by the prevalent cancer. The definition for cancer was identical in all the papers, which included all malignant neoplasms (including those of haematopoietic and lymphoid origin) and excluding basal-cell carcinoma and all carcinomas *in situ* except BC.

Missing data on Smoking- All participants with missing smoking data were excluded, this is due to smoking being a potentially strong confounder in the associations between metabolic factors and BC/UC.

Missing data on main exposures- All participants who had missing data on all main exposures were excluded since they did not add anything in the analysis, however, if they had data on at least one main exposure, they were retained.

In **paper II-IV**, we excluded women in the main analysis. The reasons for this exclusion are: lack of an association between BP (which became the main exposure of interest from **paper II** onwards) and BC risk among women in previous studies (including **paper I**), relatively low statistical power (few number of cases) among women, population composition (**paper II**) and sex-interaction in particular analysis (**paper IV**).

There were other causes of exclusions, these were specific to each paper and are included in **Figure 5**, and are described in greater detail in each individual paper.

Statistical Analysis

Cox proportional hazards regression analysis

Most of the analysis throughout the papers was conducted within a survival setting. As such, we used the Cox proportional hazards regression to investigate the association between exposure of interest and outcome. We calculated hazard ratios (HR) and their 95% confidence intervals (95% CI) to quantify such associations. We used age as the underlying time variable. In **paper II**, when investigating associations with BC-specific mortality among cases only, we used follow-up time

from the date of BC diagnosis until date of death due to BC or censoring, as the underlying time scale. We investigated BC-specific mortality in the full-population and among cases, because these two approaches have different strengths and weakness, therefore, using both approaches may mitigate the weaknesses of each individual approach. Participants were followed from baseline examination up until the date of event, or until censoring due to emigration, diagnosis of another cancer (**paper I-III**) in the analysis of BC risk (overall or separately), or until end of follow-up, whichever one occurred first. In the case where BC as an outcome was divided into 2 sub-groups (i.e. NMIBC and MIBC) follow-up in the Swedish cohorts begins on 1 January 1997 and censored individuals before that date were excluded. This date is when the Swedish National Register of Urinary Bladder Cancer (SNRUBC) was initiated, where data on tumour characteristics is reported. To assess the proportional hazards (PH) assumption, we used Schoenfield residuals and if a co-variate violated the PH assumption, they were added as strata in the model, however, in all the cases it did not materially change the effect estimates. In all the papers the main exposures did not violate the PH assumption.

Restricted cubic spline analysis

In regression analysis, an important step is to ascertain how the independent variable is related to the outcome. Restricted cubic splines (RCS) offers a method to present non-linear relationships between a continuous variable on an outcome. Across the papers, we performed RCS analysis for two main purposes. Firstly, it was used to assess the kind of relationship a continuous variable had with outcome and if a non-linear relationship was observed, to determine where to make the cut-off points when subsequently creating the categorical variable. When incorporating continuous covariates (as confounders) such as BMI and physical activity, we run cubic spline analysis, however, in all the cases, they did not significantly deviate from linearity. Secondly, it may be that the relationship between the main variable and outcome is non-linear, in such a case, demonstrating such an association in the form of a RCS may be appropriate, which was the case in **paper III** where for example, we found a non-linear association between SBP and MIBC (p-value: 0.028).

To determine whether a relationship significantly deviated from linearity, we used the likelihood ratio test (LR-test), whereby the fitted linear (constrained) model was nested within the model (unconstrained) that additionally incorporated the cubic spline. For the RCS, we placed the knots at 5^{th}, 35^{th}, 65^{th}, 95^{th} percentiles.

Heterogeneity test (Lunn-McNeil approach/duplication method)

The Lunn-McNeil (LM) approach is a method used in analysis of competing risks, but may be used to assess heterogeneity in the outcomes. This allows to detect if the

effects on the outcomes are sufficiently different so as to report them separately[138]. Unlike other approaches to competing risks, where one Cox PH regression model is fitted for each event type, in the LM approach, one model is fitted for all event types. Since only 1 model is fitted and executed once, it allows for performance additional statistical tests regarding features of competing risks, which would otherwise not be possible with other approaches for competing risks (it allows for post-estimation commands). In order to perform the LM approach, the data must be "augmented", thus, if there are *x* competing risks, the original data must be duplicated *x* times, one row for each event type of interest. Once augmented, the LR-test is used to compare the model that allowed associations to vary by outcome to the model that did not[139].

Regression dilution ratio

A variable is rarely measured with 100% precision, as such variation in the measurements is a common occurrence. This variation in the measurements may be due to measurement error or physiological (short or long term) changes and are collectively called within-person variability. This has implications when classifying the exposure and estimating associations. For example, screening for hypertension may lead to misclassification error if it is based on one BP measurement, and without accounting for within-person variability. Estimates of the BP in relation to BC risk based on the single baseline BP will underestimate estimates of "usual" BP in relation to BC risk. This phenomenon is referred to as "regression dilution bias (RDB)" due to the propensity of values that are extreme on a single measure to be less extreme upon a repetition. The extent of RDB for a specific variable can be assessed by using repeated measurements from all or a proportion of participants at baseline examination. The repeated measures may be used to estimate a quantity called the regression dilution ratio (RDR), which can then be factored in with the regression coefficient to correct for RDB. In **paper I and II**, where we had repeated observations for some participants, we corrected for RDR using a method described in Wood *et al*[140]. The RDR was factored in with the regression coefficient using the following equation: $HR_{corrected} = \exp(\text{Log}[HR_{original}]/RDR)$.

Interaction analysis

Interaction occurs when the risk of disease in the presence of two or more exposures differs from risk expected to result from their singular effects. Indeed in most diseases, the underlying causes are not discrete exposures, but interaction between them. The cascade of events that give rise to disease are often complex, interaction between the involved exposures reflects this complexity. Interaction can be assessed on a multiplicative, as well as an additive scale. Multiplicative interaction occurs when the relative risk for the joint effect is significantly greater or smaller than what would be expected by multiplying the relative risk of the individual exposures.

Because the Cox regression model is exponential, it inherently expresses associations on a multiplicative scale. Multiplicative interaction has received more focus in epidemiological studies compared to additive interaction, this is because the interaction estimate (and confidence intervals) on a multiplicative scale is immediately obtained by incorporating a product term in multiplicative models such as logistic regression and Cox regression, two widely used models. Additive interaction occurs when the relative risk of the joint effect is significantly greater or smaller than what would be expected by adding the relative risks of the individual exposures. Some epidemiologists believe that in biological models, the joint effects of two exposures appear to be consistent with interaction on an additive scale[141]. However, what is widely agreed is the role of additive interaction in public health, where it informs us the sub-group which is at highest risk or will benefit most from an intervention (**Appendix III**).

In **paper I, III and IV** we investigated the significance for multiplicative interaction using the LR-test, whereby the model without the product term was nested in the model that included the product term. Alternatively, significance for multiplicative interaction could be tested by incorporating the product in the model, then testing its coefficient using the Wald test, regardless, the two methods give similar results. We investigated additive interaction using the quantity "relative excess risk due to interaction (RERI)", the equation for RERI is as follows: RERI= RR_{11}-RR_{10}-RR_{01}+RR_{00}, where RR_{11} is the relative risk for the joint effect; RR_{10} is the relative risk for the first exposure (Exposure A); RR_{01} is the relative risk for the second exposure (Exposure B) and RR_{00} is the background risk (risk when not exposed to A or B), see **Figure 6**.

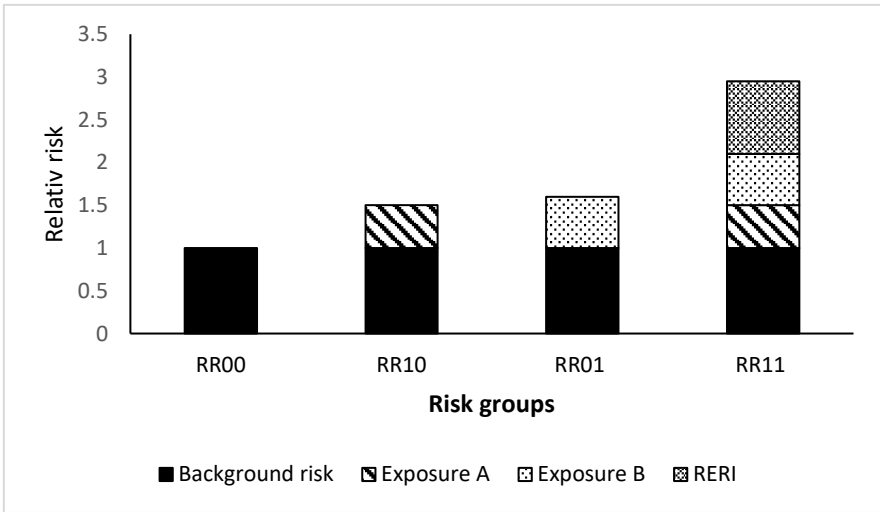

Figure 6. Illustration of additive interaction between Exposure A and Exposure B using the relative excess risk due to interaction (RERI).
Abbreviation: RR, relative risk

Since calculation of RERI is conducted outside the regression setting, confidence intervals have to be obtained through an appropriate method. We used the Delta method, which was described in detail by Lemeshow and Hosmer[142]. Alternatively, we would have used the bootstrap method among other methods, however, computer-simulated studies have shown that most of the methods give identical results[142].

Mendelian randomization analysis

MR analysis is a form of instrumental variable (IV) analysis that makes use of genetic variants to investigate a causal association between an exposure of interest and an outcome. MR has its roots in econometrics[143, 144], where it was a widely used approach for handling a phenomenon called "endogeneity". Endogeneity is a collective term for the consequences of measurement error in variables, observed and unobserved confounding, and inverse causality between the exposure and outcome. Such misspecification in the model biases the true causal effect of the exposure on the outcome. IV analysis attempts to eliminate this bias by incorporating an IV, which is a variable correlated with exposure, but immune to the effect of endogeneity. In MR analysis, genetic variants are well suited as IV for a number of reasons: firstly, genes inherited by a child are passed on from the parents in a random manner (gene recombination and chromosome segregation during meiosis [Mendel's second law]), and therefore are not affected by confounders in the exposure-outcome association. Secondly, they are formed at conception, before any disease could occur, thereby eliminating reverse causality, and finally, they are measured with high precision, thereby mitigating the effects of measurement error (and by extension, regression dilution bias)[144, 145].

With the advent of "high throughput next generation sequencing" in recent times, genetic variants are now being genotyped at an accelerated rate make MR studies more viable than before. At a conceptual level, MR analysis has been compared to a randomized clinical trial (RCT), however, one of the main differences is the interpretation of the result, where effect estimates from MR studies represent the life-long differences in the exposure. In studies where the accumulated effects of the exposure are investigated, like RCT, effect estimates tend to be larger compared to the MR counterpart. When conducting MR analysis, 3 key assumptions must be fulfilled: firstly, the instrumental variable must reliably associate with the exposure of interest; secondly, the instrumental variable must not independently associate with the outcome, all the effect of the exposure on the outcome must depend on the exposure (exclusion restriction assumption); and lastly, the instrumental variable must not be associated with confounders in the exposure-outcome association (**Figure 7**)[146].

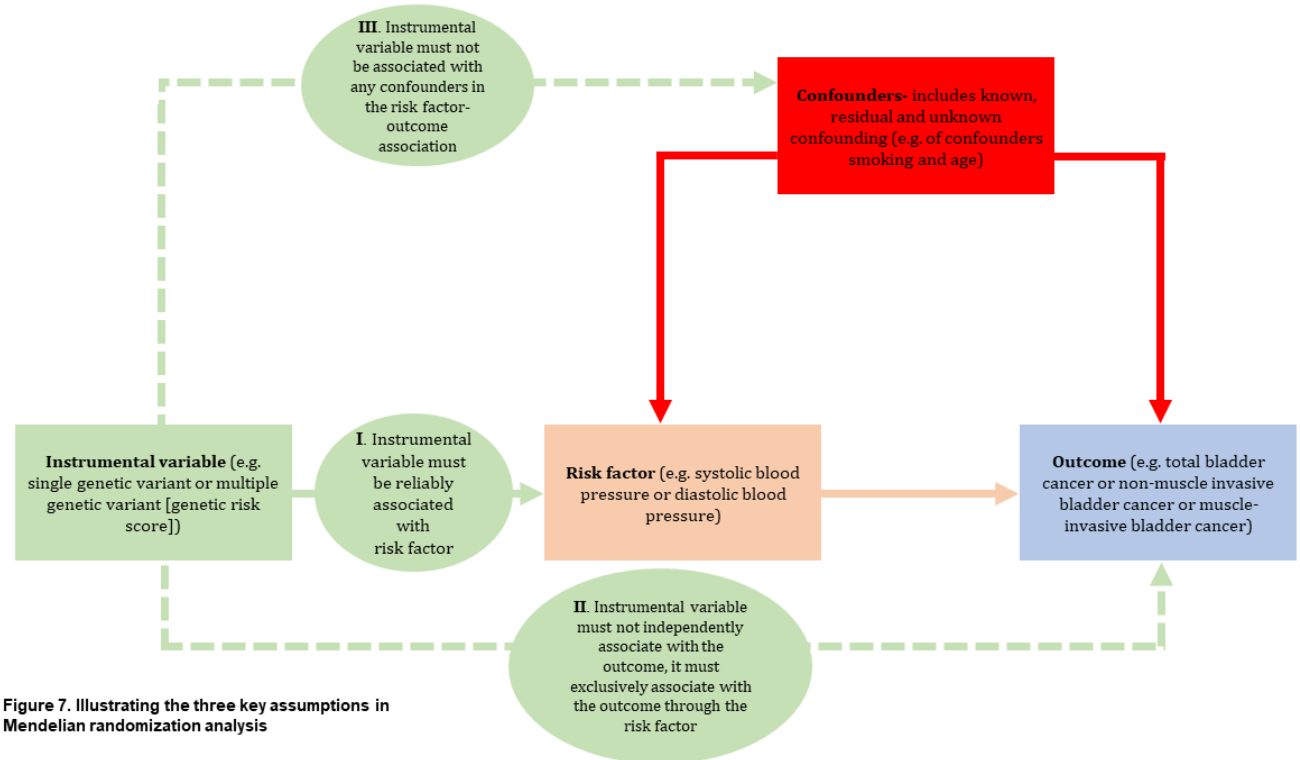

Figure 7. Illustrating the three key assumptions in Mendelian randomization analysis

In **paper II**, we investigated the association between BP and BC using MR analysis, the IV were SNPs that were aggregated into a wGRS. In the Swedish cohorts, MR analysis was conducted only in the MDCS, and not in the MPP, due the fact that genotyping in the MPP was conducted approximately 25 years after baseline. This could have introduced selection bias, since not all the participants were given an equal chance to get genotyped (those that were genotyped had to survive 25 years). Furthermore, we conducted MR analysis for SBP only in the MDCS, but additionally investigated DBP in the UK-biobank. As a supplementary analysis, we additionally performed MR analysis that included prevalent BC as the outcome. We conducted the analyses in both a one-sample and two-sample setting. In one-sample analysis, we use one sample to estimate the genetic association with the exposure and the genetic association with the outcome to estimate the causal association between the exposure and the outcome. The statistical method applied is the two stage least square (2SLS) regression analysis. In the first stage the wGRS is regressed on the exposure of interest. The predicted values (predicted genetic level of the exposure), obtained from the first stage are then used as the IV and are regressed on the outcome to estimate the causal effect of the exposure of interest on the outcome (second stage).

In a two-sample analysis, the genetic association with the exposure and the genetic association with the outcome are obtained from two similar, but non-overlapping samples. MR analysis demands good statistical power. Two-sample MR was the remedy to the power limitations faced by the earlier one-sample MR analysis; it takes advantage of large consortia being created from several GWAS and use summary estimates to investigate causal associations. In the two-sample analysis, we used the inverse-variance weighted method (IVW) to determine causal associations, which is achieved by regressing the genetic association with the exposure on the genetic association with the outcome in a linear regression, using inverse variance weights and constraining the intercept to zero in the model. In addition to a potential increase in statistical power, another advantage of two-sample MR analysis is that it permits the assessment of pleiotropy.

Pleiotropy occurs when the IV influences the outcome through other biological pathways that do not involve the exposure of interest, inclusion of pleiotropic genetic variants into the IV violates the second MR assumption (exclusion restriction assumption). We used MR-Egger and MR-PRESSO methods to assess pleiotropy. In the MR-Egger method, the estimate is similar to the IVW, with the exception that the intercept is left unconstrained. If the intercept in the Egger estimate is significantly different from zero, pleiotropy is suggested[146]. MR-PRESSO is another tool to evaluate pleiotropy, it has 3 components: the global test, which detects for pleiotropy; the outlier test, which corrects for pleiotropy by removing the outliers (pleiotropic variants); and the distortion test, which tests for differences in the causal estimates before and after removing outliers. We also conducted a "leave-one-out-analysis" as a complement to MR-Egger. Leave-one-

out-analysis is performed to assess the influence of potentially outlying genetic variants in the MR-Egger estimates. It is performed by sequentially leaving out each genetic variant in the MR analysis, thus *"X"* analysis are performed, each with *"X-1"* data points. The intention of the leave-one-out-analysis is to test the consistency of the causal estimate given the one genetic variant is left at a time, this allows us to determine if the causal effect is being driven by one (or a few) genetic variants.

While both MR-Egger and MR-PRESSO assess pleiotropy, they serve different purposes and have different strengths and weaknesses. MR-Egger is a global test for pleiotropy, and it still provides robust estimates even if all the genetic variants in the IV are pleiotropic. MR-PRESSO is an outlier test, designed for fine-tuning, as such it is more sensitive to detecting smaller pleiotropic effects, and is more suited to IVs that have few pleiotropic genetic variants. Both methods are limited by the InSIDE assumption[147].

All statistical analysis was conducted on STATA 13 and 16 (StataCorp LLC, College Station, TX), except MR-PRESSO, which was conducted on R-studio version 1.1.423.

Results

Tables showing the baseline characteristics of all the papers can be found in **Appendix IV**.

Blood pressure

Blood pressure and risk of total bladder cancer/Urothelial cancer

Table 6 shows HRs (95%CI) of BC outcomes by z-scores and per 10 mmHg of BP among men in **paper I-IV**. With the exception of a few findings in **paper II**, most associations did not significantly differ from linearity in **paper I and II**, thus the per unit presentation of the results. In **paper I**, SBP was positively associated with total BC risk among men but not women, HR per SD, 1.12 (95% CI, 1.04-1.20). In **paper III**, SBP was positively associated with total BC risk only in the Swedish cohort (HR per SD, 1.14 [1.05-1.22]), but not the UK-biobank (HR per SD, 0.93 [0.85-1.02]). There was no association between SBP and total BC/UC risk in **paper III and IV**. There was no association between DBP and BC risk in all the papers.

In **paper III**, we conducted MR analysis in addition to the conventional analysis (**Figure 8**). The quantity "r^2" obtained when the IV is regressed on the exposure of interest, represents the proportion of the variation in the exposure explained by the IV. In the MDCS, the variation of SBP explained by the wGRS was 0.6%. In the UK-biobank the variation of SBP and DBP explained by their wGRS were 0.5% and 0.7% respectively. In the MDCS, genetically predicted SBP was positively associated with risk of total BC when determined by both the 2SLS (OR per SD, 7.70 [1.92-30.9]) and the IVW (OR per SD, 3.43 [1.12-10.5]). **Figure 9** graphically illustrates the association between genetically predicted SBP and risk of total BC in the MDCS as determined by IVW and the subsequent pleiotropic analysis using MR-egger. We found no association between genetically predicted SBP and DBP and risk of BC in the UK-biobank. The MR-Egger estimates for the BP indices on risk of BC showed that in all the analyses, the MR-Egger intercepts did not significantly differ from zero. This was further consolidated by no evidence of pleiotropy and outlying genetic variants in the MR-PRESSO and leave-one-out-analysis respectively.

Table 6.Hazard ratio (95% confidence interval) of bladder cancer outcomes by z-scores and per 10mm Hg of blood pressure among men in paper I-IV

Exposure	Unit	Paper I[a]	Paper II[b]	Paper III[c]	Paper IV[d]
Total BC/UC			**HR (95%CI)**		
SBP	Per SD	1.12 (1.04-1.20)		1.14 (1.05-1.22)[i]	1.09 (0.98-1.20)
				0.93 (0.85-1.02)[j]	
	Per 10 mmHg		0.98 (0.94-1.02)[e]	1.05 (1.01-1.09 [i]	
			1.04 (1.00-1.09)[f]	0.96 (0.92-1.01)[j]	
DBP	Per SD	1.06 (1.00-1.14)		1.02 (0.95-1.09)[i]	1.00 (0.90-1.11)
				0.96 (0.91-1.01)[j]	
	Per 10 mmHg		0.95 (0.87-1.03)[e]	1.02 (0.95-1.09)[i]	
			1.08 (0.90-1.27)[f]	0.98 (0.90-1.07)[j]	
NMIBC/ Non-aggressive UC					
SBP	Per SD	1.08 (0.94-1.22)		1.06 (0.96-1.18)	1.00 (0.87-1.13)
	Per 10 mmHg		0.99 (0.96-1.02)[e]	1.02 (0.96-1.08)	
			0.98 (0.87-1.12)[f]		
DBP	Per SD	0.96 (0.85-1.10)		0.99 (0.89-1.10)	0.93 (0.82-1.07)
	Per 10 mmHg		1.00 (096-1.05)[e]	0.99 (0.89-1.10)	
			1.00 (0.79-1.30)[f]		
MIBC/Aggressive UC					
SBP	Per SD	1.26 (1.04-1.53)		1.32 (1.09-1.59)	1.27 (1.07-1.50)
	Per 10 mmHg		1.08 (0.96-1.19)[e]	1.14 (1.02-1.27)	
			1.25 (1.00-1.55)[f]		
DBP	Per SD	1.12 (0.60-1.43)		1.27 (1.04-1.55)	1.14 (0.96-1.36)
	Per 10 mmHg		1.08 (0.88-1.34)[e]	1.25 (1.03-1.53)	
			1.21 (0.75-1.93)[f]		
Bladder cancer-specific mortality					
SBP	Per SD	1.22 (1.04-1.44)			
	Per 10 mmHg		1.02 (0.94-1.11)[e,g]		
			1.04 (0.96-1.13)[e,h]		
			1.10 (1.01-1.20)[f,g]		
			1.05 (0.92-1.22)[f,h]		
DBP	Per SD	1.16 (1.02-1.47)			
	Per 10 mmHg		1.00 (0.85-1.22)[e,g]		
			1.09 (0.91-1.29)[e,h]		
			1.13 (0.96-1.32)[f,g]		
			1.05 (0.85-1.60)[f,h]		

[a] In all models attained age was used as the underlying time metric, in **paper I** models were adjusted for categories of: baseline age, date of birth, smoking and BMI. They were stratified by cohort and corrected for RDR.

[b] In **paper II**, models were adjusted for categories of age at baseline, date of birth, smoking, BMI, education and cohort and corrected for RDR.

[c] In **paper III**, models were adjusted for age at baseline, date of birth, smoking and BMI

[d] In **paper IV** models were adjusted for age at baseline, smoking, BMI, education and physical activity

[e] in the full population; [f] among never-smokers; [g] followed-up from date of baseline examination (total population); [h] followed-up from date of diagnosis (cases only). [i] in the Swedish cohorts; [j] in the UK-biobank

Abbreviations: BC, bladder cancer; HR, hazard ratio; CI, confidence interval; UC, urothelial cancer; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer

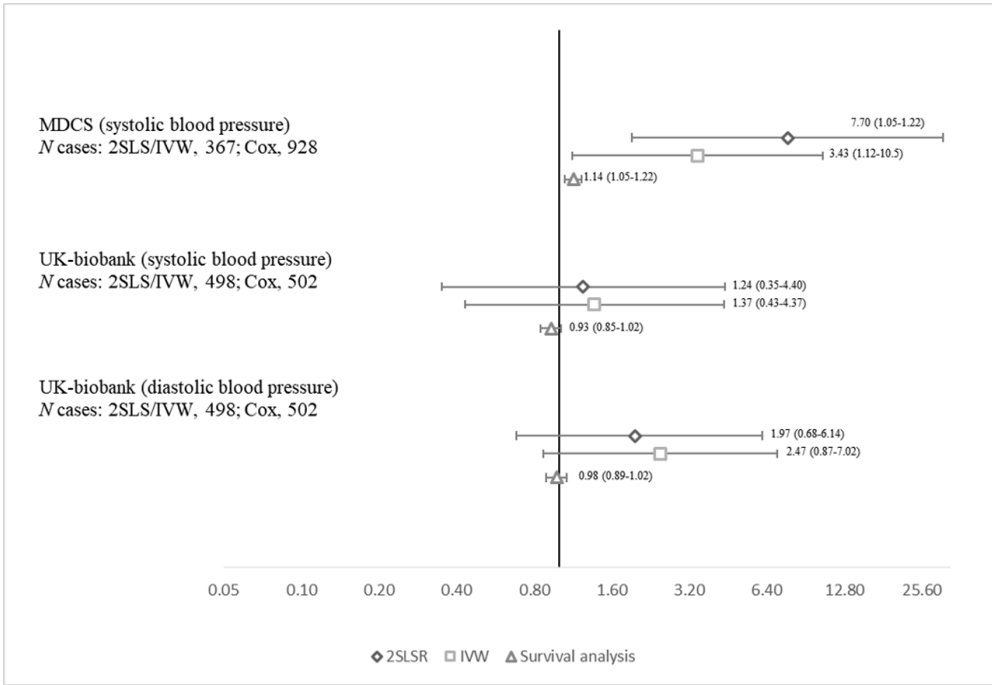

Figure 8. Relative risk (95%CI) of BC per SD of systolic and diastolic blood pressure using MR analysis 2SLS regression and IVW method and Cox regression in the MDCS* and UK-biobank.

*Also includes the Malmö Preventive Project

Abbreviations: MDCS, Malmö Diet and Cancer Study; 2SLS, 2-stage least square; IVW, inverse variance weighted

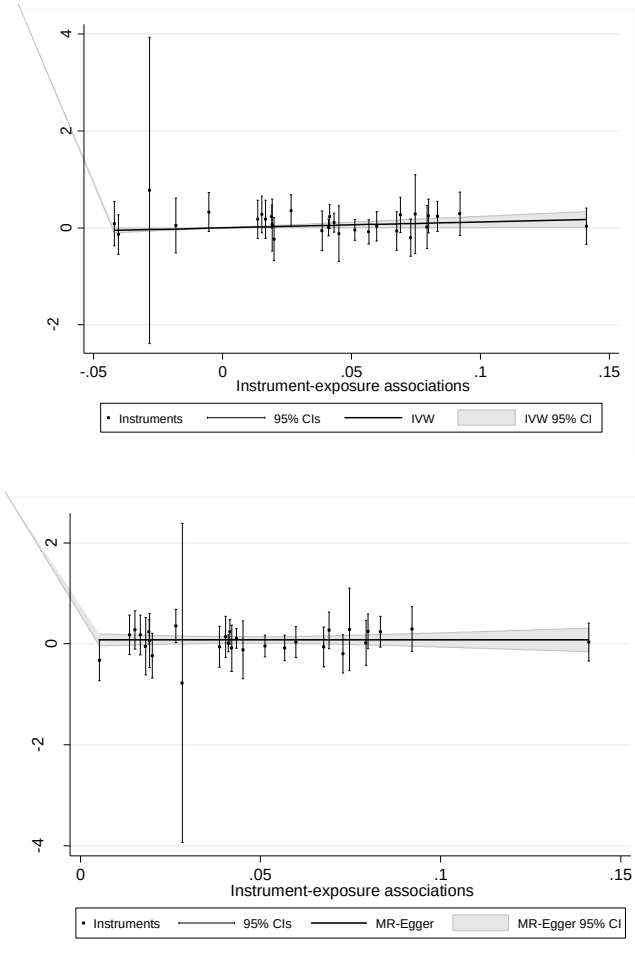

Figure 9. MR-Egger plots for the (top) inverse variance-weighted (IVW) estimate and (bottom) MR-Egger estimate for systolic blood pressure, with bladder cancer risk as the end-point in the Malmö Diet and Cancer Study.

Blood pressure and risk of non-muscle invasive bladder cancer/non-aggressive urothelial cancer

There was no association between SBP, DBP and NMIBC/non-aggressive UC risk in papers **I-IV**. In **paper II**, we additionally investigated NMIBC separately by grade and found no associations.

Blood pressure and risk of muscle invasive bladder cancer/aggressive urothelial cancer

SBP was positively associated with MIBC/aggressive UC risk among men (HR per SD, 1.26 [1.04-1.53]) in **paper I**, among never smokers in **paper II** (HR per 10 mmHg, 1.25 [1.00-1.55]), in the Swedish cohorts in **paper III** (HR per SD, 1.32 [1.09-1.59]), and in **paper IV** (HR per SD, 1.27 [1.07-1.50]). While there was no significant linear association between SBP and MIBC in the full population in **paper III**, a non-linear association was indicated (LR-test, p= 0.028). DBP was positively associated with MIBC risk in **paper III**, but there were no significant associations in **paper I, II and IV**.

BP and risk of bladder cancer-specific mortality

In **paper I**, we found a positive association between SBP and risk of BC-specific mortality among men (HR per SD, 1.22 [1.04-1.44]), but not women (HR per SD, 1.08 [0.77-1.53]). In **paper II**, we assessed risk of BC-specific mortality from baseline examination in the full-population, and from the date of diagnosis among cases. Among never-smokers, we found a positive association between SBP and risk of BC-specific mortality, when investigating from baseline examination (HR per 10 mmHg, 1.10 [1.01-1.20]), but not when investigating from the date of diagnosis among cases only. In **paper I**, DBP was positively associated with the risk of BC-specific mortality among men, but not women (HR per SD, 1.16 [1.02-1.47]). In **paper II** and in analysis of never-smokers, DBP was non-lineally associated with risk of BC-specific mortality when investigating from baseline examination (LR-test, p=0.002), the association was inverse for DBP lower than 80 mmHg (HR per 10 mmHg, 0.49 [0.32-0.73]), and positive for DBP equal or higher than 80 mmHg (HR per 10 mmHg, 1.22 [1.01-1.47]). Among cases only, and when investigating from the date of diagnosis, the association was non-linear (LR-test, p= 0.002), but only significant for DBP lower than 80 mmHg (HR per 10 mmHg, 0.09 [0.03-0.32]).

Interaction between BP, smoking, NAT2 and a weighted genetic risk score for bladder cancer in relation to bladder cancer risk

In **paper I**, we investigated interaction between mid-BP and smoking status in relation to total BC risk and found no interaction on an additive or multiplicative scale (**Figure 10**). In **paper III**, we investigated interaction between BP indices (SBP and DBP) and *NAT2* in relation to total BC risk and found no interaction on either scale. In **paper IV,** we investigated interaction between BP indices and wGRS in relation to UC risk (total and separately for non-aggressive and aggressive UC) and found a positive additive interaction between SBP and wGRS in relation to aggressive UC risk, the RERI (and 95%CI) was 0.86 (0.16; 1.58) with a p-value=0.018 (**Figure 11**). The corresponding multiplicative interaction was non-significant (LR-test, p=0.075).

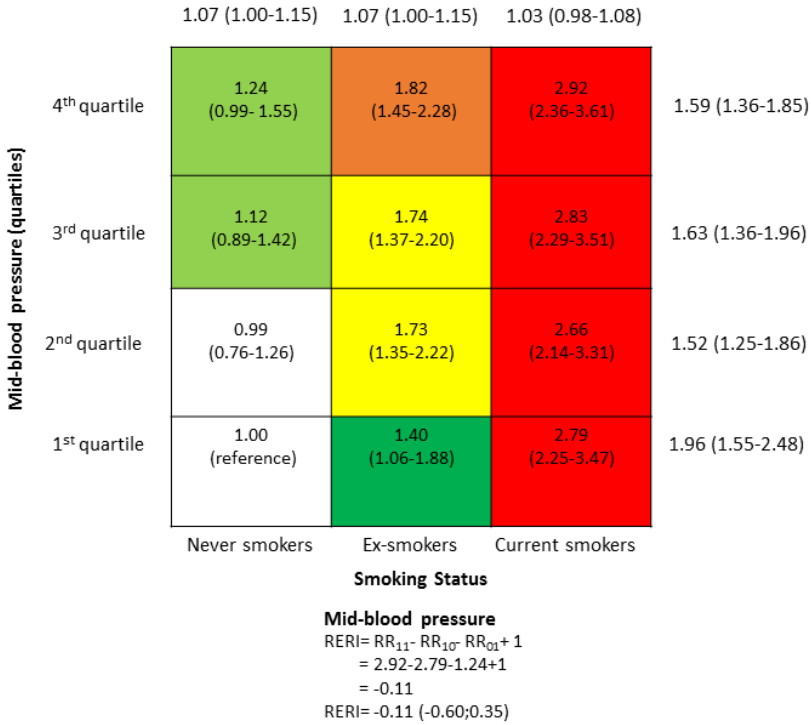

Figure 10. Hazard ratios (95%CI) for BC according to mid-blood pressure and smoking status among men. The delta method was used to obtain CI for the RERI**.** Hazard ratios were calculated by Cox regression with age as the underlying time variable. Hazard ratios were not corrected for RDR. Abbreviations: CI, confidence intervals; RERI, relative excess risk due to interaction; RDR, regression dilution ratio.

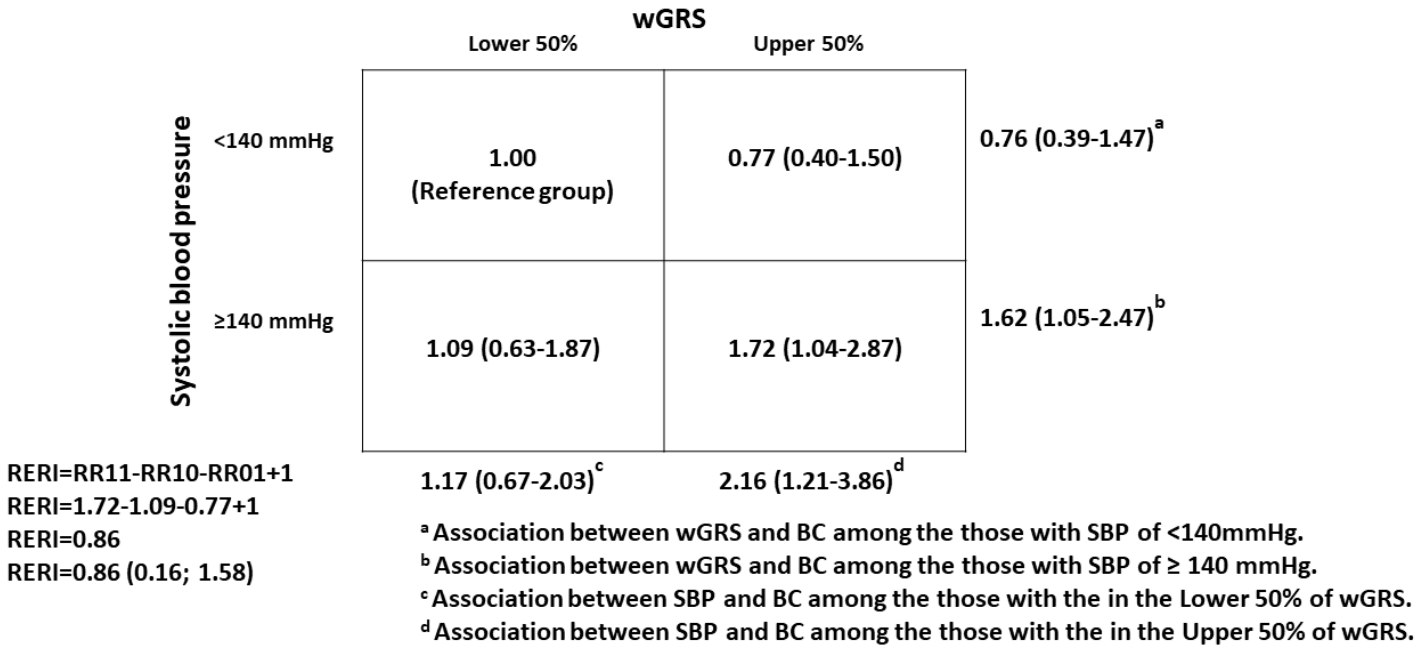

Figure 11. Additive interaction between SBP and wGRS in relation to muscle-invasive bladder cancer risk among men in the MDCS.
Boxes in the figures express hazard ratios (95% confidence interval) for MIBC risk for combinations of SBP and wGRS. RERI was calculated as RR_{11}-RR_{10}-RR_{01}+1, for which the delta method was used to obtain confidence intervals. Hazard ratios were calculated by Cox regression with attained age as the underlying time scale, adjustment for age at baseline, date of birth and smoking, BMI, physical activity and education. Abbreviations: wGRS, weighted genetic risk score; RERI, relative excess risk of interaction; SBP, systolic blood pressure; BC, bladder cancer; MIBC, muscle-invasive bladder cancer.

Body mass index

Body mass index and risk of total bladder cancer

In **paper I**, we found an inverse association between BMI and total BC risk among women (HR per SD, 0.90 [0.82-0.99]), but no association among men (HR per SD, 1.02 [0.99-1.07]) (**Table 7**). In **paper II**, we found a non-linear association between BMI and total BC risk (LR-test, p=0.035). For BMI equal or higher than 25 Kg/m^2, there was a positive association (HR per 5Kg/m^2, 1.15 [1.03-1.26]), and for BMI lower than 25 Kg/m^2, there was a non-significant inverse association (HR per 5Kg/m^2, 0.93 [0.81-1.06]), see **Figure 12**.

Table 7. Hazard ratio (95% confidence interval) of bladder cancer outcomes by z-scores and per 5 Kg/m² of BMI in paper I and II

Outcome	Model	Paper I[a]		Paper II[b]
Total bladder cancer			**HR (95%CI)**	
		Men	**Women**	**Men**
	Per SD	1.02 (0.99-1.07)	0.90 (0.82-0.99)	
	Per 5 Kg/m^2			1.02 (0.98-1.08)[c] 1.04 (0.94-1.17)[d]
Non-muscle invasive bladder cancer				
	Per SD	1.09 (1.01-1.18)	0.88 (0.75-1.04)	1.10 (1.02-1.19)[c]
	Per 5 Kg/m^2			1.14 (0.97-1.33)[d]
Muscle-invasive bladder cancer				
	Per SD	0.95 (0.82-1.08)	1.00 (0.79-1.28)	0.95 (0.85-1.09)[c]
	Per 5 Kg/m^2			1.14 (0.85-1.54)[d]
Bladder cancer-specific mortality				
	Per SD	0.92 (0.82-1.02)	0.87 (0.68-1.09)	
	Per 5 Kg/m^2			0.99 (0.88-1.10)[c,e] 1.02 (0.92-1.14)[c,f] 1.02 (0.78-1.34)[d,e] 1.06 (0.81-1.38)[d,f]

[a] In all models attained age was used as the underlying time metric, in **paper I** models were adjusted for categories of: baseline age, date of birth, smoking and BMI. They were stratified by cohort and corrected for RDR.
[b] In **paper II**, models were adjusted for categories of age at baseline, date of birth, smoking, BMI, education and cohort
[c] in the full population; [d] among never-smokers; [e] followed-up from date of baseline examination (total population); [f] followed-up from date of diagnosis (cases only).
Abbreviations: HR, hazard ratio; CI, confidence interval; SD, standard deviation

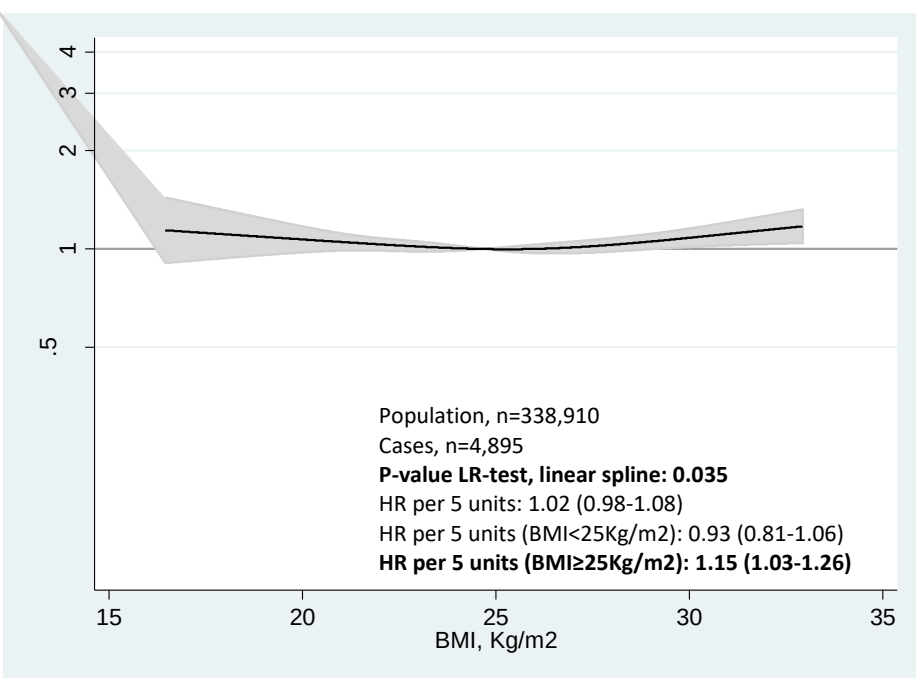

Figure 12.The Hazard ratio (HR) (black line) and 95% confidence interval (shaded area) of total BC risk by per 5 unit increase in body mass index (BMI) among men. Models were derived from restricted cubic spline regression, with knots placed at percentiles of 5, 35, 65, and 95. Participants who had values that were more extreme than the equivalent of +/-2.5 standard deviations (SD) were excluded from the analyses. P value LR test, linear-spline, refers to likelihood-ratio (LR) tests of the linear model nested in a model with the addition of splines. Abbreviations: BC, bladder cancer; SD, standard deviation

Body mass index and risk of non-muscle invasive bladder cancer

In **paper I**, we found a positive association between BMI and NMIBC risk among men, but not women (HR per SD, 1.09 [1.01-1.18]). In **paper II**, we found a positive association between BMI and NMIBC risk in the full population, but not among never-smokers (HR per 5Kg/m^2, 1.10[1.02-1.19]). Furthermore, in the full-population, BMI was positively associated with NMIBC (grade 3) risk (HR per 5Kg/m^2, 1.17 [1.01-1.34]).

Body mass index and risk of muscle-invasive bladder cancer

There was no association between BMI and MIBC risk in **paper I and II.**

Body mass index and risk of bladder cancer-specific mortality

There was no association between BMI and risk of BC-specific mortality in **paper I and II**.

Glucose, triglycerides and total cholesterol

In paper I, glucose was positively associated with MIBC risk among women (HR per SD, 1.99 [1.04-3.81]), but not among men (HR per SD, 1.19 [0.80-1.75]). Furthermore, glucose was positively associated with risk of BC-specific mortality among men, but not women (HR per SD, 1.71 [1.15-2.43]). There were no associations between glucose and total BC risk and between glucose and NMIBC risk (**Table 8**).

Among men, triglycerides were positively associated with total BC risk, NMIBC risk, and among both men and women, with risk of BC-specific mortality.

Total cholesterol was positively associated with NMIBC risk among men, but not women. However, there were no associations between total cholesterol and total BC risk, MIBC risk and risk of BC-specific mortality.

There was no evidence of additive and multiplicative interaction between glucose, triglycerides and total cholesterol, and smoking status in relation to total BC risk.

Table 8. Hazard ratio (95% confidence interval) of bladder cancer outcomes by z-scores of glucose, triglycerides and total cholesterol in paper I.

Outcome	Glucose		Triglycerides		Total Cholesterol	
	HR per SD (95%CI)[a]					
	Men	Women	Men	Women	Men	Women
Total bladder cancer						
	1.11 (0.97-1.32	1.23 (0.90-1.71)	1.17 (1.08-1.27)	1.12 (0.96-1.32)	1.65 (0.99-1.11)	1.02 (0.91-1.14)
Non-muscle invasive bladder cancer						
	1.15 (0.90-1.46)	0.83 (0.49-1.32)	1.30 (1.12-1.48)	1.30 (0.95-1.71)	1.14 (1.02-1.25)	1.12 (0.93-1.31)
Muscle-invasive bladder cancer						
	1.19 (0.80-1.75)	1.99 (1.04-3.81)	1.08 (0.87-1.39)	1.32 (0.87-2.03)	0.96 (0.80-1.14)	0.84 (0.61-1.17)
Bladder cancer-specific mortality						
	1.71 (1.15-2.43)	1.28 (0.63-2.72)	1.23 (1.02-1.48)	1.79 (1.23-2.60)	1.14 (0.99-1.30)	1.00 (0.75-1.34)

[a] In all models attained age was used as the underlying time metric, models were adjusted for categories of: baseline age, date of birth, smoking and BMI. They were stratified by cohort and corrected for RDR.

Weighted genetic risk score for bladder cancer and urothelial cancer

In **paper IV**, we found a positive association between wGRS and total UC risk and non-aggressive UC risk (HR per SD, 1.26 [1.14-1.40] and 1.34 [1.10-1.52] respectively). Furthermore, those who were in the 4th quartile of the wGRS, were at higher risk of total UC and non-aggressive UC, compared to those in the 1st quartile of wGRS (HR per SD, 1.65 [1.24-2.19] and 2.06 [1.43-2.96] respectively, p-trend for both [<0.001]). There was no association between wGRS and aggressive UC risk. Lastly, there was evidence of a positive additive interaction between SBP and wGRS in relation to aggressive UC risk (see results for SBP).

Discussion

Blood pressure and bladder cancer

In the 4 studies within this thesis, we found a positive association between SBP and total BC risk among men in **paper I and III** (through conventional analysis and by MR analysis), no association between DBP and total BC risk, and no association between any BP indices and NMIBC/non-aggressive UC. With respect to MIBC/aggressive UC, we found a positive association with SBP, howbeit, only among never-smokers in **paper II** and a positive association between DBP and MIBC only in **paper III**. We found an association between SBP and BC-specific mortality in **paper I and II**, in both papers, the positive association was found when investigated from the date of baseline examination and not from the date of BC diagnosis. Among women, BMI was inversely associated to total BC, glucose was associated with MIBC and triglycerides were associated with risk of BC-specific mortality.

Previous studies investigating the association between BP and BC outcomes are lacking, and to our knowledge, there are no previous studies investigating the association between BP and total BC risk using MR analysis. The few conventional studies that have investigated this association were within the Me-Can, the addition of our study to prior Me-Can studies was more detailed adjustment for smoking, inclusion of data on tumour characteristics and better statistical power to investigate sub-groups separately. In those studies, they found an association between BP and BC risk. In 2 of the studies, they investigated BP as mid-BP[75, 76] and in the other study[9], separately. There are a few studies on the association between hypertension and BC risk, however, categorizing a variable tends to reduce statistical power, which would diminish the capability to detect weak to moderate associations, as is suspected in the association between BP and BC. Secondly, this may not be the best approach to specify the association between BP and BC risk, because for example in cardiovascular research, studies have shown that risk of cardiovascular events does not start at BP of 140/90 (an arbitrary threshold), but rather steadily increases from BP of 115/85. While we did categorize BP in certain analysis, we primarily investigated the relationship between BP and BC outcome after having confirmed approximate linearity (per X mmHg, per SD or RCS).

Investigating BC/UC into sub-groups, by making use of tumour characteristics increased the biological specificity of findings. Our findings on BP and BC risk separately for NMIBC and MIBC or non-aggressive UC and aggressive UC, throughout the papers exemplify how investigating the associations for BC separately into pathologically and clinically informed sub-groups may bring to light unique findings. In our studies, we consistently found a null association between SBP and NMIBC/non-aggressive UC and a positive association between SBP and MIBC/aggressive UC. It is likely that the less consistent, positive association between SBP and BC risk found in our studies is driven by the MIBC/aggressive UC sub-group. This is further supported by the comparatively weaker effect estimate in the MR analysis that include prevalent cancers (which naturally comprise more indolent tumours) compared to the MR analysis that only included incident cases. This suggests SBP is associated with BC progression as opposed to BC initiation. However, biological mechanisms linking BP and BC risk (and more specifically MIBC/aggressive UC) among men is unclear. Experimental studies have suggested that the renin-angiotensin pathway maybe implicated in BC progression by promoting angiogenesis (formation of blood vessels), inhibiting apoptosis (programmed cell death) and promoting cell migration through activation of the angiotensin II type 1 receptor (AT1R), which is highly expressed on BC cells[148-150]. Activation of the angiotensin II type 2 receptor also present on tumour cells has the opposite effect of AT1R (**Figure 13**, which fits well with our hypothesis since the aforementioned effects facilitate cancer progression as opposed to cancer initiation. Such an association among men only, may be due to hormonal differences and differences in the ability to detoxify carcinogenic compounds. Some studies have speculated that androgens promote carcinogenesis while oestrogens inhibit it[10, 25, 97]. However, this is purely theoretical, other factors have to be considered and alternative explanations of the results have to be explored.

Comprehensive data on antihypertensive medicine was available in the MDCS, however, we considered it to be an effect modifier and mediator in the relationship between BP and BC risk and not as a confounder. Previous studies have explored the link between antihypertensive medication and BC[151]. Regardless, it may be difficult to separate the effect of BP in the association between antihypertensive medication and BC risk. It could also be that SBP may be an intermediate phenotype in a larger causal pathway. Confounding maybe an alternative explanation, however, this was handled in **paper II** (specifically for smoking) and **paper III** (for all confounders [known and unknown]).

In **paper II**, the association between SBP and MIBC among never-smokers is more likely to reflect a true biological association compared to an association between SBP and MIBC adjusted for smoking, since residual confounding may still persist even after the adjustment. The MR analysis employed in **paper III** was intended to produce a robust causal estimate between BP and BC risk, even in the presence of both known and unknown confounding, however, the findings were inconclusive.

This was due to low statistical power (due to weak instrumental variable) in the individual cohorts, and differences in the cohorts in terms of BP-BC association, population characteristic, reporting/recording of BC events and participation rate, which made a meta-analysis inappropriate. Although, we found no presence of pleiotropy, this may alternatively be explained by a lack of statistical power to be able to detect it.

In our studies, we found a positive association between SBP and BC-specific mortality (among never-smokers in **paper II**), which further expands our hypothesis on the role of SBP in BC progression, since it is the most aggressive BC tumours that lead to death. In addition, the association was found only when examining from the date of enrolment (baseline examination) and not when examining from the date of diagnosis (among cases only). These two approaches have different strengths and weaknesses. The main advantage of studying mortality from study enrolment is that there is no worry of selection bias, however, it is counter-intuitive to investigate for an outcome (BC-specific mortality) that the participants are not immediately at risk, secondly, in the case that the SBP is associated with BC risk, then the association between SBP and BC-specific mortality may be partially driven by the risk association, since the results from this method reflect the effect of SBP on both the risk and mortality[152]. Investigating the association from the date of cancer diagnosis has the advantage of adjusting for additional variables that may be linked to both BP and BC-specific mortality such as tumour characteristics, co-morbidity index and treatment modalities, however, this analysis is prone to collider stratification bias[153, 154]. Studies investigating BP and BC-specific mortality are severely lacking, in one study, they found a null association[75].

In addition to investigating the direct effects of BP on BC outcomes, we investigated BP in interaction with another known risk factor for BC in relation to BC risk. Interaction analysis typically requires more statistical power compared to the analysis of direct effects, as such null associations may alternatively be due to insufficient statistical power to detect interaction. In **paper IV**, we found a significant positive additive interaction between SBP and wGRS for BC in relation to MIBC risk. This suggests that the joint effect of SBP and genetic risk for BC on MIBC is greater than what would be expected when adding the individual effect of SBP and genetic risk for BC on MIBC. This additive interaction implies that SBP and genetic risk factors for BC share common pathophysiological pathways that lead to MIBC[155]. However, this finding should be interpreted with caution as the interaction may not persist in a larger sample and when using different cut-off points (for example, when we cut SBP at 130 mmHg, the interaction persisted but did not persist when SBP was cut at 150 mmHg) to categorize the risk factors. To our knowledge, there are no previous studies that investigated interaction between SBP and genetic risk for BC in relation to BC outcomes.

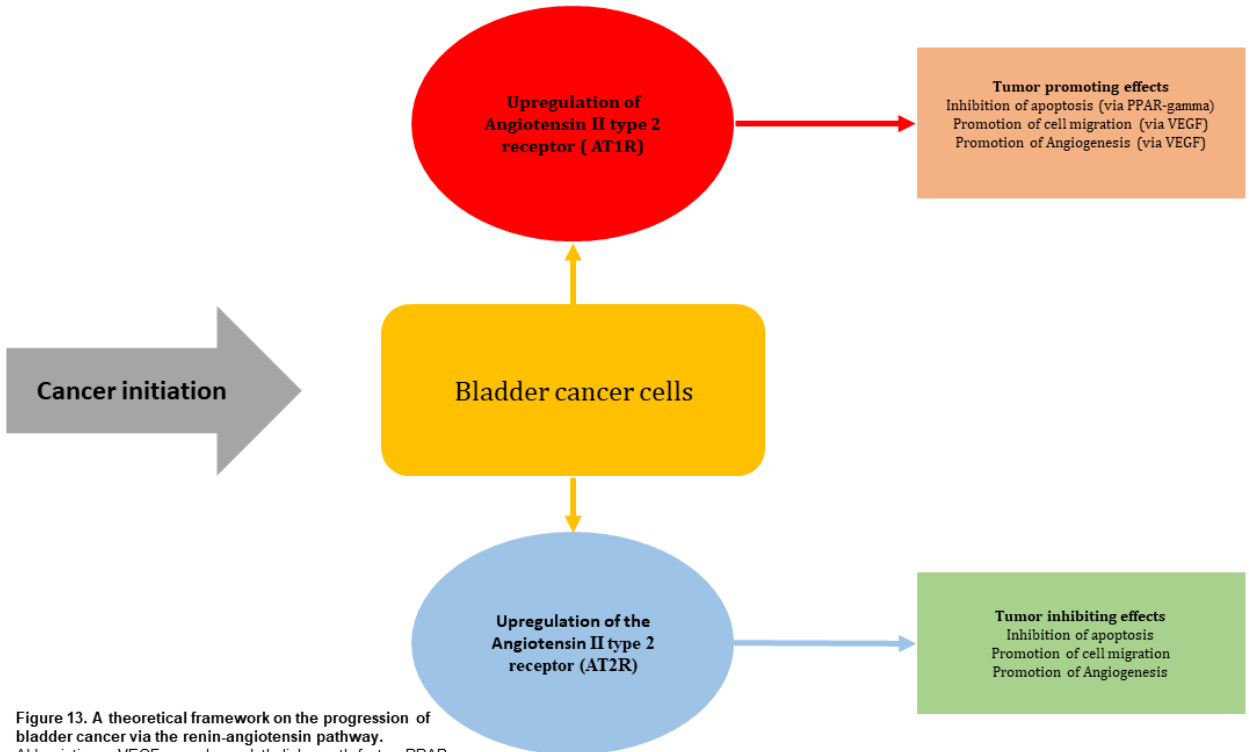

Figure 13. A theoretical framework on the progression of bladder cancer via the renin-angiotensin pathway.
Abbreviations: VEGF, vascular endothelial growth factor; PPAR-gamma, peroxisome proliferator-activator receptor gamma.

Body mass index and bladder cancer

We investigated BMI in **paper I and II**, and among men we found a positive association between BMI and NMIBC, furthermore, we found a positive association between BMI and NMIBC-grade 3 risk in **paper II**. Among women, we found an inverse association between BMI and BC risk.

Compared to other metabolic factors, previous studies investigating the relationship between BMI and risk of BC outcomes are more frequent. Three of the four meta-analysis that investigated the association between BMI and BC risk found a positive association[93-95], and one found a null association[156]. However, evidence from individual studies has been inconsistent. One of the reasons for the inconsistencies maybe due to the inability of BMI as a measure of obesity, to delineate visceral and subcutaneous adipose tissue from skeletal muscle tissue[85]. Epidemiological evidence suggest that it is visceral (abdominal) fat that is responsible for the pathogenic effects of BMI in relation to both CVD and cancer, it has been suggested that other anthropometric measures may perform better and produce more consistent estimates in the relationship between obesity and BC risk[157]. Furthermore, BMI and smoking share a unique relationship, in smoke-related cancers such as lung cancer and squamous-cell oesophageal cancer, the observed inverse association with BMI is likely due to residual confounding by smoking, because BMI values are typically lower among never-smokers compared to current smokers[156, 158]. Studies investigating the relationship between BMI and BC risk separately for NMIBC and MIBC are rare, however, two of the largest prospective studies found no association[9, 97].

There are several proposed biological mechanisms that link BMI to cancer. The first hypothesis involves the insulin resistance and insulin-like growth factor (IGF) pathway: obesity is correlated to insulin resistance and hyperinsulinemia, hyperinsulinemia inhibit the action of IGF-binding proteins, and these proteins bind to IGF and inhibit its actions, which include promoting a tumorigenic environment. The second hypothesis involves the action of adepokines and systemic inflammation. Adipose tissue produces at least 50 different types of chemical messengers referred to as adepokines, these adepokines have a myriad of effects. Accumulation of fat increases the production of pro-inflammatory adepokines (such as leptin and interleukin 6) which promote systemic inflammation, which in turn promotes carcinogenesis[92]. However, the aforementioned mechanisms apply to cancer in general, mechanisms linking BMI to BC and specifically with NMIBC are lacking.

The association between BMI and BC-specific mortality remains controversial, with some studies reporting a positive association and others reporting a null association[102, 159-162]. The reasons for the inconsistent findings include differences in study settings (clinical vs population settings) and exposure-outcome reporting

(retrospective vs prospective). Most of the studies that reported a positive association tended to be retrospective and conducted in a clinical setting[159-162], while studies that reported a null-association tended to be prospective and conducted within the general population. Furthermore, it is difficult to isolate the effect of BMI (and other metabolic factors) on BC-specific mortality when other factors such as patient frailty and response to treatment are likely to impact mortality to greater extent.

Glucose and bladder cancer

In **paper I**, we found a positive association between glucose and MIBC among women and a positive association between glucose and BC-specific death. Most previous studies that investigated the association between glucose and BC risk and mortality were Me-Can studies[9, 76, 163]. In one study, a positive association between glucose and BC risk was found among women, but not men. The relationship between type 2 diabetes and BC risk has previously been investigated. In a recent meta-analysis, they found a positive association between type 2 diabetes and BC risk and mortality among men[164]. Investigating the association between diabetes and BC is complicated by group of anti-diabetic drugs called Thiazolidinedione, of which pioglitazone is the most studied in relation to BC. In a recent meta-analysis of observational studies, a small but positive association between pioglitazone use and BC risk was found[165]. A biological mechanism linking glucose to cancer has been proposed[164]. However, with regards to BC, no biological mechanism has been proposed.

Triglycerides and bladder cancer

In **paper I**, we found a positive association between triglycerides and NMIBC among men and between triglycerides and BC-specific mortality for both men and women. Previous studies investigating triglycerides and BC risk, including a MR analysis, found no association[9, 76, 112, 166]. However, in those studies, BC was investigated as a single entity and not separated by muscle-invasiveness. In our study, and among men, we found an association for both total BC risk and NMIBC, but not MIBC, which suggests that the association with total BC risk is driven by NMIBC. Triglycerides have been linked to carcinogenesis, free fatty acids, a component of a triglyceride molecule may disrupt the mitochondrial respiratory chain in cells, leading to the production of superoxide anions, free radicals that damage DNA, increasing the risk of cancer. With BC in particular, a recent study on lipidomic profiling found that physiological states that promote the breakdown

of triglycerides into fatty acids were associated with increased risk of BC, which suggests that fatty acids maybe an important substrate (source of fuel) in BC.

Total cholesterol and bladder cancer

In **paper I**, we found a positive association between total cholesterol and NMIBC among men. Previous studies investigating total cholesterol and BC risk found no association[76, 111, 118, 148]. Findings specifically for NMIBC are lacking. Cholesterol has been linked to cancer progression, a recent paper suggested that altered cholesterol homeostasis in cancer cells results in upregulation of the LDL receptors, when aids internalization of cholesterol, which is needed to cancer cell proliferation[167]. However, biological mechanisms specifically for BC are lacking.

Strengths and limitations

Each individual paper had its unique strengths and weaknesses. In general, we used large cohorts that had a long follow-up and were linked to high quality national registers. The large sample sizes allowed us to deepen our exploration of BC by investigating BC into sub-groups based on tumour characteristics, a feat rarely seen in previous studies. By investigating BC in these sub-groups, biological specificity is increased, which in turn, may generate unique pathways of disease aetiology and pathogenesis. Secondly, the large study size allowed us to investigate associations among a group with a specific trait, e.g. in **paper III**, we investigated associations only among never-smokers, while the results from this type were likely more reflective of a truer biological mechanism, we may have had limited power in some of the analyses to detect associations. Furthermore, assessing interaction and MR analysis demand sufficient statistical power, which may have been limited in those analyses. The long follow-up time (except for the UK-biobank) were especially advantageous for BC, which tends to occur later in life (median age, 73 years). The national registers (which include the cancer and cause of death registers) in the Nordic countries generally have a high coverage, are virtually complete and are constantly being improved and updated insuring quality of data. With regards to quality registers such as the Swedish National Register of Urinary Bladder Cancer (SNRUBC), the coverage and validity vary, and while a validation study has not been conducted, the coverage for the SNRUBC approximates 97%[168].

In addition to limited statistical power in some of the analysis, not accounting for antihypertensive medication was another limitation. Although we considered antihypertensive drugs as effect modifiers in the relationship between BP and BC risk, it would have been interesting to investigate the interaction between BP and

antihypertensive medication in relation to BC risk. While most of the cohorts used had data on antihypertensive medication, the information was usually incomplete. With regards to the MR analysis in the UK-biobank, we used the base genotype dataset, which limited the number of SNPs we could include for the analysis, the imputed dataset (which we were not able to obtain) would have likely strengthened the MR results, by incorporating more SNPs in the IV.

Conclusion

In this thesis, derangement in the metabolic factors was associated with BC outcomes, triglycerides and total cholesterol were associated with NMIBC among men and glucose was associated with MIBC among women. As such, the presence, strength and direction of the associations differed depending on the subgroup of population and the specific outcome being investigated. SBP consistently showed a positive association with MIBC risk, but not sufficient to infer causality, furthermore, SBP and the genetic risk of BC positively interacted on an additive scale among men. This thesis highlights the importance of investigating associations in specific sub-groups of population and specific disease outcome, and assessing interaction to explore and clarify potential biological mechanism and inform public health. More studies, with sufficient statistical power, especially with regards to MR and interaction analysis, are need to clarify these associations.

Future perspective

Most efforts investigating risk factor relationships with BC have often assessed BC as a single entity. In more recent studies including those in this thesis, investigating BC separated into sub-groups based on tumour characteristics appreciates the possibility that the risk factors, aetiology, and pathogenesis of BC may differ depending on these sub-groups. Considering how complex and heterogeneous BC is, the traditional classification into sub-groups solely based on tumour stage and grade maybe limited and outdated. Advances in fields such as genetics and molecular immunology, the genetics-based sub-classification of BC has become increasingly studied. The molecular sub-typing of BC provides more biological information compared to the traditional sub-typing, and may help explain how tumours classified with the same sub-group (e.g. NMIBC) may have different outcomes (in terms of recurrence, progression and response to treatment). One of the solutions to the challenge of identifying and quantifying exposures such as metabolic risk factors is to have a standard classification based on molecular subtype system.

In a similar light, investigating exposures such as metabolic factors moving forward may require a more sophisticated approach for example, in studying obesity, we could investigate the biomarker insulin-like growth factor binding protein 3 (IGFBP3), a biomarker who's expression is altered in obese states. In this way, we investigate obesity in a very specific pathway. In addition, epidemiological studies predominantly investigate risk factors individually, however, individuals are exposed to multiple risk factors at the same time. Investigating biomarkers as proxies for metabolic traits as mentioned above, will give a better understanding of potential biological pathways. Another more holistic approach to better understand the association between metabolic factors and BC is to integrate them with omics approaches (such as metabolomics and epigenomics).

MR analysis is a viable method to assess causal associations between an exposure of interest and an outcome, and when well conducted, the strength of evidence approaches that of randomized clinical trials. Its greatest strength lies in its ability to deal with both known and unknown confounders in the exposure-outcome association. Smoking is a potentially strong confounder in conventional studies of metabolic factors and BC, even with detailed smoking data, residual confounding may persist and there maybe unknown confounders driving associations, making causal inference challenging. Statistical power is one of the main challenges when conducting MR analysis, however, in recent times, hundreds of SNPs discovered in GWAS, and for traits such as BP and BMI may provide the opportunity to build strong IVs. Furthermore, large consortia are being built and summary data has become more accessible making it easier to gain sufficient power.

Interaction reflects complex biological processes that take place in disease formation, joint effects may provide biological insight that may otherwise be missed in an analysis of direct effects. The excess risk from additive and multiplicative interaction is just as important in contributing to the incidence of disease as the risk from individual risk factors. Interaction studies between metabolic factors and other risk factors (genetic and environmental) are lacking and may contribute to the understanding of BC.

While up to 50% of BC is causally attributed to smoking, the aforementioned steps have the potential to: firstly delineate what proportion of that (the smoking) is due to interaction with metabolic factors, and to minimize the impact of smoking in such associations, to more effectively evaluate what proportion of BC is attributed to metabolic factors outside smoking.

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Appendix

Appendix I. Table showing the sytolic blood pressure SNPs in the Malmö Diet and Cancer Study use in the Mendelian randomization analysis (paper III)

SNP	EA	OE	EAF	Exposure_beta	Exposure_se	p_value
rs10850411	T	C	.7	.0199897	.0409328	.625
rs11191548	T	C	.91	.0192581	.0479892	.688
rs1173771	G	A	.6	.059813	.0275003	.03
rs11953630	C	T	.63	.0729499	.0350388	.037
rs12946454	T	A	.24	.074722	.0751205	.32
rs13082711	C	T	.22	-.0181249	.0518368	.727
rs13107325	C	T	.95	.1411747	.0335606	0
rs13139571	C	A	.76	.0451636	.052534	.39
rs1327235	G	A	.46	.0793004	.0401651	.048
rs1378942	C	A	.35	.0166511	.0359921	.644
rs1530440	C	T	.84	-.0419979	.0411641	.308
rs16948048	G	A	.38	.0152101	.0347053	.661
rs16998073	T	A	.35	.0512897	.0192702	.008
rs17249754	A	G	.86	.0411225	.0153865	.008
rs17367504	A	G	.85	.0136377	.0345136	.693
rs17608766	C	T	.14	.0676008	.0355089	.057
rs1799945	G	C	.14	.0798447	.0331367	.016
rs2521501	T	A	.31	.0415093	.0225838	.066
rs2932538	G	A	.75	.0920737	.0394207	.02
rs3184504	T	C	.47	.0265691	.0299942	.376
rs3774372	C	T	.17	-.0283115	.2823122	.92
rs381815	T	C	.26	.0433176	.0179166	.016
rs419076	T	C	.47	.0690467	.0331119	.037
rs4373814	C	G	.45	-.0053125	.0367197	.885
rs6015450	G	A	.12	.0567082	.02222	.011
rs633185	C	G	.72	.0190686	.0210578	.365
rs7129220	A	G	.11	.0386085	.0364733	.29
rs805303	G	A	.61	-.0403406	.0373707	.28
rs932764	G	A	.44	.0833291	.0279827	.003

Abbreviations: SNP, single nucleotide polymorphism; EA, effect allele; OE, other allele; EAF, effect allele frequency; se, standard error

Appendix IIa. Table showing the diastolic blood pressure SNPs in the UK-biobank use in the Mendelian randomization analysis (paper III)

SNP	EA	OA	EAF	Exposure_beta	Exposure_se	p_value
rs10004996	T	C	.467	.0251893	.0212441	.236
rs10741693	A	G	.205	.0463968	.0139922	.001
rs1084522	C	T	.496	.0413523	.0206508	.045
rs10850411	T	C	.7	.046548	.0141562	.001
rs11014171	C	T	.666	.0605229	.0084611	0
rs11191548	T	C	.91	.0291328	.0134291	.03
rs11556924	C	T	.616	.0681557	.0157794	0
rs1173771	G	A	.6	.0955779	.0129176	0
rs11953630	C	T	.63	.0362964	.0122305	.003
rs12627651	A	G	.288	.036019	.0180688	.046
rs13082711	C	T	.22	.0343593	.0161427	.033
rs13107325	C	T	.95	.0556265	.0091445	0
rs13139571	C	A	.76	.0553113	.0150074	0
rs1361831	C	T	.459	.0732613	.0122568	0
rs1378942	C	A	.35	.0520835	.0085126	0
rs1458038	T	C	.29	.0747652	.0080163	0
rs1530440	C	T	.815	.0653948	.0103351	0
rs1548594	G	C	.357	.0532763	.0187279	.004
rs1561468	T	C	.537	.0496952	.0131324	0
rs16982520	G	A	.12	.0505525	.0091476	0
rs17080093	C	T	.925	.0488895	.015581	.002
rs17249754	G	A	.84	.06113	.0084203	0
rs17367504	A	G	.85	.070152	.0081618	0
rs17638167	C	T	.953	.0426837	.0239129	.074
rs1799945	G	C	.14	.0530527	.0100879	0
rs2293579	A	G	.386	.0631852	.0140606	0
rs2478539	T	G	.417	.0516557	.0122657	0
rs2586886	C	T	.401	.0560214	.0133334	0
rs2692893	T	C	.335	.0813277	.016674	0
rs2923089	T	C	.466	.0944258	.0166354	0
rs2932538	G	A	.75	.0665048	.0157425	0
rs3184504	T	C	.47	.0689262	.0073734	0
rs3735533	C	T	.919	.0458996	.0141613	.001
rs3752728	A	G	.737	.0909439	.0114885	0
rs3774372	C	T	.17	.0630609	.0123344	0
rs419076	T	C	.47	.0824263	.0137516	0
rs4245739	A	C	.737	.0542924	.0152293	0
rs4247374	T	C	.857	.0746346	.0123192	0
rs4293721	A	C	.671	.0268166	.0116552	.021
rs4373814	C	G	.45	.0953509	.0153549	0
rs4590817	G	C	.84	.0664711	.0104394	0
rs6271	C	T	.928	.073289	.0135226	0
rs6795735	C	T	.57	.0744824	.0185012	0
rs6806067	A	C	.433	.0449185	.0193251	.02
rs7125196	T	C	.878	.0756095	.0137825	0
rs7129220	A	G	.11	.0848757	.0171648	0
rs7497304	T	G	.32	.0569103	.0098021	0
rs805303	G	A	.61	.0463187	.0149771	.002
rs932764	G	A	.446	.0683677	.0149734	0
rs936226	C	T	.279	.0608788	.0103993	0

Abbreviations: SNP, single nucleotide polymorphism; EA, effect allele; OE, other allele; EAF, effect allele frequency; se, standard error

Appendix IIb. Table showing the systolic blood pressure SNPs in the UK-biobank use in the Mendelian randomization analysis (paper III)

SNP	EA	OA	Exposure_beta	Exposure_se	p_value
rs10004996	T	C	.0182423	.0095399	.056
rs1053739	A	G	.0498891	.0083392	0
rs10741693	A	G	.0207398	.0090587	.022
rs10760117	T	G	.0318493	.0117309	.007
rs10850411	T	C	.0289029	.0100223	.004
rs11191548	T	C	.0277895	.0056402	0
rs11556924	C	T	.0451991	.0123692	0
rs1173771	G	A	.0645129	.0066259	0
rs11953630	C	T	.0260503	.0082619	.002
rs12046278	C	T	.0385322	.0072361	0
rs12627651	A	G	.013535	.0093442	.147
rs13107325	C	T	.0261188	.0063148	0
rs13280813	G	T	.0318715	.0086948	0
rs1361831	C	T	.0389666	.0068296	0
rs1378942	C	A	.0242346	.0057228	0
rs1458038	T	C	.0463762	.0051395	0
rs1530440	C	T	.0382574	.0074404	0
rs1540976	G	A	.0430031	.0107142	0
rs1548594	G	C	.0240692	.0113584	.034
rs16948048	G	A	.0127958	.009369	.172
rs16982520	G	A	.0294662	.0055833	0
rs17249754	G	A	.0386354	.00469	0
rs17367504	A	G	.0399412	.0048979	0
rs17608766	C	T	.0415286	.0082697	0
rs1799945	G	C	.0267223	.0072844	0
rs2293579	A	G	.037267	.010019	0
rs2396004	A	G	.0274139	.0097847	.005
rs2586886	C	T	.0389786	.0083041	0
rs2923089	T	C	.0413671	.007939	0
rs2932538	G	A	.0264955	.0096449	.006
rs3184504	T	C	.02889	.0054738	0
rs3735533	C	T	.0352017	.0078209	0
rs3741378	C	T	.0331347	.0097673	.001
rs419076	T	C	.0321379	.0080287	0
rs4247374	C	T	.0491172	.0079252	0
rs4293721	A	C	.0264073	.0088359	.003
rs4373814	C	G	.0521215	.0088893	0
rs4590817	G	C	.0330349	.0067063	0
rs6271	C	T	.0374457	.010532	0
rs6806067	A	C	.030283	.0106869	.005
rs699	G	A	.0238282	.0080782	.003
rs7125196	T	C	.0342623	.0125849	.006
rs7129220	A	G	.0398256	.0082109	0
rs7497304	T	G	.0284304	.0053634	0
rs805303	G	A	.0304312	.0089976	.001
rs932764	G	A	.0412478	.0068699	0
rs936226	C	T	.0321632	.0068127	0

Abbreviations: SNP, single nucleotide polymorphism; EA, effect allele; OE, other allele; EAF, effect allele frequency; se, standard error

Appendix Ⅲ. Showing the public health benefit of additive interaction over multiplicative interaction
Suppose that E an effective antihypertensive drug and the outcome is "normal blood pressure":
And there are 100 with G =0 and 100 with G =1 and we have 100 doses.

	E=0	E=1
G=0	0.02	0.10
G=1	0.08	0.20

The risk difference for E on those with G=0 is: 0.10 - 0.02= 0.08
The risk difference for E on those with G=1 is: 0.20 - 0.08 = 0.12

RR11 - RR10 - RR01 + RR00 = 0.20 - 0.08 - 0.10 + 0.02 = **0.04** > 0

The risk ratio for E on those with G=0 is: 0.10 / 0.02 = 5
The risk ratio for E on those with G=1 is: 0.20 / 0.08 = 2.5
RR11 / (RR10 x RR01) = 10 / (5 x 4) = 0.5 < 1

If we give the antihypertensive drug to G=0 group we cure: 100*(0.10) + 100*(0.08) = 18
If we give the antihypertensive drug to G=1 group we cure: 100*(0.02) + 100*(0.20) = 22

We should treat the G=1 group; we cure an additional **4** persons
Additive interaction (not multiplicative interaction) identifies this
The risk ratio suggests treating the G=0 group; for public health purposes we should depend on additive interaction measure to decide which subgroups to target

Appendix Ⅳa: Baseline characteristics of study participants in paper Ⅰ separated by sex

Characteristic	Men	Women
Population, *n* (%)	405,255 (49.9)	406,378 (50.1)
Baseline age (in years), mean (SD)	43 (8.8)	43 (9.6)
Fasting time (in hours), *n* (%)		
<8 hours	247,760 (61)	246,703 (61)
≥8 hours	157,495 (39)	159,675 (39)
Smoking status, *n* (%)		
Never-smoker	156,483 (39)	195,819 (48)
Ex-smoker	111,414 (27)	94,352 (23)
Current smoker	137,358 (34)	116,207 (29)
Smoking intensity (in pack years), *n* (%)[a]		
<10	34,451 (28)	46,589 (42)
10-19.9	53,384 (43)	48,680 (44)
≥20	36,783 (29)	15,858 (14)
BMI (Kg/m^2), mean (SD)[b]	26 (3.4)	25 (4.3)
Blood Pressure (mm Hg), mean (SD)[b]		
Systolic blood pressure	133 (15.9)	126 (17.6)
Diastolic blood pressure	82 (10.5)	77 (10.7)
Mid-blood pressure	107 (12.1)	102 (13.2)
Glucose (mmol/L), mean (SD)[b]	5.4 (1.3)	5.2 (1.1)
Total cholesterol (mmol/L), mean (SD)[b]	5.6 (1.2)	5.5 (1.2)
Triglycerides (mmol/L), mean (SD)[b]	1.6 (1.2)	1.2 (0.76)
Average length of follow-up time, years	19.7	19.5

Abbreviations: SD, standard deviation; BMI, body mass index.
[a] Accumulated pack-years among current smokers (excluding 17,820 current smokers with missing data on packyears).
[b] Number of missing for each metabolic factor: BMI, 3,715; systolic blood pressure, 534: diastolic blood pressure, 701; glucose, 293,647; cholesterol, 861 and triglycerides, 3,894.

Appendix IVb. Baseline characteristics of study participants in paper II separated by cohorts

Characteristic	Swedish cohorts (MDCS and MPP)	UK-biobank
Population, *n*	27,107	188,167
Baseline period	1974-1996	2006-2010
Baseline age (in years), mean (SD)	50.4 (10.7)	57.7 (8.1)
Smoking status, *n* (%)[a]		
Never-smoker	8,024 (30.6)	91735 (48.9)
Ex-smoker	7,010 (26.8)	73,528 (39.2)
Current smoker	11,172 (42.6)	22,230 (11.9)
Smoking intensity (in pack years), *n* (%)[a]		
<10	1,611 (18.8)	2,305 (13.5)
10-19.9	925 (10.8)	3,312 (19.4)
≥20	6,043 (70.4)	11,470 (67.1)
BMI (Kg/m2), mean (SD)[b]	25.4 (3.6)	27.9 (4.2)
Blood Pressure (mm Hg), mean (SD)		
Systolic blood pressure	134.9 (19.1)	143.3 (18.5)
Diastolic blood pressure	86.7 (9.9)	84.2 (10.6)
Average length of follow-up time, years	22.2	4.8

Abbreviations: MDCS, Malmö Diet and Cancer Study; MPP, Malmö Preventive Project; BMI, body mass index; SD, standard deviation.

[a] Smoking status was missing for 674 (0.4%) men in the UK-biobank and 901 men in the Swedish cohorts. Smoking intensity only includes current smokers, of which 5,143 (2.7%) and 2,593 (9.6%) has missing data for pack years in the UK-biobank and Swedish cohorts respectively.

[b] BMI data was missing for 626 men in the UK-biobank and 16 men in the Swedish cohorts.

Appendix IVc. Baseline characteristics of study participants in paper III separated by cohorts

Characteristic	VIP	MPP	CwC
Population, *n*			
Baseline period	1985-2014	1974-1992	1971-1993
Baseline age (in years), mean (SD)	52,055 (15.3)	22,276 (6.6)	264,579 (78.1)
Smoking status, *n* (%)			
Never-smoker	31,922 (61.3)	7,588 (34.1)	108,182 (40.9)
Ex-smoker	11,331 (21.8)	3,568 (16.0)	49,593 (18.7)
Current smoker	8,802 (16.9)	11,120 (49.9)	106,804 (40.4)
BMI (Kg/m2), mean (SD)	26.3 (3.7)	24.7 (3.3)	24.4 (3.1)
Blood Pressure (mm Hg), mean (SD)			
Systolic blood pressure	128.0 (16.0)	127.1 (14.9)	133.0 (14.7)
Diastolic blood pressure	79.9 (10.3)	85.5 (9.7)	81.0 (10.4)
Average length of follow-up time, years	14.1 (7.3)	29.9 (8.5)	30.7 (8.5)

Abbreviations: VIP, Västerbotten Intervention project; MPP, Malmö Preventive Project; CwC, Construction Workers Cohort; SD, standard deviation; BMI, body mass index; BC, bladder cancer.

Appendix IVd. Baseline characteristics of study participants in paper IV separated by cohorts

Characteristic	Cases	Non-cases
Population, *n*	385	10,367
Baseline age (in years), mean (SD)	60.4 (6.3)	58.9 (7.0)
Smoking status, *n* (%)		
Never-smoker	50 (13.0)	2,976 (28.7)
Ex-smoker	179 (46.5)	4,429 (42.7)
Current smoker	156 (40.5)	2,962 (28.6)
Smoking intensity (in pack years), *n* (%)		
<10	22 (14.1)	501 (16.9)
10-19.9	24 (15.4)	353 (11.9)
≥20	110 (70.5)	2,108 (71.2)
BMI (Kg/m2), mean (SD)	26.7 (3.6)	26.3 (3.5)
Blood Pressure (mm Hg), mean (SD)		
Systolic blood pressure	146 (19.7)	143.8 (19.2)
Diastolic blood pressure	88.0 (9.6)	88.0 (9.9)
Antihypertensive medication use		
Yes	79 (20.5)	2,117 (20.4)
No	306 (79.5)	8,250 (79.6)
Type of antihpyertensive medication among users, n (%)[a]		
Diuretics	21 (18.8)	502 (17.3)
Beta-blockers	45 (40.2)	1,224 (42.1)
ACE-inhibitors	11 (9.8)	488 (16.8)
Calcium channel blockers	35 (31.2)	691 (23.8)
Average length of follow-up time, years	13.9 (6.9)	20.2 (6.8)

[a] a majority of the partcipants used antihypertensive medication from different drug classes simultaneously.
Abbreviations:SD, standard deviation; BMI, body mass index; ACE, angiotensin converting enzyme.

Paper I

IJC
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Risk of bladder cancer by disease severity in relation to metabolic factors and smoking: A prospective pooled cohort study of 800,000 men and women

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Previous studies on metabolic factors and bladder cancer (BC) risk have shown inconsistent results and have commonly not investigated associations separately by sex, smoking, and tumor invasiveness. Among 811,633 participants in six European cohorts, we investigated sex-specific associations between body mass index (BMI), mid-blood pressure (BP, [systolic + diastolic]/2), plasma glucose, triglycerides, total cholesterol and risk of BC overall, non-muscle invasive BC (NMIBC) and muscle invasive BC (MIBC). Among men, we additionally assessed additive interactions between metabolic factors and smoking on BC risk. During follow-up, 2,983 men and 754 women were diagnosed with BC. Among men, triglycerides and BP were positively associated with BC risk overall (hazard ratio [HR] per standard deviation [SD]: 1.17 [95% confidence interval (CI) 1.06–1.27] and 1.09 [1.02–1.17], respectively), and among women, BMI was inversely associated with risk (HR: 0.90 [0.82–0.99]). The associations for BMI and BP differed between men and women ($p_{interaction} \leq 0.005$). Among men, BMI, cholesterol and triglycerides were positively associated with risk for NMIBC (HRs: 1.09 [95% CI 1.01–1.18], 1.14 [1.02–1.25], and 1.30 [1.12–1.48] respectively), and BP was positively associated with MIBC (HR: 1.23 [1.02–1.49]). Among women, glucose was positively associated with MIBC (HR: 1.99 [1.04–3.81]). Apart from cholesterol, HRs for metabolic factors did not significantly differ between MIBC and NMIBC, and there were no interactions between smoking and metabolic factors on BC. Our study supports an involvement of metabolic aberrations in BC risk. Whilst some associations were significant only in certain sub-groups, there were generally no significant differences in associations by smoking or tumor invasiveness.

Key words: bladder cancer, metabolic factors, smoking, non-muscle invasive bladder cancer, muscle-invasive bladder cancer
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What's new?

While associations between obesity and bladder cancer (BC) are suspected, studies to date are inconclusive. Relationships between other metabolic factors and BC are also uncertain. In this study, overall BC risk and risk of non-muscle invasive BC and muscle invasive BC (MIBC) were found to differ according to sex and metabolic factor. Elevated triglycerides and blood pressure were associated with increased BC mortality risk in men. In women, glucose was a significant factor for MIBC and body mass index was inversely associated with BC risk. No interactions were detected between metabolic factors and smoking in relation to bladder cancer.

Introduction

Bladder cancer (BC) has the fourth highest incidence rates among men and the eleventh highest incidence rates among women in high-income countries.[1] Smoking is the most important risk factor for BC, accounting for approximately 43% of the cases among men and 26% among women in Europe.[2,3] Other established environmental risk factors include occupational exposures, schistosomiasis, chronic inflammation of the bladder, and ionizing radiation.[2,4] Findings from twin studies suggest that 30% of BC is attributed to heredity, and genome-wide association studies have identified around 15 gene loci associated with BC risk.[5,6]

Obesity assessed by body mass index (BMI, kg/m^2) has been related to a range of cancer forms,[7] and was in a recent meta-analysis of 14 prospective studies related to a small, positive association with BC risk.[8] However, results from individual studies have mostly shown null associations, despite high statistical power. The weak associations may partly be due to the analysis of men and women jointly,[9] amongst whom the associations may differ.[10] However, it may also be due to the investigation of BC risk for all stages combined, without taking level of tumor invasion into account. Only one study investigated the relationship between obesity and BC by tumor invasiveness and found no difference in associations.[10] Moreover, inadequate adjustment for smoking, a potentially strong confounder, might have influenced the results in previous studies as residual confounding may persist if smoking intensity and duration are not adjusted for, and this was lacking in 9 out of the 14 included studies.[8] Additionally, smoking and obesity might interact in relation to BC risk. One study found no interaction on the multiplicative scale,[10] but no previous study has assessed additive interaction. Additive interaction is considered the most relevant interaction measure in terms of public health as it determines which sub-group is most at risk and might benefit most from an intervention.[11,12]

Metabolic factors apart from obesity have rarely been studied prospectively in relation to BC risk. Findings from existing studies are inconsistent, suffer from the aforementioned limitations in studies of obesity, and additionally have commonly been smaller.[13–16] Two large studies on cholesterol and glucose in relation to BC risk in a population of over one million Korean men and women found no associations.[17,18] In our previous study of 578,700 individuals in the Metabolic Syndrome and Cancer Project (Me-Can), we found a positive association between elevated blood pressure (BP) and a borderline association between elevated triglycerides and BC risk among men, but no association with BMI, total cholesterol and/or plasma glucose. In that study, however, we lacked data on smoking intensity and duration, and on tumor invasiveness.[19]

The aim of our study was to investigate sex-specific associations between metabolic factors, smoking and risk of non-muscle invasive (NMIBC) and muscle-invasive BC (MIBC) separately and combined, and risk of BC death, and to assess additive interaction between metabolic factors, smoking, and risk of BC.

Materials and Methods

Study population

The Me-Can 2.0 is a pooling of six cohorts from Norway (Oslo study 1, Norwegian Counties Study [NCS] and the Age 40-Programme [40-y]), Sweden (Västerbotten Intervention Project [VIP] and Malmö Preventive Project [MPP]), and Austria (Vorarlberg Health Monitoring and Prevention Programme [VHM&PP]).[19,20] It is a follow-up project for Me-Can 1.0, which has been described in full detail elsewhere.[20] Me-Can 2.0 includes additional individuals and observations in the VIP between the years 2006–2014 and in the VHM&PP between the years 2003–2005. It also includes additional time of follow-up. More variables have been added to the database, including data on smoking habit and duration. Additional information, for example on lifestyle, is available from questionnaires in some of the cohorts. Ethical committees in Norway, Sweden and Austria approved the study.

Exposure assessment

A detailed description of the protocols for measuring the metabolic factors in Me-Can have previously been published.[20] However, in the VIP, the measurement protocols for BP, total cholesterol and triglycerides have been modified as of 1 September 2009 (Supporting Information, Methods S1 and Table S1).

Follow-up and end point assessment

Cancer diagnoses and mortality were identified through linkages to the national cancer registries in Norway and Sweden, the cancer registry of the Vorarlberg province, and to national cause of death registries. For identification of emigration status,

the cohorts were linked to the respective national population registries (except in Austria). Follow-up for these linkages ended on December 31, 2012 in Norway and on December 31, 2014 in Sweden and Austria.

BC was defined according to the seventh edition of the International Classification of Diseases (ICD-7) code 181.0 and 181.6, including carcinoma *in situ*, which, according to ICD 10 is coded as C67 and D09.0. Depth of tumor invasion was based on histology from tumor biopsies, palpation and radiologic findings. Tumor data was available for 94% of the cases in the Austrian cohort (VHM&PP) and 96% of the cases in the Swedish cohorts (VIP and MPP) diagnosed after January 1, 1997, when the Swedish National Register of Urinary Bladder Cancer became nation-wide. NMIBC included non-invasive tumors (Ta), carcinoma *in situ* (Tis) and tumors that invaded no further than the lamina propria (T1). MIBC ranged from tumors that invaded the muscularis propria (T2) to tumors invading the pelvic and/or abdominal wall (T4b), and metastatic cancers (lymph node and/or distant spread) were included in this category. Death from BC was defined as BC (ICD-10 C67) recorded as the underlying cause of death in the national cause of death registries.

Selection criteria

The Me-Can 2.0 was composed of 843,531 participants with 1,557,855 observations, out of which 811,633 participants with one observation each were selected for the study (Fig. 1). Out of the 31,898 individuals excluded, the most common cause for exclusion was a prevalent cancer, defined as all malignant neoplasms, including malignant and uncertain malignant neoplasms of the lymphatic and hematopoietic tissue (ICD-7, 200–209), but excluding basaliomas and *in situ* neoplasms, before any observation (26,158 observations). Participants with missing fasting status were also excluded from the final analysis (29,842 observations), as were participants with missing smoking status (3,396 observations).

Statistical analysis

We used Cox proportional hazards regression and hazard ratios (HRs) with 95% confidence intervals (CIs) to investigate sex-specific associations between metabolic factors and BC endpoints with age as the underlying time metric. Participants were followed from baseline (the date of enrollment into the study) up until the date of event, or until censoring due to death, emigration, or diagnosis of another cancer in analysis of incident BC, or until end of follow-up, which ever occurred first. The inclusion of 527 primary BC cases as events rather than censoring at the time of diagnosis of another prior cancer diagnosis in the analysis of incident bladder did not materially change the results. Follow-up in the analysis of tumor invasiveness in the Swedish cohorts started in 1997, and censored participants before that date were excluded. We calculated HRs for metabolic factors transformed to standard scores (*z*-scores) with zero as the mean and one as standard deviation (SD). This was calculated as $z = (x - u)/\sigma$, where u is the mean, x is actual level of the exposure and σ is the SD. We transformed exposures to *z*-scores separately within each cohort and sex, and all exposures (except blood pressure), were additionally transformed within categories of fasting time. The means and SDs for metabolic factors from the separate cohorts were similar, allowing us to pool and compare their *z*-scores. Fasting time in the VHM&PP, VIP and MPP were categorized as <8 hr and ≥8 hr, and were in the Norwegian cohorts categorized as <1 hr, 1–2 hr, 3–4 hr, 4–8 hr, and >8 hr. Glucose and triglycerides displayed a right skewed distribution and were therefore logarithmically transformed (natural logarithm) prior to *z* scores transformation. In our primary analyses of blood pressure (BP) we used mid-BP ([systolic BP + diastolic BP]/2),[21] however, we also investigated systolic and diastolic BP separately. We adjusted all analyses for birth year (before 1923, 1923–1930, 1931–1938, 1939–1946, 1947–1954, 1955 and later), age at baseline (continuous), smoking status/pack-years (never smokers, ex-smokers, current smokers in quartiles of pack-years with cut-points at 3.75, 9.75, 16.25, and smokers with pack-years missing [2%]) and quartiles of BMI (except when investigating BMI). Furthermore, we stratified within all Cox regression models by cohort to allow for differences in baseline hazards between cohorts.

We investigated the shape of association between *z*-scores of metabolic factors and BC risk using restricted cubic spline regression with knots placed at percentiles 5, 35, 65, and 95. Additionally, we performed a likelihood ratio test (LR test) in which a fitted linear model was nested in the model that additionally included the cubic spline to test for the linearity of associations between metabolic factors and BC risk.

To test the proportional hazards assumption in the Cox models, we calculated Schoenfield residuals for all metabolic factors and covariates including birth year (six categories) and smoking status/intensity (seven categories). Age at measurement violated the proportional hazards assumption in some models, but including "age at measure" as a stratum in the Cox models did not materially alter HRs. Therefore, it was not retained as stratum in the models.

We corrected the HRs for within person variability and random measurement error using a method based on regression dilution ratio (RDR) as described by Wood *et al.*[22] The corrected HRs are interpreted as the expected HRs while taking the random variation of measurements into consideration. RDRs were calculated based on 133,820 individuals with 406,364 observations as described previously.[19] The calculated RDRs were for BMI, 0.902; mid blood pressure, 0.544; glucose (log), 0.278; cholesterol, 0.657; and for triglycerides (log), 0.505. All HRs were corrected for random error with the equation $HR_{corrected} = \exp(\log[HR_{original}]/RDR)$.

We measured the relative excess risk due to interaction (RERI) to investigate additive interactions between metabolic factors, smoking status and BC risk. We restricted the analysis

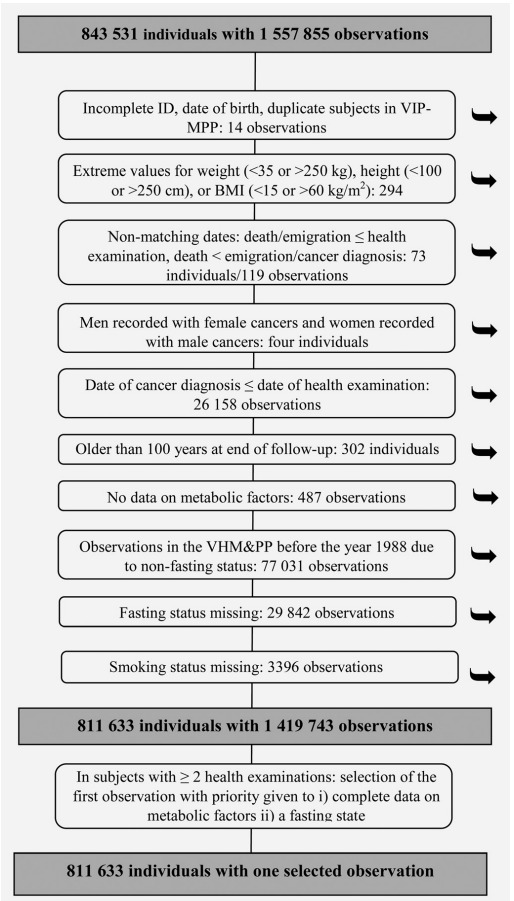

Figure 1. Denotes exclusions. Abbreviations: VIP, Västerbotten Intervention Project; MPP, Malmö Preventive Project; BMI, body mass index; VHM&PP, Vorarlberg Health Monitoring and Prevention Program.

to men, as statistical power among women was limited due to a much smaller number of cases. The RERI was based on adjusted HRs (not RDR corrected). It was calculated by RR11 – RR10 – RR01 + 1 representing the individuals in the lowest quartile of the metabolic factor and never smoker (1, reference group), lowest quartile of metabolic factor and current smoker (RR01), highest quartile of metabolic factor and never smoker (RR10), highest quartile of metabolic factor and current smoker (RR11). We obtained confidence intervals using the delta method.[23,24] In addition to additive interaction, we also calculated multiplicative interaction between continuous z-scores of metabolic factors and smoking status using the LR test. This test was also used to calculate interactions between metabolic factors and sex and cohort, respectively.

We assessed heterogeneity in HRs between NMIBC and MIBC based on competing risk models using a data duplication method.[25] We stratified the analyses for NMIBC/MIBC, thus allowing different baseline hazards for the two outcomes, and used the LR test to compare a model allowing the association with the metabolic factor of interest to vary by outcome with one that did not allow the association to vary.

In order to validate the smoking habit/intensity data, we constructed restricted cubic spline regressions of smoking intensity (in pack-years) among current smokers for risk of BC as well as for lung cancer due to its strong relationship with smoking. We additionally investigated the relationship with smoking status, with and without additional categories of pack-years among smokers. We found that smoking status

and pack-years were positively associated with risk of lung cancer and BC, as expected with a stepwise increased risk for each higher smoking category, especially for lung cancer (Supporting Information Table S2 and Fig. S1).

We evaluated the impact of anti-hypertensive medication, education, and physical activity level as potential confounders by conducting sensitivity analyses with additional adjustments for these factors within the VIP cohort.

We performed all the statistical analyses in STATA 13, (StataCorp LLC, College Station, TX), and considered p values below 0.05 as statistically significant.

Results

There were 405,255 men and 406,378 women in the study with a mean baseline age of 43 years (SD = 9; Table 1). Approximately 10% of men and women were obese (BMI ≥ 30 kg/m^2), and 34% of men and 29% of women were current smokers. Mean follow-up time was 20 years (SD = 8). During follow-up, 2,983 men and 754 women were diagnosed with BC. By use of tumor data, in the Austrian and Swedish cohorts, 929 men and 222 women were identified with NMIBC and 319 men and 94 women with MIBC. In total, 478 men and 114 women died of BC.[26–30]

The associations between the z-scores of metabolic factors and risk of BC were approximately linear (Supporting Information, Fig. S2), which supports the use of risk estimates on a continuous scale. Table 2 shows HRs of BC outcomes by continuous z-scores for metabolic factors. Adjusting for smoking and BMI did not materially alter the risk, except for BP. BP and triglycerides were positively associated with risk of BC among men. The adjusted and RDR-corrected HR per SD (95% CI) was 1.09 (1.02–1.17) for BP and 1.21 (1.10–1.32) for triglycerides (Table 2). Among women, BMI was inversely associated with risk of BC, HR 0.90 (0.82–0.99). The sensitivity analysis in the VIP cohort showed that incorporating education, anti-hypertensive medication and physical activity into the Cox models did not alter any of the associations between the metabolic factors and BC risk (Supporting Information Table S3). In relation to tumor invasiveness, positive associations were found for BP, cholesterol and triglycerides and NMIBC among men (HRs: 1.09 [95% CI 1.01–1.18], 1.14 [1.02–1.49], and 1.30 [1.12–1.48], respectively), BP and MIBC among men (HR: 1.23 [1.02–1.49]), and for glucose and MIBC among women (HR: 1.99 [1.04–3.81]). For BC mortality, there was a positive association with BP (HR 1.25 [1.06–1.49]), glucose (HR 1.71 [1.15–2.43]), and triglycerides (HR 1.23 [1.02–1.48]) among men, and with triglycerides (HR 1.79 [1.23–2.60]) among women. The associations between systolic and diastolic BP separately in relation to outcomes are shown in Supporting Information Table S4.

Significant sex-interactions were found for BMI and BP in relation to total incident BC and for BMI in relation to NMIBC ($p_{\text{interaction}} \leq 0.005$). For all associations without sex interactions, we report results for men and women combined in Supporting Information Table S5 in addition to the sex-specific results reported in Table 2. Heterogeneity tests of HRs for NMIBC versus MIBC were in case of sex-interactions performed separately among men and women but otherwise together. Significant heterogeneity was found only for cholesterol, which was positively related to NMIBC but not MIBC.

There was no statistically significant additive interaction in terms of RERI between the metabolic factors and smoking for BC risk among men (Fig. 2), and none of these interactions were significant on a multiplicative scale, except for glucose (Supporting Information Table S6). However, the proportional hazards assumption was violated for the product term of glucose and smoking, suggesting that its impact on BC risk was not constant over attained age.

Discussion

In our study, we investigated associations between metabolic factors and risk of incident BC, NMIBC, and MIBC, as well as BC mortality. We confirmed results in our previous study that BP and triglycerides were positively associated with incident BC among men.[19] In the present study, we further observed positive associations between these factors, along with glucose and BC mortality, between BP and MIBC, and between triglycerides, cholesterol, BMI and NMIBC among men. Furthermore, among women, triglycerides were positively associated with risk of BC death and glucose with MIBC, while BMI was inversely associated with risk of incident BC. The relationships between BMI, BP, and BC risk differed between men and women. However, BC risk did not significantly differ by smoking status among men, or between risks for NMIBC and MIBC, apart from cholesterol, which was positively related to NMIBC but not MIBC among men.

Elevated triglycerides were the overall strongest risk factor among men, and among women for BC mortality.[19] Two previous studies found no association between triglycerides and BC risk; however, these studies were limited by less detailed smoking adjustment and a much smaller sample size.[31,32] From a biological point of view, triglyceride-derived fatty acids could disrupt the mitochondrial respiratory chain, resulting in production of reactive oxygen species (ROS) such as superoxide anions. It is widely known that ROS, like other free radicals have the potential to damage deoxyribonucleic acid (DNA), increasing the risk of cancer.[31] Furthermore, triglycerides are associated with insulin resistance,[33] which results in increased production of insulin-like growth factor 1, a known cancer promoter.[33–35] Regarding cholesterol, a large study in Korea found no association, however, we found an association only in NMIBC.[17] Information on low-density lipoproteins (LDL) and high-density lipoproteins (HDL) would have been helpful in further elucidating the relationship between cholesterol and BC with more specificity. The potential underlying mechanism for triglycerides and cholesterol specifically in BC remains unclear.

Table 1. Baseline characteristics of the study participants in the Metabolic Syndrome and Cancer Project (Me-Can) 2.0

Characteristics	Men (n = 405,255)	Women (n = 406,378)
Cohort (year of baseline), *n* (%)		
Oslo (1972–1973)	17,856 (4)	0 (0)
NCS (1974–1988)	44,618 (11)	43,052 (11)
40y (1985–1999)	192,437 (48)	209,053 (51)
VHM&PP (1985–2005)	80,963 (20)	94,032 (23)
VIP (1992–2014)	49,244 (12)	50,614 (13)
MPP (1978–1992)	20,137 (5)	9,627 (2)
Total (1972–2014)	405,255 (100)	406,378 (100)
Baseline age, years		
Mean (SD)	43 (8.8)	43 (9.6)
Category, n (%)		
‹30	26,716 (7)	32,808 (8)
30–44	279,275 (69)	281,396 (69)
45–59	73,943 (18)	63,205 (16)
60	25,321 (6)	28,969 (7)
Fasting time, hr, *n* (%)[1]		
‹8	247,760 (61)	246,703 (61)
8	157,495 (39)	159,675 (39)
Smoking status, *n* (%)		
Never smoker	156,483 (39)	195,819 (48)
Ex-smoker	111,414 (27)	94,352 (23)
Current smoker	137,358 (34)	116,207 (29)
Smoking intensity, pack years, *n* (%)[2]		
‹10	34,451(28)	46,589 (42)
10–19.9	53,384 (43)	48,680 (44)
20	36,783 (29)	15,858 (14)
BMI, kg/m^2		
Mean (SD)	26 (3.4)	25 (4.3)
Category, n (%)[3]		
‹25 kg/m^2	188,577 (47)	253,985 (63)
25–29.9 kg/m^2	175,594 (43)	107,598 (26)
30 kg/m^2	39,360 (10)	42,804 (11)
Blood pressure, mm Hg, mean (SD)		
Systolic blood pressure	133 (15.9)	126 (17.6)
Diastolic blood pressure	82 (10.5)	77 (10.7)
Mid-blood pressure[4]	107 (12.1)	102 (13.2)
Category[3], systolic/diastolic, *n* (%)		
‹140/90 mm Hg	254,616 (63)	313,438 (77)
140/90–159/99 mm Hg	112,940 (28)	66,084 (16)
160/100 mm Hg	37,434 (9)	26,607 (7)
Glucose, mmol/L		
Mean (SD)[5]	5.4 (1.3)	5.2 (1.1)
Category[3], n (%)[6]		
‹6.1 in serum/plasma or 5.6 in whole blood	133,199 (87)	141,624 (91)
6.1–6.9 in serum/plasma or 5.6–6.0 in whole blood	13,690 (9)	10,108 (6)
7.0 in serum/plasma or 6.1 in whole blood	6,144 (4)	4,411 (3)

(Continues)

Table 1. Continued

Characteristics	Men (n = 405,255)	Women (n = 406,378)
Cholesterol, mmol/L		
Mean (SD)	5.6 (1.2)	5.5 (1.2)
Category[3], n (%)[6]		
‹5.2	61,104 (39)	70,062 (44)
5.2–6.1	52,528 (33)	50,269 (32)
6.2	43,701 (28)	39,163 (24)
Triglycerides, mmol/L		
Mean (SD)	1.6 (1.2)	1.2 (0.76)
Category[3], n (%)[6]		
‹1.7	105,794 (68)	132,194 (83)
1.7–2.2	24,020 (16)	15,954 (10)
2.3	25,472 (16)	10,604 (7)
Follow-up time, years	19.7	19.5
Category, n (%)		
‹10	43,125 (11)	37,508 (9)
10–19	167,931 (41)	175,109 (43)
20–29	150,624 (37)	166,251 (41)
30	43,575 (11)	27,510 (7)

Abbreviations: Oslo, Oslo study 1; NCS, Norwegian Counties Study; 40-y, Age 40-programme; VHM&PP, Vorarlberg Health Monitoring and Prevention Program; VIP, Västerbotten Intervention Project; MPP, Malmö Preventive Project; SD, standard deviation; BMI, body mass index.
[1] Proportion of participants with fasting time 8 hr: 3.2% in Norwegian cohorts, 98.4% in VIP, 100% in VHM&PP and 93.1% in MPP. In the Norwegian cohorts, fasting times ‹1 hour, 1–2 hours, 3–4 hours, 4–8 hours, 8 hours were available and used in analyses.
[2] Accumulated pack-years among current smokers excluding 17,820 current smokers with missing pack-year data.
[3] Sources for categories: BMI,[26] blood pressure,[27] glucose,[28] cholesterol and triglycerides.[29]
[4] (Systolic + diastolic blood pressure)/2.
[5] Fasting whole blood glucose levels in the MPP participants were converted into the equivalent of serum/plasma levels by increasing them by 12%.[30]
[6] Includes 153,033 men and 156,143 women with fasting plasma/serum/blood samples.
[g]Number of missing for each metabolic factor: BMI, 3,715; systolic blood pressure, 534; diastolic blood pressure, 701; glucose, 293,647; cholesterol, 861 and triglycerides, 3,894.

High BP was associated with an increased risk of BC and of BC death among men. Additionally, BP showed a graded increase in strength of association from BC, NMIBC, MIBC, and BC death, suggesting that BP may play a role in both cancer initiation and progression. However, formal tests of heterogeneity in BP associations by tumor invasiveness were non-significant. The putative pathophysiological mechanism between BP and BC remains unclear. Some have proposed that the mitogenic effect of angiotensin converting enzyme, vasopressin and other neuro-hormones affecting blood pressure may play a role in cancer promotion.[15,36] In our study, glucose also showed stronger associations with more advanced BC in some of the analyses, but again without significant differences by tumor invasiveness. Previous studies on glucose and risk of BC have suggested an increased risk with elevated glucose among women, but not men.[14,19] This, however, was not supported by our study that overall showed suggestive positive associations among both men and women without significant sex-interactions, though some associations were significant among men (BC mortality) or women (MIBC) only.

In relation to BMI, we observed a positive association with NMIBC among men and an inverse association with total BC incidence among women. These associations with BMI were significantly different between men and women. Additionally among men, associations with BMI were significantly positive only for NMIBC but not MIBC, and among never smokers but not current smokers. These findings may explain the inconsistent results observed in prior studies of total BC risk, for which the individual results may largely depend on the specific population investigated and the proportion of MIBC in the study.[9,37] The divergent associations with BMI by subgroups in our study were most evident between men and women, and such difference could potentially be explained by hormonal differences as has been suggested to be one potential explanation for the much larger BC incidence among men than among women.[10,38] Another possible explanation may be sex-specific differences in body composition by BMI level and thus, different associations with BC. BMI as a measure of obesity is limited owing to that it provides no information on amount and distribution of fat, with which information we could have further disentangled the results of our study.

Table 2. Hazard ratio (95% confidence interval) of bladder cancer outcomes by Z-Scores of metabolic factors

		VHM&PP, VIP, and MPP (n = 304,617)				All cohorts (n = 811,633)			
		Non-muscle invasive bladder cancer incidence[2]		Muscle-invasive bladder cancer incidence[2]		Bladder cancer incidence		Bladder cancer mortality	
Exposure	Model[1]	Men (n_{cases} = 929)	Women (n_{cases} = 222)	Men (n_{cases} = 319)	Women (n_{cases} = 94)	Men (n_{cases} = 2983)	Women (n_{cases} = 754)	Men (n_{cases} = 478)	Women (n_{cases} = 114)
BMI, kg/m²	1[3]	1.06 (0.99–1.14)	0.87 (0.75–1.02)	0.92 (0.81–1.04)	0.97 (0.78–1.21)	1.00 (0.96–1.04)	**0.87 (0.80–0.94)**	**0.88 (0.80–0.98)**	0.83 (0.67–1.02)
	2[4]	**1.08 (1.01–1.16)**	0.89 (0.77–1.04)	0.95 (0.84–1.07)	1.00 (0.81–1.25)	1.02 (0.99–1.06)	**0.91 (0.84–0.99)**	0.93 (0.84–1.02)	0.88 (0.71–1.08)
	3[5]	**1.09 (1.01–1.18)**	0.88 (0.75–1.04)	0.95 (0.82–1.08)	1.00 (0.79–1.28)	1.02 (0.99–1.07)	**0.90 (0.82–0.99)**	0.92 (0.82–1.02)	0.87 (0.68–1.09)
		p_{int} = 0.005[6]		p_{int} = 0.902[6]		p_{int} < 0.001[6]		p_{int} = 0.417[6]	
		p_{het} men = 0.265[7], p_{het} women = 0.409[7]							
Mid-blood pressure, mm Hg	1[3]	1.01 (0.95–1.07)	0.93 (0.81–1.07)	1.07 (0.96–1.19)	0.95 (0.76–1.18)	**1.03 (1.01–1.07)**	**0.92 (0.85–0.99)**	1.08 (0.99–1.18)	0.97 (0.80–1.17)
	2[4]	1.02 (0.95–1.09)	1.01 (0.87–1.16)	**1.12 (1.01–1.24)**	1.04 (0.83–1.29)	**1.05 (1.01–1.09)**	0.99 (0.91–1.06)	**1.13 (1.03–1.24)**	1.06 (0.88–1.29)
	3[5]	1.04 (0.91–1.17)	1.02 (0.77–1.31)	**1.23 (1.02–1.49)**	1.08 (0.71–1.60)	**1.09 (1.02–1.17)**	0.98 (0.84–1.11)	**1.25 (1.06–1.49)**	1.11 (0.79–1.59)
		p_{int} = 0.135[6]		p_{int} = 0.362[6]		p_{int} = 0.001[6]		p_{int} = 0.339[6]	
		p_{het} = 0.454[7]							
Glucose, mmol/l	1[3]	1.05 (0.99–1.12)	0.94 (0.82–1.08)	1.04 (0.94–1.16)	**1.20 (1.01–1.44)**	1.04 (1.00–1.08)	1.05 (0.96–1.14)	**1.14 (1.03–1.26)**	1.06 (0.86–1.30)
	2[4]	1.04 (0.97–1.11)	0.95 (0.82–1.08)	1.05 (0.94–1.17)	**1.21 (1.01–1.45)**	1.03 (0.99–1.08)	1.06 (0.97–1.16)	**1.16 (1.04–1.28)**	1.07 (0.88–1.32)
	3[5]	1.15 (0.90–1.46)	0.83 (0.49–1.32)	1.19 (0.80–1.76)	**1.99 (1.04–3.81)**	1.11 (0.97–1.32)	1.23 (0.90–1.71)	**1.71 (1.15–2.43)**	1.28 (0.63–2.72)
		p_{int} = 0.093[6]		p_{int} = 0.146[6]		p_{int} = 0.870[6]		p_{int} = 0.930[6]	
		p_{het} = 0.195[7]							
Cholesterol, mmol/l	1[3]	**1.12 (1.05–1.20)**	1.11 (0.97–1.27)	0.99 (0.88–1.11)	0.91 (0.73–1.12)	**1.07 (1.03–1.11)**	1.04 (0.97–1.12)	1.11 (1.01–1.22)	1.02 (0.85–1.23)

(Continues)

Table 2. Continued

		VHM&PP, VIP, and MPP (n = 304,617)				All cohorts (n = 811,633)			
		Non-muscle invasive bladder cancer incidence[2]		Muscle-invasive bladder cancer incidence[2]		Bladder cancer incidence		Bladder cancer mortality	
Exposure	Model[1]	Men (n_{cases} = 929)	Women (n_{cases} = 222)	Men (n_{cases} = 319)	Women (n_{cases} = 94)	Men (n_{cases} = 2983)	Women (n_{cases} = 754)	Men (n_{cases} = 478)	Women (n_{cases} = 114)
	2[4]	**1.09 (1.01–1.16)**	1.08 (0.95–1.24)	0.97 (0.86–1.09)	0.89 (0.72–1.11)	1.03 (0.99–1.07)	1.01 (0.94–1.09)	1.09 (0.99–1.19)	1.00 (0.83–1.21)
	3[5]	**1.14 (1.02–1.25)**	1.12 (0.93–1.39)	0.96 (0.80–1.14)	0.84 (0.61–1.17)	1.05 (0.99–1.11)	1.02 (0.91–1.14)	1.14 (0.99–1.30)	1.00 (0.75–1.34)
		p_{int} = 0.633[6]		p_{int} = 0.529[6]		p_{int} = 0.359[6]		p_{int} = 0.455[6]	
		p_{het} = 0.026[7]							
		Triglycerides, mmol/l	1[3]	**1.20 (1.12–1.28)**	**1.16 (1.02–1.33)**	1.07 (0.95–1.20)	1.19 (0.98–1.46)	**1.12 (1.08–1.17)**	**1.09 (1.02–1.17)**
1.11 (1.01–1.22)	**1.32**	**(1.11–1.58)**							
	2[4]	**1.14 (1.06–1.22)**	1.14 (0.99–1.31)	1.04 (0.93–1.18)	1.15 (0.93–1.43)	**1.08 (1.04–1.13)**	1.06 (0.98–1.15)	**1.11 (1.01–1.22)**	**1.34 (1.11–1.62)**
	3[5]	**1.30 (1.12–1.48)**	1.30 (0.95–1.71)	1.08 (0.87–1.39)	1.32 (0.87–2.03)	**1.17 (1.08–1.27)**	1.12 (0.96–1.32)	**1.23 (1.02–1.48)**	**1.79 (1.23–2.60)**
		p_{int} = 0.439[6]		p_{int} = 0.395[6]		p_{int} = 0.178[6]		p_{int} = 0.126[6]	
		p_{het} = 0.252[7]							

[1] Cox proportional hazards regression analysis with attained age as the underlying time scale and with stratum for cohort.
[2] Data on tumor staging only available in the VHM&PP, VIP and MPP cohorts.
[3] Model 1: adjusted for baseline age and date of birth in five categories.
[4] Model 2: as in model 1 and additionally adjusted for smoking status and intensity in six categories and BMI in four categories (except for BMI).
[5] Model 3: as in Model 2 and additionally corrected for regression dilution ratio (RDR) of the exposure, which could be transformed back to original data by: $RR_{original} = e^{\log\ (RRcorrected)_{*}RDR}$. RDR for BMI = 0.902, blood pressure = 0.544, glucose (log) = 0.278, cholesterol = 0.657, triglycerides (log) = 0.505.
[6] p Value for metabolic factor-sex interaction calculated by likelihood ratio tests.
[7] p Value for heterogeneity between NMIBC and MIBC calculated by the duplication method. Heterogeneity tests were performed for men and women jointly in the absence of sex-interaction.

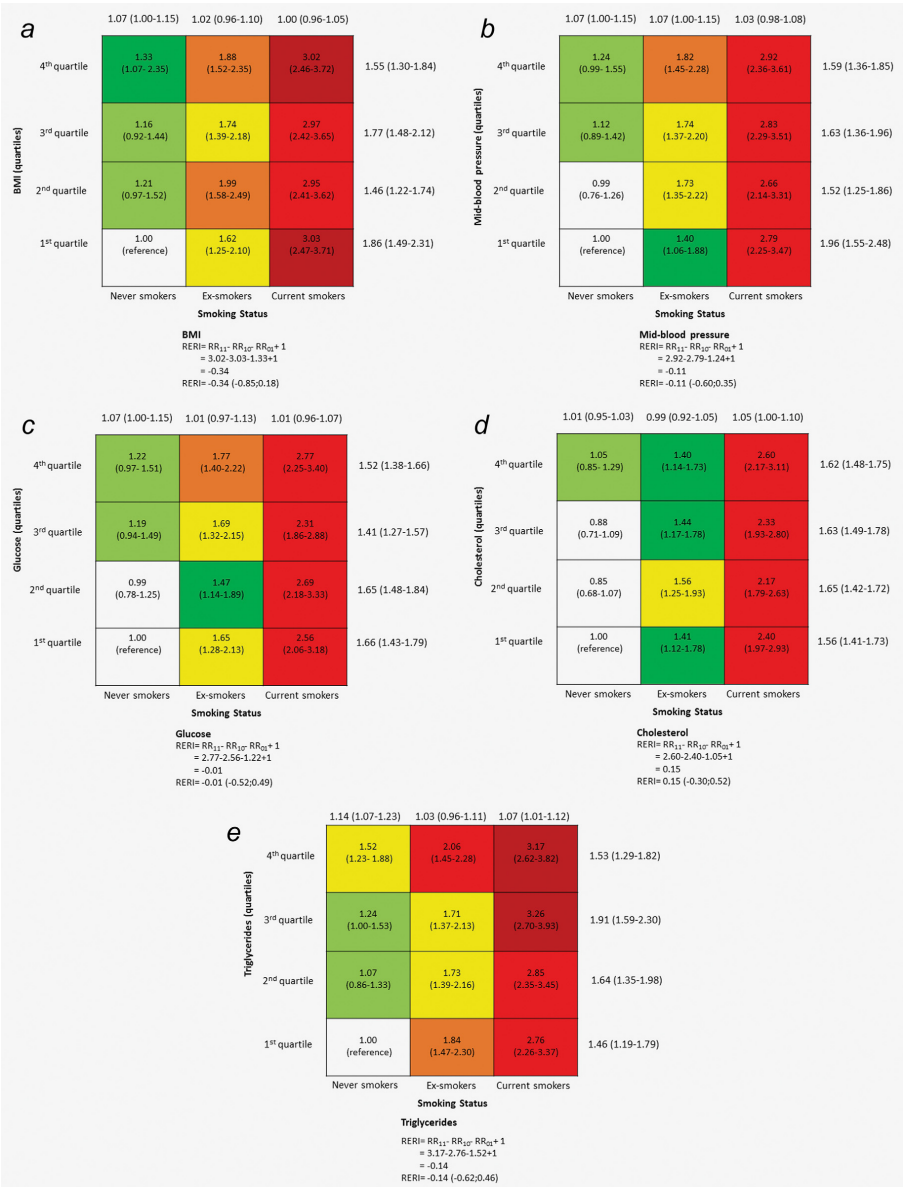

Figure 2. Hazard ratios (95% confidence interval) for bladder cancer incidence according to combinations of metabolic factor and smoking status in men. The Relative Excess Risk for Interaction (RERI) was calculated as RR11 − RR10 − RR01 + 1, for which the delta method was used to obtain confidence intervals (21). Hazard ratios were calculated by Cox regression with attained age as the underlying time scale, adjustment for BMI (except for BMI) with stratum for cohort. The hazard ratios were not corrected for RDR in the table or in the calculation of RERI. The colors displayed reflect the strength of association (from light to dark). [Color figure can be viewed at wileyonlinelibrary.com]

The search for nominally significant findings at *p*-values lower than 0.05 in our study needs consideration, on one hand in relation to the many tests performed which increases the probability of false positive findings, and on the other hand in relation to the large statistical power needed to detect interactions. Correction for multiple testing should be performed in relation to the number of hypotheses being tested, for which there is no straight forward answer in our study as metabolic factors are correlated and therefore should not be considered as exclusively separate tests, and second, because the outcomes partially included the same cases. In relation to interaction tests for sex and smoking and heterogeneity tests for tumor invasiveness, any of the four significant findings in our study might be a chance finding owing to multiple testing. However, absence of significance should also be interpreted in light of the sometimes-small sub-groups compared, and thus the limited statistical power to detect interactions despite our large study population.

The main strengths of our study were the long follow-up, use of high quality national cancer registries,[39,40] and the large sample size that allowed us to deepen the exploration of interactions compared to previous studies, and to investigate associations in several sufficiently sized sub-groups. Correcting for random error in exposure measurement and their true long-term variation further allowed for more accurate effect estimates. We incorporated smoking intensity (in pack years) in the analysis, which considerably improved the adjustment for smoking and reduced the possibility of residual confounding for this potentially strong confounder. However, aside from residual confounding, the association between metabolic factors and BC among current smokers might be underestimated due to potential random error in the measurement of smoking, which has been speculated to cause the consistently inverse association found for BMI and lung cancer.[9,41]

The study also had some weaknesses. The analysis of women lacked power, especially for MIBC and mortality. Moreover, the ascertainment of BC as primary death cause may have challenges as these patients are primarily of old age and often suffer from co-morbidities, which aggravates the ascertainment of the underlying death cause. Validation of the Swedish cause of death register has shown overall high validity, especially for cancers including prostate cancer that, like BC, affects elderly men.[42] However, validation specifically for BC as death cause is lacking, and we cannot exclude under- or overrepresentation of BC as death cause and therefore a potential influence on our results for BC mortality. We also lacked (complete) data on other potential confounders, such as drugs for hypertension, dyslipidemia and diabetes. Sensitivity analysis using the VIP cohort which had the most complete data on anti-hypertensive medication at baseline, education, and physical activity showed that including these potential confounders did not significantly alter the associations between metabolic factors and BC outcomes.

In conclusion, in this large prospective study, metabolic aberrations, especially elevated BP and triglycerides, were associated with increased risks of BC among men, whereas high BMI was associated with decreased BC risk. The associations between BMI, BP, and BC risk were significantly different between men and women. Furthermore, whilst some associations with metabolic factors were significant only in sub-groups by smoking status or exclusively in relation to MIBCs or NMIBCs, the associations were generally not significantly different. To further elucidate the findings of our study, larger studies are needed to account for the low BC incidence among women and never smokers, ideally with more specific measures of metabolic factors, such as body fat and distribution, and low and high-density lipoprotein cholesterol.

Acknowledgments

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Paper II

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ORIGINAL RESEARCH

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Association between blood pressure and BMI with bladder cancer risk and mortality in 340,000 men in three Swedish cohorts

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Abstract

Background: The relation between obesity, blood pressure (BP) and bladder cancer (BC) risk and mortality remains unclear, partially due to potential confounding by smoking, the strongest risk factor for BC, and not accounting for tumor stage and grade in such studies. We investigated body mass index (BMI) and BP in relation to BC risk by stage and grade, and BC-specific mortality, including separately among never-smokers aimed at minimizing confounding by smoking.

Methods: We analyzed 338,910 men from three Swedish cohorts, with 4895 incident BC's (940 among never-smokers) during follow-up. Cox regression was used to calculate hazard ratios (HR) and 95% confidence intervals adjusted for smoking status. HRs for BMI and BP were corrected for their regression dilution ratios, calculated from 280,456 individuals with 758,641 observations.

Results: Body mass index was positively associated with non-muscle invasive BC (NMIBC, HR per 5 kg/m^2, 1.10 [1.02–1.19]) and NMIBC grade 3 (HR 1.17 [1.01–1.34]) in the full cohort, with similar effect sizes, albeit non-significant, among never-smokers. Systolic BP was positively associated with muscle-invasive BC (MIBC, HR per 10 mmHg, 1.25 [1.00–1.55]) and BC-specific mortality (HR 1.10 [1.01–1.20]) among never-smokers, with weaker and non-significant associations in the full cohort.

Conclusions: In an analyses of BMI, BP and BC risk by stage and grade among men, we found modest positive associations between BMI and NMIBC and NMIBC grade 3. SBP was positively associated with MIBC and BC-specific mortality in an analysis of never-smokers, which may reflect the association, un-confounded by smoking, also in a broader population.

KEYWORDS
bladder cancer, blood pressure, body mass index, confounding, survival analysis

1 | INTRODUCTION

Bladder cancer (BC) is one of the most common cancer forms in developed countries, and its relationship with metabolic risk factors including obesity, commonly measured as body mass index (BMI), and blood pressure (BP), has been inconsistent.[1,2] This may partially be due to lack of statistical power and combining sub-groups with different etiology. With regards to BMI, a recent meta-analysis of 14 prospective cohort studies overall showed a small, but positive non-linear association with BC risk.[3] However, one of the largest studies so far, (including 1391 BC cases) showed that such positive association was restricted to men,[4] and our recent study (3737 BC cases) showed a positive association with BMI only for non-muscle invasive BC (NMIBC).[2] In that study, we further found positive linear associations between systolic BP (SBP) and the risk of overall BC and muscle-invasive BC (MIBC) among men, but not women. In contrast, a recent meta-analysis investigating associations between BP indices and overall BC risk found null associations. However, most of the included studies combined men and women in the analysis.[5] Moreover, classification of BC aggressiveness is usually stratified based on staging in epidemiological studies. However, grading, the extent to which the tumor cells are similar in appearance and function to the normal cells, is another dimension to measure tumor aggressiveness, harboring additional information especially for NMIBC and risk of progression.[6] Also biologically, a two-pathway theory has been proposed with stratification in NMIBC and MIBC.[7] Clinically, stratification in NMIBC and MIBC occurs in a majority of patients associated with preserving versus radical treatment, respectively. However, other factors such as tumor progression related to diagnostic delays confer increased risk of mortality beyond disease stratification in NMIBC and MIBC or even tumor stage.[8]

In relation to BC-specific mortality, studies on the association with BMI and BP, respectively, have shown inconsistent results and are few.[9,10] Most of these studies were conducted among patients undergoing radical cystectomy,[11-18] and fewer studies were conducted at population level.[19,20] Most studies investigated associations either from the time of study enrollment (in the full population), or from the time of diagnosis (among cases only), but not both. Investigating either time-line has advantages, but may also introduce certain biases that could be mitigated by investigating both time-lines.[21-24]

Smoking is the strongest known risk factor for BC and accounts for up to 50% of the cases.[25] Sufficiently accounting for such a strong risk factor in observational studies is difficult, and residual confounding may persist. Investigating risk factors in relation to BC risk separately among never-smokers may disentangle the risk factor association from smoking; however, this may be difficult to accomplish due to the challenge of achieving sufficient statistical power in an analysis restricted to never-smokers.

The aim of the study was to investigate the associations between BMI and BP and BC risk, for BC overall and separately for NMIBC and MIBC and by tumor grade. Furthermore, we aimed to investigate the association between BMI and BP and BC-specific mortality from the time of study enrollment and, among cases, from the time of diagnosis with additional adjustment for clinical characteristics. We investigated all associations in the full cohort and separately among never-smokers. Due to the population composition, we focused our analysis on men.

2 | MATERIALS AND METHODS

2.1 | Study population

The study included participants from three prospective Swedish cohorts, the Västerbotten Intervention Programme (VIP), the Malmö Preventive Project (MPP) and the Construction Workers Cohort (CWC), of which a detailed description has been published elsewhere.[26-28]

2.2 | Exposure assessment

Height and weight were measured with individuals wearing no shoes and light clothing.[29,30] BP was taken in a supine position using a standard mercury sphygmomanometer in all the cohorts; in the VIP and CWC a single reading was taken after 5 min of rest, and in the MPP, BP was recorded after an average of 2 readings taken with a 10 min interval.

2.3 | Follow-up and end point assessment

Any cancer diagnosis, death and its cause, and migration status were identified through linkage of each individual's unique identification number with Sweden's National Cancer Register, Cause of Death Register and Population Register, respectively, for events up until 31 December 2014. BC was defined according to the 10th version of the International Classification of Diseases (ICD-10) code C67 (0–9), including carcinoma in situ (D09.0). The Swedish National Register for Bladder Cancer register was used for the classifications of BC into NMIBC and MIBC, and this started in 1997, a detailed description on how BC tumors were classified by stage and grade is described in the Supporting Information (Text S1). Death due to BC (BC-specific mortality) was defined as BC (ICD-10, C67) reported as the underlying cause of death in the Swedish national cause of death registry.

2.4 | Selection criteria

The study population was initially composed of 521,896 individuals with 1,342,110 observations from the health examination data, out of which 338,910 individuals, each individual with one baseline observation, were included for the final analysis (Figure S1). For individuals with repeated observations, the first was selected as the baseline observation. Out of the 182,986 excluded individuals, the most common causes of exclusion were those younger than 20 years, women, missing smoking data, and those with any prevalent cancer which was defined as any malignant neoplasm, including malignant neoplasms of hematopoietic or lymphoid origin and other related tissues (ICD-10, C81-C96), but excluding basaliomas and all carcinomas in situ.

2.5 | Statistical analysis

Cox proportional hazards regression was used to calculate hazard ratios (HRs) with their 95% confidence intervals (95% CI) to investigate the risk of BC end-points by levels of BMI, SBP, and diastolic BP (DBP) in the full cohort, and additionally among never-smokers, using age as the underlying time scale. Participants were followed from the date of baseline examination, up until the date of event of interest or until censoring due to the diagnosis of another cancer, emigration and death, or until the end of follow-up, whichever one came first. Follow-up of NMIBC and MIBC began on 1 January 1997, and 35,860 individuals who were censored before that date were excluded from the analysis. We adjusted the analyses for smoking status (three categories), age at baseline examination (continuous), date of birth (five categories), cohort (three categories), level of education (eight categories), and BMI ([quartiles] except for the analysis of BMI). Additionally, the metabolic factors were investigated in categories of BMI, SBP and DBP in relation with BC end-points. Test for trends in these categories were performed by regressing the BC outcomes against the average in each category. We additionally investigated the shape of association between BMI (per 5 kg/m^2), SBP and DBP (per 10 mmHg), and BC end-points using restricted cubic spline regression, and we tested for the linearity of these associations with the likelihood ratio test (Figure S2–S6).

In the case-only analysis, we investigated the associations between BMI, BP, and BC-specific mortality and all-cause mortality using Cox regression with follow-up time from the date of BC diagnosis as the underlying time scale. The same adjustments were used as in the full cohort analysis; however, instead of adjusting for age at baseline examination, we adjusted for age at diagnosis and additionally for tumor grade (four categories) and stage (three categories), type of treatment (BC-specific [eight categories]) and for the Charlson co-morbidity index (four categories).[31] A detailed description of the categorical variables used in models is found in in the supplements (Table S1).

We calculated Schoenfield residuals for exposures and co-variables to test for the proportional hazards assumption in the Cox models. Depending on the end-point being analyzed, one or two co-variables were suggestive of violating this assumption; however, including them as strata in the Cox models did not materially change the HRs, thus, they were retained within the models as co-variables rather than in strata.

We corrected the HRs for intra-personal variability and measurement error using a RDR based method as described by Wood et al.[32] The values for the calculated RDRs are shown in Table S2. All HRs were corrected for RDR by: $HR_{corrected} = exponent^{(log\ [HR\ original]/RDR)}$. The corrected HRs are interpreted as the expected HRs of "usual" adult level of BMI and BP, respectively.

We performed all the statistical analyses in STATA 13, (StataCorp LLC).

3 | RESULTS

Table 1 shows the characteristics of the study participants according to smoking status. There were 147,692 never-smokers, 64,492 ex-smokers and 126,726 current smokers. On average, never-smokers, had a BMI of 24.8 (SD = 3.3), while ex-smokers had a BMI 25.3 (SD = 3.3) and current smokers had a BMI of 24.3 (SD = 3.2). With regards to BP, never-smokers had an average SBP of 131 mmHg (SD = 15) and an average DBP of 80 mmHg (SD = 11) and the corresponding values among ex-smokers and current smokers were 134 mmHg (SD = 16)/83 mmHg (SD = 10) and 133 mmHg (SD = 15)/81 mmHg (SD = 10), respectively. A breakdown of study participant characteristics according to cohort is shown in Table S3. During an average follow-up of 28 years, 4895 men had been diagnosed with BC, of which 1020 had died from BC. The associations between BMI, BP, and BC outcomes were approximately linear except for the association between BMI and overall BC, and SBP and MIBC (Figure S2–S6). There was a positive association between BMI levels above 25 kg/m^2 and BC (HR per 5 kg/m^2, 1.15 [95% CI, 1.03–1.26]), and a suggestive, non-significant, association between SBP in the lower range and risk of MIBC.

Hazard ratios and 95% CIs of BC outcomes per continuous increase in BMI and BP are shown in Figures 1–3. BMI was positively associated with risk of all NMIBC (HR per 5 kg/m^2, 1.10 [95% CI, 1.02–1.19]) and with NMIBC grade 3 risk (HR per 5 kg/m^2, 1.17 [95% CI, 1.01–1.34]). The effect sizes in these associations were similar, but did not

TABLE 1 Characteristics of the 338,910 men in the study according to smoking status

Characteristic	Never-smokers (n = 147,692)	Ex-smokers (n = 64,492)	Current Smokers (n = 126,726)	Total (n = 338,910)
Cohort, *n* (%)				
Västerbotten Intervention Programme	31,922 (21.6)	11,331 (17.6)	8802 (7.0)	52,055 (15.3)
Malmö Preventive Project	7588 (5.1)	3568 (5.5)	11,120 (8.8)	22,276 (6.6)
Construction Workers Cohort	108,182 (73.3)	49,593 (76.9)	106,804 (84.2)	264,579 (78.1)
Baseline age, years, mean (SD)	36.3 (12.4)	42.2 (12.3)	38.9 (12.3)	38.4 (12.5)
BMI, kg/m^2, mean (SD)	24.8 (3.3)	25.3 (3.3)	24.3 (3.2)	24.7 (3.3)
Category of BMI, kg/m^2, *n* (%)				
<18.5	1023 (0.7)	341 (0.5)	1667 (1.3)	3031 (0.9)
18.5–24.9	85,385 (57.8)	32,360 (50.2)	78,316 (61.8)	196,061 (57.9)
25–29.9	51,482 (34.9)	26,438 (41.0)	40,031 (31.6)	117,951 (34.8)
≥30	9802 (6.6)	5353 (8.3)	6712 (5.3)	21,867 (6.4)
Systolic BP, mmHg, mean (SD)	131.1 (14.8)	134.1 (15.8)	132.5 (15.0)	132.2 (15.1)
Diastolic BP, mmHg, mean (SD)	79.7 (10.5)	82.5 (10.3)	81.0 (10.2)	80.7 (10.4)
Category of systolic/diastolic BP, *n* (%)				
<140/90 mmHg	97,836 (66.2)	36,783 (57.0)	78,256 (61.8)	212,875 (62.8)
140/90–159/99 mmHg	39,845 (27.0)	21,093 (32.7)	38,672 (30.5)	99,610 (29.4)
≥160/100 mmHg	10,011 (6.8)	6616 (10.3)	9798 (7.7)	26,425 (7.8)
Follow-up, years, mean (SD)[a]	27.1 (10.8)	28.9 (11.1)	28.8 (10.5)	28.1 (10.8)
Incident cases of BC overall, *n*	940	1148	2807	4895
Level of invasion, *n* (%)[b]				
Non-muscle invasive	490 (78.3)	560 (76.3)	1305 (76.4)	2355 (76.7)
Muscle invasive	136 (21.7)	174 (23.7)	404 (23.6)	714 (23.3)
Grade among NMIBC, *n* (%)[c]				
Grade 1	138 (29.7)	134 (25.5)	410 (32.7)	682 (30.4)
Grade 2	185 (39.8)	216 (41.1)	504 (40.2)	905 (40.3)
Grade 3	142 (30.5)	176 (33.4)	340 (27.1)	658 (29.3)

Abbreviations: BC, bladder cancer; BMI, body mass index; BP, blood pressure; NMIBC, non-muscle invasive BC; SD, standard deviation.

[a]Follow-up until bladder cancer risk or censoring, last date of follow-up was 31 December 2014.

[b]Out of the 4895 incident bladder cancer cases, staging data were available for 3069 cases, the remaining 1826 cases either occurred before 1997 before staging data were available or staging data were available but stage could not be determined.

[c] Out of the 2355 incident NMIBC cases grading data were available for 2245 cases, for the remaining 110 cases, grade could not be determined.

reach significance in an analysis including only never-smokers. There were no other statistically significant associations between BMI and BC outcomes. BP was not associated with BC outcomes in the full cohort; however, among never-smokers, SBP was positively associated with MIBC (HR per 10 mmHg, 1.25 [95% CI, 1.00–1.55], and with BC-specific mortality in the baseline-to-event analysis (HR per 10 mmHg, 1.10 [1.01–1.20]), but not in the case-only analysis (HR per 10 mmHg, 1.05 [0.92–1.22]).

There were positive associations between BMI, SBP, and DBP, and risk of all-cause mortality, which did not reach significance in the analyses of never-smokers only (Figures 1–3). Categories of BMI and BP in relation with BC risk and mortality largely reflected the associations reported as forest plots and restricted cubic splines (Table S4–S5).

4 | DISCUSSION

In this large prospective study of nearly 340,000 men including 4900 incident BC cases, we found positive associations between BMI and NMIBC risk, especially high-grade tumors, and between SBP and MIBC among never-smokers, which are expected to display little or no confounding by smoking and may depict the smoking un-confounded association also in a broader population. SBP was further positively associated with BC-specific mortality among never-smokers, otherwise, no clear associations were observed for BMI, BP, and BC.

The association between BMI and NMIBC observed in this study was similar to the finding in our recent study,[2] which has some overlap with the present study, with the VIP

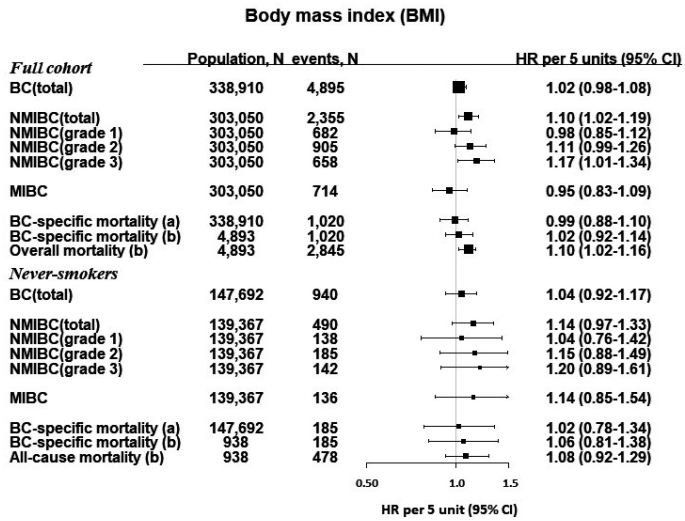

FIGURE 1 Hazard ratios and 95% confidence intervals for BC outcomes per 5 kg/m^2 increment of BMI. For BC-specific mortality, we investigated associations for (A) the time of study enrollment, and (B) among cases, from the time of diagnosis. BC, bladder cancer; kg, kilogram; m, meter; BMI, body mass index; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer. †Data on tumor characteristics were only available from 1997, therefore, the analysis for NMIBC (and by grade) and MIBC only began from 1 January 1997 and any diagnosis of BC or other censoring events (diagnosis of other cancers, emigration or death) were excluded from this analysis (35,860 participants). Two incident bladder cancer cases were excluded in the mortality analysis

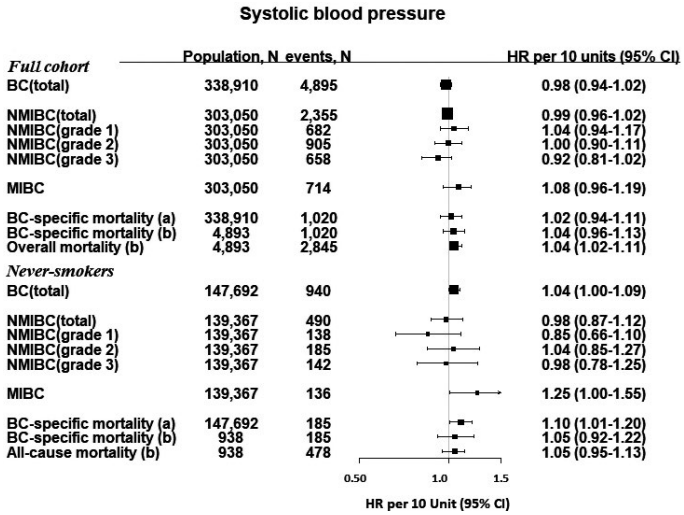

FIGURE 2 Hazard ratios and 95% confidence intervals for BC outcomes per 10 mmHg increment of systolic blood pressure. For BC-specific mortality, we investigated associations for (A) the time of study enrollment, and (B) among cases, from the time of diagnosis. BC, bladder cancer; mmHg, millimeters of mercury; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer. †Data on tumor characteristics were only available from 1997, therefore, the analysis for NMIBC (and by grade) and MIBC only began from 1 January 1997 and any diagnosis of BC or other censoring events (diagnosis of other cancers, emigration or death) were excluded from this analysis (35,860 participants). Two incident bladder cancer cases were excluded in the mortality analysis

and MPP comprising around 20% of the respective study populations. We investigated NMIBC and MIBC as two distinct entities; however, this separation may be too simplified. The relationship between NMIBC and MIBC may also be seen as a continuum, and while a majority of NMIBC are made up of low grade Ta tumors that rarely progress to severe forms, high grade NMIBC are more likely to progress into MIBC. The classification of such tumors into NMIBC and MIBC thus is affected by the time of capture, that is, diagnostic delay. In the present study, we were able to divide NMIBCs by tumor grade and found a positive linear association between BMI and NMIBC grade 3 risk. Roswall et al., in a similar analysis albeit, with fewer cases, found no such association.[4] It remains unclear why we observe an association with NMIBC and not with MIBC, and biological mechanisms linking BMI and BC also remain unclear.

We found no association between BMI and BC-specific mortality. This result remains a source of controversy[9,10] as some studies found a positive association,[11-14] while others found a null association.[15-20] One reason for inconsistent results may partially be differences in study design. Whereas most studies were conducted retrospectively and within a

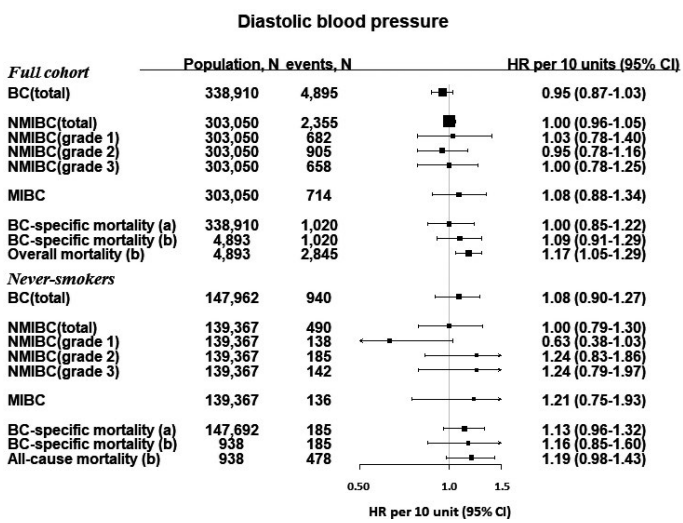

FIGURE 3 Hazard ratios and 95% confidence intervals for BC outcomes per 10 mmHg increment of diastolic blood pressure. For BC-specific mortality, we investigated associations for (A) the time of study enrollment, and (B) among cases, from the time of diagnosis. BC, bladder cancer; mmHg, millimeters of mercury; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer. †Data on tumor characteristics were only available from 1997, therefore, the analysis for NMIBC (and by grade) and MIBC only began from 1 January 1997 and any diagnosis of BC or other censoring events (diagnosis of other cancers, emigration or death) were excluded from this analysis (35,860 participants). Two incident bladder cancer cases were excluded in the mortality analysis

clinical setting,[11-14] a few studies were conducted prospectively, and these found no association.[19,20] Additionally, some studies analyzed men and women together, despite higher BC incidence rates among men (3:1 ratio)[1] and a poorer prognosis among women,[33] and their potentially differential BC etiology. Further large, well-designed and sufficiently powered studies are needed to investigate BC-specific mortality in relevant sub-groups.

We found an overall positive association between SBP and MIBC among never-smokers, which was weaker and non-significant in the full cohort. Again, these findings are consistent with our previous study, where we additionally found an association with DBP.[2] The association between BP (especially SBP) and MIBC and not with NMIBC, suggests that BP might play a role in BC progression as opposed to BC initiation. In further support of such hypothesis, we found a positive association between SBP and BC-specific mortality among never-smokers in this study, and positive associations with both SBP and DBP in our previous study. The association between SBP and MIBC could be influenced by participants being managed for hypertension in the health care system likely undergoing further tests that may lead to early detection of BC, however, such detection bias, if substantial, would also lead to an association between SBP and NMIBC, which was not found in this study.

The large study size, the virtually complete follow-up in the Swedish registers, and the RDR correction were the main strengths of our study. The large sample size enabled us to conduct analyses in different sub-groups, including analysis by tumor grade and among never-smokers only. An exposure measured on a single occasion is prone to random error due to technical error, and short-term and long-term intra-individual variability, which results in dilution of the exposure-outcome association, that is, regression dilution bias. By correcting for this bias, we investigated the association between the "usual" levels of the exposure and the outcome, which was particularly important in this study due to long follow-up. We also investigated BC-specific mortality using two approaches. In the first approach we used the full-cohort and follow-up was from the date of baseline examination. Results from this analysis reflect the influence of the metabolic factors on both the incidence and survival of BC, however, they are unlikely to suffer from selection bias.[23,24] In the second approach, we analyzed the survival of BC cases, which allowed us to adjust for tumor characteristics, co-morbidities and types of BC-related treatment; factors that have a large bearing on BC-specific survival. However, this last approach is prone to a type of selection bias called collider bias,[23,24] which may occur if the exposure is related to the risk of BC, which could explain the weaker and non-significant finding for SBP and BC-specific mortality in the case-only analysis as compared to in the baseline-to-event analysis.

The study had several limitations. First, there was no data on anti-hypertensive medication which have overall shown a positive association with BC[34] and might modify or mediate the association between BP and BC. Our results for BC-specific mortality should be interpreted with caution, as these were rather inconsistent and based on small numbers, especially among never-smokers. Lastly, the many tests performed in the analysis, may potentially attribute the significant findings to chance alone.

In conclusion, we found a positive association between BMI and NMIBC and particularly between BMI and NMIBC grade 3, and between SBP and MIBC among never-smokers.

Additionally, we found a positive association between SBP and BC-specific mortality among never-smokers. The findings on grade and among never-smokers underscore the importance of additionally investigating grade in the assessment of tumor aggressiveness, and the importance of minimizing the influence of smoking, such as analyzing never-smokers only, ideally in even larger populations than ours.

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CONFLICTS OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Stanley Teleka: Conceptualization, Data curation, Formal analysis, Writing-original draft and Writing-review and editing. Tanja Stocks: Funding acquisition, Conceptualization, Writing-original draft and Writing-review and editing. Sylvia H J Jochems: Data curation and Writing-review and editing. Angela M Wood: Writing-review and editing. Bengt Järvholm: Writing-review and editing. Marju Orho-Melander: Writing-review and editing. Fredrik Liedberg: Writing-review and editing.

ETHICS APPROVAL

The ethics committee at Umeå University approved the study (no. 2012-354-31M, 2014-162-32M, 2014-267-32M and 2015-7-32M). This study was performed in accordance with the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from https://www.malmo-kohorter.lu.se/ for the Malmö Preventive Project, from https://www.umu.se/en/biobank-research-unit/research/northern-sweden-health-and-disease-study-vip-monica-and-the-mammography-screening-project/ for the VIP, and from https://www.umu.se/en/research/projects/work-retirement-and-health/ for the CwC, with ethical approval and permission from the respective steering committee through registration and study approval. Restrictions apply to the availability of the data, appropriate procedures were followed to obtain data for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Paper III

PLOS ONE

RESEARCH ARTICLE

Blood pressure and bladder cancer risk in men by use of survival analysis and in interaction with *NAT2* genotype, and by Mendelian randomization analysis

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Abstract

The association between blood pressure (BP) and bladder cancer (BC) risk remains unclear with confounding by smoking being of particular concern. We investigated the association between BP and BC risk among men using conventional survival-analysis, and by Mendelian Randomization (MR) analysis in an attempt to disconnect the association from smoking. We additionally investigated the interaction between BP and N-acetyltransferase-2 (*NAT2*) rs1495741, an established BC genetic risk variant, in the association. Populations consisting of 188,167 men with 502 incident BC's in the UK-biobank and 27,107 men with 928 incident BC's in two Swedish cohorts were used for the analysis. We found a positive association between systolic BP and BC risk in Cox-regression survival analysis in the Swedish cohorts, (hazard ratio [HR] per standard deviation [SD]: 1.14 [95% confidence interval 1.05–1.22]) and MR analysis (odds ratio per SD: 2-stage least-square regression, 7.70 [1.92–30.9]; inverse-variance weighted estimate, 3.43 [1.12–10.5]), and no associations in the UK-biobank (HR systolic BP: 0.93 [0.85–1.02]; MR OR: 1.24 [0.35–4.40] and 1.37 [0.43–4.37], respectively). BP levels were positively associated with muscle-invasive BC (MIBC) (HRs: systolic BP, 1.32 [1.09–1.59]; diastolic BP, 1.27 [1.04–1.55]), but not with non-muscle invasive BC, which could be analyzed in the Swedish cohorts only. There was no interaction between BP and *NAT2* in relation to BC on the additive or multiplicative scale. These results suggest that BP might be related to BC, more particularly MIBC. There was no evidence to support interaction between BP and *NAT2* in relation to BC in our study.

Introduction

Elevated blood pressure (BP) is an established risk factor for cardiovascular diseases [1]. Owing to shared risk factors and pathophysiological pathways, several hypotheses have been

reference number 'UK Biobank Main Application 42410'. For the Swedish cohort, due to ethical and legal restrictions related to the Swedish Biobanks in Medical Care Act (2002:297) and the Personal Data Act (1998:204), data are available upon request from the data access group of Malmo Diet and Cancer study and the Malmö Preventive Program by contacting Anders Dahlin (anders.dahlin@med.lu.se).

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Competing interests: The authors have declared that no competing interests exist.

formed linking BP with cancer [2]. Regarding bladder cancer (BC), studies in human experimental biology have speculated that the angiotensin-renin system, a physiologic pathway responsible for the regulation of BP, may be involved in BC carcinogenesis [3, 4]. We recently reported epidemiologic support of this hypothesis in a large prospective study that showed a positive association between BP and BC risk, but only among men [5]. Other observational studies of BP and BC risk have shown conflicting results, with some studies showing a positive association [5–8], and others showing no association [2, 9–11], altogether resulting in null results in a meta-analysis that included studies predating our previous study [9]. However, most included studies were hampered by limited sample size and a combined analysis of men and women, who could have different risk profiles as indicated by the results in our study [5] and by the substantially higher BC incidence among men than among women [5, 12]. Further, factors interacting with BP in relation to BC might also have caused inconsistent results between studies. N-acetyltransferase 2 (*NAT2*) is a gene that codes for a carcinogen-metabolizing enzyme. The polymorphism that phenotypically expresses "slow acetylation" has been associated with BC, and the interaction between *NAT2* and smoking in relation to BC is well documented [13, 14]. It has been stated that if two exposures are associated with a common outcome, then they must interact either on a multiplicative or additive scale [15]. A potential interaction between BP and *NAT2* in relation to BC has not been investigated.

Mendelian randomization (MR) analysis is a methodological approach that makes use of genetic variants as an instrumental variable (IV) to, under certain assumptions, study the causal association between an exposure of interest and an outcome [16, 17]. A valid IV must fulfill three key assumptions: it must 1) be associated with the exposure of interest, 2) associate with the outcome exclusively through the exposure of interest, and 3) not be associated with confounders in the exposure-outcome association. When these assumptions are met, MR analysis overcomes the major limitations such as residual and unknown confounding, reverse causation and measurement error that are inherent to other observational studies [16, 17]. In relation to BP and BC risk, residual confounding by tobacco smoking, the strongest risk factor for BC [18], is of particular concern. To our knowledge, there are no MR studies on BP and BC risk.

The aim of the study was to investigate the association between BP and BC risk using both conventional survival analysis and MR analysis, and to study the interaction between BP and *NAT2* (rs1495741) in the association. Due to limited statistical power among women in the interaction analysis and MR analysis, which were the added novelty of this study compared to prior studies, we undertook the main investigation among men only.

Materials and methods

Study populations

The study included participants from two cohorts in the city of Malmö, in the southernmost part of Sweden, the Malmö Diet and Cancer Study (MDCS) and the Malmö Preventive Project (MPP), and the UK-biobank from the United Kingdom. The MDCS is a population-based cohort of 30,447 participants aged between 45 and 73 years, who underwent a health examination in 1991–96. The MPP is also population-based and included 33,346 men and women who had a health examination in 1974–1992. Detailed descriptions of the Malmö cohorts are published elsewhere [19, 20]. The UK-biobank is a publicly available research resource in the form of a population-based cohort of men and women aged between 40 and 69 years. The project recruited 502,627 individuals nationally between 2006 and 2011. A detailed description of the cohort is published elsewhere [21].

Ethical considerations

This study was performed in accordance with the Declaration of Helsinki. Participants provided a written consent at baseline physical examination to have their data used for research. The ethics committee at Lund University approved the study of the MDCS and the MPP (Dnr 2014/830). The UK-biobank's research ethics committee and Human Tissue Authority Research Bank approved this study (application number 42410) [22].

BP assessment

In the MDCS and MPP, BP was measured twice in a recumbent position after a rest of 5 (MDCS) or 10 (MPP) minutes using a standard mercury sphygmomanometer on the right arm, the average of these two values was recorded as the actual levels of BP. In the UK-biobank, two BP readings were taken with the participant seated, with 1-minute interval between readings. An Omron 7015 IT electronic BP monitor (OMRON Healthcare, Europe B.V. Kruisweg 577 2132 NA Hoofddorp) was used to take the readings.

Follow-up and outcome assessment

In the MDCS and MPP, participants were linked to the national cancer register, cause of death register and the total population register, through their civil registration number, unique to all inhabitants of Sweden. These registers identified cancer diagnoses, death and emigration, respectively. Follow-up for these linkages ended on 31 December 2016. In the UK-biobank, linkages to the UK national cancer registers and cause of death registers were used to identify cancer diagnoses and cause of death, respectively. Information on emigration was obtained from several sources, including the National Health Service. BC was defined according to the ninth edition of the International Classification of Diseases (ICD-9) code 188 [0–9], and ICD-10 code C67 [0–9], including carcinoma in situ (D090). TNM-classification based on histology, palpation and radiology reported to the Swedish National Register of Urinary BC (SNRUBC) was available in the Swedish cohorts. The SNRUBC became nationwide in 1997, and since then has covered on average 97% of BC cases as compared to the Swedish Cancer Register [23]. BC tumors are divided into two groups, based on depth of invasion: 1) Non-muscle invasive BC: Ta, Tis and T1, and 2) Muscle invasive BC: T2, T3, and T4. Death was defined as BC (ICD-10, C67) if recorded as the primary cause of death in the national cause of death registers.

Genotyping

In the MDCS cohort, a MALDI-TOF mass spectrometer (Sequenom MassArray, Sequenom, San Diego, CA, USA) was used to genotype DNA samples using Sequenom reagents and protocols. In the case where a candidate SNP failed the genotyping, a "proxy SNP" was used in its place. Proxy SNPs were identified using SNAP version 2.2.2 when commercial primers were not available. SNPs that failed Sequenom genotyping were alternatively genotyped individually using TaqMan, KASPar allelic discrimination on an ABI 7900HT (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), per manufacturer's instructions. In the MPP, blood samples were taken, on average, 25 years after study baseline, and was thus excluded from the MR analysis to avoid collider bias [24, 25]. In the UK-biobank, Affymetrix (ThermoFisher Scientifics) performed genotype calling on two closely related, but custom-designed arrays. Approximately 50,000 participants were ran on UK BiLEVE Axiom array and the remaining 450,000 were ran on UK-Biobank Axiom array. A detailed description of the genotype process and internal quality control is described elsewhere [21].

Mendelian randomization analysis-assumptions

In Mendelian randomization analysis, three key assumptions regarding the IV must be fulfilled. Firstly, it must be associated with the exposure of interest. Secondly, it must be associated with the outcome exclusively through the exposure of interest, and thirdly, it must not be associated with confounders in the exposure-outcome association. In this study, we addressed the first assumption by only using genetic variants that have shown an association with BP in genome-wide association studies (GWAS). Pleiotropy occurs when the IV affects the outcome through a different biological pathway from the exposure of interest. Inclusion of pleiotropic SNPs violates the second assumption, which may lead to biased causal estimates [26]. We investigated for pleiotropy using MR-Egger and MR-PRESSO. Lastly, we addressed the third assumption by investigating the association between the IV and confounders in the BP-BC association, due to the importance of smoking as a confounder, we additionally investigated for potential overlap of genetic variants between the IV and smoking using the most recent GWAS on smoking [27].

Selection of genetic variants for the systolic BP (SBP) and diastolic BP (DBP) genotype risk scores

Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation in humans. We used a genetic score of BP SNPs as IV in our MR analysis. In the MDCS cohort, a SBP instrument of 29 SNPs with established associations from two large consortia (International consortium of BP genome-wide association studies [ICBP] and the CHARGE consortium) of European ancestry was created [28–30]. Previous MR studies on BP in the MDCS based their IVs on these 29 SNPs [31–33] and a detailed description of the genotype process is reported therein. In the UK-biobank, we created a SBP instrument of 47 SNPs and a DBP instrument of 50 SNPs. The SNPs were obtained from the results provided by the ICBP and 14 other consortia. All SNPs were discovered in populations of European ancestry and outside the UK-biobank [28–30], the latter in order to avoid biased causal estimates towards the confounded observational association, due to the overlap that occurs between the sample that was used to discover the SNP, and the sample used in the MR analysis [16]. We initially found 67 SBP SNPs and 71 DBP SNPs that underwent a rigorous selection process to be included in the instruments; the details are documented in Supplementary information (S1 and S2 Files). In brief, we removed SNPs that were highly correlated (linkage disequilibrium [LD] $\geq$ 0.8), had low genotype rate (<95%), had low minor allele frequency ($\leq$1%), or were out of HWE (threshold calculated as 0.05/number of SNPs tested). Where necessary, a suitable proxy SNP (LD$\geq$0.8) was used for candidate SNPs not available in the UK-biobank. LDlink, a web-based interactive tool was used to find suitable proxy SNPs [34, 35]. The quality control was performed on PLINK v1.9 [36]. To avoid false-positive findings and winner's curse, all the included SNPs had been validated through an independent replication process.

NAT2 genotype

To investigate *NAT2* in interaction with BP and BC, we use the SNP"rs1495741 (A/G)". *NAT2* was genotyped in the same way as the BP SNPs per cohort. The polymorphism "A/A" represented fast acetylation, "A/G" represented intermediate acetylation and "G/G" represented slow acetylation (risk variant). In the analysis, we combined fast and intermediate acetylators to investigate *NAT2* polymorphism as a dichotomy.

Selection of study participants

The combination of MDCS and MPP resulted in 50,670 participants from which 27,107 were included in the final analysis (S1 Fig). The causes of exclusion were cohort overlap, female sex and missing data on SBP, DBP and smoking status. The UK-biobank overall contained 502,543 individuals. In order to mitigate the effects of population stratification, 92,909 individuals who were of Non-European ancestry were excluded from this study. This was achieved through a Principal Component Analysis conducted in all 502,543 participants[22] the causes of exclusion were female sex and missing data on SBP, DBP and smoking status, after which 188,167 participants were retained in the study. In our primary analysis, prevalent BC cases at the time of baseline examination were excluded (44 in the Malmö cohort and 514 in the UK-biobank). In an additional MR analysis, we included prevalent BC cases and women, respectively. The exclusion of women in the main analysis was due to very weak statistical power owing to only 182 incident BCs among women in the MDCS and 129 in the UK-biobank. Furthermore, findings from the largest prospective studies indicated no association among women [5, 7]. A description of the baseline characteristics among women is shown in the supplementary material (S1 Table).

Statistical analysis

In survival analysis of BP level and BC risk, participants were followed from the baseline examination until the date of event, or until censoring due to diagnosis of another cancer, emigration, or death, whichever one occurred first. The analysis of NMIBC and MIBC in the Swedish cohorts started on 1 January 1997, and censored participants before then were excluded. We used Cox proportional hazards regression to calculate hazard ratios (HR) for BC by SBP and DBP standard transformed (z-scores), per 10 mmHg, and in quartiles. Attained age was used as the underlying time variable, and we adjusted for smoking in five categories (never-smoker, ex-smoker, and tertiles of pack-years among current-smokers), BMI (quintiles), age at baseline (categories) and date of birth (categories). Models in the MDCS and MPP were tested for the additional inclusion of anti-hypertensive medication, physical activity and education; however, adding these co-variables to the model did not change the results, so for consistency with analyses in the UK-biobank, these variables were excluded from further analyses. We tested the proportional hazards assumption using Schoenfield residuals, and found that "age at baseline" and "date of birth" violated the PH assumption; however, inclusion of these variables in the stratum did not materially change the results, so the final models were left un-stratified. The Swedish cohorts combined and the UK-biobank were analyzed separately due to markedly different associations between BP and BC risk. In relation to these findings, we also performed an ad hoc Kaplan-Meier analysis to compare BC-specific survival in the two cohorts to detect any major differences in the proportion of MIBC (S2 Fig). With average length of follow-up of 22 years and 5 years in the Swedish cohorts and UK-biobank, respectively, the leading time between measurement of BP and BC diagnosis likely differed between these cohorts. We therefore calculated the average age at diagnosis among BC cases and performed a lag-time analysis to investigate potential reverse causation in the association between SBP and BC.

We used the quantity "relative excess risk of interaction" (RERI) as our measure of additive interaction between BP and *NAT2* in relation to BC risk, which was based on adjusted HRs. It was calculated by RR11—RR10—RR01 + 1, reflecting the individuals in the lower half of BP and fast/intermediate *NAT2* acetylation (1, reference group), upper half of BP and fast/intermediate *NAT2* acetylation (RR10), lower half of BP and slow *NAT2* acetylation (RR01), and upper half of BP and slow *NAT2* acetylation (RR11). Confidence intervals were obtained using the delta method by Hosmer and Lemeshow. In addition, we investigated multiplicative

interaction between BP and *NAT2* in relation to BC risk using the likelihood ratio test. For the interaction tests, BP and *NAT2* were assessed as categorical variables.

MR analysis can be performed in a one-sample setting, or in a two-sample setting. We first employed the one-sample, 2-stage least square (2SLS) method to estimate associations between genetic scores of the BP indices and BC risk. In the first stage, a weighted genetic score was created as follows: each SNP was coded 0, 1, 2 according to the number of BP-increasing alleles, then that value was weighted according to its effect estimate (β-coefficient) obtained from the aforementioned genome-wide association studies (GWAS), then the weighted value of each SNP were summed up (weighted score = $[\beta_1 \times SNP_1 + \beta_2 \times SNP_2 + \ldots \beta_n \times SNP_n]$/number of SNPs). Next, we regressed the weighted genetic score on the z-transformed BP levels (SBP or DBP). The predicted values, corresponding to the predicted z-transformed genetic level of SBP or DBP, were used as IV in MR analyses of BC risk. Additionally, we performed MR in a two-sample setting, with the added advantage of formally testing for pleiotropy. We used the inverse-variance weighted (IVW) estimation to investigate the association between BP and BC using two-sample MR analysis. It is obtained from the linear regression of the genetic associations with BC on the genetic associations with BP indices using inverse variance weights and the intercept restrained to zero in the model. To detect pleiotropy, we performed the MR-Egger test and MR-PRESSO. The MR-Egger estimate is similar to the IVW except that the intercept is left unrestrained. It provides accurate estimates even in the presence of an invalid instrument, but is limited by the InSIDE (Instrumental strength independent of direct effects) assumption and can only detect the direction of pleiotropy (cannot detect presence of pleiotropy in opposing direction) [17]. Pleiotropy is suggested if the Egger intercept is significantly different from zero. MR-PRESSO is a tool designed to evaluate horizontal pleiotropy in a two-sample setting. It has three components and the first component (MR-PRESSO global test) detects horizontal pleiotropy [37]. Additionally, we evaluated the influence of any potentially outlying SNPs in the MR-Egger estimates using a leave-one out analysis. The two-sample analyses were performed using the STATA package "mrrobust" [38] and R packages "TwoSampleMR" and "MR-PRESSO" [37]. We also investigated the associations between the IVs and potential confounders, and between the BP indices and potential confounders, by linear/logistic regression (S2 Table). Some IVs were associated with body mass index (BMI); however, the variance explained for BMI by the BP GSs was only 0.02–0.05%. Furthermore, we searched for other traits associated with the SNPs that may be linked with BC through other biological pathways. These analyses were performed on phenoscanner v2 [39], an online, publicly available database containing results from large-scale genetic associations in humans. In phenoscanner, genetic variants are cross-referenced for associations with a wide-range of other traits. All the statistical analyses were performed in STATA 13, (StataCorp LLC, College Station, TX) and RStudio version 1.1.423.

Results

There were 27,107 men in the Swedish cohorts and 188,167 men in the UK-biobank. Mean age at baseline was 58 years (SD = 8) amongst men in the UK-biobank and 50 years (SD = 11, Table 1) in the Swedish cohorts. Approximately 12% of men in the UK-biobank were current smokers at baseline, compared to 43% of men in the Swedish cohorts. On average, men in the UK-biobank had a SBP level of 143 mmHg (SD = 19) and a DBP level of 84 mmHg (SD = 11), and the corresponding in the Swedish cohorts were 135 mmHg (SD = 19) and 87 mmHg (SD = 10), respectively. Furthermore, 58% of the men in the UK-biobank had hypertensive BP levels (SBP/DBP $\geq$140/90) compared to 53% in the Swedish cohorts, and 26% of the men the UK-biobank were obese (BMI $\geq$30 kg/m^2) compared to only 10% in the Swedish cohorts.

Table 1. Baseline characteristics of the study participants included in the assessment of the risk of bladder cancer in relation to blood pressure.

Characteristic	MDCS and MPP (n = 27,107)	UK-biobank (n = 188,167)
Baseline year, range	1974–1996	2006–2010
Baseline age, years, mean (SD)	50.4 (10.7)	57.7 (8.1)
Category, n (%)		
<30	533 (2.0)	0 (0.0)
30–44	7,168 (26.4)	17,904 (9.5)
45–59	13,273 (49.0)	81,881 (43.5)
≥60	6,133 (22.6)	88,382 (47.0)
Smoking status, n (%)*		
Never smoker	8,024 (30.6)	91,735 (48.9)
Ex-smoker	7,010 (26.8)	73,528 (39.2)
Current smoker	11,172 (42.6)	22,230 (11.9)
Pack years among current smokers, n (%)*		
<10	1,611 (18.8)	2,305 (13.5)
10–19.9	925 (10.8)	3,312 (19.4)
≥20	6,043 (70.4)	11,470 (67.1)
Blood pressure, mm Hg, mean (SD)		
Systolic blood pressure	134.9 (19.1)	143.3 (18.5)
Diastolic blood pressure	86.7 (9.9)	84.2 (10.6)
Category, systolic/diastolic, n (%)		
<140/90 mm Hg	12,678 (46.8)	78,832 (41.9)
140/90-159/99 mm Hg	9,304 (34.3)	70,676 (37.6)
≥160/100 mm Hg	5,125 (18.9)	38,659 (20.5)
BMI, kg/m^2, mean (SD)†	25.4 (3.6)	27.9 (4.2)
<18.5	280 (1.0)	422 (0.2)
18.5–24.9	12,891 (47.6)	46,418 (24.8)
25–29.9	11,286 (41.6)	92,943 (49.6)
≥30	2,634 (9.8)	47,758 (25.6)
Mean follow-up time, years (SD)	22.2 (11.5)	4.8 (3.9)
Follow-up time, n (%)		
<5	2,192 (8.1)	53,878 (28.6)
5–9	2,224 (8.2)	134,289 (71.4)
10–14	2,668 (9.8)	0 (0.0)
≥15	20,023 (73.9)	0 (0.0)

* Smoking status was missing for 674 (0.4%) men in the UK-biobank and for 901 (3.3%) men in the MDCS and MPP combined. Includes accumulated pack-years among current smokers,
Excluding 2 593 (9.6%) and 5 143 (2.7%) current smokers with missing pack-years data in the MPP and MDC combined and UK-biobank respectively.

† BMI data were missing for 626 men in the UK-biobank and 16 men in MDCS and MPP combined.

Abbreviations: MDCS, Malmö Diet and Cancer Study; MPP, Malmö Preventive Program; BMI, body mass index.

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During a mean follow-up time of five years (SD = 4) in the UK-biobank, 502 incident BCs occurred, and during a mean follow-up time of 22 years (SD = 12) in the Swedish cohorts, 928 incident BCs occurred.

Table 2 shows the HRs for BC overall and separately for NMIBC and MIBC (in the Swedish cohorts only) by continuous z-scores, per 10 mmHg and in quartiles of SBP and DBP. SBP,

Table 2. Hazard ratio (95% confidence interval)* of bladder cancer outcomes by levels of systolic and diastolic blood pressure among men.

		MDCS & MPP (N = 27,107)			UK-biobank (N = 188,167)
		Muscle-invasive bladder cancer	Non-muscle invasive bladder cancer	Bladder cancer incidence	Bladder cancer incidence
Exposure	**Exposure level**	**(N_{cases} = 105)** †	**(N_{cases} = 425)** †	**(N_{cases} = 928)**	**(N_{cases} = 498)**
SBP, mm Hg	Per SD	**1.32 (1.09–1.59)**	1.06 (0.96–1.18)	**1.14 (1.05–1.22)**	0.93 (0.85–1.02)
	Per 10mm Hg	**1.14 (1.02–1.27)**	1.02 (0.96–1.08)	**1.05 (1.01–1.09)**	0.96 (0.92–1.01)
	Quartiles				
	Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Q2	1.08 (0.60–1.94)	1.16 (0.87–1.53)	**1.23 (1.01–1.49)**	1.04 (0.80–1.35)
	Q3	1.12 (0.65–1.92)	1.21 (0.91–1.62)	**1.36 (1.12–1.66)**	0.94 (0.73–1.22)
	Q4	1.82 (0.97–3.39)	1.17 (0.86–1.59)	**1.24 (1.00–1.52)**	0.86 (0.67–1.13)
DBP, mm Hg	Per SD	**1.27 (1.04–1.55)**	0.99 (0.89–1.10)	1.02 (0.95–1.09)	0.96 (0.91–1.01)
	Per 10mm Hg	**1.25 (1.03–1.53)**	0.99 (0.89–1.10)	1.02 (0.95–1.09)	0.98 (0.90–1.07)
	Quartiles				
	Q1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
	Q2	1.08 (0.60–1.94)	0.99 (0.74–1.32)	0.96 (0.78–1.17)	1.04 (0.82–1.32)
	Q3	1.12 (0.65–1.92)	1.16 (0.90–1.49)	1.16 (0.98–1.38)	1.09 (0.85–1.39)
	Q4	1.38 (0.81–2.33)	0.96 (0.73–1.26)	0.96 (0.80–1.16)	0.92 (0.71–1.20)

* Hazard ratios were calculated using Cox proportional hazards regression models with attained age as the underlying time scale, adjusted for smoking (categories), age at baseline (categories), date of birth (categories), and BMI (quintiles).

† Data on tumor staging was only available in the MDCS and MPP cohorts, it was obtained from the Swedish National Register of Urinary BC (SNRUBC), which originated in 1997. As a result all tumors that occurred before 1997, which were available for the analysis on total incidence, were not included in the analysis for NMIBC and MIBC.

Abbreviations: MDCS, Malmö diet and cancer study; MPP, Malmö preventive project; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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but not DBP, was positively associated with overall incidence of BC in the Swedish cohorts, the HR per SD (95% CI) was 1.14 (1.05–1.22). Furthermore, the association between SBP and BC risk overall, in the Swedish cohorts, was stronger for those in the second, third and fourth quartile compared to those in the first quartile. SBP and DBP were both positively associated with MIBC, the HRs per SD were 1.32 (1.09–1.59) and 1.27 (1.04–1.55), respectively. In the UK-biobank, SBP and DBP were not associated with BC risk.

There was no statistically significant additive interaction between BP and *NAT2* in relation to BC in the UK-biobank and MDCS when using RERI as the measure of interaction (Fig 1). Likewise, there was no statistically significant interaction on a multiplicative scale using the LR test; the p-value was 0.82 in the UK-biobank and 0.67 in the MDCS.

The associations between SBP and DBP with BC risk in the MDCS and UK-biobank, determined by 2SLS regression and IVW estimation, are shown in Fig 2. Genetically predicted elevation in SBP was associated with higher BC risk in the MDCS, the odds ratio (OR) (95%CI) per SD was 7.70 (1.92–30.9) for the 2SLS and 3.43 (1.12–10.5) for IVW. Similar to measured BP levels, there were no associations between genetically predicted SBP and DBP levels and BC risk in the UK-biobank. S3–S5 Figs of MR-Egger estimates for SBP and DBP in relation to BC risk showed that the intercept did not significantly differ from zero in any of the analysis assessing for pleiotropy. This was further supported by no evidence of horizontal pleiotropy and outlying SNPs in the MR-PRESSO and leave-one out analysis respectively (S6–S8 Figs). The MR-PRESSO global test had p-values of 0.65, 0.16 and 0.37 for systolic BP in the MDCS, and systolic and diastolic BP in the UK-biobank, respectively. When including prevalent BC

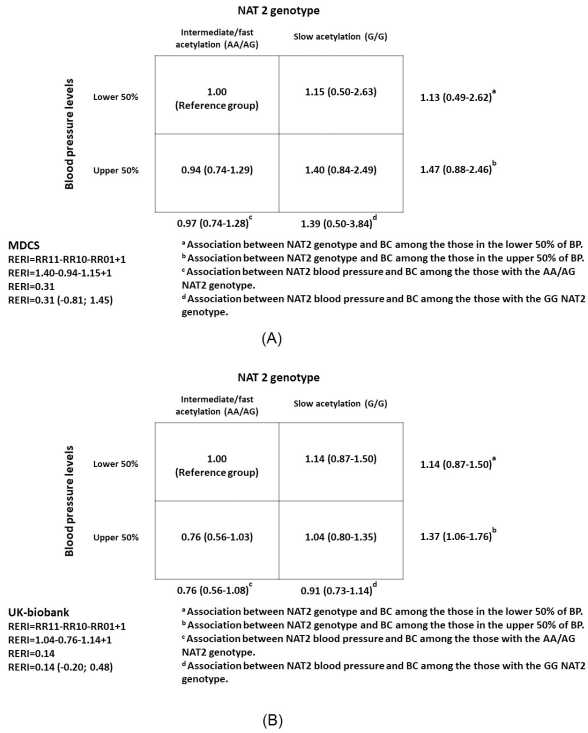

Fig 1. Additive interaction between blood pressure and *NAT2* in relation to bladder cancer risk in the (A) Malmö Diet and Cancer Study (MDCS; $N_{participants}$ = 7 749; N_{cases} = 282) and (B) UK-biobank ($N_{participants}$ = 187 688; N_{cases} = 498).

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cases (S3 Table) or women (S4 Table) in the MR analysis, the associations tended to be weaker, although confidence intervals for these results largely overlapped the results for incident BC among men only.

Further investigation followed to understand potential explanations for the different findings between the Swedish cohorts and the UK-biobank. The average age at BC diagnosis was 76 years for the Swedish cohorts and 66 years for the UK-biobank, which could possibly translate to BCs of different tumor characteristics. However, survival curves of incident BC cases in the UK-biobank and the MDCS were similar (p-value for the log-rank test = 0.092) and thus, did not provide a clear explanation for the different findings between the cohorts (S2 Fig). The HRs per SD (95% CI), in the lag-time analysis for SBP and BC risk in the UK-biobank were closer to 1 than the original: 0.97 (0.87–1.09) and 1.00 (0.84–1.19) for 3 and 5 years respectively. Relatively few cases were omitted for the respective analysis in the Swedish cohorts (1.3% for 3 years and 5.6% for 5 years), resulting in no material change in HRs.

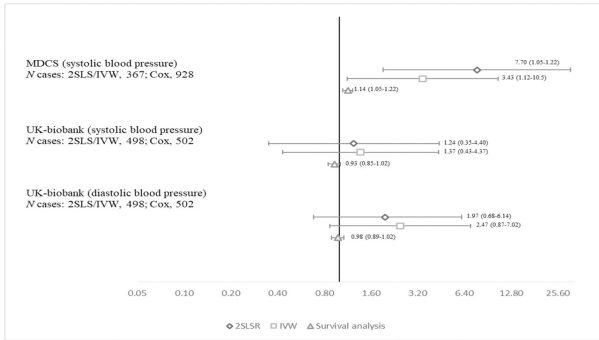

Fig 2. Relative risk (95% confidence interval) of bladder cancer per standard deviation of systolic and diastolic blood pressure using Mendelian randomization two stage least square regression (2SLSR) regression and inverse variance weighted (IVW) method, and Cox regression*, in the Malmö Diet and Cancer Study (MDCS) and UK-biobank. *Also includes the Malmö Preventive Project.

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Discussion

In this study, we investigated the association between SBP, DBP and BC risk among men in cohorts in Sweden and the UK-biobank, using conventional and MR analysis. In conventional survival analysis, we found that SBP was positively associated with BC risk overall in the Swedish cohorts, but not in the UK-biobank, and both SBP and DBP were positively associated with MIBC, but not NMIBC, which was investigated in the Swedish cohorts only. We further observed a positive association between SBP and BC risk by MR analysis of men in the MDCS, but not in the UK-biobank. Additionally, we investigated additive and multiplicative interaction between BP and *NAT2* (rs1495741) in relation with BC risk, but did not find any support for such interaction.

The different findings between the cohorts may have several explanations. Participant characteristics of the cohorts differed at large, both with regards to blood pressure levels, BMI and smoking, which altogether might limit the capacity of applying external validity between the two cohorts. Secondly, low participation rate remains a concern in the MDCS, where the participation was 41% [40], but even more so in the UK-biobank, which is known as a very selective population with a participation rate of only 5% [25]. Furthermore, the difference in the average age at diagnosis between the cohorts may suggest a difference in the type of BC occurring. Although survival analysis of BC cases did not indicate major differences in disease aggressiveness between the cohorts, different etiology of BC and the relative importance of risk factors such as BP in younger vs. older age could in part contribute to the different findings. Lastly, the lag-time analysis for 3 and 5 years respectively in the UK-biobank slightly changed HRs, potentially suggesting the influence of reverse causation.

The null association between BP and NMIBC risk and a positive association between BP and MIBC risk in the Swedish cohorts, may suggest that the positive association between BP and BC risk overall in conventional and MR analysis of the Swedish cohorts are largely driven by MIBC tumors. This is further supported by a somewhat weaker association between BP and BC risk in the MR analysis that included prevalent cases, which inherently comprise more indolent BC's. However, the positive association between SBP and BC observed in the MR analysis of the MDCS must be interpreted with caution. On one hand, the result is consistent

with findings from the conventional analysis in this and our previous, larger study [5], and in some other previous observational studies [6–8]. However, the association may also be driven by low study power and pleiotropy. In our study, the MR-Egger test, MR-PRESSO and leave-one out analysis did not indicate pleiotropy, which may be a true reflection, but may also be a result of insufficient statistical power. The use of a stronger IV to predict BP would have been desirable for increased statistical power; however, in the largest BP GWAS to date of 535 loci associated with BP, 325 SNPs were discovered in the UK-biobank. Including SNPs discovered in the UK-biobank would led to sample overlap, which is strongly discouraged in a two-sample analysis due to the high risk of obtaining biased estimates [16, 41]. Furthermore, the 210 remaining SNPs had not been validated, increasing the potential for false positive findings, if included. To validate our findings in the MDCS, further studies are needed based on stronger IVs and a larger number of validated BC cases, ideally separated by muscle invasiveness.

A potential biological mechanism linking BP to BC remains unclear. Studies from experimental biology on human BC cells have suggested that the angiotensin-renin pathway may play a role in BC carcinogenesis [3, 4]. From these studies, it is suggested that the angiotensin-renin pathway might play a role in BC progression, which would support an association between BP and BC driven by MIBC. However, these findings need to be replicated and validated in other population studies.

Despite the use of large cohorts, statistical power was the main weakness of this study. The study was large enough to examine main associations between BP and BC risk in the conventional analysis, but interaction analysis requires more power, which may explain the null interaction observed between BP and *NAT2*. With sufficient power, we expected to see interaction either on an additive or multiplicative scale or both since *NAT2*, through smoking, is a known risk factor for BC, and BP is a potentially independent risk factor for BC. Likewise, limited statistical power in the MR analysis did not allow us to detect effect estimates nearly as low as the estimates in the conventional survival analyses. This would have been counteracted by a meta-analysis of the results from the MDCS and the UK-biobank, which, however, we considered inappropriate given the different findings between the cohorts. The main strengths of the study were the large sample size for the observational analysis, the detailed smoking data, and the investigation of three separate cohorts, which allowed us to investigate the reliability of our results from one cohort on the other.

In conclusion, in this study of BP and BC risk among men, SBP was positively associated with BC risk in both conventional and MR analysis of Swedish cohorts, but not in the UK-biobank. However, the population characteristics differed at large between the cohorts. There was no evidence to support interaction between BP and *NAT2* in relation with BC. The heterogeneous results between the cohorts and low study power in some of the analyses calls for more epidemiological studies in the field.

Supporting information

S1 File. Systolic blood pressure SNP selection.
(XLSX)

S2 File. Diastolic blood pressure SNP selection.
(XLSX)

S1 Fig. Selection of participants in the Malmö Diet and Cancer Study (MDCS), Malmö Preventive Project (MPP) and UK-biobank.
(TIF)

S2 Fig. Kaplan Meier curves for bladder cancer-specific survival among incident bladder cancer cases since time of diagnosis by study population.
(TIF)

S3 Fig. MR-Egger plots for the (a) inverse variance-weighted (IVW) estimate and (b) MR-Egger estimate for systolic blood pressure, with bladder cancer as the end-point in the Malmö Diet and Cancer Study.
(TIF)

S4 Fig. MR-Egger plots for the (a) inverse variance-weighted (IVW) estimate and (b) MR-Egger estimate for systolic blood pressure, with bladder cancer as the end-point in the UK-biobank.
(TIF)

S5 Fig. MR-Egger plots for the (a) inverse variance-weighted (IVW) estimate and (b) MR-Egger estimate for diastolic blood pressure, with bladder cancer as the end-point in the UK-biobank.
(TIF)

S6 Fig. Leave–one out analysis of 29 systolic blood pressure single nucleotide polymorphisms (SNPs) in the Malmö Diet and Cancer Study.
(TIF)

S7 Fig. Leave–one out analysis of 47 systolic blood pressure single nucleotide polymorphisms (SNPs) in the UK–Biobank.
(TIF)

S8 Fig. Leave–one out analysis of 50 diastolic blood pressure single nucleotide polymorphisms (SNPs) in the UK–Biobank.
(TIF)

S1 Table. Baseline characteristics women in the Swedish cohorts and the UK-biobank.
(PDF)

S2 Table. Association between per standard deviation of measured and instrumental variables of systolic and diastolic blood pressure, and potential confounders in the relationship between blood pressure and bladder cancer risk. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; IV, instrumental variable; MDCS, Malmö diet and Cancer Study; OR, odds ratio; BMI, body mass index. [a] For age at baseline, date of birth, BMI, smoking, and education, we used linear regressions to investigate the association with blood pressure indices and their respective genetic scores. For physical activity and antihypertensive medication, we used logistic regression.
(PDF)

S3 Table. Odds ratio (95% confidence interval) from Mendelian randomization analysis of incident bladder cancer, and incident and prevalent bladder cancers combined, for systolic and diastolic blood pressure in the Malmö Diet and Cancer Study and UK-biobank.
(PDF)

S4 Table. Two stage least square regression and inverse variance weighted method for systolic and diastolic blood pressure in relation to bladder cancer incidence for men and women combined in the Malmö Diet and Cancer Study and UK-biobank. Abbreviations: MDCS, Malmö Diet and Cancer Study; OR, odd ratio; CI, confidence intervals; BP, blood pressure; 2SLS, two-stage least square regression; IVW, inverse-variance weighted. [a] R^2 is the

proportion of BP variance that is explained the genetic score.
(PDF)

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Paper IV

Interaction between blood pressure and genetic score for bladder cancer, and risk of urothelial carcinoma

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Abstract

Background

The genetic predisposition to bladder cancer (BC) accounts for 31% of the cases, however the risk of BC from BC genetic risk score (GRS) has only been investigated with a few genetic variants. Blood pressure (BP) has been positively associated with BC risk in men, but the potential interaction with a GRS for BC is unknown.

Methods

We investigated 10,795 men, with 385 incident urothelial cancers (UCs) during follow-up. Cox regression was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) to investigate main associations between weighted GRS (wGRS), constructed from 18 BC genetic variants, BP and risk. We used the relative excess risk for interaction (RERI) to investigate additive interaction and the likelihood ratio test to investigate multiplicative interaction.

Results

Systolic BP (SBP) was positively associated with aggressive UC (HR per SD, 1.27 [1.07-1.50]). The wGRS was positively associated with UC overall and non-aggressive UC (HR per SD, 1.26 [95% CI, 1.14-1.40], and 1.26 [1.14-1.40] respectively). There was evidence of additive interaction between SBP and wGRS in relation to aggressive UC risk (HR, 1.70 [95%CI, 1.02-2.84], RERI = 0.85 [95%CI, 0.17; 145]).

Conclusions

Our findings support an association between SBP and aggressive UC, between wGRS and UC overall and non-aggressive UC, and a potential additive interaction between wGRS and SBP in relation to aggressive UC. The findings on interaction may provide biological insight, but require replication in larger studies.

Introduction

Urothelial carcinoma (UC) is a cancer that originates from the mucosal surfaces (termed "urothelium") of renal collecting tubules, calyces, pelvis, ureters, urethra and the bladder. Urothelial bladder cancer (BC) has by far the highest frequency of occurrence, comprising between 90-95% of all UC[1]. BC is a heterogeneous disease with known genetic and environmental risk factors[2, 3]. With regards to the genetic predisposition, 31% of BCs can be attributed to genetic variation, and previous studies have reported a 2-fold increased risk among first degree relatives with BC[4-6]. While rare germline mutations with strong effect on disease risk, such as the DNA-mismatch repair protein 2 (*MSH2*) mutation in Lynch syndrome have been found[7], the genetic mechanisms behind a majority of BC is assumed to be polygenic, whereby individual genetic variants each have a small effect on disease risk[8]. Single nucleotide polymorphism (SNP) is the most common type of genetic variation in humans, and at least 28 SNPs related to BC have been discovered, most through genome-wide association studies (GWAS) [7, 9]. A weighted genetic risk score (wGRS) is the sum total of weighted genotypes on a trait or disease of interest[10]. In a polygenic disease, where a single variant may not be informative, a genetic risk score can be generated to sufficiently identify those at high risk[11]. Associations between GRS for BC and BC risk have been investigated, however, the GRS comprised only a few genetic variants[12, 13].

The association between blood pressure (BP) and cancer is an area of investigation that has received increased attention in recent times. The most consistent evidence linking BP to a site–specific cancer is for renal cell carcinoma[14]. With respect to BC, evidence from the largest prospective studies report a positive association only among men, and a stronger association with muscle invasive BC (MIBC) [15-17].

Gene-environment interaction may provide insight into biological mechanisms of a disease, and can be assessed on an additive and multiplicative scale[18]. BC, being a complex disease, is an ideal setting to investigate the complex interplay between genetic and environmental risk factors[3]. The most established gene-environment interaction in relation to BC include smoking and N-acetyltransferase 2 (*NAT2*), and smoking and glutathione S-transferase-mu 1 (*GSTM1*)[6, 8, 19]. Other environmental risk factors investigated in such interactions include occupational carcinogens and caffeine[2, 20-22]. Potential interaction between BP and genetic variants related to BC in relation to UC has not been investigated. Herein, we investigated a

bladder cancer wGRS, BP, and their interaction, in relation to UC risk overall and separately for aggressive and non-aggressive tumors in men.

Methods

Study population

This study included participants from the Malmö Diet and Cancer Study, a population-based prospective cohort study from Malmö, a city in southern Sweden. The cohort included 30,447 men and women aged between 45-73 years, who underwent a baseline health examination between 1991 and 1996. A full description of the cohort is published elsewhere[23].

Exposure assessment

A standard mercury sphygmomanometer placed on the right arm was used to obtain the BP levels. BP was taken twice, in a supine position with a rest of 5 minutes between the readings. The average value between the two readings was then reported as the actual BP level. To obtain BMI, height and weight were taken with no shoes and only with light indoor clothing. Information on smoking habits, physical activity during leisure time, and highest level of attained education was obtained from a questionnaire asked at baseline health examination[23].

Selection of SNPs and genotyping

Genetic variants associated with BC were identified from published genome-wide association studies, which extend from 2008 to 2017[7, 24]. SNPs included in this study were discovered and validated in a population of European ancestry, SNPs discovered through other study designs/methods, and from populations of other ancestries were not included. Genotyping for the study participants was performed using the Illumina GSA v1 genotyping array. An internal quality control check excluded samples with a low call rate (<90%), SNPs that were out of Hardy-Weinberg equilibrium, and those that exhibited discordance between reported and genetically inferred sex[25]. The Haplotype Reference Consortium, a large reference panel of human haplotypes was used to perform the genotype imputation[26].

To generate the wGRS (of 18 SNPs), the genotype dosage for each SNP (coded as 0, 1and 2 for each risk increasing allele) was multiplied by its respective weight (beta-coefficient from the association of each SNP with BC) obtained from GWAS of BC, followed by summation across all the variants according to the following equation (wGRS for each individual = [$\beta_1 \times SNP_1 + \beta_2 \times SNP_2 + \ldots \beta_x \times SNP_x$]/number of SNPs). For GWAS of BC that expressed the

association between SNP and BC as odds ratios, the natural log (ln) was used to convert the odds ratio to beta-coefficient.

Follow-up and end-point assessment

Any diagnosis of cancer, cause of death and emigration status were identified through linkage of each study participants' unique civil registration number with the National Cancer Register, Cause of Death Register, and Population Register respectively. Follow-up of these linkages ended on 31 December 2018. UC was defined according to the tenth edition of the International Classification of Diseases (ICD-10) code C64-68 [0–9], including carcinoma in situ (D09 [0-1]). Specimens taken from the UC cases underwent histopathological evaluation. The pathological stage of the primary tumor (pT) was based on the TNM classification. Pathological staging acquires its evidence from surgery and histopathological evaluation, it is more accurate that the clinical staging (evidence obtained at initial evaluation- physical examination, endoscopy [and biopsy], radiology and other relevant diagnostics) since it requires a complete evaluation of the entire bladder wall to accurately assess the highest possible T stage[27]. We classified tumor aggressiveness based on whether the tumor invaded the muscularis layer and UC-specific mortality. Non-aggressive tumors included non-muscle invasive (Ta, Tis, and T1) tumors and aggressive tumors included muscle-invasive (T2-T4) tumors and metastatic tumors (distal [M1] and/or lymph node spread [from N1]). We initially considered to define tumor aggressiveness by including Tis and T1 (including any grade 3, according to WHO [1999]) tumors in the "aggressive" group; however, we opted for muscle-invasiveness as the base for classification because these groups showed a greater difference in association with BP and wGRS (hazard ratios) and with UC-specific mortality plotted with Kaplan-Meier curves. Because non-muscle invasive tumors is a heterogeneous group of tumors of which some will be lethal, we also included UC-specific mortality in the aggressive group defined as UC recorded as the underlying cause of death in Sweden's national cause of death registry within 10 years after diagnosis.

Selection criteria

From a study population of 30,440 participants, 10,752 men were included in the final analysis **(Figure 1)**. Female sex was the main cause of exclusion (n=18,323). The reasons for excluding women in the analysis were a sex-interaction with SBP (p-value=0.04 for aggressive UC) and no association between BP and BC risk in women in the largest prospective studies, and low statistical power (177 incident UC cases) for a separate analysis

of women. After follow-up and histopathological re-evaluation of tumors, we identified 10,752 men with incident UC (385 bladder cancers), out of which 129 were categorized as aggressive and 246 as non-aggressive (10 UC had missing tumor data).

Statistical analysis

We calculated hazard ratios (HRs) and their 95% confidence intervals (95%CI) using Cox proportional hazards regression to investigate the association between BP (SBP and DBP separately), wGRS and UC risk (overall, and separately for aggressive and non-aggressive tumors). Age was used as the underlying time metric and participants were followed from the date of baseline health examination until date of UC diagnosis, or until censoring due to migration or death, whichever one came first. The actual levels of SBP, DBP and wGRS were transformed to z-scores calculated as $z = (x - u)/\sigma$, where x is the actual level, u the mean, and σ the SD. Additionally, we investigated the associations based on categories for each exposure (SBP [<140, 140-149, 150-159, ≥160 mmHg], DBP [<90, 90-94, 95-99, ≥100 mmHg], wGRS [quartiles]). Models were adjusted for smoking in 5 categories (never-smokers, ex-smokers and tertiles of current smokers [tertile based on pack years]), BMI (quartiles), physical activity (tertiles), and level of education (8 categories). The p-value for trend across categories was investigated by incorporating the categories of SBP, DBP and wGRS as a continuous variable in the regression model and testing its coefficient using the Wald test. We tested for the Cox proportional hazards assumption using Schoenfield residuals, which showed no violation of the proportional hazards assumption.

To investigate additive interaction between BP and wGRS in relation to UC, we tested whether the joint effect of BP and wGRS was larger than the sum of individual effects of BP and wGRS, as illustrated by **Figure 2**. This was achieved by using the quantity "relative excess risk of interaction" (RERI) expressed as RR11—RR10—RR01 + 1, where: RR_{00} (or 1, reference group) represented individuals with normal BP (<140/90) and lower 50% of the BC genetic risk; RR_{10} represented those with hypertension (≥140/90) and lower 50% of BC genetic risk: RR_{01} represented those with normal BP and upper 50% of BC genetic risk; and RR_{11} representing those with hypertension and upper 50% of BC genetic risk. The confidence intervals for the additive interaction were obtained using the delta method by Hosmer and Lemeshow[28]. To investigate the corresponding multiplicative interaction, we used the likelihood ratio test whereby the restricted model (without the product term) was nested in the model that additionally included the product term.

All statistical analyses was performed in STATA 16 (StataCorp LLC, College Station, TX), and regarded *p* values <0.05 as statistically significant.

Results

The participants were on average 59.0 (SD=7.0) years old at baseline and were followed for on average 20.0 (SD=6.9) years. **Table 1** shows the characteristics of the participants separated by case status. Cases were more often current smokers compared with non-cases (41% for cases, 29% for non-cases).

The associations between SBP, DBP, wGRS and UC outcomes are shown in **Table 2**. SBP was positively associated with aggressive UC risk (HR per SD, 1.27 [95% CI, 1.07-1.50]), but not with UC (overall) and non-aggressive UC risk. There was a step-wise increased risk of overall and non-aggressive UC by increasing quartile level of the wGRS (p-trend <0.001), and those in the fourth quartile of the wGRS had a significantly higher risk for UC (overall) (HR per SD, 1.65 [95% CI, 1.24-2.19]) and non-aggressive UC (HR per SD, 2.06 [95% CI, 1.43-2.96]) compared to those in the first quartile. The association per SD wGRS were 1.26 (95% CI, 1.14-1.40) for UC overall, 1.26 (95% CI, 1.14-1.40) for non-aggressive UC, and 1.19 (95% CI, 1.00-1.41) for aggressive UC. There was no association between DBP and risk of UC outcomes.

Figure 3 show HRs and additive and multiplicative interactions for combinations of SBP and DBP with wGRS, with respect to UC outcomes. In relation to total and aggressive UC, high SBP (≥140 mm Hg) combined with high wGRS composed the highest risk, HR per SD 1.54 (95%CI, 1.13-2.09) and 1.70 (95%CI, 1.02-2.84) respectively, compared to the low SBP-low wGRS group. There was a positive additive interaction between SBP and wGRS in relation to aggressive UC risk (RERI, 0.85 [95% CI, 0.16; 1.65], p=0.028), but not in relation to UC overall and non-aggressive UC risk, and not for multiplicative interaction. To assess the robustness in our findings for aggressive UC, we repeated the analysis using SBP 130 and 150 mm Hg as cut-points. The HR for high SBP-high wGRS was 1.34 (95% CI, 0.72-2.51) using the 130 mm Hg cut-point, and the RERI was 0.81 (95% CI, 0.17; 1.45), p=0.013). Using the 150 mm Hg cut-point, the corresponding HR was 1.91 (95% CI, 1.17-3.10) and the RERI was 0.48 (95% CI, -0.43; 1.39), p=0.304. There was no significant interaction (additive or multiplicative) between DBP and wGRS in relation to UC outcomes.

Discussion

In this prospective study of nearly 11,000 men and 400 UC cases, we found a positive association between SBP and aggressive UC risk, and between a wGRS and UC overall and non-aggressive disease. Additionally, we found a positive additive interaction between SBP and wGRS in relation to aggressive UC, suggesting that the joint risk increase by high SBP and wGRS is greater than the sum of their individually contributing risks.

The association between SBP and aggressive UC among men is consistent with findings from previous studies based on muscle invasiveness (NMIBC and MIBC) [15-17, 29]. The association between SBP and aggressive UC and not with non-aggressive cancer might suggest that SBP may play a role in cancer progression as opposed to cancer initiation. However, the reasons to why the association is only among men, only for SBP and not DBP, and whether this association is causal, remains unclear. In a previous much larger study of men in Sweden, we found a positive association between SBP and MIBC among never-smokers, in which any residual confounding by smoking, the main potential confounder in the association, should be minimal. However, potential biological mechanisms linking SBP and BC, and more especially aggressive BC, has not yet been elucidated. Studies for experimental science have speculated that, the renin-angiotensin system may play a role in carcinogenesis[30, 31].

Prospective cohort studies have shown that GRSs can contribute to the risk of developing disease, thus, the consistent association between the wGRS and BC with previous studies was not surprising[12, 13]. However, in previous studies, the wGRS was constructed from a fewer number of SNPs and the association was investigated with total BC, which combined both aggressive and non-aggressive tumors[12, 13]. We further investigated the association separately for non-aggressive and aggressive tumors, where we found an association for non-aggressive UC, but not aggressive UC, which, however, had lower statistical power.

We found an additive interaction between SBP and wGRS in relation to aggressive UC, with a risk increase of 85% among men with high SBP and high wGRS. This interaction suggests that genetics and SBP may have a stronger joint effect than the sum of each risk factor individually in relation to aggressive UC. Furthermore, the excess risk that is due to the interaction between genetics and SBP, suggests that they share common pathways that lead to aggressive UC[32]. This result should however, be interpreted with caution, as the interaction may not persist with different cut-off points for the risk factors and in larger samples. We previously found an association between SBP and BC that changed at lower levels than 140

mm Hg, which we used as the primary cut-point in the present study[29]. When applying a cut point for SBP at 130 mm Hg, the additive interaction with wGRS persisted, whilst it did not when applying a cut point at 150 mm Hg. Interaction on the additive scale is rarely studied in epidemiological studies, yet it is widely regarded to be a reflection on an underlying biological interaction[32, 33]. While the biological insight provided by additive interaction may still be in question, its importance in public health is consensus, since it helps to identify the sub-group which is at most risk or will benefit most from an intervention[33]. Studies on gene-environmental interaction in relation to UC are common[4, 8, 20, 34], however, to our knowledge, there are no prior studies on the interaction between SBP and genetics.

The main strengths of the study were the long and complete follow-up of the cohort, and use of pathologically-verified tumor data. Furthermore, the wGRS incorporated most SNPs discovered in GWAS of European ancestry to date. A main limitation of the study is statistical power which was reflected in the fewer number of cases in some of the sub-groups. Furthermore, although we had data on antihypertensive medication, which we consider to be an effect modifier or potentially a mediator in the relationship between BP and UC, we were unable to investigate associations separately by antihypertensive intake due to limited numbers in the antihypertensive user group.

In conclusion, our findings support an association between SBP and aggressive UC, between wGRS and UC (overall) and non-aggressive, and additive interaction between SBP and wGRS in relation to aggressive UC. The findings on interaction may provide biological insight, but the findings have to be replicated in larger studies.

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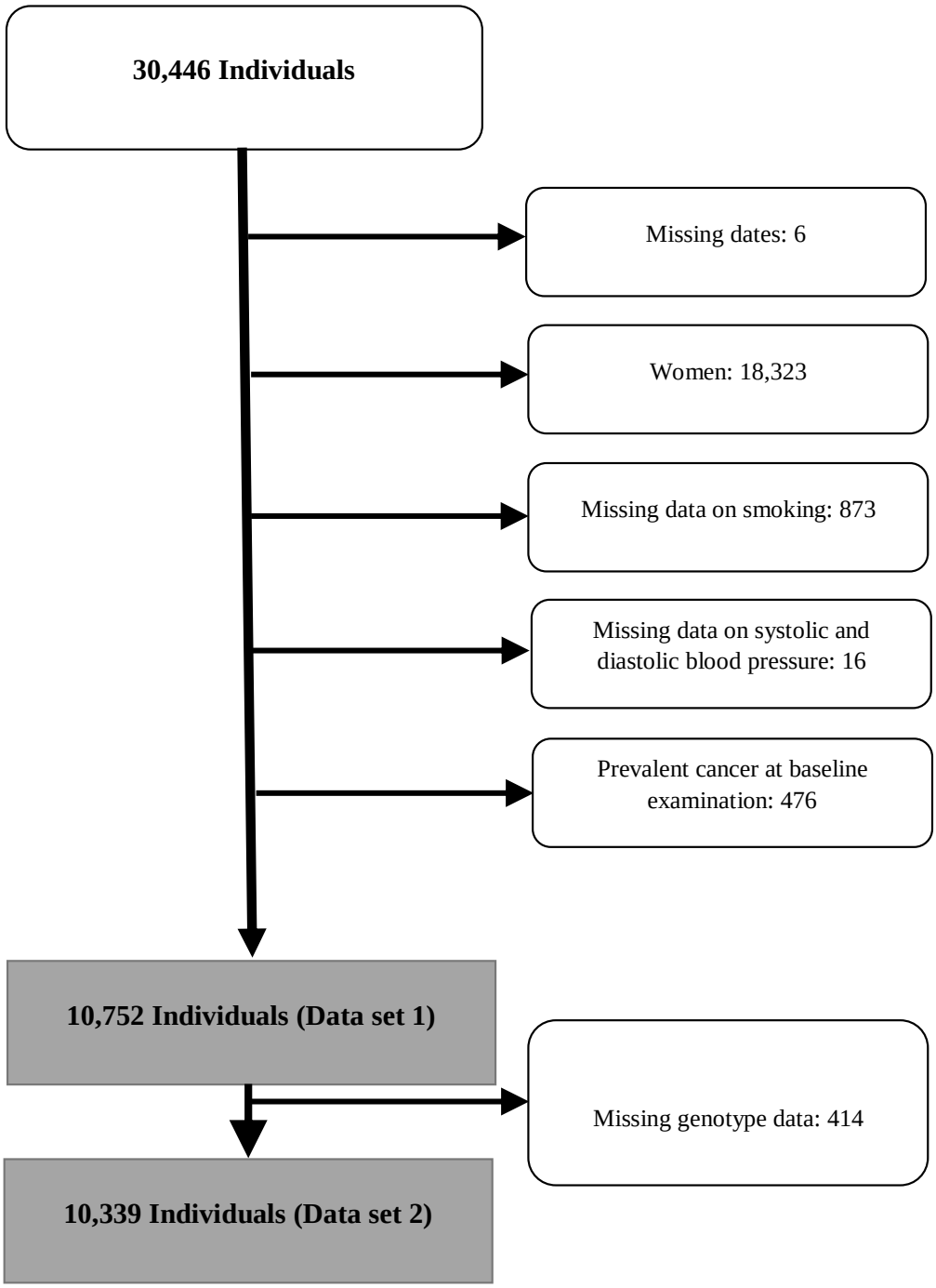


Figure 1: Flow chart showing selection of the study participants. Data set 1 was used for associations between the blood pressure indices and BC outcomes. Data set 2 was used for association between the weighted genetic risk score for bladder cancer and bladder cancer outcomes and the interaction analysis.

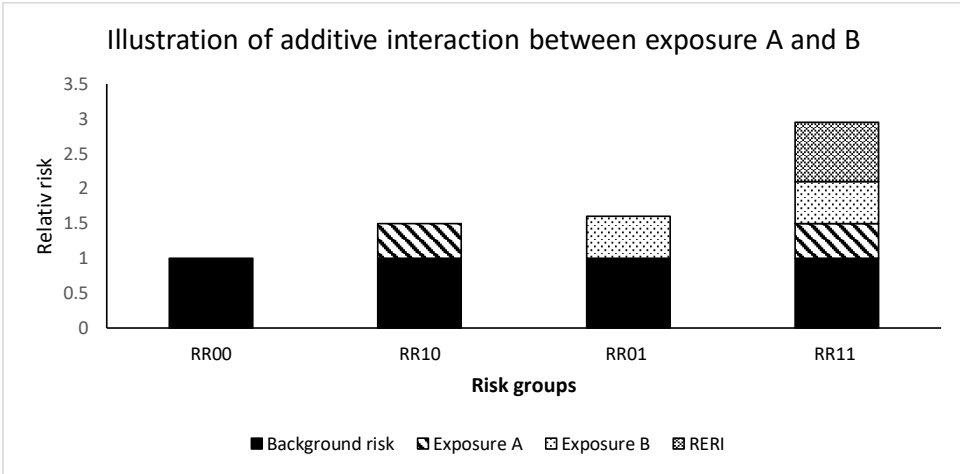
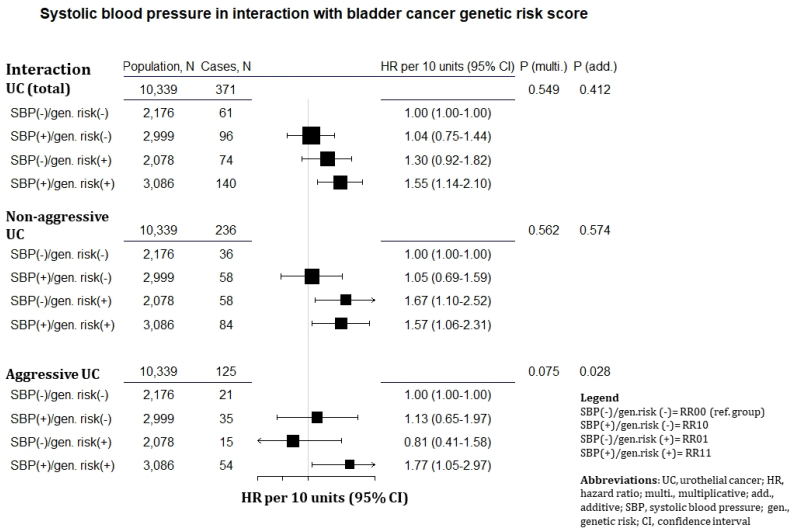


Figure 2: An illustration of additive interaction between Exposures A and B. RR_{00} represents the relative risk among those not exposed to A and B (also known as the background risk), RR_{10} represents the relative risk among those exposed to A only, RR01 represents the relative risk among those exposed to B only, RR11 represents the relative risk when exposed to both A and B, RR11 may additionally contain the relative excess risk of interaction (RERI), which is the excess risk that only occurs when exposure A interacts with exposure B additively.

A)



B)

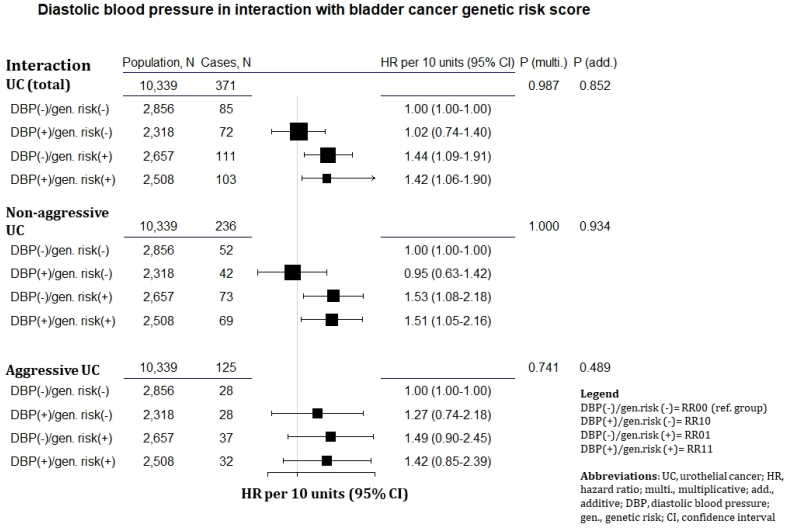


Figure 3: Hazard ratios (95% confidence interval) by groups of: **A)** systolic blood pressure (SBP); and **B)** diastolic blood pressure (DBP), and bladder cancer genetic score, including their multiplicative and additive interaction p-values, in relation to risk of urothelial cancer outcomes in the Malmö Diet and Cancer Study (MDCS; $N_{participants}$=10,339; N_{cases}= 371). Hazard ratios were calculated by Cox regression with attained age as the underlying time scale, with adjustment for smoking, BMI, physical activity and level of education. The Relative excess risk for interaction (RERI) was calculated as RR_{11}-RR_{10}-RR_{01}+1, where: RR_{00} (or 1, reference group) represented individuals with normal SBP/DBP (<140/90) and lower 50% of the BC genetic risk; RR_{10} represented those with high SBP/DBP (≥140/90) and lower 50% of BC genetic risk: RR_{01} represented those with normal SBP and upper 50% of BC genetic risk; and RR_{11} representing those with high SBP and upper 50% of BC genetic risk. The confidence intervals for RERI were obtained using the delta method, the p-value for additive interaction (p-value [add.]) was obtained from the RERI model. Multiplicative interaction was calculated using the likelihood ratio test (LR test). P-value (multi.) is the p-value for the multiplicative interaction obtained from the LR test.

Table 1: Characteristics of the 10,795 men in the Malmö Diet and Cancer Study

Characteristic	Cases	Non-cases	Total
Population, n	385	10,367	10,752
Age at baseline, mean (SD)	60.4 (6.3)	58.9 (7.0)	59.0 (7.0)
Categories, n (%)			
<50	22 (5.7)	1,116 (10.8)	1,138 (10.6)
50-54	68 (17.7)	2,459 (23.7)	2,527 (23.5)
55-59	78 (20.3)	2,146 (20.7)	2,224 (20.7)
≥60	217 (56.3)	4,646 (44.8)	4,863 (45.2)
Smoking status, n (%)			
Never-smokers	50 (13.0)	2,976 (28.7)	3,026 (28.1)
Ex-smokers	179 (46.5)	4,429 (42.7)	4,608 (42.9)
Current smokers	156 (40.5)	2,962 (28.6)	3,118 (29.0)
Pack years among current smokers, n (%)			
<10	22 (14.1)	501 (16.9)	523 (16.8)
10-19	24 (15.4)	353 (11.9)	377 (12.1)
≥20	110 (70.5)	2,108 (71.2)	2,218 (71.1)
Blood pressure, mm Hg, mean (SD)			
Systolic blood pressure	146 (19.7)	143.8 (19.2)	143.8 (19.3)
Diastolic blood pressure	88.0 (9.6)	88.0 (9.9)	88.0 (9.9)
Categories, systolic/diastolic, n (%)			
<140/90 mm Hg	113 (29.4)	3,618 (34.9)	3,731 (34.7)
140/90-159/99 mm Hg	161 (41.8)	3,907 (37.7)	4,068 (37.8)
≥160/100 mm Hg	111 (28.8)	2,842 (27.4)	2,953 (27.5)
Antihypertensive medication use			
Yes	79 (20.5)	2,117 (20.4)	2,196 (20.4)
No	306 (79.5)	8,250 (79.6)	8,556 (79.6)
Type of Antihypertensive medication among users, n*			
Diuretics	21	502	523
Beta blockers	45	1,224	1,269
ACE inhibitors	11	488	499
Calcium channel blockers	35	691	726
BMI, kg/m^2, mean (SD)^	26.7 (3.6)	26.3 (3.5)	26.3 (3.5)
BMI, categories, n (%)			
>18.5	0 (0.0)	57 (0.5)	57 (0.5)
18.5-24.9	127 (33.0)	3,836 (37.1)	3,963 (36.9)
25-29.9	195 (50.6)	5,121(49.5)	5,316 (49.5)
≥30	63 (16.4)	1,341(13.0)	1,404 (13.1)
Mean follow-up time, years (SD)	13.9 (6.9)	20.2 (6.8)	20.0 (6.9)
Follow-up time, n (%)			
<5	52 (13.5)	456 (4.4)	508 (4.7)
5-9	68 (17.7)	746 (7.2)	814 (7.6)
10-14	80 (20.8)	1,100 (10.6)	1,180 (11.0)
≥15	185 (48.0)	8,065 (77.8)	8,250 (76.7)

*****A majority of the participants used antihypertensive medication from different drug classes.

^BMI 12 missing

Table 2: Hazard ratios and 95% confidence interval for systolic and diastolic blood pressure and a bladder cancer weighted genetic risk score in relation to risk of urothelial carcinoma outcomes

Exposure	Analysis	UC (overall)	Non-aggressive UC [b]	Aggressive UC [b]
		Population, n (Cases, n)	**Population, n (Cases, n)**	**Population, n (Cases, n)**
		10,752 (385)	10,752 (246)	10, 752 (129)
		HR (95 % CI)[a]	**HR (95 % CI)[a]**	**HR (95 % CI)[a]**
Systolic blood pressure	**<140 mm Hg (reference)**	1.00	1.00	1.00
	140-149 mm Hg	0.99 (0.75-1.31)	0.89 (0.63-1.27)	1.31 (0.80-2.15)
	150-159 mm Hg	1.13 (0.84-1.51)	0.91 (0.62-1.34)	1.78 (1.08-2.91)
	≥160 mm Hg	1.15 (0.89-1.50)	1.02 (0.74-1.42)	1.51 (0.95-2.40)
	p-trend	0.237	0.871	0.081
	Per SD [d]	1.09 (0.98-1.20)	1.00 (0.87-1.13)	**1.27 (1.07-1.50)**
Diastolic blood pressure	**<90 mm Hg (reference)**	1.00	1.00	1.00
	90-94 mm Hg	1.11 (0.84-1.46)	1.06 (0.75-1.49)	1.18 (0.73-1.90)
	95-99 mm Hg	1.05 (0.80-1.39)	1.17 (0.83-1.63)	0.82 (0.49-1.39)
	≥100 mm Hg	0.98 (0.73-1.32)	0.77 (0.52-1.13)	1.46 (0.91-2.34)
	Per SD	1.00 (0.90-1.11)	0.93 (0.82-1.07)	1.14 (0.96-1.36)
	p-trend	0.872	0.297	0.215
Weighted genetic risk score [c]	**1st Quartile (reference)**	1.00	1.00	1.00
	2nd Quartile	1.05 (0.77-1.43)	1.18 (0.79-1.77)	0.89 (0.53-1.51)
	3rd Quartile	1.24 (0.92-1.68)	1.34 (0.90-1.99)	1.18 (0.72-1.94)
	4th Quartile	**1.65 (1.24-2.19)**	**2.06 (1.43-2.96)**	1.24 (0.76-2.02)
	p-trend	**<0.001**	**<0.001**	0.267
	Per SD	**1.26 (1.14-1.40)**	**1.34 (1.10-1.52)**	1.19 (1.00-1.41)

Abbreviations: SD, standard deviation; UC, urothelial cancer; HR, hazard ratio; CI, confidence interval.

[a] Hazard ratios were calculated by Cox regression with age as the underlying time metric. Models were adjusted for categories of smoking, BMI, education, and physical activity.

[b] 10 individuals with incident UC cases had missing tumor data on muscle invasiveness.

[c] 413 (14 cases) individuals had missing genotype data (not included in analysis involving bladder cancer genetic risk score).

[d] The p-value for trend across categories was investigated by incorporating the categories of SBP, DBP and wGRS as a continuous variable in the regression model and testing its coefficient using the Wald test.

Supplementary table 1: List of bladder cancer single nucleotide polymorphisms (SNPs) used to create the weighted genetic risk score.

SNP	Effect allele	Other allele	EAF (%) reference	Odds ratio	ln (Odds ratio)
rs1014971	T	C	34.4	1.18	0.165514
rs10775480	T	C	45.5	1.13	0.122218
rs10936599	C	T	74.2	1.15	0.139762
rs11892031	A	C	91.9	1.17	0.157004
rs1495741	A	G	27.3	1.13	0.122218
rs17674580	T	C	36.9	1.17	0.157004
rs2294008	T	C	42.4	1.13	0.122218
rs2736098	A	G	31.3	1.16	0.14842
rs401681	C	T	57.8	1.12	0.113329
rs4907479	A	G	27.8	1.13	0.122218
rs6104690	A	G	42.4	1.07	0.067659
rs62185668	A	C	25.8	1.19	0.173953
rs710521	A	G	70.2	1.18	0.165514
rs7238033	C	T	45.5	1.2	0.182322
rs798766	T	C	79.3	1.2	0.182322
rs8102137	C	T	28.8	1.13	0.122218
rs907611	A	G	33.3	1.15	0.139762
rs9642880	T	G	40.9	1.21	0.19062

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