GERMLINE PROGNOSTIC MARKERS FOR URINARY BLADDER CANCER

By

NADEŽDA LIPUNOVA, MSc

A thesis submitted to

University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY

Institute of Cancer and Genomic Sciences

College of Medical and Dental Sciences

University of Birmingham

October 2019

ABSTRACT

The majority of urinary bladder cancer (UBC) cases are diagnosed as non-muscle invasive malignancies, having a favourable prognosis in terms of overall 5-year survival. However, non-muscle invasive bladder cancer (NMIBC) cases show high recurrence and progression rates and inconsistencies within the NMIBC risk group, resulting in a substantial burden on patients and health systems.

The evidence for genetic risk factors having a role in NMIBC susceptibility and prognosis make NMIBC a good candidate for personalised medicine approaches; however, multiple genome-wide association studies (GWAS) have primarily focused on NMIBC risk alone, even though investigating prognostic factors would arguably yield more benefit.

It is recognised that genetic variation contributes to complex traits in the form of multiple effects of low-penetrance, as well as interacting not only with each other, but with various environmental factors as well, resulting in a complex problem to resolve.

In a setting of a bladder cancer patient cohort, our project aims to identify genetic variants of genome-wide significance that might be associated with certain NMIBC characteristics at diagnosis, and potential gene-environment interaction effects with smoking. Furthermore, we aim to address the importance of replication in genetic association studies by utilising a resource of UK Biobank, whilst introducing a novel approach for identifying prognostic events from routinely collected data.

In conclusion, the current thesis provides additional evidence to the field of bladder cancer genetics and suggests further research topics of interest that could lead to optimising NMIBC patients' care.

ACKNOWLEDGMENTS

This thesis has been written between nine airports, four homes, three offices, and an infinite amount of trains and coffee shops. The final piece is a result of efforts by many good people, to whom I am genuinely grateful.

My academic supervisors have provided me a great deal of guidance, but I am especially thankful to those who have surpassed their supervisory role and became true mentors. There is no doubt I highly value the passed-on knowledge. However, the moments I cherish the most are the ones filled with insight, kindness, and encouragement.

To my absolutely amazing parents, for letting me to discover life and become my own person. There were many losses and triumphs in doing so, but I was never without a safe haven. The love and connection between us has been the most defining force of my character, and I am lucky to have our family as the reference point to what genuinely matters. И спасибо за генетический подарок в форме носа с амбициями, который сую куда не надо (в докторскую диссертацию, например). Jūs nuostabūs.

To all of my extended non-genetic family, aka the most beautiful people that I have met in Britain and The Netherlands, and to those whom I always long for in Vilnius and the rest of the globe. Lady luck is someone I rarely rely on, but she has been more than generous in this context. Most importantly, to Nika, Igor, and B. You are my home. We all know I am a restless neurotic, eager to simultaneously live in the past, present, and future. But with you, only now exists, and I cannot imagine a better place in all of time and space.

To weightlifting! For keeping me sane and reminding there are many things (literally) much heavier than a PhD or any other issue for that matter. It has also never failed to reiterate the true value of simply trying, even if one more time is all there is to give (CORE TIGHT, BACK STRAIGHT, GO!).

I will avoid being meek and not say I could not have done it without you. But the journey would have been lonely and bleak; whereas now it was vivid and full of colour, all in HD.

One thing holds true – at the dawn of my beautiful 30th (no sarcasm whatsoever), I am the happiest I have ever been. And *this* would have been impossible without each and every single one of you.

As in the words of Kurt Vonnegut; So it goes.

CONTENT LISTING

- Table of contents
- List of Figures
- List of Tables
- List of Supplementary Data in the Appendix
- List of papers and conference proceedings

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTARY DATA IN THE APPENDIX

Supplementary Table 6.2. Previously reported polymorphisms in association with bladder cancer death.

Supplementary Table 6.3. Previously reported polymorphisms in association with age at the time of bladder cancer diagnosis.

Supplementary Table 6.4. Previously reported polymorphisms in association with bladder cancer progression.

Supplementary Table 6.5. Minor allele frequencies and imputation scores (info) for tested SNPs.

LIST OF PAPERS AND CONFERENCE PROCEEDINGS

During my PhD study, different chapters of work have resulted in manuscripts and/or accepted as conference abstracts. The full list of disseminated research is listed below.

Accepted publications

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Galesloot TE, Kiemeney L, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer. Eur Urol Oncol. 2019;2(4):381-9.

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Cazier JB, Bryan RT, Zeegers MP. Systematic Review: Genetic associations for Prognostic Factors of Urinary Bladder Cancer. Biomark Cancer. 2019; doi:10.1177/1179299X19897255.

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ**,** Cazier JB**,** Bryan RT, Zeegers MP. External replication of urinary bladder cancer prognostic polymorphisms in the UK Biobank. Front Oncol. 2019; 9:1082; doi: 10.3389/fonc.201901082.

In Press

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier, JB Zeegers MP. Gene-environment interaction with smoking for increased non-muscle-invasive bladder cancer tumour size. Transl Androl Urol. 2019.

Abstracts

Lipunova N, Bryan RT, Cazier JB, Wesselius A, Cheng KK, van Schooten FJ, Zeegers MP. **GWAS for tumour size, grade, stage, and age of onset in NMIBC patients in West Midlands Bladder Cancer Prognosis Programme.** Accepted for poster presentation at the 16th Centre for Genomic Regulation (CRG) Symposium: Seventh International Workshop on Genomic Epidemiology. September 2017.

Lipunova N, Cazier JB, Zeegers MP, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT. **GWAS for tumour size, grade, stage, and age in NMIBC patients in the West Midlands Bladder Cancer Prognosis Programme.** Accepted for poster presentation at the 11th Genomics of Common Diseases conference, September 2017.

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Zeegers MP. **Gene-environment interaction with smoking on non-muscle-invasive bladder cancer size at the time of diagnosis.** Accepted for poster presentation at the 27th International Genetic Epidemiology Society Annual Meeting, October 2018.

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Zeegers MP. **Use of Hospital Episode Statistics in the UK Biobank to aid independent replication of genetic associations.** Accepted for poster presentation at the Health Data Science 2019 Conference, June 2019.

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Zeegers MP. **Independent Replication of Genetic Associations with Urinary Bladder Cancer Prognosis in the UK Biobank using Hospital Record Data.** Accepted for poster presentation at the 28th International Genetic Epidemiology Society Annual Meeting, October 2019.

Other Presentations

2018 Institute of Cancer & Genomic Sciences PGR Festival. Best 2nd Year PGR presentation.

2019 Institute of Cancer & Genomic Sciences PGR Festival. Best 3rd Year PGR presentation.

College of Medical and Dental Sciences Research Festival 2019. 3 Minute Thesis competition, 1st Runner up

CHAPTER 1.

GENERAL INTRODUCTION

1.1 EPIDEMIOLOGY OF URINARY BLADDER CANCER

Urinary bladder cancer (UBC) accounts for approximately 430,000 new cases and 165,000 deaths worldwide annually [1], placing as the 9th most common cancer (6th among men and 19th among women) [2]. Importantly, the management of UBC places burden on medical systems due to high monetary costs, and UBC has been estimated to account for 3% of all cancer-related healthcare costs in the European Union in 2012 [3].

The incidence of UBC varies significantly between and within populations, as well as across different sets of characteristics. As in the case of many cancers, age is one of the strongest predictors for developing UBC. The risk of disease increases steadily during lifetime, reaching a median age at the time of diagnosis of 65-70 years [1, 4]. Males are consistently observed to have a higher UBC incidence in comparison to females, no matter the geographic region (gender ratio of roughly 3.5 to 1) [1, 2].

UBC is mostly a disease of countries with high human development index (HDI), with 55% of all new cases and 45% of all deaths being registered in North America and Europe [1, 2]. Some African and Asian regions also exhibit high UBC rates, which are mostly attributed to *Schistosoma* infections [2, 5]. Divergent disease aetiology reflects the distribution of UBC cancer types between these regions; urothelial carcinoma is the prevailing type in HDI countries, whilst African regions observe a much higher rate of other histological types, mainly squamous-cell carcinomas [5]. Overall, global incidence and mortality rates have a temporal decreasing trend, except for countries undergoing rapid economic development [2]. Recent changes have been mostly attributed to reduced prevalence of smoking, the most significant risk factor for UBC [2, 5]. It is unknown whether the decline will be sustained, as it is also heavily

influenced by other counteracting factors. For example, the old age at the time of diagnosis is expected to cause a slight incidence surge in the upcoming decades. As life expectancy increases, the population at-risk becomes larger, which highlights that the health burden caused by UBC is likely to persist [5].

Nonetheless, a direct comparison of UBC incidence rates poses a challenge due to the large disparity in cancer registration practices. Globally, most registries tend to include both invasive and non-invasive forms of bladder cancer [6]. Non-muscle-invasive bladder cancer (NMIBC) is diagnosed more often and has a much better prognosis in comparison to tumours that have invaded into the detrusor muscle. Hence, comparing UBC distribution, especially in terms of mortality and prevalence, is subject to a careful evaluation on whether the considered countries and/or regions have included all UBC cases or have limited their reports to only muscle-invasive bladder cancer (MIBC) [6].

1.2. RISK FACTORS FOR DEVELOPING UBC

1.2.1. Smoking

The principal risk factor for UBC development is tobacco use, which is estimated to account for almost 50% of all new UBC cases [5-7]. Smoking results in high exposure to many carcinogenic substances with DNA-damaging capabilities [8]. Among those, aromatic amines have been specifically linked to bladder cancer [6-9]. Aromatic amines are powerful DNA-damaging compounds, and since they are excreted via the urinary system, the carcinogenic effect on the bladder epithelium is substantial [6, 7]. When not repaired, the aromatic amine-induced cellular DNA damage may lead to mutations, resulting in dysregulation of key biological processes and, ultimately, urothelial cell transformation [8, 9]. In addition to these genotoxic effects, aromatic

amines, like many other chemical carcinogens, induce epigenetic changes that are also involved in bladder carcinogenesis [10, 11].

Given the widespread use of tobacco in many countries, it poses a serious public health problem. Moreover, as the latency period between smoking exposure and UBC development spans over two to three decades [5, 7], the true consequences are delayed and hence difficult to assess. Current estimates suggest smoking results in three- to four-fold increase in UBC risk, when compared to never-smokers [5, 7]. In addition, smokers of black (air-cured) tobacco have a higher bladder cancer risk than smokers of blond tobacco (flue-cured) due to the higher levels of the aromatic amines in black tobacco smoke in comparison to blond tobacco smoke [9]. Former tobacco users have a slightly lower risk of developing UBC (Relative Risk (RR)≈2) [5, 7], which shows smoking cessation interventions are a promising tactic in reducing UBC incidence and mortality. For example, the slight reduction in global UBC incidence and mortality rates worldwide has been attributed to decreasing use of tobacco in many Western countries. However, some Eastern European and Baltic countries have had a slower reduction of tobacco use prevalence, and a correspondent UBC incidence decline is expected only after a few more decades [2].

1.2.2. Occupational exposure

Smoking is not the only source of aromatic amines, and additional exposure can be related to various workplaces, for instance, during the production of textiles, dyes, paints, inks, rubbers, cables, solvents, and leather dusts [6, 7]. However, occupational exposures are not limited to aromatic amines, and are also found to be rich with other carcinogens, namely benzidine, metal working fluids, polyaromatic hydrocarbons, diesel exhaust, 2-naphtylamine, 2-chloroaniline, 4-aminobiphenyl, ortho-toluidine,

tetrachloroethylene, and combustion products from natural gas [6, 7, 12]. The occupation-related attributable risk for bladder cancer varies, and is reported to be as low as 5% [6] and as high as 20% [7]. The exact extent of occupation exposure influence on UBC development is problematic for many reasons – the average workplace has multiple factors affecting health, and measurement of all exposures is subject to a significant error. Nonetheless, the evidence is consistent with some industries (tobacco, metallurgy, dye) being associated with an increased risk of UBC (lowest reported RR:1.72 (tobacco workers), highest RR=13.4 (dye workers)) [6].

1.2.3. Dietary factors

The role of nutrition in bladder cancer has been investigated in numerous studies; however, findings remain inconsistent, mostly due to the difficulty of accurately measuring the effect of specific nutrients, individual foods, or product groups.

Water intake has been hypothesised to have a protective effect on bladder cancer, by increasing urine volume and daily excretion frequency, thus reducing carcinogen exposure to the urothelium [6, 7]. Alternatively, chlorinated tap water may actually introduce carcinogens - trihalomethanes - and contribute to an increased risk of UBC [13]. Moreover, the effect of various beverages, such as alcohol, tea, coffee, cola, and dairy products has been investigated separately to adjust for active ingredients in each product. The estimates between studies vary [14], but meta-analyses of observational studies indicate there is little to suggest that any of the mentioned fluids have a convincing link with bladder cancer risk [6, 7, 15].

Conflicting results are also observed for specific foods and dietary patterns. Not surprisingly, higher fruit and vegetable consumption is linked with lower risk of urinary

bladder cancer [16, 17], but the protective effect is not supported by all reported meta-analyses [6, 18, 19].

Pooling studies on red meat consumption and bladder cancer risk has shown no significant effect, but high intake of processed meat is suggestive of an increased rate of UBC [20, 21].

Overall, it is widely recognised that diet plays an essential role in human health. However, when considering all published evidence as a whole, it seems that the generic dietary advice [22] provided for prevention of all - communicable and non-communicable diseases – is also relevant for bladder cancer. More studies on dietary patterns with consistent results are needed before any targeted recommendations can be made for preventive use of diet for bladder cancer. Nevertheless, such studies can be challenging to conduct.

Research on micronutrients in relation to bladder cancer is assisted by a few randomised controlled trials (RCTs). Supplementation with various compounds has been reported in the literature, mostly on vitamins A, B, C, D, E, beta-carotene, and selenium) [6, 7, 23]. However, a recent meta-analysis of 14 RCTs found no effect of any supplementation [23], as well as a Cochrane review on selenium [24]. Interestingly, subgroup analyses consisting of 3 RCTs showed beta-carotene may have a harmful effect and increase the risk of UBC (RR=1.44, 95%CI: 1.00-2.09). In addition, observational studies investigating baseline levels of various micronutrients have also failed to show consistent associations with bladder cancer risk [6].

1.2.4. Non-modifiable risk factors

UBC is also associated with a variety of non-modifiable factors. The most prominent of such factors is gender. Bladder cancer is diagnosed almost three times more

frequently in men than women [1, 2]. Extensive previous research on causes for the inter-gender gap has mostly considered sex-specific smoking behaviours and hormones [7], but they fail to fully explain the observed patterns [25]. Fortunately, new evidence shows the cause may be linked to a gene on the X chromosome, *KDM6A* [26]. *KDM6A* escapes the process of X chromosome inactivation and is highly expressed in women. Evidence suggests *KDM6A* acts as a tumour suppressor and reduces the likelihood of developing UBC [26]. Moreover, women with low expression of *KDM6A* have worse prognostic UBC outcomes. Importantly, an elegant study by Kaneko et al. [26] highlights sex chromosomes and hormones as having independent roles that amplify each other's effects, further enhancing UBC disparities between males and females.

The concept of genetics playing a role in bladder cancer was first recognised having observed almost a two-fold increased risk of developing UBC among first-degree family members [27]. The genetic contribution to UBC diagnosis has been further investigated in larger datasets, estimating the heritability to be roughly 12% [28]. Importantly, investigations into smoking-related cancers have shown that genetic loci related to tobacco use also make a significant contribution to bladder cancer heritability (~3%) [28]. This finding implies that some people have a genetic tendency to smoke or smoke in higher quantities, and this behaviour contributes to developing bladder cancer. In concordance with these observations, the heritability of UBC is slightly lower among never-smokers than in those with any history of smoking [28]. These patterns underscore the complex nature of genetic determinants, since some act upon the outcome directly, whilst others may exert effects in a more complex path [28].

The number of currently reported loci for genetic predisposition to bladder cancer is such that the word limitations of the current thesis do not allow the full discussion of each. Most consistently validated associations include genes *GSTM1* and *NAT2,* responsible for regulating detoxification of various substances [29, 30]. Although GSTM1 and NAT2 proteins are both stage II-detoxifiers, they seem to play different roles in bladder cancer. *NAT2* is heavily involved in tobacco-related compound clearance, and an impaired function leads to longer carcinogen exposure. This hypothesis is supported by studies on gene-environment (GxE) interaction between *NAT2* polymorphisms and smoking, which conclude that *NAT2* mutations only contribute to UBC risk among ever smokers [30]. On the other hand, *GSTM1-null* genotype has been associated with an increased UBC risk regardless of smoking behaviour, suggesting it is involved in the detoxification of carcinogens not limited to tobacco [29].

Moreover, the presence of gene-gene interaction has also been implied in multiple studies, showing variants in some genes have a different combined effect in comparison to their individual consequences. Specifically, there have been suggested multiplicative interactions between *NAT2* slow-acetylator and *GSTM1*-null [31], and between *GSTM1*- and *GSTT1*-null genotypes [32].

Multiple other genes have been shown to alter the risk of bladder cancer, but only a handful have been analysed in meta-analyses. When multiple studies have been considered, evidence shows that the implicated genes vary widely in their function. Some regulate overarching processes, such as DNA repair (*ERCC2*/*XPD* [33-36], *XRCC1* [37-40], *XRCC3* [41-43], *XRCC4* [44], *XPC* [45-48], *NBS1* [49]), cell cycle progression (*CCND1* [45, 50]*, MYC* [51, 52]), telomere integrity (*TERT-CLPTM1L* [53,

54]), mRNA translation (*MIR146A* [55]), apoptosis (*Survivin/BIRC5* [56, 57]), and cell adhesion (*CDH1* [58]).

Other genes are implicated in specific mechanisms, such as regulating immune function (*IL10* [59], *IL6* [60]*, TNF-α* [61], *CCR2* [62]), prostate-related protein coding (*PSCA* [52, 63, 64]), sulphate conjugation of many compounds (*SULT1A1* [65, 66]), detoxification (*NQO1* [67-70] , *GSTP1* [71, 72], *GPX1* [73, 74], *UGT1A7* [75], nitric oxide production (*eNOS/NOS3* [76]), extracellular matrix breakdown (*MMP1, MMP2, MMP7* [77, 78]), catecholamine metabolism (*COMT* [79]), drug metabolism and production of steroids, lipids, and cholesterol (*CYP1A1* [80], *CYP2E1* [81], and regulating well-known tumour suppressor proteins (*TP53* [82, 83]).

In addition, pooling the published studies is also useful in clarifying which associations are likely to be false positives. Specifically, meta-analyses report various polymorphisms in genes *ERCC6* [84], *MSH3* [85], *MDM2* [86], *APE1* [87], *CYP1A2* [88], *XRRC7* [89], *NKFB1* [90], *HIF-1α* [91], *XPG/ERCC5* [92], *CYP1B1* [93], and *TGFBR1*6A/9A* [94] have little to suggest their importance in UBC development.

However, the direct interpretation of any reports, including meta-analyses, is burdened by many limitations, such as comparing different ethnicities and the use of divergent methodologies. Several loci were found to be associated with bladder cancer only among people of certain ethnic backgrounds, for example, single nucleotide polymorphisms (SNPs) in *MTHFR* [95], *SULT1A1* [65], *CYP2E1* [81], *MDM2* [96], *BIRC5* [56], *XRCC3* [43], *UGT1A7* [75], and *TP53* [97-99]. These reports carry even further uncertainty, as some meta-analyses have found variants (e.g. *XRCC1*) to be significant only in ethnic subgroups [39, 40]), whilst others have concluded the effect is present regardless of ethnicity [100].

Group-specific loci may also become apparent when considering gene-environment interactions with various external exposures: the genetic associations will be evident only in specific populations, but not overall, for example, SNPs in *hOGG1 (*[101, 102]) and *NAT2* [30].

Moreover, meta-analyses on variants differ extensively in their strength. For example, one meta-analysis on *CYP1B1* concluded an overall association with urinary cancers, alongside an acknowledged presence of publication bias and a very small number of studies [103]. The persisting challenges are further reflected in reports contradicting each other. For example, a few meta-analyses have stated that a mutation in *CYP1A2* (rs762551) has a protective effect on bladder cancer [88, 104], and another one concluded that there is no evidence to support such findings [105]. The same inconsistency is present for rs1048943 in *CYP1A1* [80, 106], rs1801133 in *MTHFR* [95, 107, 108], and rs1800566 in *NQO1* [109, 110].

Finally, several polymorphisms in the same gene have been observed to have associations in different directions, even for the same outcome. For example, rs25487 (R399Q) in *XRCC1* seems to be protective for bladder cancer among smokers, but variants rs1799782 (R194W) and rs25489 (R280H) show increased risk of UBC among Asians [38].

To conclude, various inconsistencies in genetic association studies demonstrate the topic is multi-faceted and complex. Further investigations, accompanied by advances in methodology, will be essential to calibrate our current knowledge of the relationship between genetics and UBC development.

1.3. BLADDER CANCER PATHOPHYSIOLOGY

1.3.1. Molecular pathophysiology

Although NMIBC and MIBC share topography, there is substantial evidence for them to be considered separate types of cancers [5, 111, 112]. Current research shows that they may develop in distinct pathways – papillary (NMIBC) and non-papillary (MIBC) – that overlap to a degree, but clinically result in two different forms of UBC [111] (**Figure 1.1**).

Papillary cancer is often characterised by mutations in *FGFR3*, alongside deletions on chromosome 9 (9q). These genomic changes are considered to be a precursor for urothelial hyperplasia, that later give rise to NMIBC or remain benign and can be discovered as urothelial papilloma [5, 113].

In contrast, MIBC mostly develops via a non-papillary pathway and makes up for 20-25% of all new UBC cases [5]. A precursor for MIBC is thought to be urothelial dysplasia and/or carcinoma *in situ* (CIS), which in time may develop into high-grade and non-papillary urothelial cancer [111, 113, 114]. These tumours have a high propensity for penetrating the detrusor muscle and forming distant metastases, which signals an unfavourable outcome [111]. In addition, MIBC shows distinct genetic mutations, mostly in genes functioning as tumour suppressors – *TP53*, *PTEN*, and *RB1*, among other [5, 112, 113] (**Figure 1.1**)

It is important to mention these pathways are not entirely independent; for example, roughly 15% of low-grade Ta tumours become genetically unstable and develop into high-grade Ta tumours. These tumours show signs of both hyperplasia and dysplasia, and have alterations in *RB1* and *TP53* genes [5, 112]. Moreover, *FGFR3* mutations are not exclusive to NMIBC; as 40% of MIBC tumours also demonstrate *FGFR3* overexpression [5, 113].

1.3.2. Molecular subtypes

In practice, bladder cancer is often present not just as a single tumour, but as multiple neoplasms, scattered across the bladder lining [5]. Multifocality raises the question of whether all tumours are independent of one another or have a single cell of origin. Whole-bladder mapping studies have shown UBC is a highly heterogeneous disease, and it is most likely a result of multiple pathogenic processes, working in parallel [112].

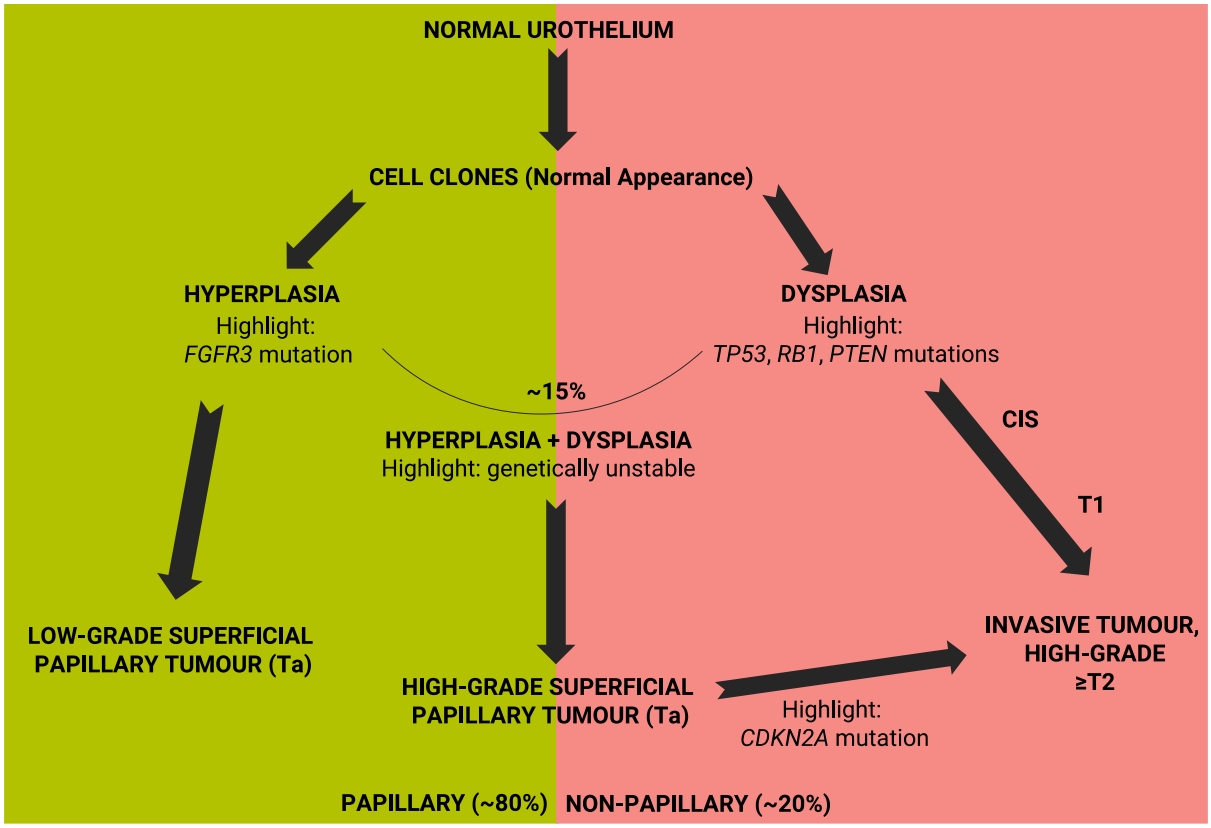


Figure 1.1. Molecular pathophysiology of bladder cancer. CIS-carcinoma in situ.

The urothelium consists of three main tissue layers – basal, intermediate, and luminal. The basal layer is considered to harness stem cells that are capable of self-regeneration and show least differentiation. Intermediate cells show a higher level of differentiation, but the proliferation potential is much lower than that of the basal layer. Finally, the luminal cells are fully differentiated and make up the top layer of the urothelium. Each cell type has a specific protein expression profile, which makes it

possible to infer the developmental origin of bladder tumours [112]. In fact, a few important studies investigated transcriptional profiles of both NMIBC and MIBC and have provided guidelines for UBC molecular subtyping. Although the exact categories identified by each group differ (**Figure 1.2**), there are a few overlapping patterns that are likely to be pivotal in future UBC management [5, 112].

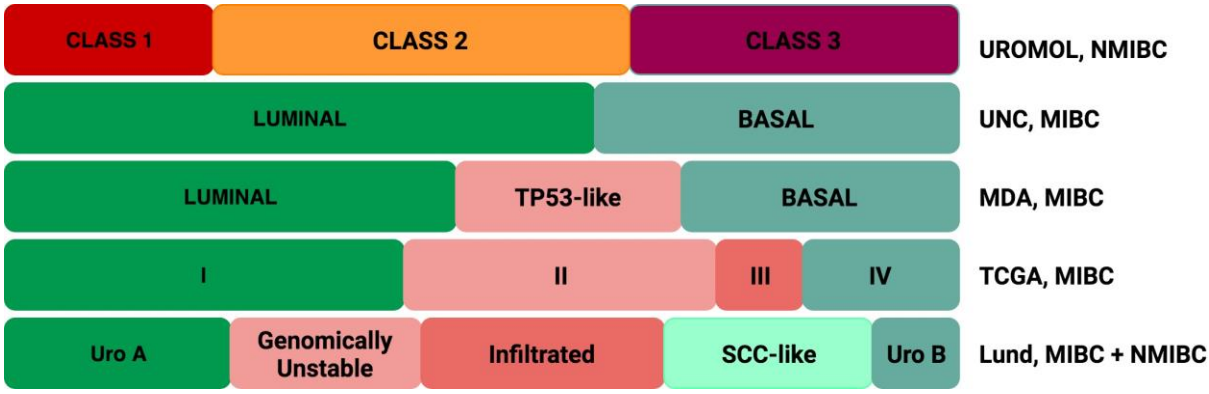


Figure 1.2. Molecular subtypes of urinary bladder cancer. Adapted from Sanli et al. [5]. MDA–MD Anderson, MIBC–muscle-invasive bladder cancer, NMIBC–non-muscle-invasive bladder cancer, SCC-squamous cell carcinoma, TCGA-The Cancer Genome Atlas Program, UNC-University of North Carolina.

NMIBC samples have been included in two studies [115, 116], and both show low-grade papillary tumours (Ta) can be defined by markers of high differentiation, early-cell cycle and *FGFR3* gene signatures [5, 115, 116]. Alternatively, high-grade and T1 NMIBC has been observed to retain markers of high differentiation, but were also genetically unstable and expressed markers of late cell-cycles [5, 115, 116].

Molecular subtypes of MIBC have been exclusively reported by three studies [5, 117-119]. The number of subtypes ranges from two [119] to four [117], but the main similarity among all is distinguishing luminal and basal MIBC. Similarly to NMIBC, luminal invasive bladder cancer expresses markers of cell differentiation, early cell cycle genes, and *FGFR3* signature. In contrast, basal tumours are accompanied by markers of basal layer of the urothelium [5].

Overall, these data provide important insights into the landscape of UBC. Firstly, there seems to be two broad categories of luminal and basal cancers, mostly corresponding to papillary and non-papillary pathways, respectively [112]. Non-invasive cancers are usually present as luminal and have a better prognosis, whilst muscle-invasive tumours can be either of basal origin or a mixture of basal and luminal. Invasive UBC of luminal type is likely to represent tumours that have initially developed as papillary low-grade tumours, but have lost genetic stability and have progressed into high-grade papillary cancer [112]. Importantly, although basal cancers are intrinsically more aggressive, they are also seemingly more responsive to cisplatin-based chemotherapy. At the same time, MIBC type that represents a cross-over of luminal and basal types, tagged “p53-like”, were less aggressive, but were resistant to the same chemotherapy agents [120].

To conclude, a growing body of evidence suggests that combining NMIBC and MIBC groups in observational and experimental research may be incorrect.

1.4. URINARY BLADDER CANCER IN A CLINICAL SETTING

In Western societies, urothelial or transitional cell carcinoma is diagnosed in 75-90% of all UBC cases [112]. Other histological types, such as squamous cell carcinoma, are associated with an aggressive disease, and usually require specific management that cannot be guided with group-level recommendations [5]. In a clinical setting, UBC is categorised into broad groups of NMIBC and MIBC, as the level of penetration into the detrusor muscle still represents the most important prognostic factor [114] (**Figure 1.3**).

NMIBCs comprise the majority of UBC cases (70-80%) and are usually diagnosed as papillary carcinoma [5, 114, 121].

Papillary carcinoma of NMIBC is generally associated with a better outcome, especially in terms of mortality. However, the recurrence rates are very high despite local therapy (5-year recurrence rate: 50-70%, 5-year progression rate: 10-30%) [7, 111, 122].

Painless haematuria is the most common clinical symptom of UBC, which is either observed during routine urine tests or by patients themselves [5, 122]. Sometimes other, non-specific, urinary tract symptoms (e.g. increased urination frequency, dysuria, etc.) may also indicate the presence of a malignant tumour, and are more often associated with CIS [5, 122].

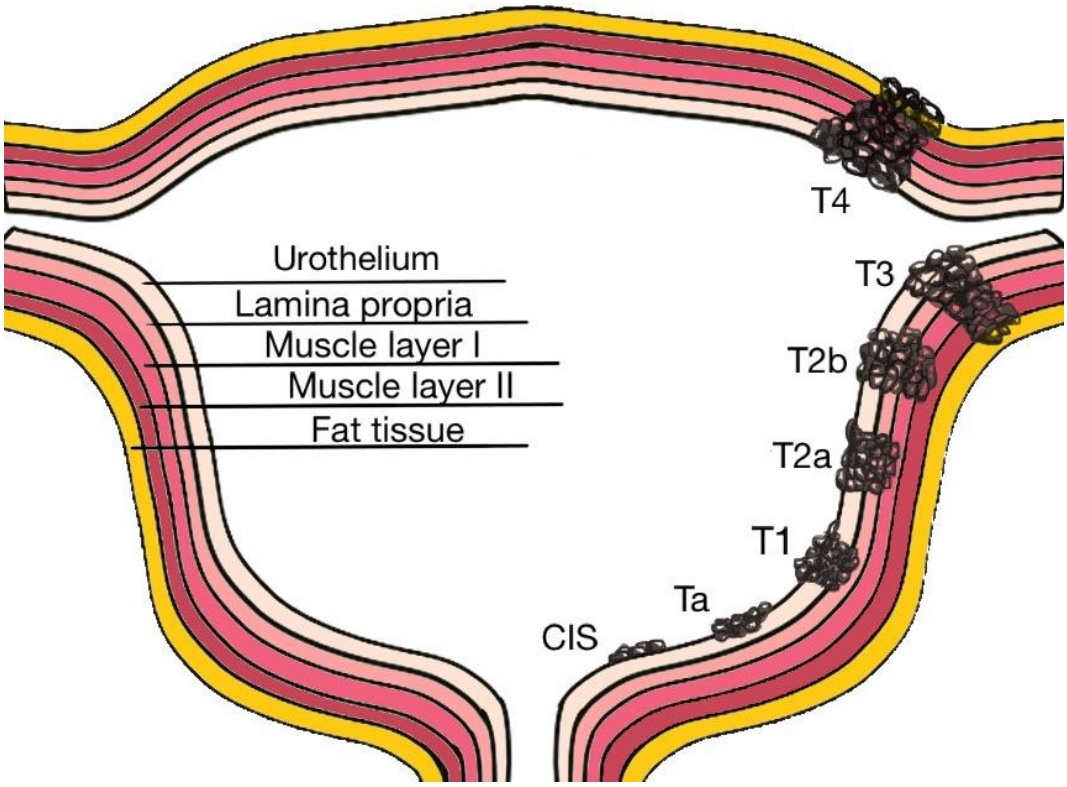


Figure 1.3. Urinary bladder cancer staging. CIS-Carcinoma in situ.

The long-standing gold standard for UBC diagnosis is cystoscopy, performed under local anaesthesia. A flexible endoscope is inserted into the bladder via the urethra, which allows a visual examination of the bladder lining. Suspicious lesions deviating from the normal urothelium are biopsied for histological investigation, which further

dictates management strategy [5]. Cystoscopy is an invasive and expensive procedure, and uncomfortable for patients. Hence, many efforts are directed towards finding an alternative diagnostic tool. As tumour cells and DNA are shed into the urine, it can theoretically be used for UBC detection. Regardless of the potential, no urine-based biomarkers have yet reached sufficient sensitivity and specificity metrics, leaving as cystoscopy the principal diagnostic tool for the time-being [123].

If cystoscopy is suspicious of cancer, the first order of action is a transurethral resection of bladder tumour (TURBT) (unless cystoscopy suggests MIBC; in that case, additional staging techniques might be used before TURBT, such as Computed Tomography (CT) or Magnetic Resonance Imaging (MRI)) [124]. During TURBT, not only is the tumour removed, but additional investigation is carried out to better describe the disease. Urothelium is assessed for multifocal tumours, lesion size, and a biopsy of detrusor muscle deep to the tumour is taken to rule out an invasive cancer [124].

Treatment differs substantially for MIBC and NMIBC cases. MIBC is a high-risk disease, and, therefore, necessitates early radical treatment. Based on specific characteristics of individual cases, radical therapy includes either full removal of the urinary bladder (cystectomy) or radiotherapy [124]. In addition, neoadjuvant cisplatin-based chemotherapy may be administered prior to radical treatment if appropriate [124].

NMIBC is a slightly more complex entity, and the treatment depends on a defined risk category (low, intermediate, or high) [122, 124]. To assign the risk category, factors of stage, grade, size, multifocality, number of previous recurrences, presence of concomitant CIS, histological variants, and level of invasion into the lamina propria are considered (**Figure 1.4**). For low-risk NMIBC, TURBT and a one-time instillation of chemotherapy (mitomycin C or similar chemotherapeutic) into the bladder is usually

sufficient. Cancers falling into the intermediate-risk category require TURBT and multiple installations of mitomycin C or Bacillus Calmette-Guerin (BCG) treatment into the bladder, but are usually not recommended for more than one year [122].

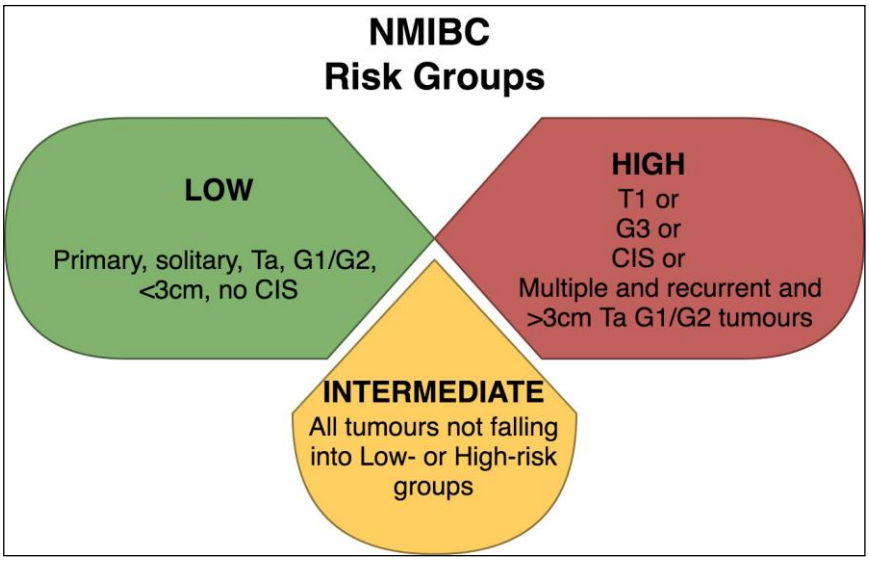


Figure 1.4 NMIBC Risk Groups [122].

Finally, high-risk NMIBC is treated with a TURBT and intravesicular instillations of BCG for a maximum of three years [122, 124]. Some guidelines include consideration of a radical cystectomy approach for high-risk NMIBC [124]. All NMIBC cases are subject to rigorous post-treatment monitoring, albeit at different frequencies. Patients with low-risk NMIBC are advised a cystoscopy at three and 12 months after the diagnosis. Intermediate-risk patients should have a cystoscopy at three, nine, and 18 months after the NMIBC diagnosis, and an annual follow-up until five years. Cases of high-risk NMIBC require cystoscopies every three months for the first two years, every six months until five years, and once a year for the remaining time (**Figure 1.5**) [122, 124].

However, it is important to highlight there are several guidelines for NMIBC management, and although they overlap to a high degree, differences between all of the approaches remain [125].

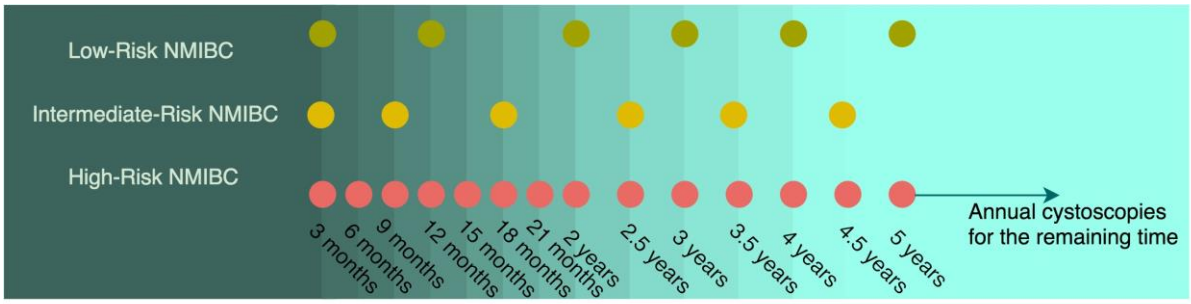


Figure 1.5. NMIBC patient cystoscopy follow-up schedule.

1.5. INDIVIDUAL PREDICTIVE AND PROGNOSTIC TOOLS FOR URINARY BLADDER CANCER OUTCOME

As the management of NMIBC cases is not a homogenous process and differs based on various characteristics, there have been multiple attempts for developing instruments that would best predict individual outcomes and optimise treatment strategies.

The European Organization for Research and Treatment of Cancer (EORTC) has developed a tool to predict individual outcomes for patients who only underwent a single intervention of TURBT. The scoring system estimates per-person probability rates of recurrence and progression events, and relies on six clinical factors: tumour size, stage, grade, presence of CIS, number of tumours, and prior recurrence rate (**Table 1.1**) [122]. Every factor is assigned a different weight, indicating the relative importance of each (**Table 1.2**).

A separate scoring system has been jointly developed by the Club Urologico Espanolde Tratamiento Oncologico (CUETO) and EORTC, that only applies to BCG-treated patients [126]. The system considers gender, age, recurrence status, number of tumours, T stage, presence of concomitant CIS, and tumour grade (**Table 1.3**). These factors are then transferred into an algorithm that estimates recurrence and progression probabilities at one, two, and five years.

Table 1.1. Probability of urinary bladder cancer recurrence and progression based on the EORTC score [122, 127].

	Probability of the event at 1 year		Probability of the event at 5 years	
	%	95% CI	%	95% CI
Recurrence score:				
0	15	10-19	31	24-37
1-4	24	21-26	46	42-49
5-9	38	35-41	62	58-65
10-17	61	55-67	78	73-84
Progression score:				
0	0.2	0-0.7	0.8	0-1.7
2-6	15	0.4-1.6	62	5-8
7-13	5	4-7	17	14-20
14-23	17	10-24	45	35-55

CI=confidence interval.

Table 1.2. Weights used to calculate NMIBC recurrence and progression scores [122, 127].

Factor	Levels	Recurrence	Progression
Number of tumours	**Single**	0	0
	2-7	3	3
	≥8	6	3
Tumour diameter, cm	**<3**	0	0
	≥3	3	3
Prior recurrence rate	**Primary**	0	0
	≤ 1 recurrence/year	2	2
	> 1 recurrence/year	4	2
T stage	**Ta**	0	0
	T1	1	4
Concomitant CIS	**No**	0	0
	Yes	1	6
Grade	**G1**	0	0
	G2	1	0
	G3	2	5
Total scores		**0-17**	**0-23**

CIS-Carcinoma in situ; NMIBC-non-muscle-invasive bladder cancer.

On average, the CUETO scoring system estimates lower progression and recurrence rates, which most likely reflects better outcomes as a result of administering BCG [122, 128].

Table 1.3. CUETO scoring system for BCG-treated bladder cancer patients [126].

Factor	Levels	Recurrence	Progression
Gender	**Male**	0	0
	Female	3	0
Age, years	**< 60**	0	0
	60-70	1	0
	> 70	2	2
Recurrent tumour	**No**	0	0
	Yes	4	2
Number of tumours	**≤3**	0	0
	>3	2	1
T stage	**Ta**	0	0
	T1	0	2
Concomitant CIS	**No**	0	0
	Yes	2	1
Grade	**G1**	0	0
	G2	1	2
	G3	3	6
Total scores			

BCG-Bacillus Calmette–Guérin; CIS-Carcinoma in situ.

Although the current used prognostic tools are useful, the individual accuracy of estimates varies considerably, receiving fair criticism for the lack of precision [129, 130]. Additional characteristics were investigated to upgrade the current tools; however, none of those satisfy criteria of robustness and accuracy to be used in practice [131, 132]. Other studies were carried out in specific populations (**Table 1.4**) and hence are difficult to generalise for guidelines [122].

1.6. OTHER POTENTIAL FACTORS FOR NMIBC PROGNOSTICATION

1.6.1. Smoking

Smoking is the most important external risk factor for developing UBC, that accounts for approximately 50% of all new cases [4, 7, 114, 133]. Besides increasing the risk of UBC, smoking also has been shown to worsen UBC prognosis, and is associated with higher risks of NMIBC recurrence and MIBC mortality [134-136].

Table 1.4. Additional prognostic factors for NMIBC [122].

Sample	Prognostic factor
In patients with T1 tumours	Lymphovascular invasion [137], level of invasion to lamina propria [138-141]
In patients with T1G2 tumours treated with TURBT	Recurrence at 3 months was the most important predictor of progression [142]
In patients with T1G3 tumours:	Bladder (pseudo) diverticulum because of an absence of muscle layer in the diverticular wall [143];
those treated with an induction course of BCG	Female sex, CIS in the prostatic urethra in patients treated with an induction course of BCG [144];
those treated with BCG	Age, tumour size, and concurrent CIS [145];
In patients with high-risk disease	Tumour stage at the time of the second TURBT [146, 147];
In patients treated with TURBT	Presence of lymphovascular invasion [148];
All UBC patients	Histological variants other than papillary (micropapillary, plasmocytoid, nested, sarcomatoid, squamous, adenocarcinoma) [149-153];
All UBC patients	Molecular markers, particularly *FGFR3* mutation status, are promising but need further evaluation [140, 154-157].

BCG-Bacillus Calmette–Guérin; CIS-carcinoma in situ; UBC-urinary bladder cancer; TURBT-transurethral resection of bladder tumour.

NMIBC progression and mortality have also shown to be affected by smoking, although to a lesser extent [134, 136]. Moreover, lifetime smoking has been associated with specific baseline characteristics at the time of UBC diagnosis that contribute towards a worse prognosis, namely higher stage and grade, larger tumour size, and lower age at the time of diagnosis [158].

1.6.2. Diet and diet-related factors

Various dietary components have been investigated for their potential effect on NMIBC prognosis. As elegantly summarised in a recent review [159], there is no good-standing evidence for the effect of various dietary components (e.g. Vitamins A, E, cruciferous vegetables, total fluid intake, etc.) or supplements (e.g. multivitamins) on reduced likelihood of NMIBC recurrence, progression, or overall survival. However, diet's contribution to NMIBC prognosis cannot be ruled out, and additional studies will

further advance the current knowledge. In fact, it is likely that dietary patterns (e.g. a generic Western diet, high in fried foods and red meat), rather than individual components, may propose a more robust prediction [160].

Furthermore, high body mass index (BMI), has been shown to be a predictor of NMIBC recurrence, but not progression [159].

1.6.3. Genetic determinants

As the interest in genetics expands beyond the identification of risk variants, studies have also investigated germline variation having an influence on UBC outcomes, rather than only risk. Initially, UBC susceptibility polymorphisms were also queried for their association with UBC outcomes. The results are not straightforward – none of the UBC susceptibility loci have shown association with NMIBC recurrence; whether overall or in groups of low- and high-risk NMIBC [161]. Being an exception, a SNP in *MYC* (8q24, rs9642880) has shown promise in predicting NMIBC progression. *FGFR3* potentially alters risk of low-grade NMIBC recurrence, but the results are inconsistent [161, 162].

Candidate-gene (CG) studies have also investigated UBC outcomes directly, resulting in over 100 reported associations with various endpoints [161, 163-175]. Nevertheless, very few have been successfully replicated in independent samples [163, 176, 177]. A recent study showed only six of the 114 SNPs could have been successfully validated [163] (NMIBC progression: rs6678136 (*RGS4*) and rs11585883 (*RGS5*); NMIBC recurrence among BCG-treated: rs1799793 (*ERCC2*) and rs187238 (*IL18*); MIBC overall survival: rs12035879 (*RGS5*) and rs2075786 (*TERT*)).

Previously reported genetic associations for NMIBC outcomes cannot necessarily be viewed as false positives if they lack validation, and cannot be held positively true if

validation was successful. The only way to calibrate existing knowledge is to add more evidence from other discovery and replication studies to further guide scientifically valid hypotheses. The fast-evolving field of genetics has shown that translation of germline variation into an observed phenotype is anything but straightforward. It is now widely recognised that genetic associations are highly dependent on outcome definition and external exposures, making the issue far more complex.

1.6.4. Gene-environment interactions for UBC prognosis

Smoking is the strongest single risk factor for UBC [178], and it has been shown to interact with UBC susceptibility loci [179]. It is important to know whether prognostic genetic variants bear the same relevance for people of different smoking background, as it may eventually alter policies of clinical NMIBC management.

Moreover, GxE interaction for NMIBC outcomes has already been observed previously. Increased risk of NMIBC recurrence among smokers was observed in multiple studies, associations mapping to genes responsible for phase II detoxification processes (*GSTM1*, *UGT1A1* [180]*, NAT2* [161]). Additionally, never-smokers have exhibited lower rates of recurrence with an allele change in *FGFR3* (4p16.3, rs798766) [161].

Reports on GxE for NMIBC remain scarce, and it is difficult to form current evidence into a comprehensive summary. Moreover, it is virtually unknown whether any GxE with tobacco is present for baseline characteristics of prognostic relevance (e.g. grade, stage). This represents an important gap in previous analyses, as there might be genetic associations with some, but not all constituents of more complex prognostic outcomes.

1.7. THIS THESIS

As UBC mostly comprises of highly-recurrent NMIBC cases, the main burden of UBC comes from NMIBC being suboptimally managed [121], leading to high monetary and emotional costs [3]. Diagnosis of NMIBC requires an ongoing, expensive, and burdensome patient-surveillance scheme, that makes use of frequent, sometimes lifelong, cystoscopies [121, 122, 181]. Economic evaluations have showed that one of the main routes to reducing the high costs of bladder cancer would be advances in managing individual NMIBC cases [181]. As such, accurate disease prognostication is a highly-prioritised research field, where developments in current practice would greatly benefit both medical systems and patients.

The currently-used prognostic NMIBC tools carry a promise of the useful categorisation of patients, permitting application of an evidence-based treatment strategy. In reality, that promise is mainly unfulfilled, since the assumption of such tools being accurate is has not yet been met [182]. These inaccuracies do not call for discontinuing their use; instead, they serve as a rationale for further tool calibration, whilst using previously unavailable data (e.g. genomics) and analytical techniques.

The current thesis aims to broaden our knowledge of the role of genetic variation in NMIBC prognosis. As the methodology of genetic association studies is meticulous, detail-oriented, and is a great predictor of research validity, quality control (QC) and data preparation are described first in **Chapter 2**.

To best summarise the current evidence on genetic associations and prognostic factors of UBC, a systematic review of relevant published studies is presented in **Chapter 3**.

Chapter 4 describes a genome-wide association study (GWAS) on clinical NMIBC characteristics (tumour stage, grade, size, and patient's age) that have prognostic properties. Although these characteristics carry important weight in clinical decision-making, none of those were investigated as primary outcomes of interest, especially in a GWAS setting.

It is clear that bladder cancer is one of few cancers for which GxE interaction has been observed repeatedly. However, GxE interaction with smoking for developing a tumour with specific features (e.g. larger size) has not yet been studied. **Chapter 5** describes our investigation of whether the discovered variants from a GWAS analysis (**Chapter 4**) may have a differential effect on tumour and patient characteristics at the time of NMIBC diagnosis among people of different smoking habits.

The strength of new evidence is always tested under the scrutiny of replication and consistency. To explore the validity of genetic associations with bladder cancer prognosis, **Chapter 6** describes efforts for result replication. The previously reported associations that were summarised in the systematic review (**Chapter 3**) are tested in a UBC cohort that was identified using data from the UK Biobank. This work also highlights the possibility of modelling outcomes using Hospital Episode Statistics (HES) that are not collected routinely (e.g. UBC recurrence). A newly developed algorithm can be used in other studies, and is hoped to help extend the use of large publicly-available clinical datasets in the future.

Chapter 7 aims to best summarise all described analyses, contextualise the results, explore future directions, and provide final conclusions for the readers of this thesis.

REFERENCES

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Patterns in Globocan 2012. International journal of cancer. 2015;136(5):E359-86.

[2] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. European urology. 2017;71(1):96-108.

[3] Leal J, Luengo-Fernandez R, Sullivan R, Witjes JA. Economic Burden of Bladder Cancer across the European Union. Eur Urol. 2016;69(3):438-47.

[4] Kirkali Z, Chan T, Manoharan M, Algaba F, Busch C, Cheng L, Kiemeney L, Kriegmair M, Montironi R, Murphy WM, Sesterhenn IA, Tachibana M, Weider J. Bladder Cancer: Epidemiology, Staging and Grading, and Diagnosis. Urology. 2005;66(6, Supplement 1):4-34.

[5] Sanli O, Dobruch J, Knowles MA, Burger M, Alemozaffar M, Nielsen ME, Lotan Y. Bladder Cancer. Nature reviews Disease primers. 2017;3:17022.

[6] Cumberbatch MGK, Jubber I, Black PC, Esperto F, Figueroa JD, Kamat AM, Kiemeney L, Lotan Y, Pang K, Silverman DT, Znaor A, Catto JWF. Epidemiology of Bladder Cancer: A Systematic Review and Contemporary Update of Risk Factors in 2018. European urology. 2018;74(6):784-95.

[7] Burger M, Catto JWF, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and Risk Factors of Urothelial Bladder Cancer. European Urology. 2013;63(2):234-41.

[8] Ma B, Stepanov I, Hecht SS. Recent Studies on DNA Adducts Resulting from Human Exposure to Tobacco Smoke. Toxics. 2019;7(1).

[9] Besaratinia A, Tommasi S. Genotoxicity of Tobacco Smoke-Derived Aromatic Amines and Bladder Cancer: Current State of Knowledge and Future Research Directions. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2013;27(6):2090-100.

[10] Vantaku V, Amara CS, Piyarathna DWB, Donepudi SR, Ambati CR, Putluri V, Tang W, Rajapakshe K, Estecio MR, Terris MK, Castro PD, Ittmann MM, Williams SB, Lerner SP, Sreekumar A, Bollag R, Coarfa C, Kornberg MD, Lotan Y, Ambs S, Putluri N. DNA Methylation Patterns in Bladder Tumors of African-American Patients Point to Distinct Alterations in Xenobiotic Metabolism. Carcinogenesis. 2019.

[11] Schulz WA, Goering W. DNA Methylation in Urothelial Carcinoma. Epigenomics. 2016;8(10):1415-28.

[12] Kiriluk KJ, Prasad SM, Patel AR, Steinberg GD, Smith ND. Bladder Cancer Risk from Occupational and Environmental Exposures. Urologic oncology. 2012;30(2):199-211.

[13] Villanueva CM, Cantor KP, King WD, Jaakkola JJ, Cordier S, Lynch CF, Porru S, Kogevinas M. Total and Specific Fluid Consumption as Determinants of Bladder Cancer Risk. International journal of cancer. 2006;118(8):2040-7.

[14] Yu EY, Wesselius A, van Osch F, Stern MC, Jiang X, Kellen E, Lu CM, Pohlabeln H, Steineck G, Marshall J, Allam MF, La Vecchia C, Johnson KC, Benhamou S, Zhang ZF, Bosetti C, Taylor JA, Zeegers MP. The Association between Coffee Consumption and Bladder Cancer in the Bladder Cancer Epidemiology and Nutritional Determinants (Blend) International Pooled Study. Cancer causes & control : CCC. 2019;30(8):859-70.

[15] Hong X, Xu Q, Lan K, Huang H, Zhang Y, Chen S, Chi Z, Lin J, Zhou Y, Wu W, Liu G, Lin W, Zhang Y. The Effect of Daily Fluid Management and Beverages Consumption on the Risk of Bladder Cancer: A Meta-Analysis of Observational Study. Nutrition and cancer. 2018;70(8):1217-27.
[16] Liu H, Wang XC, Hu GH, Guo ZF, Lai P, Xu L, Huang TB, Xu YF. Fruit and Vegetable Consumption and Risk of Bladder Cancer: An Updated Meta-Analysis of Observational Studies. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP). 2015;24(6):508-16.
[17] Yao B, Yan Y, Ye X, Fang H, Xu H, Liu Y, Li S, Zhao Y. Intake of Fruit and Vegetables and Risk of Bladder Cancer: A Dose-Response Meta-Analysis of Observational Studies. Cancer causes & control : CCC. 2014;25(12):1645-58.
[18] Xu C, Zeng XT, Liu TZ, Zhang C, Yang ZH, Li S, Chen XY. Fruits and Vegetables Intake and Risk of Bladder Cancer: A Prisma-Compliant Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies. Medicine. 2015;94(17):e759.
[19] Vieira AR, Vingeliene S, Chan DS, Aune D, Abar L, Navarro Rosenblatt D, Greenwood DC, Norat T. Fruits, Vegetables, and Bladder Cancer Risk: A Systematic Review and Meta-Analysis. Cancer medicine. 2015;4(1):136-46.
[20] Li F, An S, Hou L, Chen P, Lei C, Tan W. Red and Processed Meat Intake and Risk of Bladder Cancer: A Meta-Analysis. International journal of clinical and experimental medicine. 2014;7(8):2100-10.
[21] Wang C, Jiang H. Meat Intake and Risk of Bladder Cancer: A Meta-Analysis. Medical oncology (Northwood, London, England). 2012;29(2):848-55.
[22] Ahmed T, Nawaz S, Noreen R, Bangash KS, Rauf A, Younis M, Anwar K, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R, Ali SHB. A 3' Untranslated Region Polymorphism Rs2304277 in the DNA Repair Pathway Gene Ogg1 Is a Novel Risk Modulator for Urothelial Bladder Carcinoma. Annals of human genetics. 2018;82(2):74-87.
[23] Park SJ, Myung SK, Lee Y, Lee YJ. Effects of Vitamin and Antioxidant Supplements in Prevention of Bladder Cancer: A Meta-Analysis of Randomized Controlled Trials. Journal of Korean medical science. 2017;32(4):628-35.
[24] Vinceti M, Filippini T, Del Giovane C, Dennert G, Zwahlen M, Brinkman M, Zeegers MP, Horneber M, D'Amico R, Crespi CM. Selenium for Preventing Cancer. The Cochrane database of systematic reviews. 2018;1:Cd005195.
[25] Dobruch J, Daneshmand S, Fisch M, Lotan Y, Noon AP, Resnick MJ, Shariat SF, Zlotta AR, Boorjian SA. Gender and Bladder Cancer: A Collaborative Review of Etiology, Biology, and Outcomes. European urology. 2016;69(2):300-10.
[26] Kaneko S, Li X. X Chromosome Protects against Bladder Cancer in Females Via a Kdm6a-Dependent Epigenetic Mechanism. Science advances. 2018;4(6):eaar5598.
[27] Aben KK, Witjes JA, Schoenberg MP, Hulsbergen-van de Kaa C, Verbeek AL, Kiemeney LA. Familial Aggregation of Urothelial Cell Carcinoma. International journal of cancer. 2002;98(2):274-8.
[28] Sampson JN, Wheeler WA, Yeager M, Panagiotou O, Wang Z, Berndt SI, Lan Q, Abnet CC, Amundadottir LT, Figueroa JD, Landi MT, Mirabello L, Savage SA, Taylor PR, De Vivo I, McGlynn KA, Purdue MP, Rajaraman P, Adami HO, Ahlbom A, Albanes D, Amary MF, An SJ, Andersson U, Andriole G, Jr., Andrulis IL, Angelucci E, Ansell SM, Arici C, Armstrong BK, Arslan AA, Austin MA, Baris D, Barkauskas DA, Bassig BA, Becker N, Benavente Y, Benhamou S, Berg C, Van Den Berg D, Bernstein L, Bertrand KA, Birmann BM, Black A, Boeing H, Boffetta P, Boutron-

Ruault MC, Bracci PM, Brinton L, Brooks-Wilson AR, Bueno-de-Mesquita HB, Burdett L, Buring J, Butler MA, Cai Q, Cancel-Tassin G, Canzian F, Carrato A, Carreon T, Carta A, Chan JK, Chang ET, Chang GC, Chang IS, Chang J, Chang-Claude J, Chen CJ, Chen CY, Chen C, Chen CH, Chen C, Chen H, Chen K, Chen KY, Chen KC, Chen Y, Chen YH, Chen YS, Chen YM, Chien LH, Chirlaque MD, Choi JE, Choi YY, Chow WH, Chung CC, Clavel J, Clavel-Chapelon F, Cocco P, Colt JS, Comperat E, Conde L, Connors JM, Conti D, Cortessis VK, Cotterchio M, Cozen W, Crouch S, Crous-Bou M, Cussenot O, Davis FG, Ding T, Diver WR, Dorronsoro M, Dossus L, Duell EJ, Ennas MG, Erickson RL, Feychting M, Flanagan AM, Foretova L, Fraumeni JF, Jr., Freedman ND, Beane Freeman LE, Fuchs C, Gago-Dominguez M, Gallinger S, Gao YT, Gapstur SM, Garcia-Closas M, García-Closas R, Gascoyne RD, Gastier-Foster J, Gaudet MM, Gaziano JM, Giffen C, Giles GG, Giovannucci E, Glimelius B, Goggins M, Gokgoz N, Goldstein AM, Gorlick R, Gross M, Grubb R, 3rd, Gu J, Guan P, Gunter M, Guo H, Habermann TM, Haiman CA, Halai D, Hallmans G, Hassan M, Hattinger C, He Q, He X, Helzlsouer K, Henderson B, Henriksson R, Hjalgrim H, Hoffman-Bolton J, Hohensee C, Holford TR, Holly EA, Hong YC, Hoover RN, Horn-Ross PL, Hosain GM, Hosgood HD, 3rd, Hsiao CF, Hu N, Hu W, Hu Z, Huang MS, Huerta JM, Hung JY, Hutchinson A, Inskip PD, Jackson RD, Jacobs EJ, Jenab M, Jeon HS, Ji BT, Jin G, Jin L, Johansen C, Johnson A, Jung YJ, Kaaks R, Kamineni A, Kane E, Kang CH, Karagas MR, Kelly RS, Khaw KT, Kim C, Kim HN, Kim JH, Kim JS, Kim YH, Kim YT, Kim YC, Kitahara CM, Klein AP, Klein RJ, Kogevinas M, Kohno T, Kolonel LN, Kooperberg C, Kricker A, Krogh V, Kunitoh H, Kurtz RC, Kweon SS, LaCroix A, Lawrence C, Lecanda F, Lee VH, Li D, Li H, Li J, Li YJ, Li Y, Liao LM, Liebow M, Lightfoot T, Lim WY, Lin CC, Lin D, Lindstrom S, Linet MS, Link BK, Liu C, Liu J, Liu L, Ljungberg B, Lloreta J, Di Lollo S, Lu D, Lund E, Malats N, Mannisto S, Le Marchand L, Marina N, Masala G, Mastrangelo G, Matsuo K, Maynadie M, McKay J, McKean-Cowdin R, Melbye M, Melin BS, Michaud DS, Mitsudomi T, Monnereau A, Montalvan R, Moore LE, Mortensen LM, Nieters A, North KE, Novak AJ, Oberg AL, Offit K, Oh IJ, Olson SH, Palli D, Pao W, Park IK, Park JY, Park KH, Patiño-Garcia A, Pavanello S, Peeters PH, Perng RP, Peters U, Petersen GM, Picci P, Pike MC, Porru S, Prescott J, Prokunina-Olsson L, Qian B, Qiao YL, Rais M, Riboli E, Riby J, Risch HA, Rizzato C, Rodabough R, Roman E, Roupret M, Ruder AM, Sanjose S, Scelo G, Schned A, Schumacher F, Schwartz K, Schwenn M, Scotlandi K, Seow A, Serra C, Serra M, Sesso HD, Setiawan VW, Severi G, Severson RK, Shanafelt TD, Shen H, Shen W, Shin MH, Shiraishi K, Shu XO, Siddiq A, Sierrasesúmaga L, Sihoe AD, Skibola CF, Smith A, Smith MT, Southey MC, Spinelli JJ, Staines A, Stampfer M, Stern MC, Stevens VL, Stolzenberg-Solomon RS, Su J, Su WC, Sund M, Sung JS, Sung SW, Tan W, Tang W, Tardón A, Thomas D, Thompson CA, Tinker LF, Tirabosco R, Tjønneland A, Travis RC, Trichopoulos D, Tsai FY, Tsai YH, Tucker M, Turner J, Vajdic CM, Vermeulen RC, Villano DJ, Vineis P, Virtamo J, Visvanathan K, Wactawski-Wende J, Wang C, Wang CL, Wang JC, Wang J, Wei F, Weiderpass E, Weiner GJ, Weinstein S, Wentzensen N, White E, Witzig TE, Wolpin BM, Wong MP, Wu C, Wu G, Wu J, Wu T, Wu W, Wu X, Wu YL, Wunder JS, Xiang YB, Xu J, Xu P, Yang PC, Yang TY, Ye Y, Yin Z, Yokota J, Yoon HI, Yu CJ, Yu H, Yu K, Yuan JM, Zelenetz A, Zeleniuch-Jacquotte A, Zhang XC, Zhang Y, Zhao X, Zhao Z, Zheng H, Zheng T, Zheng W, Zhou B, Zhu M, Zucca M, Boca SM, Cerhan JR, Ferri GM, Hartge P, Hsiung CA, Magnani C, Miligi L, Morton LM, Smedby KE, Teras LR, Vijai J, Wang SS, Brennan P, Caporaso NE, Hunter DJ, Kraft P, Rothman N, Silverman DT, Slager SL, Chanock SJ, Chatterjee N. Analysis of Heritability and Shared

Heritability Based on Genome-Wide Association Studies for Thirteen Cancer Types. J Natl Cancer Inst. 2015;107(12):djv279.
[29] Zhou T, Li HY, Xie WJ, Zhong Z, Zhong H, Lin ZJ. Association of Glutathione S-Transferase Gene Polymorphism with Bladder Cancer Susceptibility. BMC cancer. 2018;18(1):1088.
[30] Ma C, Gu L, Yang M, Zhang Z, Zeng S, Song R, Xu C, Sun Y. Rs1495741 as a Tag Single Nucleotide Polymorphism of N-Acetyltransferase 2 Acetylator Phenotype Associates Bladder Cancer Risk and Interacts with Smoking: A Systematic Review and Meta-Analysis. Medicine. 2016;95(31):e4417.
[31] Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Castano-Vinyals G, Yeager M, Welch R, Chanock S, Chatterjee N, Wacholder S, Samanic C, Tora M, Fernandez F, Real FX, Rothman N. Nat2 Slow Acetylation, Gstm1 Null Genotype, and Risk of Bladder Cancer: Results from the Spanish Bladder Cancer Study and Meta-Analyses. Lancet. 2005;366(9486):649-59.
[32] Lee SJ, Cho SH, Park SK, Kim SW, Park MS, Choi HY, Choi JY, Lee SY, Im HJ, Kim JY, Yoon KJ, Choi H, Shin SG, Park TW, Rothman N, Hirvonen A, Kang D. Combined Effect of Glutathione S-Transferase M1 and T1 Genotypes on Bladder Cancer Risk. Cancer letters. 2002;177(2):173-9.
[33] Li SX, Dai QS, Chen SX, Zhang SD, Liao XY, Deng X, Chi HB, Li FJ, Zhu JH, Jiang YY. Xeroderma Pigmentosum Complementation Group D (Xpd) Gene Polymorphisms Contribute to Bladder Cancer Risk: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(4):3905-15.
[34] Wu Y, Yang Y. Complex Association between Ercc2 Gene Polymorphisms, Gender, Smoking and the Susceptibility to Bladder Cancer: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(6):5245-57.
[35] Li S, Zeng XT, Ruan XL, Liu TZ, Wang XH. Association between Xpd Lys751gln Polymorphism and Bladder Cancer Susceptibility: An Updated and Cumulative Meta-Analysis Based on 6,836 Cases and 8,251 Controls. Molecular biology reports. 2014;41(6):3621-9.
[36] Xiong T, Yang J, Wang H, Wu F, Liu Y, Xu R, Lv Z, Xue P, Cao W, Zhang Y. The Association between the Lys751gln Polymorphism in the Xpd Gene and the Risk of Bladder Cancer. Molecular biology reports. 2014;41(4):2629-34.
[37] Zhang M, Li W, Hao Z, Zhou J, Zhang L, Liang C. Association between Twelve Polymorphisms in Five X-Ray Repair Cross-Complementing Genes and the Risk of Urological Neoplasms: A Systematic Review and Meta-Analysis. EBioMedicine. 2017;18:94-108.
[38] Li S, Peng Q, Chen Y, You J, Chen Z, Deng Y, Lao X, Wu H, Qin X, Zeng Z. DNA Repair Gene Xrcc1 Polymorphisms, Smoking, and Bladder Cancer Risk: A Meta-Analysis. PloS one. 2013;8(9):e73448.
[39] Yang D, Liu C, Shi J, Wang N, Du X, Yin Q, Wang Y. Association of Xrcc1 Arg399gln Polymorphism with Bladder Cancer Susceptibility: A Meta-Analysis. Gene. 2014;534(1):17-23.
[40] Liu C, Yin Q, Li L, Jiao G, Wang M, Wang Y. Xrcc1 Arg194trp and Arg280his Polymorphisms in Bladder Cancer Susceptibility: A Meta-Analysis. Critical reviews in eukaryotic gene expression. 2013;23(4):339-54.
[41] Ma Q, Zhao Y, Wang S, Zhang X, Zhang J, Du M, Li L, Zhang Y. Genetic Polymorphisms of Xrcc3 Thr241met (C18067t, Rs861539) and Bladder Cancer Risk:

A Meta-Analysis of 18 Research Studies. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(2):1473-80.
[42] Peng Q, Mo C, Tang W, Chen Z, Li R, Zhai L, Yang S, Wu J, Sui J, Li S, Qin X. DNA Repair Gene Xrcc3 Polymorphisms and Bladder Cancer Risk: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(3):1933-44.
[43] He XF, Wei W, Li JL, Shen XL, Ding DP, Wang SL, Liu ZZ, Qin JB, Wu LX, Xie DL. Association between the Xrcc3 T241m Polymorphism and Risk of Cancer: Evidence from 157 Case-Control Studies. Gene. 2013;523(1):10-9.
[44] Shao N, Jiang WY, Qiao D, Zhang SG, Wu Y, Zhang XX, Hua LX, Ding Y, Feng NH. An Updated Meta-Analysis of Xrcc4 Polymorphisms and Cancer Risk Based on 31 Case-Control Studies. Cancer biomarkers : section A of Disease markers. 2012;12(1):37-47.
[45] Wang Y, Li Z, Liu N, Zhang G. Association between Ccnd1 and Xpc Polymorphisms and Bladder Cancer Risk: A Meta-Analysis Based on 15 Case-Control Studies. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(4):3155-65.
[46] Dou K, Xu Q, Han X. The Association between Xpc Lys939gln Gene Polymorphism and Urinary Bladder Cancer Susceptibility: A Systematic Review and Meta-Analysis. Diagnostic pathology. 2013;8:112.
[47] Dai QS, Hua RX, Zeng RF, Long JT, Peng ZW. Xpc Gene Polymorphisms Contribute to Bladder Cancer Susceptibility: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(1):447-53.
[48] Sankhwar M, Sankhwar SN, Bansal SK, Gupta G, Rajender S. Polymorphisms in the Xpc Gene Affect Urinary Bladder Cancer Risk: A Case-Control Study, Meta-Analyses and Trial Sequential Analyses. Scientific reports. 2016;6:27018.
[49] Zhang Y, Huang YS, Lin WQ, Zhang SD, Li QW, Hu YZ, Zheng RL, Tang T, Li XZ, Zheng XH. Nbs1 Glu185gln Polymorphism and Susceptibility to Urinary System Cancer: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(11):10723-9.
[50] Li J, Luo F, Zhang H, Li L, Xu Y. The Ccnd1 G870a Polymorphism and Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(1):171-7.
[51] Zhao Y, Qi JG, Yang N, Lin YL, Liang J, Zhu X. Association between Myc Rs9642880[T] Allele and Bladder Cancer Risk: A Meta-Analysis. Genetics and molecular research : GMR. 2015;14(4):14745-51.
[52] Gao J, Yang PT, Diao Y, Kang HF, Zhao Y, Lin S, Wang ZM, Wang M, Wang XJ, Dai ZJ. Effects of Psca Rs2294008 (C/T) and C-Myc Rs9642880 (G/T) Polymorphisms on Bladder Cancer: Evidence from a Meta-Analysis. International journal of clinical and experimental medicine. 2015;8(2):2156-64.
[53] Zhang M, Wu X, Lu W, Ge Y, Wang X, Cai Z, Wu S. Rs401681 Polymorphism in Tert-Clptm1l Was Associated with Bladder Cancer Risk: A Meta-Analysis. Iranian journal of basic medical sciences. 2015;18(11):1130-6.
[54] Yin J, Li Y, Yin M, Sun J, Liu L, Qin Q, Li X, Long L, Nie S, Wei S. Tert-Clptm1l Polymorphism Rs401681 Contributes to Cancers Risk: Evidence from a Meta-Analysis Based on 29 Publications. PloS one. 2012;7(11):e50650.

[55] Nikolic ZZ, Savic Pavicevic DL, Vucic NL, Romac SP, Brajuskovic GN. Association between a Genetic Variant in the Hsa-Mir-146a Gene and Cancer Risk: An Updated Meta-Analysis. Public health genomics. 2015;18(5):283-98.
[56] Xu L, Zhou X, Xu L, Yin R. Survivin Rs9904341 (G>C) Polymorphism Contributes to Cancer Risk: An Updated Meta-Analysis of 26 Studies. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(2):1661-9.
[57] Zhu Y, Li Y, Zhu S, Tang R, Liu Y, Li J. Association of Survivin Polymorphisms with Tumor Susceptibility: A Meta-Analysis. PloS one. 2013;8(9):e74778.
[58] Wang Y, Kong CZ, Zhang Z, Yang CM, Li J. Role of Cdh1 Promoter Polymorphism and DNA Methylation in Bladder Carcinogenesis: A Meta-Analysis. DNA and cell biology. 2014;33(4):205-16.
[59] Shi X, Xie X, Xun X, Jia Y, Li S. Associations of Il-10 Genetic Polymorphisms with the Risk of Urologic Cancer: A Meta-Analysis Based on 18,415 Subjects. SpringerPlus. 2016;5(1):2034.
[60] Xu B, Niu XB, Wang ZD, Cheng W, Tong N, Mi YY, Min ZC, Tao J, Li PC, Zhang W, Wu HF, Zhang ZD, Wang ZJ, Hua LX, Feng NH, Wang XR. Il-6 -174g>C Polymorphism and Cancer Risk: A Meta-Analysis Involving 29,377 Cases and 37,739 Controls. Molecular biology reports. 2011;38(4):2589-96.
[61] Cai J, Yang MY, Hou N, Li X. Association of Tumor Necrosis Factor-Alpha 308g/a Polymorphism with Urogenital Cancer Risk: A Systematic Review and Meta-Analysis. Genetics and molecular research : GMR. 2015;14(4):16102-12.
[62] Huang Y, Chen H, Wang J, Bunjhoo H, Xiong W, Xu Y, Zhao J. Relationship between Ccr2-V64i Polymorphism and Cancer Risk: A Meta-Analysis. Gene. 2013;524(1):54-8.
[63] Zhao Y, Gui ZL, Liao S, Gao F, Ge YZ, Jia RP. Prostate Stem Cell Antigen Rs2294008 (C>T) Polymorphism and Bladder Cancer Risk: A Meta-Analysis Based on Cases and Controls. Genetics and molecular research : GMR. 2014;13(3):5534-40.
[64] Geng P, Li J, Wang N, Ou J, Xie G, Liu C, Zhao X, Xiang L, Liao Y, Liang H. Psca Rs2294008 Polymorphism with Increased Risk of Cancer. PloS one. 2015;10(8):e0136269.
[65] Su CM, Chen MC, Lin IC, Chen HA, Huang MT, Wu CH, Shen KH, Wang YH. Association between the Sult1a1 Arg213his Polymorphism and the Risk of Bladder Cancer: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(7):7147-53.
[66] Li W, Gu M. Sult1a1 Arg213his Polymorphism Is Associated with Bladder Cancer Risk: A Meta-Analysis. Medical science monitor : international medical journal of experimental and clinical research. 2014;20:1590-5.
[67] Mandal RK, Dubey S, Panda AK, Mittal RD. Genetic Variants of Nqo1 Gene Increase Bladder Cancer Risk in Indian Population and Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(7):6415-23.
[68] Gong M, Yi Q, Wang W. Association between Nqo1 C609t Polymorphism and Bladder Cancer Susceptibility: A Systemic Review and Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013;34(5):2551-6.

[69] Yang S, Jin T, Su HX, Zhu JH, Wang DW, Zhu SJ, Li S, He J, Chen YH. The Association between Nqo1 Pro187ser Polymorphism and Bladder Cancer Susceptibility: A Meta-Analysis of 15 Studies. PloS one. 2015;10(1):e0116500.
[70] Zhang Y, Yang D, Zhu JH, Chen MB, Shen WX, He J. The Association between Nqo1 Pro187ser Polymorphism and Urinary System Cancer Susceptibility: A Meta-Analysis of 22 Studies. Cancer investigation. 2015;33(2):39-40.
[71] Wang Z, Xue L, Chong T, Li H, Chen H, Wang Z. Quantitative Assessment of the Association between Glutathione S-Transferase P1 Ile105val Polymorphism and Bladder Cancer Risk. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013;34(3):1651-7.
[72] Wu K, Wang X, Xie Z, Liu Z, Lu Y. Glutathione S-Transferase P1 Gene Polymorphism and Bladder Cancer Susceptibility: An Updated Analysis. Molecular biology reports. 2013;40(1):687-95.
[73] Men T, Zhang X, Yang J, Shen B, Li X, Chen D, Wang J. The Rs1050450 C > T Polymorphism of Gpx1 Is Associated with the Risk of Bladder but Not Prostate Cancer: Evidence from a Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(1):269-75.
[74] Cao M, Mu X, Jiang C, Yang G, Chen H, Xue W. Single-Nucleotide Polymorphisms of Gpx1 and Mnsod and Susceptibility to Bladder Cancer: A Systematic Review and Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(1):759-64.
[75] Han SX, Wang L, Wu DQ. The Association between Ugt1a7 Polymorphism and Cancer Risk: A Meta-Analysis. Cancer epidemiology. 2012;36(4):e201-6.
[76] Gao X, Wang J, Wang W, Wang M, Zhang J. Enos Genetic Polymorphisms and Cancer Risk: A Meta-Analysis and a Case-Control Study of Breast Cancer. Medicine. 2015;94(26):e972.
[77] Yan Y, Liang H, Li T, Li M, Li R, Qin X, Li S. The Mmp-1, Mmp-2, and Mmp-9 Gene Polymorphisms and Susceptibility to Bladder Cancer: A Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(4):3047-52.
[78] Tao L, Li Z, Lin L, Lei Y, Hongyuan Y, Hongwei J, Yang L, Chuize K. Mmp1, 2, 3, 7, and 9 Gene Polymorphisms and Urinary Cancer Risk: A Meta-Analysis. Genetic testing and molecular biomarkers. 2015;19(10):548-55.
[79] Zhou Q, Wang Y, Chen A, Tao Y, Song H, Li W, Tao J, Zuo M. Association between the Comt Val158met Polymorphism and Risk of Cancer: Evidence from 99 Case-Control Studies. OncoTargets and therapy. 2015;8:2791-803.
[80] Wang Y, Kong CZ, Zhang Z, Yang CM, Li J. Relationships between Cyp1a1 Genetic Polymorphisms and Bladder Cancer Risk: A Meta-Analysis. DNA and cell biology. 2014;33(3):171-81.
[81] Deng XD, Gao Q, Zhang B, Zhang LX, Zhang W, Mu Er ZE, Xie Y, Ma Y, Liu Y. Functional Rsai/Psti Polymorphism in Cytochrome P450 2e1 Contributes to Bladder Cancer Susceptibility: Evidence from a Meta-Analysis. Asian Pacific journal of cancer prevention : APJCP. 2014;15(12):4977-82.
[82] Li DB, Wei X, Jiang LH, Wang Y, Xu F. Meta-Analysis of Epidemiological Studies of Association of P53 Codon 72 Polymorphism with Bladder Cancer. Genetics and molecular research : GMR. 2010;9(3):1599-605.

[83] Jiang DK, Ren WH, Yao L, Wang WZ, Peng B, Yu L. Meta-Analysis of Association between Tp53 Arg72pro Polymorphism and Bladder Cancer Risk. Urology. 2010;76(3):765.e1-7.
[84] Hong Z, Wu J, Li Q, Zhang S, Shi Z. Meta-Analysis Reveals No Significant Association between Ercc6 Polymorphisms and Bladder Cancer Risk. The International journal of biological markers. 2017;32(1):e113-e7.
[85] Miao HK, Chen LP, Cai DP, Kong WJ, Xiao L, Lin J. Msh3 Rs26279 Polymorphism Increases Cancer Risk: A Meta-Analysis. International journal of clinical and experimental pathology. 2015;8(9):11060-7.
[86] Ding H, Dai Y, Ning Z, Fan N, Wang Z, Li P, Zhang L, Tao Y, Wang H. Murine Double Minute 2 Snp T309g Polymorphism and Urinary Tract Cancer Risk: A Meta-Analysis. Medicine. 2016;95(12):e2941.
[87] Zhong JH, Zhao Z, Liu J, Yu HL, Zhou JY, Shi R. Association between Ape1 Asp148glu Polymorphism and the Risk of Urinary Cancers: A Meta-Analysis of 18 Case-Control Studies. OncoTargets and therapy. 2016;9:1499-510.
[88] Sun WX, Chen YH, Liu ZZ, Xie JJ, Wang W, Du YP, Chen Y, Shen XL, He XF, Wu LX, Wei W, Zhang L. Association between the Cyp1a2 Polymorphisms and Risk of Cancer: A Meta-Analysis. Molecular genetics and genomics : MGG. 2015;290(2):709-25.
[89] Xiao M, Shen Y, Chen L, Liao Z, Wen F. The Rs7003908 (T>G) Polymorphism in the Xrcc7 Gene and the Risk of Cancers. Molecular biology reports. 2014;41(6):3577-82.
[90] Duan W, Wang E, Zhang F, Wang T, You X, Qiao B. Association between the Nfkb1-94ins/Del Attg Polymorphism and Cancer Risk: An Updated Meta-Analysis. Cancer investigation. 2014;32(7):311-20.
[91] Hu X, Fang Y, Zheng J, He Y, Zan X, Lin S, Li X, Li H, You C. The Association between Hif-1alpha Polymorphism and Cancer Risk: A Systematic Review and Meta-Analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(2):903-16.
[92] Liu C, Yin Q, Hu J, Weng J, Wang Y. Quantitative Assessment of the Association between Xpg Asp1104his Polymorphism and Bladder Cancer Risk. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(2):1203-9.
[93] Liu Y, Lin CS, Zhang AM, Song H, Fan CC. The Cyp1b1 Leu432val Polymorphism and Risk of Urinary System Cancers. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4719-25.
[94] Liao RY, Mao C, Qiu LX, Ding H, Chen Q, Pan HF. Tgfbr1*6a/9a Polymorphism and Cancer Risk: A Meta-Analysis of 13,662 Cases and 14,147 Controls. Molecular biology reports. 2010;37(7):3227-32.
[95] Shi R, Zhao Z, Zhou H, Zhou J, Tan W. Lack of Association between Mthfr Ala222val and Glu429ala Polymorphisms and Bladder Cancer Risk: A Meta-Analysis of Case-Control Studies. Biomedical reports. 2014;2(3):396-403.
[96] Wang HG, Wu QY, Zhou H, Peng XS, Shi MJ, Li JM, Zhou YF. The Mdm2 Snp309t>G Polymorphism Increases Bladder Cancer Risk among Caucasians: A Meta-Analysis. Asian Pacific journal of cancer prevention : APJCP. 2014;15(13):5277-81.
[97] Xu T, Xu ZC, Zou Q, Yu B, Huang XE. P53 Arg72pro Polymorphism and Bladder Cancer Risk--Meta-Analysis Evidence for a Link in Asians but Not

Caucasians. Asian Pacific journal of cancer prevention : APJCP. 2012;13(5):2349-54.
[98] Yang Z, Nie S, Zhu H, Wu X, Jia S, Luo Y, Tang W. Association of P53 Arg72pro Polymorphism with Bladder Cancer: A Meta-Analysis. Gene. 2013;512(2):408-13.
[99] Liu ZH, Bao ED. Quantitative Assessment of the Association between Tp53 Arg72pro Polymorphism and Bladder Cancer Risk. Molecular biology reports. 2013;40(3):2389-95.
[100] Mao Y, Xu X, Lin Y, Chen H, Wu J, Hu Z, Zhu Y, Xu X, Xie L. Quantitative Assessment of the Associations between Xrcc1 Polymorphisms and Bladder Cancer Risk. World journal of surgical oncology. 2013;11:58.
[101] Wenjuan C, Jianzhong L, Chong L, Yanjun G, Keqing L, Hanzhang W, Zhiping W. The Hogg1 Ser326cys Gene Polymorphism and Susceptibility for Bladder Cancer: A Meta-Analysis. International braz j urol : official journal of the Brazilian Society of Urology. 2016;42(5):883-96.
[102] Ji C, Liu Z, Chen H, Guo H, Liu C. An Association between Hogg1 Ser326cys Polymorphism and the Risk of Bladder Cancer in Non-Smokers: A Meta-Analysis. BMC cancer. 2012;12:335.
[103] Jiang W, Sun G, Xiong J, Xi X, Shi Z. Association of Cyp1b1 L432v Polymorphism with Urinary Cancer Susceptibility: A Meta-Analysis. Diagnostic pathology. 2014;9:113.
[104] Zeng Y, Jiang HY, Wei L, Xu WD, Wang YJ, Wang YD, Liu C. Association between the Cyp1a2 Rs762551 Polymorphism and Bladder Cancer Susceptibility: A Meta-Analysis Based on Case-Control Studies. Asian Pacific journal of cancer prevention : APJCP. 2015;16(16):7249-54.
[105] Vukovic V, Ianuale C, Leoncini E, Pastorino R, Gualano MR, Amore R, Boccia S. Lack of Association between Polymorphisms in the Cyp1a2 Gene and Risk of Cancer: Evidence from Meta-Analyses. BMC cancer. 2016;16:83.
[106] Lu Y, Zhang XL, Xie L, Li TJ, He Y, Peng QL, Deng Y, Wang J, Qin X, Li S. Lack of Association between Cyp1a1 Polymorphisms and Risk of Bladder Cancer: A Meta-Analysis. Asian Pacific journal of cancer prevention : APJCP. 2014;15(9):4071-7.
[107] You W, Li Z, Jing C, Qian-Wei X, Yu-Ping Z, Weng-Guang L, Hua-Lei L. Mthfr C677t and A1298c Polymorphisms Were Associated with Bladder Cancer Risk and Disease Progression: A Meta-Analysis. DNA and cell biology. 2013;32(5):260-7.
[108] Xu W, Zhang H, Wang F, Wang H. Quantitative Assessment of the Association between Mhtfr C677t (Rs1801133, Ala222val) Polymorphism and Susceptibility to Bladder Cancer. Diagnostic pathology. 2013;8:95.
[109] Lajin B, Alachkar A. The Nqo1 Polymorphism C609t (Pro187ser) and Cancer Susceptibility: A Comprehensive Meta-Analysis. British journal of cancer. 2013;109(5):1325-37.
[110] Zhang H, Wen X, Lu X, Zhang H. Association between Nad(P)H:Quinone Oxidoreductase 1 Rs1800566 Polymorphism and Risk of Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013;34(6):3377-81.
[111] Czerniak B, Dinney C, McConkey D. Origins of Bladder Cancer. Annual review of pathology. 2016;11:149-74.
[112] Guo CC, Czerniak B. Bladder Cancer in the Genomic Era. Archives of pathology & laboratory medicine. 2019;143(6):695-704.

[113] Knowles MA, Hurst CD. Molecular Biology of Bladder Cancer: New Insights into Pathogenesis and Clinical Diversity. Nature reviews Cancer. 2015;15(1):25-41.
[114] Eble J.N. SG, Epstein J.I., Sesterhenn I.A. (Eds.). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs: IARC Press: Lyon; 2004.
[115] Sjodahl G, Lauss M, Lovgren K, Chebil G, Gudjonsson S, Veerla S, Patschan O, Aine M, Ferno M, Ringner M, Mansson W, Liedberg F, Lindgren D, Hoglund M. A Molecular Taxonomy for Urothelial Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2012;18(12):3377-86.
[116] Hedegaard J, Lamy P, Nordentoft I, Algaba F, Hoyer S, Ulhoi BP, Vang S, Reinert T, Hermann GG, Mogensen K, Thomsen MBH, Nielsen MM, Marquez M, Segersten U, Aine M, Hoglund M, Birkenkamp-Demtroder K, Fristrup N, Borre M, Hartmann A, Stohr R, Wach S, Keck B, Seitz AK, Nawroth R, Maurer T, Tulic C, Simic T, Junker K, Horstmann M, Harving N, Petersen AC, Calle ML, Steyerberg EW, Beukers W, van Kessel KEM, Jensen JB, Pedersen JS, Malmstrom PU, Malats N, Real FX, Zwarthoff EC, Orntoft TF, Dyrskjot L. Comprehensive Transcriptional Analysis of Early-Stage Urothelial Carcinoma. Cancer cell. 2016;30(1):27-42.
[117] Comprehensive Molecular Characterization of Urothelial Bladder Carcinoma. Nature. 2014;507(7492):315-22.
[118] Choi W, Porten S, Kim S, Willis D, Plimack ER, Hoffman-Censits J, Roth B, Cheng T, Tran M, Lee IL, Melquist J, Bondaruk J, Majewski T, Zhang S, Pretzsch S, Baggerly K, Siefker-Radtke A, Czerniak B, Dinney CP, McConkey DJ. Identification of Distinct Basal and Luminal Subtypes of Muscle-Invasive Bladder Cancer with Different Sensitivities to Frontline Chemotherapy. Cancer cell. 2014;25(2):152-65.
[119] Damrauer JS, Hoadley KA, Chism DD, Fan C, Tiganelli CJ, Wobker SE, Yeh JJ, Milowsky MI, Iyer G, Parker JS, Kim WY. Intrinsic Subtypes of High-Grade Bladder Cancer Reflect the Hallmarks of Breast Cancer Biology. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(8):3110-5.
[120] McConkey DJ, Choi W, Shen Y, Lee IL, Porten S, Matin SF, Kamat AM, Corn P, Millikan RE, Dinney C, Czerniak B, Siefker-Radtke AO. A Prognostic Gene Expression Signature in the Molecular Classification of Chemotherapy-Naive Urothelial Cancer Is Predictive of Clinical Outcomes from Neoadjuvant Chemotherapy: A Phase 2 Trial of Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin with Bevacizumab in Urothelial Cancer. Eur Urol. 2016;69(5):855-62.
[121] Kamat AM, Hahn NM, Efstathiou JA, Lerner SP, Malmstrom PU, Choi W, Guo CC, Lotan Y, Kassouf W. Bladder Cancer. Lancet (London, England). 2016;388(10061):2796-810.
[122] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.
[123] D'Costa JJ, Goldsmith JC, Wilson JS, Bryan RT, Ward DG. A Systematic Review of the Diagnostic and Prognostic Value of Urinary Protein Biomarkers in Urothelial Bladder Cancer. Bladder Cancer (Amsterdam, Netherlands). 2016;2(3):301-17.
[124] Bladder Cancer: Diagnosis and Management of Bladder Cancer: (C) Nice (2015) Bladder Cancer: Diagnosis and Management of Bladder Cancer. BJU international. 2017;120(6):755-65.

[125] Power NE, Izawa J. Comparison of Guidelines on Non-Muscle Invasive Bladder Cancer (Eau, Cua, Aua, Nccn, Nice). Bladder cancer (Amsterdam, Netherlands). 2016;2(1):27-36.
[126] Fernandez-Gomez J, Madero R, Solsona E, Unda M, Martinez-Pineiro L, Gonzalez M, Portillo J, Ojea A, Pertusa C, Rodriguez-Molina J, Camacho JE, Rabadan M, Astobieta A, Montesinos M, Isorna S, Muntanola P, Gimeno A, Blas M, Martinez-Pineiro JA. Predicting Nonmuscle Invasive Bladder Cancer Recurrence and Progression in Patients Treated with Bacillus Calmette-Guerin: The Cueto Scoring Model. The Journal of urology. 2009;182(5):2195-203.
[127] Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW, Kurth K. Predicting Recurrence and Progression in Individual Patients with Stage Ta T1 Bladder Cancer Using Eortc Risk Tables: A Combined Analysis of 2596 Patients from Seven Eortc Trials. European urology. 2006;49(3):466-5; discussion 75-7.
[128] Fernandez-Gomez J, Madero R, Solsona E, Unda M, Martinez-Pineiro L, Ojea A, Portillo J, Montesinos M, Gonzalez M, Pertusa C, Rodriguez-Molina J, Camacho JE, Rabadan M, Astobieta A, Isorna S, Muntanola P, Gimeno A, Blas M, Martinez-Pineiro JA. The Eortc Tables Overestimate the Risk of Recurrence and Progression in Patients with Non-Muscle-Invasive Bladder Cancer Treated with Bacillus Calmette-Guerin: External Validation of the Eortc Risk Tables. European urology. 2011;60(3):423-30.
[129] Sylvester RJ. How Well Can You Actually Predict Which Non-Muscle-Invasive Bladder Cancer Patients Will Progress? Eur Urol. 2011;60(3):431-3; discussion 3-4.
[130] Xylinas E, Kent M, Kluth L, Pycha A, Comploj E, Svatek RS, Lotan Y, Trinh QD, Karakiewicz PI, Holmang S, Scherr DS, Zerbib M, Vickers AJ, Shariat SF. Accuracy of the Eortc Risk Tables and of the Cueto Scoring Model to Predict Outcomes in Non-Muscle-Invasive Urothelial Carcinoma of the Bladder. British journal of cancer. 2013;109(6):1460-6.
[131] van Rhijn BW, Zuiverloon TC, Vis AN, Radvanyi F, van Leenders GJ, Ooms BC, Kirkels WJ, Lockwood GA, Boeve ER, Jobsis AC, Zwarthoff EC, van der Kwast TH. Molecular Grade (Fgfr3/Mib-1) and Eortc Risk Scores Are Predictive in Primary Non-Muscle-Invasive Bladder Cancer. European urology. 2010;58(3):433-41.
[132] Alkhateeb SS, Neill M, Bar-Moshe S, Rhijn BV, Kakiashvili DM, Fleshner N, Jewett M, Petein M, Schulman C, Hanna S, Bostrom PJ, Roumeguere T, Shariat SF, Rorive S, Zlotta AR. Long-Term Prognostic Value of the Combination of Eortc Risk Group Calculator and Molecular Markers in Non-Muscle-Invasive Bladder Cancer Patients Treated with Intravesical Bacille Calmette-Guerin. Urology annals. 2011;3(3):119-26.
[133] Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Abnet CC. Association between Smoking and Risk of Bladder Cancer among Men and Women. JAMA : the journal of the American Medical Association. 2011;306(7):737-45.
[134] van Osch FH, Jochems SH, van Schooten FJ, Bryan RT, Zeegers MP. Significant Role of Lifetime Cigarette Smoking in Worsening Bladder Cancer and Upper Tract Urothelial Carcinoma Prognosis: A Meta-Analysis. The Journal of urology. 2016;195(4p1):872-9.
[135] Crivelli JJ, Xylinas E, Kluth LA, Rieken M, Rink M, Shariat SF. Effect of Smoking on Outcomes of Urothelial Carcinoma: A Systematic Review of the Literature. European urology. 2014;65(4):742-54.

[136] Murta-Nascimento C, Schmitz-Drager BJ, Zeegers MP, Steineck G, Kogevinas M, Real FX, Malats N. Epidemiology of Urinary Bladder Cancer: From Tumor Development to Patient's Death. World journal of urology. 2007;25(3):285-95.
[137] Cho KS, Seo HK, Joung JY, Park WS, Ro JY, Han KS, Chung J, Lee KH. Lymphovascular Invasion in Transurethral Resection Specimens as Predictor of Progression and Metastasis in Patients with Newly Diagnosed T1 Bladder Urothelial Cancer. The Journal of urology. 2009;182(6):2625-30.
[138] Orsola A, Trias I, Raventos CX, Espanol I, Cecchini L, Bucar S, Salinas D, Orsola I. Initial High-Grade T1 Urothelial Cell Carcinoma: Feasibility and Prognostic Significance of Lamina Propria Invasion Microstaging (T1a/B/C) in Bcg-Treated and Bcg-Non-Treated Patients. European urology. 2005;48(2):231-8; discussion 8.
[139] Andius P, Johansson SL, Holmang S. Prognostic Factors in Stage T1 Bladder Cancer: Tumor Pattern (Solid or Papillary) and Vascular Invasion More Important Than Depth of Invasion. Urology. 2007;70(4):758-62.
[140] van Rhijn BW, Liu L, Vis AN, Bostrom PJ, Zuiverloon TC, Fleshner NE, van der Aa MN, Alkhateeb SS, Bangma CH, Jewett MA, Zwarthoff EC, Bapat B, van der Kwast TH, Zlotta AR. Prognostic Value of Molecular Markers, Sub-Stage and European Organisation for the Research and Treatment of Cancer Risk Scores in Primary T1 Bladder Cancer. BJU international. 2012;110(8):1169-76.
[141] van Rhijn BW, van der Kwast TH, Alkhateeb SS, Fleshner NE, van Leenders GJ, Bostrom PJ, van der Aa MN, Kakiashvili DM, Bangma CH, Jewett MA, Zlotta AR. A New and Highly Prognostic System to Discern T1 Bladder Cancer Substage. European urology. 2012;61(2):378-84.
[142] Palou J, Rodriguez-Rubio F, Millan F, Algaba F, Rodriguez-Faba O, Huguet J, Villavicencio H. Recurrence at Three Months and High-Grade Recurrence as Prognostic Factor of Progression in Multivariate Analysis of T1g2 Bladder Tumors. Urology. 2009;73(6):1313-7.
[143] Golijanin D, Yossepowitch O, Beck SD, Sogani P, Dalbagni G. Carcinoma in a Bladder Diverticulum: Presentation and Treatment Outcome. The Journal of urology. 2003;170(5):1761-4.
[144] Palou J, Sylvester RJ, Faba OR, Parada R, Pena JA, Algaba F, Villavicencio H. Female Gender and Carcinoma in Situ in the Prostatic Urethra Are Prognostic Factors for Recurrence, Progression, and Disease-Specific Mortality in T1g3 Bladder Cancer Patients Treated with Bacillus Calmette-Guerin. European urology. 2012;62(1):118-25.
[145] Gontero P, Sylvester R, Pisano F, Joniau S, Vander Eeckt K, Serretta V, Larre S, Di Stasi S, Van Rhijn B, Witjes AJ, Grotenhuis AJ, Kiemeney LA, Colombo R, Briganti A, Babjuk M, Malmstrom PU, Oderda M, Irani J, Malats N, Baniel J, Mano R, Cai T, Cha EK, Ardelt P, Varkarakis J, Bartoletti R, Spahn M, Johansson R, Frea B, Soukup V, Xylinas E, Dalbagni G, Karnes RJ, Shariat SF, Palou J. Prognostic Factors and Risk Groups in T1g3 Non-Muscle-Invasive Bladder Cancer Patients Initially Treated with Bacillus Calmette-Guerin: Results of a Retrospective Multicenter Study of 2451 Patients. European urology. 2015;67(1):74-82.
[146] Dalbagni G, Vora K, Kaag M, Cronin A, Bochner B, Donat SM, Herr HW. Clinical Outcome in a Contemporary Series of Restaged Patients with Clinical T1 Bladder Cancer. European urology. 2009;56(6):903-10.
[147] Bishr M, Lattouf JB, Latour M, Saad F. Tumour Stage on Re-Staging Transurethral Resection Predicts Recurrence and Progression-Free Survival of Patients with High-Risk Non-Muscle Invasive Bladder Cancer. Canadian Urological

Association journal = Journal de l'Association des urologues du Canada. 2014;8(5-6):E306-10.
[148] Kim HS, Kim M, Jeong CW, Kwak C, Kim HH, Ku JH. Presence of Lymphovascular Invasion in Urothelial Bladder Cancer Specimens after Transurethral Resections Correlates with Risk of Upstaging and Survival: A Systematic Review and Meta-Analysis. Urologic oncology. 2014;32(8):1191-9.
[149] Comperat E, Roupret M, Yaxley J, Reynolds J, Varinot J, Ouzaid I, Cussenot O, Samaratunga H. Micropapillary Urothelial Carcinoma of the Urinary Bladder: A Clinicopathological Analysis of 72 Cases. Pathology. 2010;42(7):650-4.
[150] Kamat AM, Gee JR, Dinney CP, Grossman HB, Swanson DA, Millikan RE, Detry MA, Robinson TL, Pisters LL. The Case for Early Cystectomy in the Treatment of Nonmuscle Invasive Micropapillary Bladder Carcinoma. The Journal of urology. 2006;175(3 Pt 1):881-5.
[151] Amin A, Epstein JI. Noninvasive Micropapillary Urothelial Carcinoma: A Clinicopathologic Study of 18 Cases. Human pathology. 2012;43(12):2124-8.
[152] Wasco MJ, Daignault S, Zhang Y, Kunju LP, Kinnaman M, Braun T, Lee CT, Shah RB. Urothelial Carcinoma with Divergent Histologic Differentiation (Mixed Histologic Features) Predicts the Presence of Locally Advanced Bladder Cancer When Detected at Transurethral Resection. Urology. 2007;70(1):69-74.
[153] Blochin EB, Park KJ, Tickoo SK, Reuter VE, Al-Ahmadie H. Urothelial Carcinoma with Prominent Squamous Differentiation in the Setting of Neurogenic Bladder: Role of Human Papillomavirus Infection. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2012;25(11):1534-42.
[154] Fristrup N, Ulhoi BP, Birkenkamp-Demtroder K, Mansilla F, Sanchez-Carbayo M, Segersten U, Malmstrom PU, Hartmann A, Palou J, Alvarez-Mugica M, Zieger K, Borre M, Orntoft TF, Dyrskjot L. Cathepsin E, Maspin, Plk1, and Survivin Are Promising Prognostic Protein Markers for Progression in Non-Muscle Invasive Bladder Cancer. The American journal of pathology. 2012;180(5):1824-34.
[155] Palou J, Algaba F, Vera I, Rodriguez O, Villavicencio H, Sanchez-Carbayo M. Protein Expression Patterns of Ezrin Are Predictors of Progression in T1g3 Bladder Tumours Treated with Nonmaintenance Bacillus Calmette-Guerin. European urology. 2009;56(5):829-36.
[156] van Rhijn BW, van der Kwast TH, Liu L, Fleshner NE, Bostrom PJ, Vis AN, Alkhateeb SS, Bangma CH, Jewett MA, Zwarthoff EC, Zlotta AR, Bapat B. The Fgfr3 Mutation Is Related to Favorable Pt1 Bladder Cancer. The Journal of urology. 2012;187(1):310-4.
[157] Pan CC, Chang YH, Chen KK, Yu HJ, Sun CH, Ho DM. Prognostic Significance of the 2004 Who/Isup Classification for Prediction of Recurrence, Progression, and Cancer-Specific Mortality of Non-Muscle-Invasive Urothelial Tumors of the Urinary Bladder: A Clinicopathologic Study of 1,515 Cases. American journal of clinical pathology. 2010;133(5):788-95.
[158] van Roekel EH, Cheng KK, James ND, Wallace DM, Billingham LJ, Murray PG, Bryan RT, Zeegers MP. Smoking Is Associated with Lower Age, Higher Grade, Higher Stage, and Larger Size of Malignant Bladder Tumors at Diagnosis. International journal of cancer. 2013;133(2):446-54.
[159] Westhoff E. Body Mass Index, Diet-Related Factors, and Bladder Cancer Prognosis: A Systematic Review and Meta-Analysis.4(1):91-112.
[160] Westhoff E, Wu X, Kiemeney LA, Lerner SP, Ye Y, Huang M, Dinney CP, Vrieling A, Tu H. Dietary Patterns and Risk of Recurrence and Progression in Non-

Muscle-Invasive Bladder Cancer. International journal of cancer. 2018;142(9):1797-804.
[161] Grotenhuis AJ, Dudek AM, Verhaegh GW, Witjes JA, Aben KK, van der Marel SL, Vermeulen SH, Kiemeney LA. Prognostic Relevance of Urinary Bladder Cancer Susceptibility Loci. PloS one. 2014;9(2):e89164.
[162] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.
[163] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.
[164] Masson-Lecomte A, Lopez de Maturana E, Goddard ME, Picornell A, Rava M, Gonzalez-Neira A, Marquez M, Carrato A, Tardon A, Lloreta J, Garcia-Closas M, Silverman D, Rothman N, Kogevinas M, Allory Y, Chanock SJ, Real FX, Malats N. Inflammatory-Related Genetic Variants in Non-Muscle-Invasive Bladder Cancer Prognosis: A Multimarker Bayesian Assessment. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2016;25(7):1144-50.
[165] Lopez de Maturana E, Picornell A, Masson-Lecomte A, Kogevinas M, Marquez M, Carrato A, Tardon A, Lloreta J, Garcia-Closas M, Silverman D, Rothman N, Chanock S, Real FX, Goddard ME, Malats N. Prediction of Non-Muscle Invasive Bladder Cancer Outcomes Assessed by Innovative Multimarker Prognostic Models. BMC cancer. 2016;16:351.
[166] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).
[167] Lenormand C, Couteau J, Nouhaud FX, Maillet G, Bou J, Gobet F, Pfister C. Predictive Value of Nramp1 and Hgpx1 Gene Polymorphism for Maintenance Bcg Response in Non-Muscle-Invasive Bladder Cancer. Anticancer research. 2016;36(4):1737-43.
[168] O'Donnell PH, Alanee S, Stratton KL, Garcia-Grossman IR, Cao H, Ostrovnaya I, Plimack ER, Manschreck C, Ganshert C, Smith ND, Steinberg GD, Vijai J, Offit K, Stadler WM, Bajorin DF. Clinical Evaluation of Cisplatin Sensitivity of Germline Polymorphisms in Neoadjuvant Chemotherapy for Urothelial Cancer. Clinical genitourinary cancer. 2016;14(6):511-7.
[169] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be

Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.
[170] Deng X, Yang X, Cheng Y, Liu X, Li X, Zhao R, Qin C, Lu Q, Yin C. Gstp1 and Gsto1 Single Nucleotide Polymorphisms and the Response of Bladder Cancer Patients to Intravesical Chemotherapy. Scientific reports. 2015;5:14000.
[171] Li P, Zhang X, Deng X, Tao J, Qin C, Yang X, Cheng Y, Lu Q, Wang Z, Yin C. Pharmacogenetic Association between Xrcc1 Polymorphisms and Improved Outcomes in Bladder Cancer Patients Following Intravesical Instillation of Epirubicin. International journal of clinical and experimental medicine. 2015;8(7):11167-73.
[172] Zhou B, Zhang P, Tang T, Liao H, Zhang K, Pu Y, Chen P, Song Y, Zhang L. Polymorphisms and Plasma Levels of Il-27: Impact on Genetic Susceptibility and Clinical Outcome of Bladder Cancer. BMC cancer. 2015;15:433.
[173] Buffen K, Oosting M, Quintin J, Ng A, Kleinnijenhuis J, Kumar V, van de Vosse E, Wijmenga C, van Crevel R, Oosterwijk E, Grotenhuis AJ, Vermeulen SH, Kiemeney LA, van de Veerdonk FL, Chamilos G, Xavier RJ, van der Meer JW, Netea MG, Joosten LA. Autophagy Controls Bcg-Induced Trained Immunity and the Response to Intravesical Bcg Therapy for Bladder Cancer. PLoS pathogens. 2014;10(10):e1004485.
[174] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[175] Teo MT, Dyrskjot L, Nsengimana J, Buchwald C, Snowden H, Morgan J, Jensen JB, Knowles MA, Taylor G, Barrett JH, Borre M, Orntoft TF, Bishop DT, Kiltie AE. Next-Generation Sequencing Identifies Germline Mre11a Variants as Markers of Radiotherapy Outcomes in Muscle-Invasive Bladder Cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2014;25(4):877-83.
[176] Chen M, Hildebrandt MA, Clague J, Kamat AM, Picornell A, Chang J, Zhang X, Izzo J, Yang H, Lin J, Gu J, Chanock S, Kogevinas M, Rothman N, Silverman DT, Garcia-Closas M, Grossman HB, Dinney CP, Malats N, Wu X. Genetic Variations in the Sonic Hedgehog Pathway Affect Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Cancer prevention research (Philadelphia, Pa). 2010;3(10):1235-45.
[177] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.
[178] Wu X, Ros MM, Gu J, Kiemeney L. Epidemiology and Genetic Susceptibility to Bladder Cancer. BJU international. 2008;102(9 Pt B):1207-15.
[179] Lubin JH, Kogevinas M, Silverman D, Malats N, Garcia-Closas M, Tardon A, Hein DW, Garcia-Closas R, Serra C, Dosemeci M, Carrato A, Rothman N. Evidence for an Intensity-Dependent Interaction of Nat2 Acetylation Genotype and Cigarette Smoking in the Spanish Bladder Cancer Study. Int J Epidemiol. 2007;36(1):236-41.
[180] Lacombe L, Fradet V, Levesque E, Pouliot F, Larue H, Bergeron A, Hovington H, Caron A, Nguile-Makao M, Harvey M, Fradet Y, Guillemette C. Phase Ii Drug-Metabolizing Polymorphisms and Smoking Predict Recurrence of Non-Muscle-Invasive Bladder Cancer: A Gene-Smoking Interaction. Cancer prevention research (Philadelphia, Pa). 2016;9(2):189-95.

[181] Svatek RS, Hollenbeck BK, Holmang S, Lee R, Kim SP, Stenzl A, Lotan Y. The Economics of Bladder Cancer: Costs and Considerations of Caring for This Disease. Eur Urol. 2014;66(2):253-62.
[182] Zamboni S, Moschini M, Simeone C, Antonelli A, Mattei A, Baumeister P, Xylinas E, Hakenberg OW, Aziz A. Prediction Tools in Non-Muscle Invasive Bladder Cancer. Translational andrology and urology. 2019;8(1):39-45.

CHAPTER 2.

PREPARATION OF THE DATA: QUALITY CONTROL AND GENOTYPE IMPUTATION

2.1. STUDY PARTICIPANTS

The West Midlands Bladder Cancer Prognosis Programme (BCPP)

Subjects for the main study were obtained from the West Midlands Bladder Cancer Prognosis Programme [1]. BCPP is a prospective cohort initiated by the Cancer Research UK Bladder Cancer Group at the University of Birmingham that focuses on investigating prognostic factors for urinary bladder cancer (UBC). Besides identifying determinants for UBC recurrence and progression, the initiative aimed to use the collected data for developing a more accurate prognostic tool for predicting adverse UBC outcomes.

Subjects were initially recruited from the West Midlands region during the period between 2005 to 2011, resulting in 1,544 eligible participants. Patients were identified at haematuria clinics, and were deemed eligible if they had cystoscopy that was suggestive of a UBC diagnosis. All enrolled participants had a pathological UBC diagnosis confirmation. Patients with cancer diagnosis of the urinary system (bladder, urethra, ureter, renal pelvis) within the last decade were excluded in the recruitment process.

BCPP has three broad aims, each focusing on different determinants for UBC prognosis: modifiable exposures (e.g. smoking, diet), quality of life, and molecular markers. Data on lifestyle factors and various exposures (e.g. smoking, occupation, diet, medication use, social descriptors) were collected via semi-structured face-to-face interviews and questionnaires. Trained research nurses carried out the interviews at baseline, whilst additional ongoing information was provided by patients submitting questionnaires by post afterwards [1]. Specific clinical characteristics on tumour stage and grade were retrieved from medical records to complete the pathological data. The

size of the largest tumour was established visually whilst performing TURBT. Biological samples included urine, tumour tissue, and blood specimens, all collected at baseline. In total, blood samples for 888 UBC patients were genotyped using Illumina HumanOmniExpress BeadChip at deCODE Genetics [2] (Reykjavik, Iceland). All analyses in BCPP were limited to the non-muscle-invasive bladder cancer (NMIBC) patients (N=712), corresponding to stages pTa, pT1, and pTis.

Nottingham Multi-Centre Research Ethics Committee provided the ethical approval for the BCPP study (reference number: 06/MRE04/65; clinicaltrials.gov registration number: NCT00553215). Informed consent at baseline was obtained from all participants.

The Nijmegen Bladder Cancer Study (NBCS)

Participants for the NBCS were selected from the regional Cancer Registry, covering the Eastern part of the Netherlands. Recruitment took place between years 1995 and 2006, and targeted UBC cases under the age of 75.

Clinical data were obtained via medical records, whilst other information was collected using self-administered lifestyle questionnaires.

Following informed consent, patients also provided blood samples for genotyping, which was carried out on the on the HumanHap300 and HumanHapCNV370 BeadChip panel (Illumina, Inc., San Diego, USA). Initially, full genotype and clinical data were available for circa 1,200 UBC patients, all of European descent. Additional quality control and the thresholds used for data cleaning are discussed in the initial study [3].

UK Biobank

UK Biobank is a large, population-based cohort in the United Kingdom, containing data on more than 500,000 participants [4]. Recruitment took place 2006-2010 and included people aged 40-69 years. The cohort includes a variety of data sources, which allowed us to analyse a sample of 1,534 UBC cases via the data from Cancer Registry. The codes used to identify bladder cancer patients included C67.0-C67.9 (International Classification of Diseases (ICD) 10) and 1880, 1882, 1884, 1886, 1888, 1889, 2337 (ICD9).

Clinical data were obtained from Hospital Episode Statistics (HES), whilst demographic data were recorded via self-administered questionnaires. Genotyping was carried out on a specifically-developed UK Biobank Axiom Array [5] . All analyses were limited to White British participants, as defined by the UK Biobank study. Our use of the UK Biobank data has been registered under Application Number 42772. Additional procedures on data collection and processing are described in detail elsewhere [4, 5].

2.2 QUALITY CONTROL (QC)

Pre-imputation data cleaning was divided into two main steps: per-individual and per-marker QC. This approach helps to retain a higher number of genetic markers used in the analyses, as exclusion of a participant virtually has little impact on the count of total single nucleotide polymorphism (SNP) panel; however, marker exclusion prevents testing them for an association in the whole sample. Hence, individuals with overall flawed data are identified first.

All QC procedures were carried out using PLINK v1.90 (released 17th November 2016) [6, 7].

2.2.1 Per-individual QC

Per-individual QC [8-10] covers the following steps:

a) Genetic sex check;

b) Estimation of missing genotypes per individual;

c) Evaluating genotype heterozygosity rate;

d) Identifying related and duplicated individuals;

e) Detecting population stratification.

a) A genetic sex check primarily yields to identify the discordance between phenotypically-assigned and de-facto gender. The procedure is very useful for identifying potential sample swaps or accidental errors and allows to prevent further misclassification. Moreover, it can help to identify individuals with karyotypic chromosome abnormalities that may have a significant impact on study results.

Genetic sex is determined by the X chromosome homozygosity rate, otherwise called an F statistic. The rationale is that females have two X chromosomes and are expected to be highly heterozygous in their genotypes (due to the presence of many two-allele combinations from both chromosomes). Hence, females' X chromosome genotypes are expected to follow a Hardy-Weinberg equilibrium (HWE) and result in an F statistic of homozygosity <0.2. Males, on the other hand, have only one X chromosome and are expected to be mostly homozygous (reaching F>0.8) [9-11].

However, there are some occasions in which individuals fall in between those thresholds, yielding an "inconclusive" genetic sex call. That might be a result of multiple causes: low individual genotyping rate, mosaicism (where only a fraction of cells carry an abnormal karyotype, whilst the rest are normal), loss of heterozygosity

in females' X chromosome, or karyotype anomalies (i.e. males with Kleinfelter syndrome (XXY), females with Turner syndrome (XO)) [10]. Whilst the homozygosity rate is a good indication for identifying individuals that might skew the results of an analysis, it is worth clarifying the F statistic itself does not provide specifics on the cause of the discrepancy. However, it can be investigated further with access to clinical (e.g. checking for any reported genetic conditions) or array data (e.g. X chromosome probe intensity plots, referred to as LogR ratios in Illumina platform or probe intensity in Affymetrix) [10].

Since our dataset did not have gender assigned phenotypically, no sample swaps could have been identified, even if those had taken place. However, the procedure identified three individuals with inconclusive calls on gender (yielding F statistic values of 0.77, 0.56, and 0.62). Although a definite reason for the discordance is difficult to give, it is worth noting that these three samples also come up in sequential QC steps as well, most likely indicating low-quality samples. Therefore, the three samples were added to the list for exclusion.

b) High missing genotype rate per individual indicates low sample quality and might bias the association result (e.g. if missingness differs for the categories of a studied phenotype). It is more often observed in case-control or multi-centre studies, where different sets of samples were collected and/or genotyped separately. Cohort studies, such as BCPP, suffer less from this problem; however, they are not immune to low-quality samples. An exact threshold level for excluding individuals varies on a study-to-study basis; it was usual to apply a threshold of 3-7% for missing genotypes per individual for exclusion [10], whereas nowadays it is reasonable to apply a more stringent criterion. In our sample, 49 individuals have failed the QC step due to having more than 2% of their genotype missing. Missing genotype fraction per individual is

also presented in a histogram (**Figure 2.1**), which shows most individuals have a high genotype rate (approximately 99%).

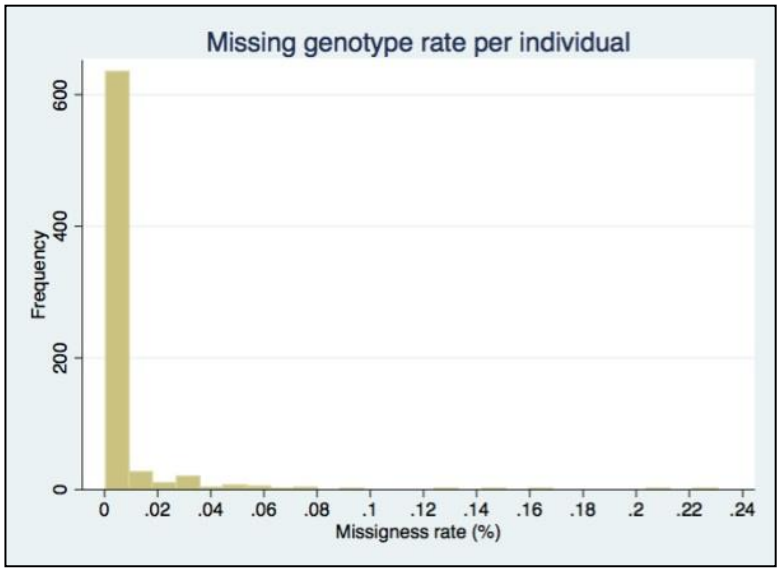


Figure 2.1. Missing genotype rate per individual.

c) Excessively increased or reduced proportion of heterozygous genotypes in the sample might also signal the presence of sample contamination or inbreeding. Mean genotype heterozygosity is calculated using the individual-level information on genotype non-missingness (*N(NM)*) and the number of homozygous genotypes (*E(HOM)*), which can then be used to calculate observed heterozygosity rate per person (*N(NM)-E(HOM))/N(NM)*). Although an exact threshold for excluding individuals varies on a study-to-study basis, it is usual to exclude those with observed heterozygosity fluctuating 3 standard deviations (SDs) from the mean [8]. In the case of BCPP, the mean was equal to 0.3168287, SD=0.0065949; hence all individuals exceeding heterozygosity rate of 0.3366134 and having a lower rate than 0.2970440 were identified for further exclusion (N=12). **Figure 2.2** shows the sample distribution under thresholds of excessive missingness and heterozygosity.

d) Genetic association study assumes all tested individuals are non-related, and failure to adjust for cryptic relatedness may produce clusters of genotypes in an

otherwise random collection of participants. For that reason, any related individuals and duplicates should be identified and excluded from the sample.

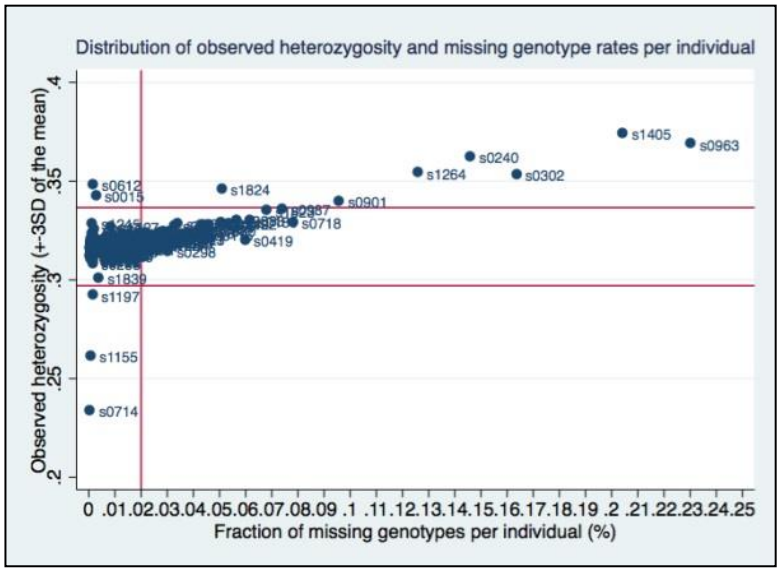


Figure 2.2. Observed heterozygosity and missing genotype rates (red lines indicate applied thresholds).

Even though all people share genomic regions (referred to as identical-by-state (IBS)), being related increases the magnitude of IBS regions. In return, estimated IBS metric provides information on the level of relatedness, i.e. identity-by-decent (IBD). An IBS equal to 1 indicates sample duplication, whilst IBS of 0.5 signifies first-degree relative [8]. Before calculating IBS, it is essential to first exclude regions of high linkage-disequilibrium (LD) [12] and correlated regions (R^2>0.2), as those result in identification of falsely-related participants [8]. Afterwards, participant pairs exceeding IBS of 0.1875 (showing relatedness halfway between third- and second-degree relatives) are identified. As only one individual from a pair is enough to be excluded to prevent sample sub-structure, the participant with a lower genotyping rate is listed for further data cleaning.

Relatedness analysis in the BCPP dataset revealed two pairs of participants who had a greater-than-expected IBS level. Out of each pair of related individuals, participants with a lower genotype rate were added to the exclusion list.

e) Population stratification is one of the most important forms of confounding in a genome-wide association study (GWAS). Allele frequencies differ between the populations, and consecutively, so do distributions in phenotypic traits. In a GWAS with subpopulations present, detected differences in outcomes might be wrongly attributed to a certain genotype when in fact it is caused by population stratification [13]. To address the issue, we have carried out a multi-stage principal component analysis (PCA). PCA identifies genetic clusters (eigenvectors), which explain proportions of genetic variation within the sample. Additionally, all individuals are assigned a score (an eigenvalue) for every eigenvector.

Importantly, BCPP contains self-reported data on ethnic background, but its validity was never verified. To investigate if these records are accurate, we have firstly compared the self-reported ethnicity records in the BCPP to a publicly-available reference panel of 1,000 Genomes (Phase 3, released 2nd May 2013) [14]. Autosomal 1,000 Genomes data were pruned of high-LD regions and merged with the BCPP genome dataset. Afterwards, a PCA analysis was conducted on a combined sample of 3,216 people (NMIBC cases in the BCPP (N=712) and 1,000 genomes (N=2,504)). **Figure 2.3** shows the distribution of individuals across the first 2 principal components (PCs) in both samples. Self-reported ethnic background in the BCPP represented five categories: Black (Caribbean), Indian, Pakistani, White, and Other; whereas data in 1000 Genomes can be stratified into five superpopulations: East Asians (EAS), Europeans (EUR), Africans (AFR), Ad-mixed Americans (AMR), and South Asians

(SAS). As shown in **Figure 2.3,** the self-reported ethnicities are in an overall agreement with the 1,000 Genomes reference panel.

Furthermore, superpopulations in 1,000 Genomes can be further stratified into smaller groups, each of those containing from five to seven ethnic origins:

- ***EAS***: Chinese Dai in Xishuangbanna (China (CDX)); Han Chinese in Beijing (China (CHB)), Southern Han Chinese (CHS), Japanese in Tokyo (Japan (JPT)), Kinh in Ho Chi Minh City (Vietnam (KHV));

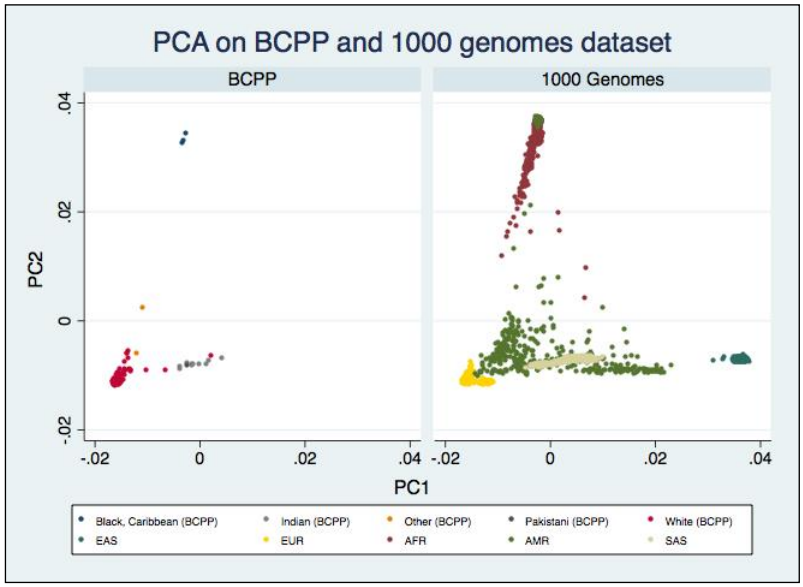


Figure 2.3. Distribution of individuals against first 2 PCs of a joint PCA analysis, presented separately for BCPP and 1,000 Genomes Phase 3 samples.

- ***EUR***: Utah Residents (CEPH) with Northern and Western European Ancestry (CEU), Finnish in Finland (FIN), British in England and Scotland (GBR), Iberian Population in Spain (IBS), Toscani in Italia (TSI);
- ***AFR***: African Caribbeans in Barbados (ACB), Americans of African Ancestry in SW USA (ASW), Esan in Nigeria (ESN), Gambian in Western Divisions in the Gambia (GWD), Luhya in Webuye (Kenya (LWK)), Mende in Sierra Leone (MSL), Yoruba in Ibadan (Nigeria (YRI));

- ***AMR***: Colombians from Medellin (Colombia (CLM)), Mexican Ancestry from Los Angeles USA (MXL), Peruvians from Lima (Peru (PEL)), Puerto Ricans from Puerto Rico (PUR);
- ***SAS***: Bengali from Bangladesh (BEB), Gujarati Indian from Houston (Texas (GIH)), Indian Telugu from the UK (ITU), Punjabi from Lahore (Pakistan (PJL)), Sri Lankan Tamil from the UK (STU).

Figures 2.4 and **2.5** show the eigenvalue distribution for populations of European and African origins, respectively. In **Figure 2.4,** self-reported "White" participants plot similarly to European populations in the 1,000 Genomes. Expectedly, most eigenvalues in the BCPP correspond to the population of GBR in 1,000 Genomes.

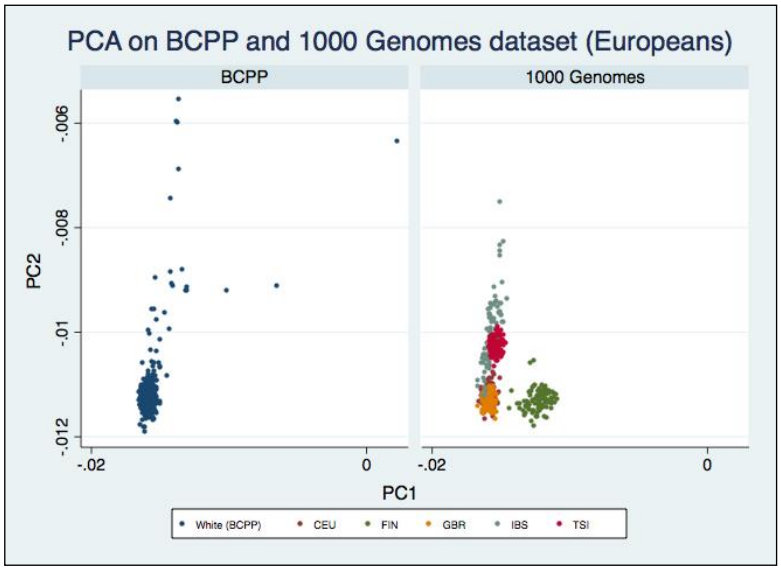


Figure 2.4. Distribution of individuals of European decent against first 2 PCs of joint PCA analysis, presented separately for BCPP and 1,000 Genomes Phase 3 samples.

As shown in **Figure 2.5**, BCPP self-identified "Black, Caribbean" participants cluster similarly to the ACB population in the 1,000 Genomes (**Figure 2.5**).

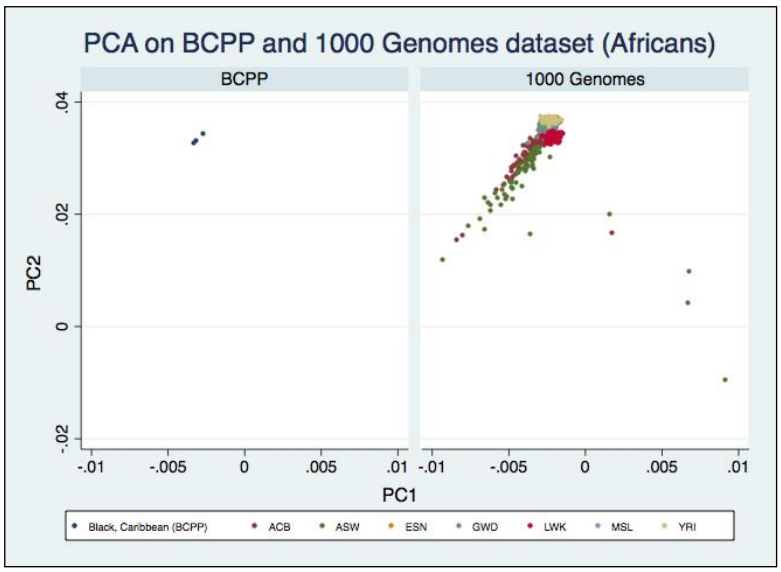


Figure 2.5. Distribution of individuals of African descent against first 2 PCs of a joint PCA analysis, presented separately for BCPP and 1,000 Genomes Phase 3 samples.

Adding a third PC to visualise genetic distances across individuals in a merged dataset shows little new information is added with an additional PC; hence, the first 2 PCs are enough to make a decision on population structure (**Figure 2.6** and **2.7**).

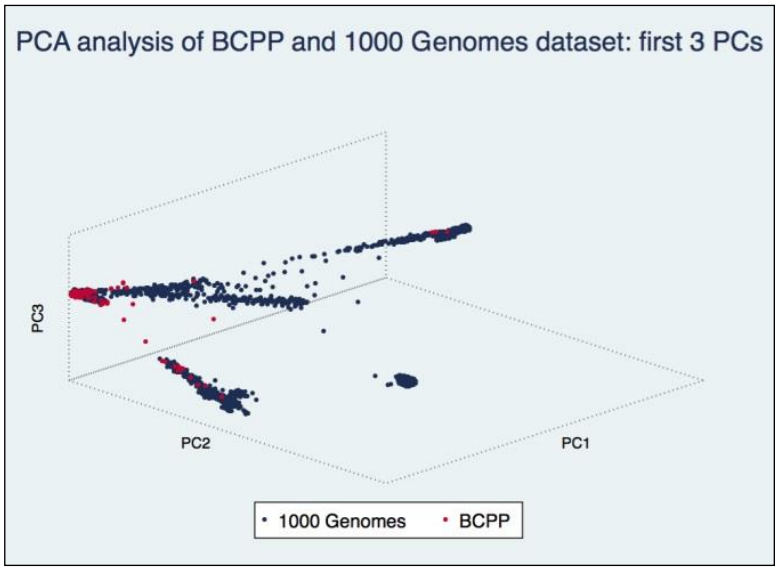


Figure 2.6. First three principal components of merged BCPP and 1,000 Genomes Phase 3 genotypes.

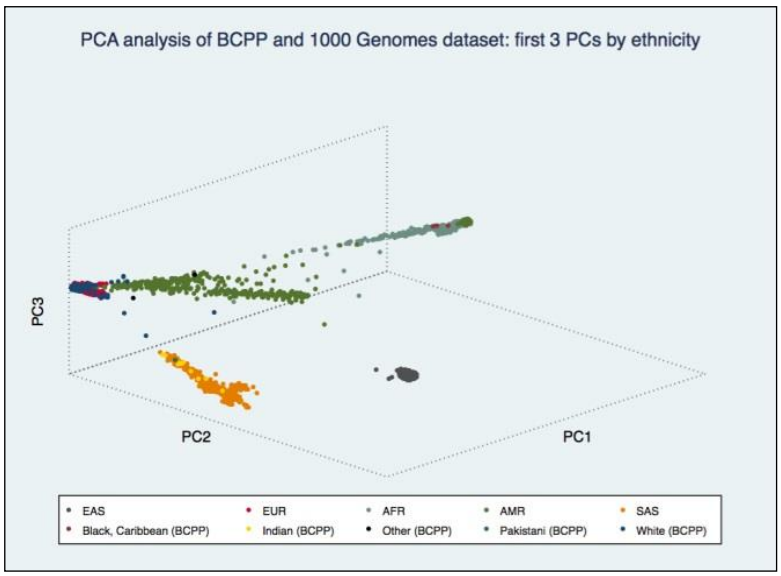


Figure 2.7. First three principal components of merged BCPP and 1,000 Genomes Phase 3 genotypes; stratified by self-reported ethnicity (BCPP) and superpopulation (1,000 Genomes).

After confirming that the self-reported information on ethnicity in the BCPP is valid, we conducted a PCA on BCPP dataset only to clearly identify existing outliers. Plotting the two top genetic PCs in BCPP shows at least four individuals do not fall into the general cluster, which were added to the list of participants to exclude from further analyses (**Figure 2.8**).

At the end of all per-individual QC procedures, 59 unique individuals are identified (70, including 11 duplicates) and excluded from the dataset, leaving 653 NMIBC cases.

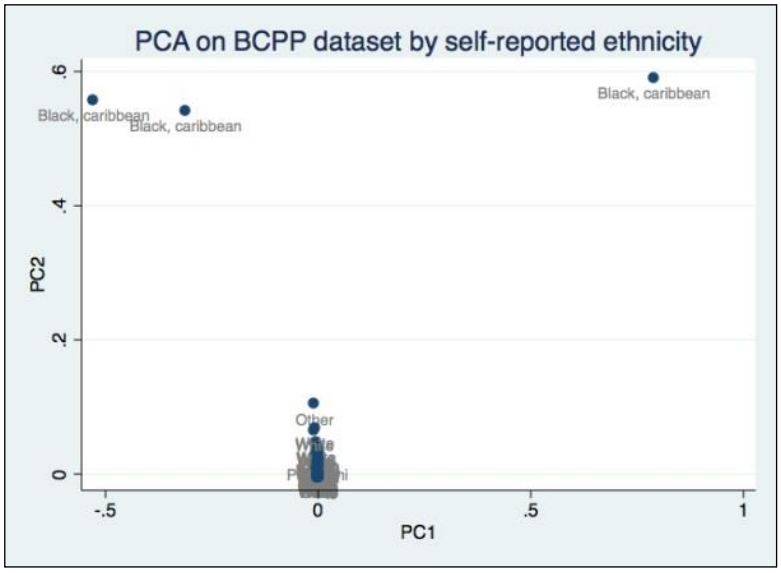


Figure 2.8. Top two principal genetic components in BCPP cohort with self-reported ethnicity.

2.2.2. Per-marker QC

Generic marker-specific QC consists of the following steps:

a) Identifying markers with an excessive missing genotype rate;

b) Checking genotype calls for cases and controls;

c) Checking for HWE;

d) Filtering markers with low minor allele frequencies (MAF).

a) Excluding markers with high missing genotype rate is essential to avoid having low-quality SNPs in the dataset. Markers that are missing for a large fraction of tested individuals might result in a false-positive association and/or reduced power of the study, as fewer SNPs are tested overall. It is usual to filter out SNPs with a missing rate of 5% or higher [8]; however, in recent years, a more strict threshold of 2% has been favoured, which is also used in our study.

b) If the variant is missing at significantly different rates for cases and controls, it creates a substructure within the sample, which might be a source of bias in genetic association studies. However, our study is designed to consist of bladder cases only, without having clearly defined case and control groups. Instead, there are multiple outcomes of both categorical and continuous nature, hence this step is omitted from QC.

c) HWE describes a distribution of alleles (and genotypes) that remain stable in subsequent generations under a specific set of conditions. Deviation from the

equilibrium might occur due to events of mutations or inbreeding, but can also indicate genotyping errors or population substructure [10, 15]. In case-control designs, only controls are tested to see if they comply to HWE, as cases might deviate from the distribution if some variants are strongly associated with an outcome [8, 15]. As our cohort consists fully of cases, HWE assumption is tested for everyone in the sample.

d) Markers with low MAF are usually excluded, since they are prone to falsely appear as significant results just because they are rare. Otherwise, a very large sample size is needed to provide enough power for those variants to be analysed. Our study is underpowered to discover rare variants; hence, all SNPs with MAFs of $\leq$1% are excluded [8].

A separate QC pipeline was developed for the X chromosome, as handling variants on non-autosomal regions calls for a different approach [16, 17]. Firstly, the X chromosome data was split by gender. It is expected that genetic variants on male X chromosome will naturally not comply to the HWE; therefore, compliance to HWE is only tested in the female group. Both – males' and females' - X chromosome variants have been additionally pruned for monomorphic and/or rare variants with a MAF threshold of 1%, whilst the threshold for genotype missingness was set at 2%.

All per-marker exclusion criteria have resulted in an exclusion of 158 SNPs due to deviations from HWE, 37,912 SNPs with low MAFs, and 14,631 SNPs with missing rate higher than or equal to 2% across all individuals. The impact of each criterion on a chromosome level can be seen in **Table 2.1**.

After completing both quality control steps for individuals and SNPs, a clean dataset consisting of 653 individuals and 597,764 markers was available for further analyses.

Table 2.1. SNP count before and after applying per-marker quality control procedures.

Chromosome	SNP count before QC	Total call rate (%)	SNPs lost due to high missingness (≥2%)	HWE (<0.0001)	MAF (≤1%)	SNP count after QC
1	52489	0.997383	934	11	3773	47771
2	51369	0.997316	980	14	2972	47403
3	42468	0.997163	855	12	2456	39145
4	36597	0.996885	857	6	1953	33781
5	38053	0.997269	747	13	1734	35559
6	43409	0.997101	956	12	2185	40256
7	34086	0.997152	735	5	1602	31744
8	33260	0.997375	582	3	1551	31124
9	29810	0.997347	562	6	1526	27716
10	35201	0.997455	606	13	2236	32346
11	32884	0.997385	593	8	1957	30326
12	32055	0.99726	644	6	1850	29555
13	25015	0.996979	576	6	1574	22859
14	21001	0.997341	429	5	1342	19225
15	19493	0.997579	333	9	1185	17966
16	20030	0.997704	284	6	1052	18688
17	17743	0.997411	338	5	1088	16312
18	19450	0.99718	410	6	1204	17830
19	12977	0.997142	284	5	698	11990
20	16383	0.997477	302	2	861	15218
21	9103	0.997152	191	2	464	8446
22	9245	0.997522	167	3	479	8596
Total (22)	**632121**	**0.997281**	**12365**	**158**	**35742**	**583856**
X (Females)	15253	0.997493	450	0	1078	13725
X (Males)	15253	0.9908	1816	N/A	1092	12345
Total (22, X)	**647374**	**0.995895**	**14631**	**158**	**37912**	**597764***

*(the number does not equal sum of all above fields due to SNP overlap on X chromosome).
HWE-Hardy-Weinberg Equilibrium; MAF-minor allele frequency; SNP-single nucleotide polymorphism; QC-quality control.

After obtaining the final set of individuals to proceed with further analyses, it was considered worthy of an additional investigation on residual population stratification in the BCPP sample. The exclusion of individuals based on PCA results can be considered subjective, and an effort to maintain the balance between high QC and an excessive dataset reduction might not always be well preserved. To investigate this, a basic preliminary association analysis was undertaken for all outcomes of interest in PLINK software to estimate the genomic inflation factor (λ) value, which, if

substantially inflated (λ>1.1 [18], or as recently more strict criteria in GWAS have been introduced, λ>1.05 [19]), indicates potential population stratification. The preliminary analysis showed that the lambda metric did not exceed 1.05 for all of the tested outcomes (tumour size (cm), grade (G3/G2+G1), stage (T1+Tis/Ta), and patient's age (years), further described in detail in **Chapter 4**).

2.3 IMPUTATION OF THE BCPP DATA

Despite genetic epidemiology techniques undergoing a remarkable improvement in recent years, genotyping arrays usually assay somewhere between 300,000 and 1,000,000 single-nucleotide variants [20]. Considering that the human genome consists of approximately 3 billion base-pairs, a vast majority of the genome is usually missing from the analyses, potentially preventing a significant discovery. Genotype imputation techniques, which entail predicting a missing genotype based on a reference genotype panel (that is much more dense), are very useful in increasing study power, fine-mapping observed associations, and conducting meta-analyses [21].

A reference panel of 1000 Genomes Phase 3 (released 2nd May 2013) [14] was chosen to carry out imputation in the genome build 19 (GRCh37/hg19). Even though the 1000 Genomes data has been remapped into the newest reference of genome build (GRCh38/hg38), it is still difficult to obtain all corresponding files needed for a successful imputation (e.g. legend files, that carry a specific format). For that reason, BCPP data (annotated in the GRCh38 assembly), has been converted into an earlier format of GRCh37 (hg19), using a freely-accessible liftOver software [22].

The most commonly used imputation software is BEAGLE [23], IMPUTE2 [24], and MACH [25]. After referring to detailed reports in the literature comparing each tool's strengths and weaknesses [21, 26, 27], it has been decided to use IMPUTE2. Imputation takes the form of a two-step process: genotype phasing and imputing. The software tool for phasing recommended by IMPUTE2 is SHAPEIT [28, 29]. However, we have chosen to use a newly-released software, Eagle v2.3.2 [30] (latest release used: March 2017), as it has been recently estimated to increase in computational speed by 20% and haplotype assignment accuracy by approximately 10%, in comparison to SHAPEIT [30]. Due to an efficient algorithm, it has also become a default phasing software used in publicly available imputation servers [20, 31].

As with pre-imputation QC, there are several steps taken to evaluate the quality of imputation:

a) Estimating accuracy (concordance rate);

b) Filtering variants with low MAF (≤1%).

a) A gold standard measure of imputation quality is the concordance rate between imputed and genotyped calls [32]. Concordance rate, or accuracy, makes use of a masked analysis [27]. Initially, all directly-genotyped markers are masked and are imputed by making a best-guess generated by the algorithm. Afterwards, imputed results are compared to the actual genotype to provide a concordance rate in the form of a percentage. The accuracy is presented in two ways: as an overall measure for all tested SNPs (written in a summary file of IMPUTE2) and as a per-marker score (presented in an "info" output in IMPUTE2**).** It is usually regarded that the overall concordance rate of 95% or more indicates high imputation accuracy [27].

Imputation accuracy is dependent on the use of a reference panel. Evidence shows that the Haplotype Research Consortium (HRC) panel, consisting of more than 32,000

haplotypes [31] is the most suitable reference for European-population based samples. HRC also includes 1,000 Genomes panel, among many other, and has been reported to have a substantial increase in imputing variants with low MAF [33]. Significant improvements in imputation quality have already been documented, during a global transition from using HapMap to much bigger 1,000 Genomes data as a reference panel [34]. However, time constraints, computational limits, and ethical consideration for using an external imputation server have prevented us from using the HRC in our analyses. Instead, the optimal choice was to rely on a manual imputation process using 1,000 Genomes Phase 3 dataset, containing 2,504 samples. Once imputed, the dataset was filtered for SNPs with an info score (i.e. the concordance rate) of >0.3 and MAFs of >1%, resulting in a dataset containing 11,914,228 markers available for genetic association analyses.

2.4 POST-HOC STATISTICAL POWER CALCULATIONS

Our analyses are subject to many limitations, and pots-hoc power calculations provide a better context whilst interpreting all results, summarised in chapters hereafter.

Genome-wide association analyses

To estimate the power of our analyses, we have used methods described in a publication by Moore et al [35], which has been implemented in an R package "genpwr".

All calculations assume an additive model and a statistical significance level of 5e-08.

Tumour size as a continuous outcome

Sample size=653

Assumed effect allele frequency (lowest observed in the analyses) = 0.01

Effect size as estimated in the linear regression=0.9 (centimetres of tumour size) and standard deviation of 0.5.

Estimated power at alpha level of 5e-08 = 87%.

Age at diagnosis as a continuous outcome

Sample size=653

Assumed effect allele frequency (lowest observed in the analyses) = 0.04

Effect size as estimated in the linear regression =0.5 (age in years) and standard deviation of 0.5.

Estimated power at alpha level of 5e-08 = 96%.

Grade as a categorical variable

Sample size=653 (N(cases)=207, N(controls)=436)

Assumed effect allele frequency (lowest observed in the analyses) = 0.06

Odds ratio as estimated in logistic regression=3.6

Estimated power at alpha level of 5e-08 = 46%.

Stage as a categorical variable

Sample size=653 (N(cases)=209, N(controls)=444)

Assumed effect allele frequency (lowest observed in the analyses) = 0.02

Odds ratio as estimated in logistic regression=0.02

Estimated power at alpha level of 5e-08 = 13%.

Age as a categorical variable

Sample size=653 (N(cases)=355, N(controls)=298)

Assumed effect allele frequency (lowest observed in the analyses) = 0.16

Odds ratio as estimated in logistic regression=2.5

Estimated power at alpha level of 5e-08 = 66%.

Gene environment interaction with smoking for tumour size as an outcome

Environment status is assumed to be binary in nature (ever smokers versus never smokers). Statistical significance level is set at alpha=0.05.

Total sample size=546.

Assumed effect allele frequency (lowest observed in the analyses) = 0.01

Effect size of the genotype in linear regression=0.5 (centimetres of tumour size)

Effect size of the gene-environment interaction term in linear regression=4.1 (median of all effect sizes reported in our gene-environment interaction analyses)

Estimated power at alpha level of 0.05 = 47%.

REFERENCES

[1] Zeegers MP, Bryan RT, Langford C, Billingham L, Murray P, Deshmukh NS, Hussain S, James N, Wallace DM, Cheng KK. The West Midlands Bladder Cancer Prognosis Programme: Rationale and Design. BJU international. 2010;105(6):784-8.

[2] Hakonarson H, Gulcher JR, Stefansson K. Decode Genetics, Inc. Pharmacogenomics. 2003;4(2):209-15.

[3] Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KK, Stacey SN, Gudmundsson J, Jakobsdottir M, Bergthorsson JT, Sigurdsson A, Blondal T, Witjes JA, Vermeulen SH, Hulsbergen-van de Kaa CA, Swinkels DW, Ploeg M, Cornel EB, Vergunst H, Thorgeirsson TE, Gudbjartsson D, Gudjonsson SA, Thorleifsson G, Kristinsson KT, Mouy M, Snorradottir S, Placidi D, Campagna M, Arici C, Koppova K, Gurzau E, Rudnai P, Kellen E, Polidoro S, Guarrera S, Sacerdote C, Sanchez M, Saez B, Valdivia G, Ryk C, de Verdier P, Lindblom A, Golka K, Bishop DT, Knowles MA, Nikulasson S, Petursdottir V, Jonsson E, Geirsson G, Kristjansson B, Mayordomo JI, Steineck G, Porru S, Buntinx F, Zeegers MP, Fletcher T, Kumar R, Matullo G, Vineis P, Kiltie AE, Gulcher JR, Thorsteinsdottir U, Kong A, Rafnar T, Stefansson K. Sequence Variant on 8q24 Confers Susceptibility to Urinary Bladder Cancer. Nat Genet. 2008;40(11):1307-12.

[4] Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. Uk Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS medicine. 2015;12(3):e1001779.

[5] Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The Uk Biobank Resource with Deep Phenotyping and Genomic Data. Nature. 2018;562(7726):203-9.

[6] Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-Generation Plink: Rising to the Challenge of Larger and Richer Datasets. GigaScience. 2015;4:7.

[7] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. American journal of human genetics. 2007;81(3):559-75.

[8] Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data Quality Control in Genetic Case-Control Association Studies. Nature protocols. 2010;5(9):1564-73.

[9] Weale ME. Quality Control for Genome-Wide Association Studies. Methods in molecular biology (Clifton, NJ). 2010;628:341-72.

[10] Turner S, Armstrong LL, Bradford Y, Carlson CS, Crawford DC, Crenshaw AT, de Andrade M, Doheny KF, Haines JL, Hayes G, Jarvik G, Jiang L, Kullo IJ, Li R, Ling H, Manolio TA, Matsumoto M, McCarty CA, McDavid AN, Mirel DB, Paschall JE, Pugh EW, Rasmussen LV, Wilke RA, Zuvich RL, Ritchie MD. Quality Control Procedures for Genome-Wide Association Studies. Current protocols in human genetics. 2011;Chapter 1:Unit1.19.

[11] Marees AT, de Kluiver H, Stringer S, Vorspan F, Curis E, Marie-Claire C, Derks EM. A Tutorial on Conducting Genome-Wide Association Studies: Quality Control and Statistical Analysis. International journal of methods in psychiatric research. 2018;27(2):e1608.

[12] Price AL, Weale ME, Patterson N, Myers SR, Need AC, Shianna KV, Ge D, Rotter JI, Torres E, Taylor Kent D, Goldstein DB, Reich D. Long-Range Ld Can Confound Genome Scans in Admixed Populations. American Journal of Human Genetics. 2008;83(1):132-5.
[13] Cardon LR, Palmer LJ. Population Stratification and Spurious Allelic Association. Lancet (London, England). 2003;361(9357):598-604.
[14] Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, Tasse AM, Flicek P. The International Genome Sample Resource (Igsr): A Worldwide Collection of Genome Variation Incorporating the 1000 Genomes Project Data. Nucleic acids research. 2017;45(D1):D854-d9.
[15] Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Rational Inferences About Departures from Hardy-Weinberg Equilibrium. Am J Hum Genet. 2005;76(6):967-86.
[16] Konig IR, Loley C, Erdmann J, Ziegler A. How to Include Chromosome X in Your Genome-Wide Association Study. Genetic epidemiology. 2014;38(2):97-103.
[17] Gao F, Chang D, Biddanda A, Ma L, Guo Y, Zhou Z, Keinan A. Xwas: A Software Toolset for Genetic Data Analysis and Association Studies of the X Chromosome. Journal of Heredity. 2015;106(5):666-71.
[18] Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Mägi R, Ferreira T, Fall T, Graff M, Justice AE, Luan J, Gustafsson S, Randall JC, Vedantam S, Workalemahu T, Kilpeläinen TO, Scherag A, Esko T, Kutalik Z, Heid IM, Loos RJF. Quality Control and Conduct of Genome-Wide Association Meta-Analyses. Nature protocols. 2014;9(5):1192-212.
[19] Power RA, Parkhill J, de Oliveira T. Microbial Genome-Wide Association Studies: Lessons from Human Gwas. Nature reviews Genetics. 2017;18(1):41-50.
[20] Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, Schlessinger D, Stambolian D, Loh PR, Iacono WG, Swaroop A, Scott LJ, Cucca F, Kronenberg F, Boehnke M, Abecasis GR, Fuchsberger C. Next-Generation Genotype Imputation Service and Methods. Nature genetics. 2016;48(10):1284-7.
[21] Marchini J, Howie B. Genotype Imputation for Genome-Wide Association Studies. Nature reviews Genetics. 2010;11(7):499-511.
[22] Hinrichs AS, Karolchik D, Baertsch R, Barber GP, Bejerano G, Clawson H, Diekhans M, Furey TS, Harte RA, Hsu F, Hillman-Jackson J, Kuhn RM, Pedersen JS, Pohl A, Raney BJ, Rosenbloom KR, Siepel A, Smith KE, Sugnet CW, Sultan-Qurraie A, Thomas DJ, Trumbower H, Weber RJ, Weirauch M, Zweig AS, Haussler D, Kent WJ. The Ucsc Genome Browser Database: Update 2006. Nucleic Acids Research. 2006;34(Database issue):D590-8.
[23] Browning BL, Browning SR. Haplotypic Analysis of Wellcome Trust Case Control Consortium Data. Human genetics. 2008;123(3):273-80.
[24] Howie BN, Donnelly P, Marchini J. A Flexible and Accurate Genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. PLoS genetics. 2009;5(6):e1000529.
[25] Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. Mach: Using Sequence and Genotype Data to Estimate Haplotypes and Unobserved Genotypes. Genetic epidemiology. 2010;34(8):816-34.
[26] Pei YF, Li J, Zhang L, Papasian CJ, Deng HW. Analyses and Comparison of Accuracy of Different Genotype Imputation Methods. PLoS ONE. 2008;3(10):e3551.
[27] Verma SS, de Andrade M, Tromp G, Kuivaniemi H, Pugh E, Namjou-Khales B, Mukherjee S, Jarvik GP, Kottyan LC, Burt A, Bradford Y, Armstrong GD, Derr K, Crawford DC, Haines JL, Li R, Crosslin D, Ritchie MD. Imputation and Quality

Control Steps for Combining Multiple Genome-Wide Datasets. Frontiers in Genetics. 2014;5.
[28] Delaneau O, Marchini J, Zagury JF. A Linear Complexity Phasing Method for Thousands of Genomes. Nature methods. 2011;9(2):179-81.
[29] Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and Accurate Genotype Imputation in Genome-Wide Association Studies through Pre-Phasing. Nature genetics. 2012;44(8):955-9.
[30] Loh PR, Danecek P, Palamara PF, Fuchsberger C, Y AR, H KF, Schoenherr S, Forer L, McCarthy S, Abecasis GR, Durbin R, A LP. Reference-Based Phasing Using the Haplotype Reference Consortium Panel. Nature genetics. 2016;48(11):1443-8.
[31] McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM, Fuchsberger C, Danecek P, Sharp K, Luo Y, Sidore C, Kwong A, Timpson N, Koskinen S, Vrieze S, Scott LJ, Zhang H, Mahajan A, Veldink J, Peters U, Pato C, van Duijn CM, Gillies CE, Gandin I, Mezzavilla M, Gilly A, Cocca M, Traglia M, Angius A, Barrett JC, Boomsma D, Branham K, Breen G, Brummett CM, Busonero F, Campbell H, Chan A, Chen S, Chew E, Collins FS, Corbin LJ, Smith GD, Dedoussis G, Dorr M, Farmaki AE, Ferrucci L, Forer L, Fraser RM, Gabriel S, Levy S, Groop L, Harrison T, Hattersley A, Holmen OL, Hveem K, Kretzler M, Lee JC, McGue M, Meitinger T, Melzer D, Min JL, Mohlke KL, Vincent JB, Nauck M, Nickerson D, Palotie A, Pato M, Pirastu N, McInnis M, Richards JB, Sala C, Salomaa V, Schlessinger D, Schoenherr S, Slagboom PE, Small K, Spector T, Stambolian D, Tuke M, Tuomilehto J, Van den Berg LH, Van Rheenen W, Volker U, Wijmenga C, Toniolo D, Zeggini E, Gasparini P, Sampson MG, Wilson JF, Frayling T, de Bakker PI, Swertz MA, McCarroll S, Kooperberg C, Dekker A, Altshuler D, Willer C, Iacono W, Ripatti S, Soranzo N, Walter K, Swaroop A, Cucca F, Anderson CA, Myers RM, Boehnke M, McCarthy MI, Durbin R. A Reference Panel of 64,976 Haplotypes for Genotype Imputation. Nature genetics. 2016;48(10):1279-83.
[32] Lin P, Hartz SM, Zhang Z, Saccone SF, Wang J, Tischfield JA, Edenberg HJ, Kramer JR, A MG, Bierut LJ, Rice JP. A New Statistic to Evaluate Imputation Reliability. PLoS ONE. 2010;5(3).
[33] Mitt M, Kals M, Parn K, Gabriel SB, Lander ES, Palotie A, Ripatti S, Morris AP, Metspalu A, Esko T, Magi R, Palta P. Improved Imputation Accuracy of Rare and Low-Frequency Variants Using Population-Specific High-Coverage Wgs-Based Imputation Reference Panel. European journal of human genetics : EJHG. 2017.
[34] Nho K, Shen L, Kim S, Swaminathan S, Risacher SL, Saykin AJ. The Effect of Reference Panels and Software Tools on Genotype Imputation. AMIA Annual Symposium proceedings AMIA Symposium. 2011;2011:1013-8.
[35] Moore CM, Jacobson S, Fingerlin TE. Power and Sample Size Calculations for Genetic Association Studies in the Presence of Genetic Model Misspeacification. American Society of Human Genetics. October 2018, San Diego.

CHAPTER 3.

SYSTEMATIC REVIEW: GENETIC ASSOCIATIONS FOR PROGNOSTIC FACTORS OF URINARY BLADDER CANCER

Contents of this chapter have been published:

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Cazier JB, Bryan RT, Zeegers MP. Systematic Review: Genetic associations for Prognostic Factors of Urinary Bladder Cancer. Biomark Cancer. 2019; doi:10.1177/1179299X19897255.

ABSTRACT

INTRODUCTION

Many germline associations have been reported for urinary bladder cancer (UBC) outcomes and prognostic characteristics. It is unclear if there are overlapping genetic patterns for various prognostic endpoints. Our objective was to review contemporary literature on genetic associations with UBC prognostic outcomes and to identify potential overlap in reported genes.

METHODS

EMBASE, MEDLINE, and PubMed databases were queried for relevant articles in English language without date restrictions.

RESULTS

The initial search identified 1,346 articles. After exclusions, 112 studies have been summarized. Cumulatively, 316 single nucleotide polymorphisms (SNPs) were reported across prognostic outcomes (recurrence, progression, death) and characteristics (tumour stage, grade, size, age, risk group). There were considerable differences between studied outcomes in the context of genetic associations. The most commonly reported SNPs were located in *OGG1*, *TP53*, and *MDM2*. For outcomes with the highest number of reported associations (i.e. recurrence and death), functional enrichment annotation yields different terms, potentially indicating separate biological mechanisms.

CONCLUSIONS

In our analysis, UBC prognostic outcomes show significant genetic heterogeneity and it might be valuable they are studied as distinct phenotypes. Further validation of most-promising observations is essential for including the genetic component into predicting UBC patient outcomes.

INTRODUCTION

Urothelial bladder cancer (UBC) results in considerable clinical input and necessitates ongoing research to reduce the burden of patients and healthcare providers [1]. Current era of genomics offers new insights into UBC pathogenesis [2]. However, due to the complex nature of genetics, many studies are difficult to summarize into clear recommendations for future research and clinical practice.

UBC is most frequently diagnosed as a non-muscle-invasive bladder cancer (NMIBC), accounting for 70-80 percent of all new cases [3]. NMIBC management is complex with appropriate treatment dependent upon multiple clinical and pathological components. Importantly, a significant proportion of patients are prone to tumour recurrence and/or progression, both events difficult to predict. Previously developed multifactorial prognostic NMIBC tools [4] have been useful to describe populations, but lack accuracy for individual outcomes and require further advances [5]. Muscle-invasive bladder cancer (MIBC) cases are equally complex to treat with various permutations of chemotherapy, radiotherapy, and cystectomy [6], with an addition of recent initiatives in molecular-genomic subtyping [2].

Although multiple studies have addressed the potential role of genetic variation in UBC prognosis, the findings are yet to be implemented into clinical practice. For the most part, genetic associations are often reported in small samples and their validity is difficult to establish. In addition, the interpretation of the biological relevance over many reports is challenging. Furthermore, it is not yet clear if genetic associations overlap within and between the groups of direct (recurrence, progression, survival) and indirect (stage, grade, tumour size, age at the time of diagnosis) prognostic endpoints. Identifying existing genetic similarities between prognostic outcomes would

help to potentially decipher underlying pathological mechanisms and guide promising directions in research on UBC.

In the current review, our objective is to summarize genetic associations for UBC prognostic phenotypes and to describe any overlap or existing patterns that would clarify their pertinence for future clinical practice.

METHODS

The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [7] (**Supplementary Table 3.1**).

We queried EMBASE, Medline, and PubMed with the following search term: (("urinary bladder neoplasms" OR "bladder cancer" OR "urothelial carcinoma") AND (prognosis OR survival OR recurrence OR progression OR grade OR stage OR "tumour size" OR age) AND (polymorphi* OR SNP OR germline)). The search was limited to articles published prior to the 13th November 2018, written in English and describing human research only. A detailed flowchart on the selection and search process is presented in **Figure 3.1.** Reference lists of included manuscripts were checked for potentially missing reports. Study eligibility was determined by the main author (NL).

Inclusion criteria were as follows:

1) Studies assessing single germline single-nucleotide polymorphism (SNP) variants (not somatic mutations, insertion/deletions, microsatellites, haplotype analyses, dinucleotide polymorphism associations, multiple-SNP prediction models);
2) Original reports (not meta-analyses, reviews, letters, case reports, other);

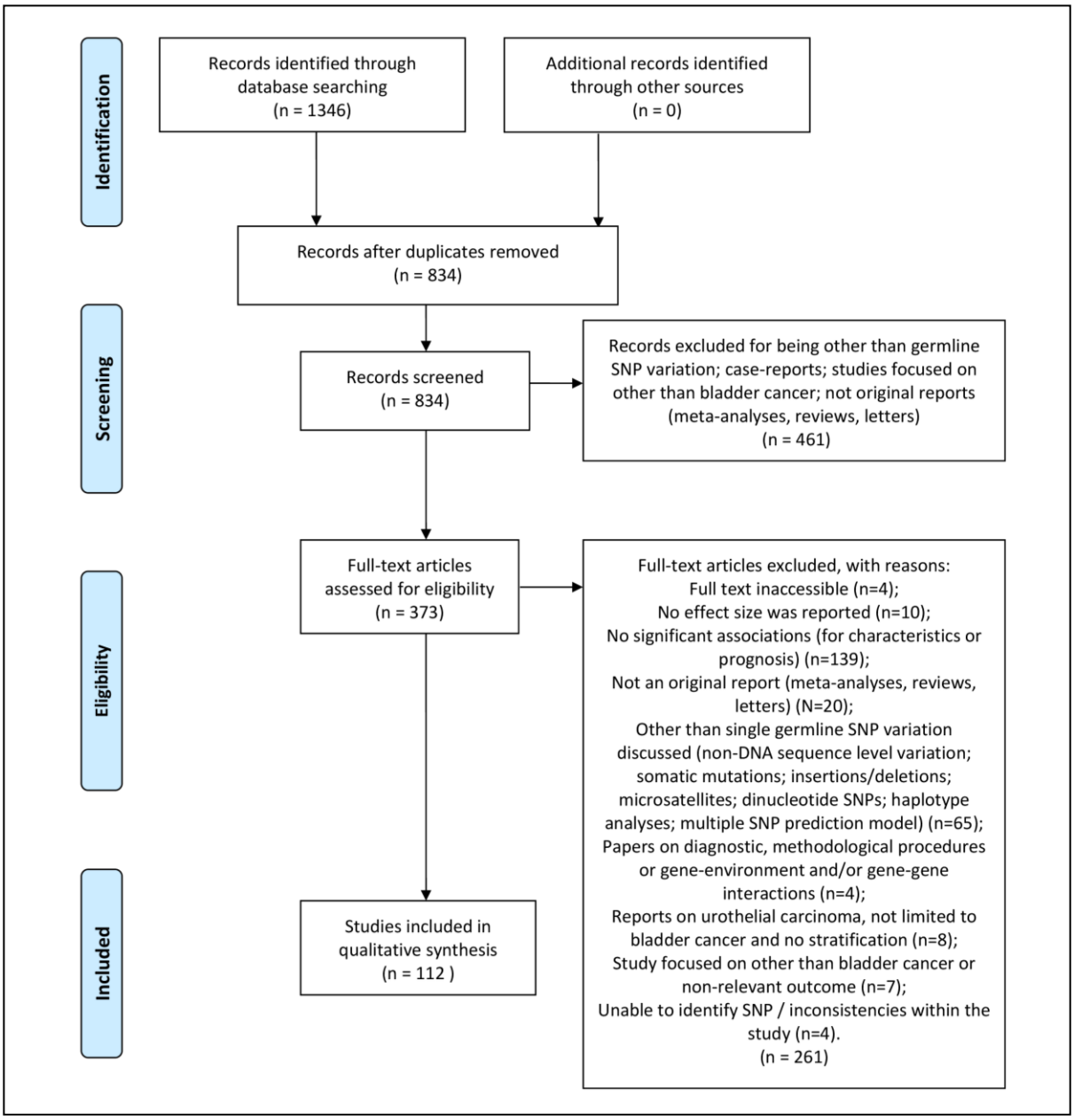


Figure 3.1. Flow diagram of the study selection used in evidence synthesis. SNP-single nucleotide polymorphism.

3) Studies focused on UBC or where UBC data are described distinctly from a broader urothelial carcinoma cohort;
4) Studies reporting an effect size;
5) Studies reporting significant associations (for characteristics or prognosis);
6) DNA sequence level variation described;
7) The described SNPs could be identified.

Studies describing diagnostic, methodological procedures, gene-gene and gene-environment interactions were excluded.

Each study was assessed for quality by evaluating reporting adequacy. Inconsistency was regarded as mismatching data within the study (e.g. different SNP IDs reported in article sections). Data completeness was verified if all relevant data fields for a genetic associations study [8] were available to extract from the report. The quality criteria were part of the study selection process (e.g. studies stating variant relevance for an outcome without providing an effect size were regarded as having low quality and excluded from further evaluation).

Data extraction

Further information was extracted from each eligible study: year of publication, first author, patient subgroup (UBC, MIBC, NMIBC, or other), cancer subtype (urothelial carcinoma (UC) or other), ethnicity, sample size, SNP ID, locus, gene, effect allele, reference allele, effect allele frequency, effect size, corresponding 95% confidence intervals, p-value.

Summarizing overlap in genetic associations and outcomes

To see if previously reported genes may play a role across multiple UBC outcomes, results were put in a ranked table. Genes associated with many UBC endpoints are ranked high, whilst genes that were reported for one or few of the outcomes are ranked low. As such, we are able to suggest genes that are important for UBC prognosis overall and which genes are more likely to be outcome-specific (e.g. only associated with cancer recurrence).

The resulting ranking acted as a guideline for identifying genes that were commonly observed for most of the prognostic outcomes and characteristics. Outcomes with at

least 20 genes were chosen and their functional roles were further described in additional detail.

Functional annotation

After summarizing the overlap, some outcomes have been associated with multiple genes. Every biological process is polygenic, and having bigger sets of identified genes helps to elucidate biological pathways behind the studied phenotype. We chose outcomes with the largest number of reported genes and submitted those sets to the DAVID Functional Annotation Tool [9]. The tool groups genes by their functional similarity, using information from well-known databases, such as Gene Ontology (GO) for biological mechanisms and KEGG for pathways, among others. Gene clustering was carried out with setting the highest level of classification stringency. A high level of stringency generates fewer clusters, but genes within them are associated more tightly. Moreover, to reduce the likelihood of describing false-positive clusters, only gene groups containing pathways with false discovery rates (FDR) of <5% were interpreted as valid results.

Statistical analysis

Overall, our search has resulted in multiple genes corresponding to various outcomes. As such, the resulting data is very difficult to describe in a comprehensive manner. To reduce the dimensionality of current data, we have performed a Principal Component Analysis (PCA). PCA can be seen as a form of an exploratory analysis to identify group-level correlations in the sample. It is a useful tool for improving the interpretation of data, as it allows visualizing similarities between groups with regard to chosen characteristics. In our analysis, we aimed to investigate the similarity between UBC outcomes in terms of their genetic background.

We have constructed a binary matrix for UBC outcomes and associated genes. Every gene and outcome combination took a value of '1' in case association has been reported, and a value of '0' if no associations were published in the literature. As such, clinical outcomes that share genes would plot more closely, whilst an outcome that does not share any genes with other endpoints would plot far from other groups. In the currently reviewed literature, some outcomes have been investigated more often (e.g. recurrence and death), hence we have adjusted the size of data points in a PCA plot to represent the number of associated genes. First two principal components were plotted for all studied endpoints.

RESULTS

For the current review, 373 full-text articles were evaluated in-depth, resulting in a final set of 112 articles for further summary (**Figure 3.1**). In total, 316 associations were extracted across all investigated outcomes (age (N=12, [10-21]), stage (N=79, [10, 12, 17, 19, 22-65], tumour size (N=2 [66, 67]), grade (N=49 [10, 17, 26, 28, 31, 32, 34-36, 41, 46, 49, 53, 57, 61, 65, 66, 68-74]), risk groups (N=15 [11, 13, 16, 23, 24, 29, 30, 32, 39, 75, 76]), recurrence (N=81 [13, 22, 24, 29, 30, 39, 42, 48, 49, 51, 52, 68, 70, 77-106]), progression (N=24, [25, 32, 45, 86-88, 106-111]), and death (N(cancer-specific)= 12 [33, 42, 45, 100, 106, 112-116], N(overall)=42 [33, 42, 49, 55, 88, 89, 110, 111, 117-121]).

There was considerable heterogeneity across all associations, including assumed patterns of inheritance, studied ethnic populations, and outcome definitions.

Age was investigated using multiple year cut-offs, namely: 50 [12], 56 [16], 60 [10, 15, 21], 65 [11, 14, 17-20], and once as a continuous variable [13] (**Supplementary Table 3.2**).

Tumour size was investigated either as using a cut-off of ≥3 cm [66] or defined as a large tumour, corresponding to stages T1-T4 [67] (**Supplementary Table 3.3**).

Tumour stage was analysed using multiple combinations. Broadly, we have differentiated between stage corresponding to NMIBC and MIBC cases. For studies reporting on NMIBC, following endpoints were used: tumours of Tis [60, 64], T1 [36], Ta+T1 [12, 17, 35, 38, 53, 57, 61-63, 65], and Ta+T1+Tis [31, 58, 59] (**Supplementary Table 3.4**). As for MIBC, most studies have defined the primary outcome as T2+ staged tumours [10, 19, 22-31, 33-56]. However, some associations have been reported for a merged group of T2+ and T1 stages [32, 33].

Most reports on grade can be roughly categorized into containing either low- or high-grade UBC cases. Low-grade UBC definitions were as follows: G1 [17, 53, 61, 69], G2 [34, 57, 61, 69], G1+G2 [35], low-grade [68, 70], and G1+G2+papilloma [31]. High-grade UBC was usually defined as grade 3 UBC [10, 26, 28, 31, 35, 36, 46, 49, 66, 69, 71-74], a combination of G2 and G3 NMIBC [53, 65], and some studies have reported estimates for grade 4 tumours, without a reference for the grading system used (G3+G4 [41]) and G2+G3+G4 [32]) (**Supplementary Table 3.5**).

It was common for studies to classify UBC as a disease of low- or high-risk, that correspond to various combinations of clinical stage and grade. For low-risk, researchers used the following definitions: TaG2 [32, 33], TaG1 [32], TaG1-2 [11, 13, 16]. In contrast, high-risk tumours were defined as: TaG2-3+T1G1-3 [24, 29, 30, 39, 75], TaG3+T1G2-3 [23], G2-3 with T1-4 [76], and TaG3+T1 [32]. (**Supplementary Table 3.6**).

For genetic associations with tumour recurrence, studies mostly focused on NMIBC cases (except for few reports considering UBC group overall [48] or MIBC [49, 70]). NMIBC recurrence was investigated as an overall outcome [48, 68, 77, 88, 89, 93, 96,

103, 105], or in specific groups: patients younger than 64 years [97], patients not treated with Bacillus Calmette-Guérin (BCG) therapy: [39, 42]; BCG-treated patients: [22, 24, 29, 30, 39, 42, 51, 52, 78-82, 84-86, 92, 94, 95, 98, 99, 101, 102, 106], patients treated only with transurethral urinary bladder resection (TURBT): [87, 100]; patients who have received both TURBT and BCG treatments: [90, 91]; patients having received treatments of TURBT and epirubicin [104], and recurrence only among low-risk NMIBC: [13, 83] (**Supplementary Table 3.7**).

Progression was defined as an increase of stage in NMIBC group [32, 108, 109] or UBC [107] overall. Also, transition from NMIBC to MIBC or metastatic disease [86-88] was considered a disease progression, sometimes expanding the latter definition to include cancer-specific death [106, 111]. In other cases, alternative definitions were considered, namely occurrence of metastases [25, 45] and a confirmed relapse among MIBC [110] (**Supplementary Table 3.8**).

In terms of death outcomes, there were two broad groups of overall- [32, 42, 55, 88, 89, 111, 117-120] and cancer-specific [33, 42, 45, 100, 106, 112-116] survival endpoints (**Supplementary Table 3.9**).

Retrieved data and detailed study characteristics, including outcome definition for each study, are presented in **Supplementary Tables 3.2-9**.

Overlap between the outcomes

A summary table of existing overlap between outcomes and associated genes is presented in **Table 3.1**. *OGG1* (rs2304277, rs1052133) was the most commonly reported gene, having been associated with patient age [18], tumour stage [53], grade [53], recurrence [39, 77], and risk group [39]. Associations on *OGG1* and UBC did not cluster within a clearly defined subgroup and instead showed relationships with various characteristics: increased age at diagnosis (>65 years) [18] and elevated risks

of the following: non-muscle-invasive and invasive UBC [53], low- and high-grade tumours [53], rate of recurrence [39, 77], and high-risk tumours [39].

Table 3.1. Overlap between reported outcomes and mapped genes.

Outcomes	Number of overlapping genes	Mapped genes
Age / Grade / Recurrence / Risk group / Stage	1	OGG1
Death / Grade / Recurrence / Risk group / Stage	2	TP53, MDM2
Age / Grade / Risk group / Stage	1	CCND1
Age / Recurrence / Risk group / Stage	1	XRCC7(PRKDC)
Age / Death / Recurrence / Stage	1	XRCC1
Grade / Progression / Risk group / Stage	1	HRAS
Death / Grade / Recurrence / Stage	2	PDCD6, XPD(ERCC2)
Age / Grade / Stage	1	H19
Age / Death / Stage	1	EGFR
Grade / Risk group / Stage	1	MSH6
Death / Risk group / Stage	1	NQO1
Progression / Recurrence / Stage	1	MIR146A
Death / Grade / Recurrence	1	IL6
Grade / Recurrence / Tumour size	1	TSP-1(THBS1)
Death / Progression / Recurrence	1	NOS3
Age / Recurrence / Risk group	1	TACC3/FGFR3
Death / Grade / Stage	2	RAD51, MTHFR
Recurrence / Risk group / Stage	2	CASP9, IL18
Grade / Recurrence / Stage	4	CCR2, PPARG, GSTP1, XPC
Risk group / Stage	1	XRCC5
Progression / Stage	1	IL4
Grade / Recurrence	1	IL31
Progression / Recurrence	1	RGS1
Age / Risk group	2	CASC11, TP63
Death / Stage	2	TLR10, IL27
Death / Recurrence	3	RGS2, GSTO1, XPF(ERCC4)
Grade / Stage	4	LEPR, IGFBP3, XPG(ERCC5), PSCA
Recurrence / Stage	4	IL17A, TNFA, GPX1, NAMPT
Death / Progression	4	NOD2, BCL2, RGS5, ERCC1
Tumour size	1	WISP1(CCN4)
Risk group	2	POLG2, BRCA2
Age	3	PCAT1, POR, HOTAIR
Grade	6	MIR143_CARMN, TMEM129_TACC3_FGFR3, CLPTM1L, MYC, TNFRSF10A (TRAILR1, DR4), CCNE1
Progression	9	DGCR8, NOS2, CDKN2A, TGFB1, RGS4, RGS7, IL10, UNG, RGS14
Stage	15	CD44, SDF1(CXCL12), CXCR4, SLC23A1, MATR3, DNAJC18, C13ORF31(LACC1), CD4, CFH, XRCC3, IL22, MMP12, COX2(PTGS2), P21(CDKN1A), PMS2
Death	20	IL8RB(CXCR2), RPTOR, RGS12, GSTO2, MRE11, RB1CC1, EPHX1, BCL2L1, GATA3, UGT1A1, XRCC4, PIK3R1, DRD4, RGS3, TERT, CD80, AURKA, AKT2, TGFBR1, GNB3
Recurrence	26	VDR, Survivin(BIRC5), MMP2, GPX4, NFKBIA, CDH1, IGF1, GLI2, NEIL2, GLI3, RNASEN(DROSHA), IL8(CXCL8), ICAM1, IFN-G, SHH, RGS13, RGS16, RGS10, DDX20, GSS,

		CWC27, SOD1, ERCC6, NRAMP1(SLC11A1), ALDH2, TNFRSF10A (TRAILR1, DR4)

A set of two genes (*TP53* (rs1042522, rs1154065) and *MDM2* (rs2279744) have also been reported for multiple endpoints, specifically UBC grade [41, 68], stage [24, 40, 41], recurrence [24, 68, 100], survival [100, 116], and risk group [24, 75]. For most outcomes (death, risk category, grade, stage) the associations for *MDM2*- and *TP53*-related variants were in opposite directions.

In terms of number of genes corresponding to a single endpoint, tumour recurrence was the outcome with the highest sum of genes (N=28) showing associations; followed by death (N=21) (**Table 3.1**).

To elucidate any unifying pathways between these genes, gene sets for recurrence and death were submitted to the functional annotation tool DAVID [9].

For recurrence, DAVID identified has identified two gene clusters of similar functions that contained pathways with acceptable FDR values (**Supplementary Table 3.10**). The first group (enrichment score=2.72) was formed entirely of *RGS* family genes (*RGS10*, *RGS13*, *RGS16*). The second cluster (enrichment score=2.42) was formed by *GLI2*, *GLI3*, and *SHH* genes. Out of ten functional terms within the cluster, one was of satisfactory FDR and reached Bonferroni-adjusted <0.05, termed "*hindgut morphogenesis*".

For individual enriched pathways, 20 have yielded FDR<5% and are listed in **Supplementary Table 3.11.** Three functional terms – "*hindgut morphogenesis*", "*Pathways in cancer*", and "*positive regulation of transcription from RNA polymerase II promoter*" have shown both low FDR rates and were also below the conventional level of statistical significance (p<0.05) after multiple-comparison adjustment.

For genes associated with UBC survival, the submitted set retrieved six functional clusters in total; however, no individual terms had acceptable FDR values.

Nonetheless, there were multiple individual functional pathways with FDR<5% were identified instead (**Supplementary Table 3.12**). One term, "*Pancreatic cancer*", has reached a Bonferroni-adjusted statistical significance (p=0.05).

Finally, a performed PCA analysis for previously-reported genetic associations showed UBC recurrence to be the most distinct outcome (**Figure 3.2**), with tumour stage and grade also showing significant deviations from other endpoints.

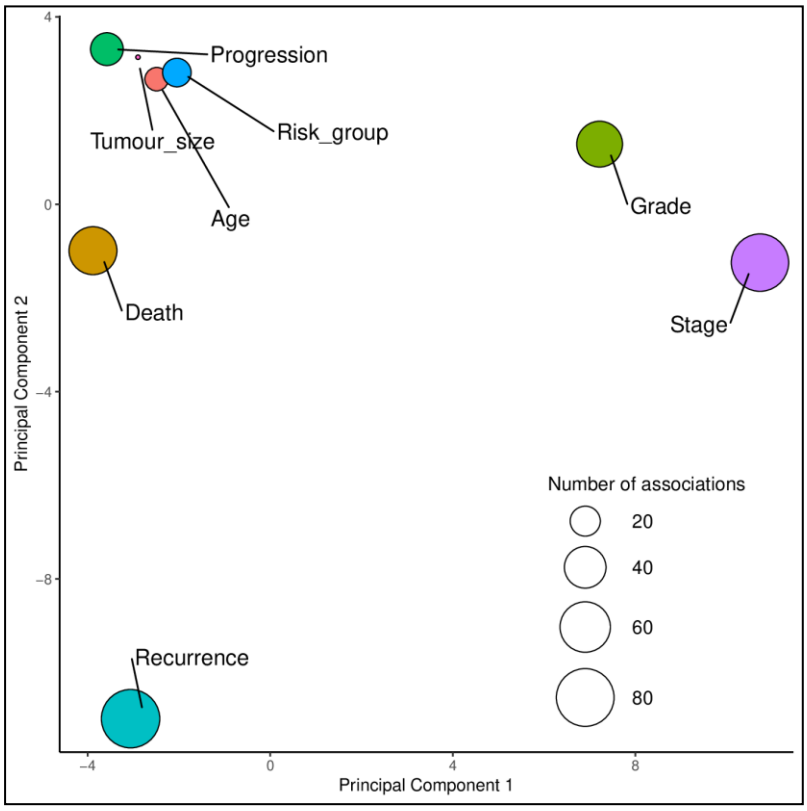


Figure 3.2. Principal Component Analysis for Genetic Associations with Urinary Bladder Outcomes. Data point sizes are indicative of the number of associated genes with each outcome.

DISCUSSION

In the current review, we have summarized existing evidence for single-SNP genetic associations with UBC characteristics (tumour size, stage, grade, patient's age) and

prognostic outcomes (recurrence, progression, survival). There were multiple associations for considered endpoints with limited overlap. Based on these data, we have made several observations.

It is widely accepted that complex disease genetic architecture is highly polygenic [122]. However, currently-summarized list of associations for UBC outcomes and characteristics is far from exhaustive. It is essential to note future studies with higher per-study power will contribute additional associations and will clarify the validity of those already reported.

Importantly, our review underscores the sensitivity of outcome definition in genetic studies. It has been demonstrated that genetic variants for UBC risk are unlikely to be relevant for prognosis [123], and our report implies prognostic outcomes demonstrate further within-group heterogeneity. Interestingly, the PCA revealed the largest differences for direct prognostic outcomes: UBC death and progression showed similar characteristics, whilst UBC recurrence significantly deviated from the group. From a biological perspective, cancer recurrence is not an equivalent to progression or death, and it is likely the mechanisms involved are triggered and organised via different pathways. Similarly, tumour characteristics (grade, stage, size) and patient characteristics (age) are likely different entities in terms of genetic contribution.

When trying to elucidate unifying pathways for multiple genes involved in certain outcomes, UBC recurrence was found to be associated with terms that relate to formation of a new tissue (e.g. *"hindgut morphogenesis"*). In contrast, functional pathway terms were different for death as an outcome and indicate a separate biological mechanism. Interestingly, the most promising associated term for death was "*Pancreatic cancer*", which exhibits very low survival rates in comparison to cancers of any other site [124].

In the light of our analyses, UBC prognosis may represent a complex phenotype, and the current review indicates different outcomes imply distinct genetic associations. The genetic relationships may overlap but, nonetheless, should be treated as independent endpoints.

Importantly, the review identifies a number of commonly-reported genes, specifically *OGG1*, *TP53*, and *MDM2*. Given that they are reported most often, we would suggest these targets might be of important interest to investigate in further studies. *OGG1* encodes a protein involved in base excision repair (BER) pathways to protect cells from oxidative stress [125]. Although having a clear role in mutagenic processes, *OGG1*-null mice showed only moderate increases in malignancy rate, likely due to effective alternative damage repair pathways [126]. Evidence from multiple meta-analyses [127-129] of *OGG1* involvement in UBC cancerogenesis is contradictory; and if having a genuine effect, is more likely to play a supporting role in a multi-stage process rather than being the main cause of it [126]. It is also probable that the establishment of the type and direction of genetic associations requires larger populations (underscoring sufficient sample sizes for different ethnicities), not yet available to researchers.

Additionally, the link between *TP53* and *MDM2* genes has been extensively reported in the literature, offering an attractive pharmacological target in cancer treatment [130]. P53 protein acts as a tumour suppressor, which is negatively regulated by MDM2 oncoprotein. The pattern is somewhat mirrored in observed associations, where variation in SNPs of the two genes seemed to correspond to effects in opposite direction (e.g. SNPs in *TP53* increased the risk of T2+ stage, whist alterations in *MDM2* showed reduced risk of invasive tumours).

Collectively, *OGG1*, *TP53*, and *MDM2* are relevant for multiple essential DNA-preserving cellular mechanisms, and hence would be expected to have importance for a variety of UBC characteristics and outcomes, as observed in our review.

The limitations of our study are important to acknowledge. Many reports have analysed different ethnicities, which alone does not undermine the reported associations, but makes inter-population relevance improbable due to differencing allele frequencies [131]. Moreover, assumed genetic patterns of inheritance (e.g. recessive, dominant, additive) differed highly between the studies, without a clear preference for the chosen model. Usually, the reported model was chosen *ad hoc* as a consequence of being statistically significant, making it difficult to be confident the reported model reflected true genetic architecture of the association. Since associations were highly heterogeneous, we were unable to carry out a meta-analysis (which would have provided a preferred summary of these data). Furthermore, the majority of included studies were of candidate-gene design; we would expect different results if all studies followed an agnostic genome-wide association approach. Finally, sample sizes were limited, and it is difficult to establish whether all reported associations are robust.

It is important to note we were only able to analyse reported associations, and it is assumed many studies have not been published due to negative results. We aimed to include all available publications, but anticipate our results are affected by some level of publication bias. The lack of external replication studies for genetic associations is detrimental to translating science into practice, as many genetic findings are likely to be false-positive [132]. Optimally, only validated variants would be included in review studies. We underscore the importance of validation efforts for future studies to be able summarizing only unambiguous variants.

To conclude, we have summarized existing genetic associations for tumour and patient characteristics and disease prognosis for UBC. Multiple loci have been identified that demonstrate little consensus and highlight the possibility of UBC prognostic outcomes being unique entities in the context of genetic contribution. We recommend that further replication of previously identified SNPs should be undertaken. Consecutive formal reviews of existing associations will help facilitate their potential use in clinical practice.

REFERENCES

[1] Svatek RS, Hollenbeck BK, Holmang S, Lee R, Kim SP, Stenzl A, Lotan Y. The Economics of Bladder Cancer: Costs and Considerations of Caring for This Disease. Eur Urol. 2014;66(2):253-62.

[2] Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, Castro MAA, Gibb EA, Kanchi RS, Gordenin DA, Shukla SA, Sanchez-Vega F, Hansel DE, Czerniak BA, Reuter VE, Su X, de Sa Carvalho B, Chagas VS, Mungall KL, Sadeghi S, Pedamallu CS, Lu Y, Klimczak LJ, Zhang J, Choo C, Ojesina AI, Bullman S, Leraas KM, Lichtenberg TM, Wu CJ, Schultz N, Getz G, Meyerson M, Mills GB, McConkey DJ, Weinstein JN, Kwiatkowski DJ, Lerner SP. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell. 2017;171(3):540-56.e25.

[3] Kamat AM, Hahn NM, Efstathiou JA, Lerner SP, Malmstrom PU, Choi W, Guo CC, Lotan Y, Kassouf W. Bladder Cancer. Lancet (London, England). 2016;388(10061):2796-810.

[4] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.

[5] Sylvester RJ. How Well Can You Actually Predict Which Non-Muscle-Invasive Bladder Cancer Patients Will Progress? Eur Urol. 2011;60(3):431-3; discussion 3-4.

[6] Alfred Witjes J, Lebret T, Comperat EM, Cowan NC, De Santis M, Bruins HM, Hernandez V, Espinos EL, Dunn J, Rouanne M, Neuzillet Y, Veskimae E, van der Heijden AG, Gakis G, Ribal MJ. Updated 2016 Eau Guidelines on Muscle-Invasive and Metastatic Bladder Cancer. European urology. 2017;71(3):462-75.

[7] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The Prisma Statement. PLoS medicine. 2009;6(7):e1000097.

[8] Sagoo GS, Little J, Higgins JP. Systematic Reviews of Genetic Association Studies. Human Genome Epidemiology Network. PLoS medicine. 2009;6(3):e28.

[9] Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. The David Gene Functional Classification Tool: A Novel Biological Module-Centric Algorithm to Functionally Analyze Large Gene Lists. Genome biology. 2007;8(9):R183.

[10] Hua Q, Lv X, Gu X, Chen Y, Chu H, Du M, Gong W, Wang M, Zhang Z. Genetic Variants in Lncrna H19 Are Associated with the Risk of Bladder Cancer in a Chinese Population. Mutagenesis. 2016;31(5):531-8.

[11] Wang M, Wang M, Zhang W, Yuan L, Fu G, Wei Q, Zhang Z. Common Genetic Variants on 8q24 Contribute to Susceptibility to Bladder Cancer in a Chinese Population. Carcinogenesis. 2009;30(6):991-6.

[12] Kelsey KT, Park S, Nelson HH, Karagas MR. A Population-Based Case-Control Study of the Xrcc1 Arg399gln Polymorphism and Susceptibility to Bladder Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(8):1337-41.

[13] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F,

Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.
[14] Xiao X, Ma G, Li S, Wang M, Liu N, Ma L, Zhang Z, Chu H, Zhang Z, Wang SL. Functional Por A503v Is Associated with the Risk of Bladder Cancer in a Chinese Population. Scientific reports. 2015;5:11751.
[15] Lin Y, Ge Y, Wang Y, Ma G, Wang X, Liu H, Wang M, Zhang Z, Chu H. The Association of Rs710886 in Lncrna Pcat1 with Bladder Cancer Risk in a Chinese Population. Gene. 2017;627:226-32.
[16] Stern MC, Van Den Berg D, Yuan JM, Conti DV, Gago-Dominguez M, Pike MC, Xiang YB, Gao YT, Cortessis VK. Sequence Variant on 3q28 and Urinary Bladder Cancer Risk: Findings from the Los Angeles-Shanghai Bladder Case-Control Study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(11):3057-61.
[17] Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, Zhang Z. Cyclin D1 G870a Polymorphism Is Associated with Risk and Clinicopathologic Characteristics of Bladder Cancer. DNA and cell biology. 2010;29(10):611-7.
[18] Ma L, Chu H, Wang M, Shi D, Zhong D, Li P, Tong N, Yin C, Zhang Z. Hogg1 Ser326cys Polymorphism Is Associated with Risk of Bladder Cancer in a Chinese Population: A Case-Control Study. Cancer science. 2012;103(7):1215-20.
[19] Chu H, Wang M, Jin H, Lv Q, Wu D, Tong N, Ma L, Shi D, Zhong D, Fu G, Yuan L, Qin C, Yin C, Zhang Z. Egfr 3'utr 774t>C Polymorphism Contributes to Bladder Cancer Risk. Mutagenesis. 2013;28(1):49-55.
[20] Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD, Liu QZ. Genetic Variants of the Xrcc7 Gene Involved in DNA Repair and Risk of Human Bladder Cancer. International journal of urology : official journal of the Japanese Urological Association. 2008;15(6):534-9.
[21] Wang X, Wang W, Zhang Q, Gu D, Zhang K, Ge Y, Chu H, Du M, Xu B, Wang M, Lv X, Zhang Z, Yuan L, Gong W. Tagging Snps in the Hotair Gene Are Associated with Bladder Cancer Risk in a Chinese Population. Gene. 2018;664:22-6.
[22] Ahirwar DK, Mandhani A, Dharaskar A, Kesarwani P, Mittal RD. Association of Tumour Necrosis Factor-Alpha Gene (T-1031c, C-863a, and C-857t) Polymorphisms with Bladder Cancer Susceptibility and Outcome after Bacille Calmette-Guerin Immunotherapy. BJU international. 2009;104(6):867-73.
[23] Guey LT, Garcia-Closas M, Murta-Nascimento C, Lloreta J, Palencia L, Kogevinas M, Rothman N, Vellalta G, Calle ML, Marenne G, Tardon A, Carrato A, Garcia-Closas R, Serra C, Silverman DT, Chanock S, Real FX, Malats N. Genetic Susceptibility to Distinct Bladder Cancer Subphenotypes. Eur Urol. 2010;57(2):283-92.
[24] Gangwar R, Mittal RD. Association of Selected Variants in Genes Involved in Cell Cycle and Apoptosis with Bladder Cancer Risk in North Indian Population. DNA and cell biology. 2010;29(7):349-56.

[25] Deng S, Wang W, Li X, Zhang P. Common Genetic Polymorphisms in Pre-Micrornas and Risk of Bladder Cancer. World journal of surgical oncology. 2015;13:297.
[26] Safarinejad MR, Shafiei N, Safarinejad SH. The Association between Bladder Cancer and a Single Nucleotide Polymorphism (Rs2854744) in the Insulin-Like Growth Factor (Igf)-Binding Protein-3 (Igfbp-3) Gene. Archives of toxicology. 2011;85(10):1209-18.
[27] Lin HH, Ke HL, Hsiao KH, Tsai CW, Wu WJ, Bau DT, Chang LL. Potential Role of Ccnd1 G870a Genotype as a Predictor for Urothelial Carcinoma Susceptibility and Muscle-Invasiveness in Taiwan. The Chinese journal of physiology. 2011;54(3):196-202.
[28] Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Association of Genetic Polymorphism of Glutathione S-Transferase (Gstm1, Gstt1, Gstp1) with Bladder Cancer Susceptibility. Urologic oncology. 2013;31(7):1193-203.
[29] Gangwar R, Mandhani A, Mittal RD. Caspase 9 and Caspase 8 Gene Polymorphisms and Susceptibility to Bladder Cancer in North Indian Population. Annals of surgical oncology. 2009;16(7):2028-34.
[30] Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of Il-12, Il-18 Variants and Serum Il-18 with Bladder Cancer Susceptibility in North Indian Population. Gene. 2013;519(1):128-34.
[31] Ali SHB, Bangash KS, Rauf A, Younis M, Anwar K, Khurram R, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R. Identification of Novel Potential Genetic Predictors of Urothelial Bladder Carcinoma Susceptibility in Pakistani Population. Familial cancer. 2017;16(4):577-94.
[32] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.
[33] Sanyal S, Ryk C, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Nqo1 and the Clinical Course of Urinary Bladder Neoplasms. Scandinavian journal of urology and nephrology. 2007;41(3):182-90.
[34] Wang S, Tang J, Wang M, Yuan L, Zhang Z. Genetic Variation in Psca and Bladder Cancer Susceptibility in a Chinese Population. Carcinogenesis. 2010;31(4):621-4.
[35] Safarinejad MR, Shafiei N, Safarinejad S. Genetic Susceptibility of Methylenetetrahydrofolate Reductase (Mthfr) Gene C677t, A1298c, and G1793a Polymorphisms with Risk for Bladder Transitional Cell Carcinoma in Men. Medical oncology (Northwood, London, England). 2011;28 Suppl 1:S398-412.
[36] Pandith AA, Shah ZA, Khan NP, Baba KM, Wani MS, Siddiqi MA. Hras T81c Polymorphism Modulates Risk of Urinary Bladder Cancer and Predicts Advanced Tumors in Ethnic Kashmiri Population. Urologic oncology. 2013;31(4):487-92.
[37] Zhou B, Zhang P, Wang Y, Shi S, Zhang K, Liao H, Zhang L. Interleukin-17 Gene Polymorphisms Are Associated with Bladder Cancer in a Chinese Han Population. Molecular carcinogenesis. 2013;52(11):871-8.
[38] Chen WC, Wu HC, Hsu CD, Chen HY, Tsai FJ. P21 Gene Codon 31 Polymorphism Is Associated with Bladder Cancer. Urologic oncology. 2002;7(2):63-6.
[39] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Do DNA Repair Genes Ogg1, Xrcc3 and Xrcc7 Have an Impact on Susceptibility to Bladder Cancer in the North Indian Population? Mutation research. 2009;680(1-2):56-63.

[40] Lin HY, Huang CH, Yu TJ, Wu WJ, Yang MC, Lung FW. P53 Codon 72 Polymorphism as a Progression Index for Bladder Cancer. Oncology reports. 2012;27(4):1193-9.
[41] Pandith AA, Shah ZA, Khan NP, Rasool R, Afroze D, Yousuf A, Wani S, Siddiqi M. Role of Tp53 Arg72pro Polymorphism in Urinary Bladder Cancer Predisposition and Predictive Impact of Proline Related Genotype in Advanced Tumors in an Ethnic Kashmiri Population. Cancer genetics and cytogenetics. 2010;203(2):263-8.
[42] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.
[43] Kader AK, Liu J, Shao L, Dinney CP, Lin J, Wang Y, Gu J, Grossman HB, Wu X. Matrix Metalloproteinase Polymorphisms Are Associated with Bladder Cancer Invasiveness. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(9):2614-20.
[44] Kucukgergin C, Sanli O, Amasyali AS, Tefik T, Seckin S. Genetic Variants of Mnsod and Gpx1 and Susceptibility to Bladder Cancer in a Turkish Population. Medical oncology (Northwood, London, England). 2012;29(3):1928-34.
[45] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.
[46] Kucukgergin C, Isman FK, Dasdemir S, Cakmakoglu B, Sanli O, Gokkusu C, Seckin S. The Role of Chemokine and Chemokine Receptor Gene Variants on the Susceptibility and Clinicopathological Characteristics of Bladder Cancer. Gene. 2012;511(1):7-11.
[47] Wen H, Feng CC, Fang ZJ, Xia GW, Jiang HW, Xu G, Huang XD, Ding Q. Study on Bladder Cancer Susceptibility and Genetic Polymorphisms of Xpc, Xpg, and Cyp in Smokers and Non-Smokers. Actas urologicas espanolas. 2013;37(5):259-65.
[48] Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y, Song Y, Zhang L. Genetic Variants in Nampt Predict Bladder Cancer Risk and Prognosis in Individuals from Southwest Chinese Han Group. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4031-40.
[49] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[50] Weng WC, Huang YH, Yang SF, Wang SS, Kuo WH, Hsueh CW, Huang CH, Chou YE. Effect of Cd44 Gene Polymorphisms on Risk of Transitional Cell Carcinoma of the Urinary Bladder in Taiwan. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;37(5):6971-7.
[51] Mittal RD, Singh R, Manchanda PK, Ahirwar D, Gangwar R, Kesarwani P, Mandhani A. Xrcc1 Codon 399 Mutant Allele: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Cancer biology & therapy. 2008;7(5):645-50.

[52] Gangwar R, Mandhani A, Mittal RD. Xpc Gene Variants: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Journal of cancer research and clinical oncology. 2010;136(5):779-86.
[53] Ahmed T, Nawaz S, Noreen R, Bangash KS, Rauf A, Younis M, Anwar K, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R, Ali SHB. A 3' Untranslated Region Polymorphism Rs2304277 in the DNA Repair Pathway Gene Ogg1 Is a Novel Risk Modulator for Urothelial Bladder Carcinoma. Annals of human genetics. 2018;82(2):74-87.
[54] Gangwar R, Mandhani A, Mittal RD. Functional Polymorphisms of Cyclooxygenase-2 (Cox-2) Gene and Risk for Urinary Bladder Cancer in North India. Surgery. 2011;149(1):126-34.
[55] Zhou B, Zhang P, Tang T, Liao H, Zhang K, Pu Y, Chen P, Song Y, Zhang L. Polymorphisms and Plasma Levels of Il-27: Impact on Genetic Susceptibility and Clinical Outcome of Bladder Cancer. BMC cancer. 2015;15:433.
[56] Mittal RD, Mandal RK. Genetic Variation in Nucleotide Excision Repair Pathway Genes Influence Prostate and Bladder Cancer Susceptibility in North Indian Population. Indian journal of human genetics. 2012;18(1):47-55.
[57] Sobti RC, Kaur S, Sharma VL, Singh SK, Hosseini SA, Kler R. Susceptibility of Xpd and Rad51 Genetic Variants to Carcinoma of Urinary Bladder in North Indian Population. DNA and cell biology. 2012;31(2):199-210.
[58] Verhaegh GW, Verkleij L, Vermeulen SH, den Heijer M, Witjes JA, Kiemeney LA. Polymorphisms in the H19 Gene and the Risk of Bladder Cancer. Eur Urol. 2008;54(5):1118-26.
[59] Zhao T, Wu X, Liu J. Association between Interleukin-22 Genetic Polymorphisms and Bladder Cancer Risk. Clinics (Sao Paulo, Brazil). 2015;70(10):686-90.
[60] Ito M, Habuchi T, Watanabe J, Higashi S, Nishiyama H, Wang L, Tsuchiya N, Kamoto T, Ogawa O. Polymorphism within the Cyclin D1 Gene Is Associated with an Increased Risk of Carcinoma in Situ in Patients with Superficial Bladder Cancer. Urology. 2004;64(1):74-8.
[61] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Influence of Xpd and Ape1 DNA Repair Gene Polymorphism on Bladder Cancer Susceptibility in North India. Urology. 2009;73(3):675-80.
[62] Wen H, Ding Q, Fang ZJ, Xia GW, Fang J. Population Study of Genetic Polymorphisms and Superficial Bladder Cancer Risk in Han-Chinese Smokers in Shanghai. International urology and nephrology. 2009;41(4):855-64.
[63] Ichimura Y, Habuchi T, Tsuchiya N, Wang L, Oyama C, Sato K, Nishiyama H, Ogawa O, Kato T. Increased Risk of Bladder Cancer Associated with a Glutathione Peroxidase 1 Codon 198 Variant. The Journal of urology. 2004;172(2):728-32.
[64] Lima L, Silva J, Amaro T, Morais A, Lopes C, Medeiros R, Videira PA, Santos L. Il-4 and Tnf-Alpha Polymorphisms Are Associated with Risk of Multiple Superficial Tumors or Carcinoma in Situ Development. Urologia internationalis. 2011;87(4):457-63.
[65] Sakano S, Kumar R, Larsson P, Onelov E, Adolfsson J, Steineck G, Hemminki K. A Single-Nucleotide Polymorphism in the Xpg Gene, and Tumour Stage, Grade, and Clinical Course in Patients with Nonmuscle-Invasive Neoplasms of the Urinary Bladder. BJU international. 2006;97(4):847-51.
[66] Gu J, Tao J, Yang X, Li P, Yang X, Qin C, Cao Q, Cai H, Zhang Z, Wang M, Gu M, Lu Q, Yin C. Effects of Tsp-1-696 C/T Polymorphism on Bladder Cancer Susceptibility and Clinicopathologic Features. Cancer genetics. 2014;207(6):247-52.

[67] Lee HL, Chiou HL, Wang SS, Hung SC, Chou MC, Yang SF, Hsieh MJ, Chou YE. Wisp1 Genetic Variants as Predictors of Tumor Development with Urothelial Cell Carcinoma. Urologic oncology. 2018;36(4):160.e15-.e21.
[68] Xie L, Sun Y, Chen T, Tian D, Li Y, Zhang Y, Ding N, Shen Z, Xu H, Nian X, Sha N, Han R, Hu H, Wu C. Association between Mdm2 Snp309 T>G Polymorphism and the Risk of Bladder Cancer: New Data in a Chinese Population and an Updated Meta-Analysis. OncoTargets and therapy. 2015;8:3679-90.
[69] Rothman N, Garcia-Closas M, Chatterjee N, Malats N, Wu X, Figueroa JD, Real FX, Van Den Berg D, Matullo G, Baris D, Thun M, Kiemeney LA, Vineis P, De Vivo I, Albanes D, Purdue MP, Rafnar T, Hildebrandt MA, Kiltie AE, Cussenot O, Golka K, Kumar R, Taylor JA, Mayordomo JI, Jacobs KB, Kogevinas M, Hutchinson A, Wang Z, Fu YP, Prokunina-Olsson L, Burdett L, Yeager M, Wheeler W, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Andriole G, Jr., Grubb R, 3rd, Black A, Jacobs EJ, Diver WR, Gapstur SM, Weinstein SJ, Virtamo J, Cortessis VK, Gago-Dominguez M, Pike MC, Stern MC, Yuan JM, Hunter DJ, McGrath M, Dinney CP, Czerniak B, Chen M, Yang H, Vermeulen SH, Aben KK, Witjes JA, Makkinje RR, Sulem P, Besenbacher S, Stefansson K, Riboli E, Brennan P, Panico S, Navarro C, Allen NE, Bueno-de-Mesquita HB, Trichopoulos D, Caporaso N, Landi MT, Canzian F, Ljungberg B, Tjonneland A, Clavel-Chapelon F, Bishop DT, Teo MT, Knowles MA, Guarrera S, Polidoro S, Ricceri F, Sacerdote C, Allione A, Cancel-Tassin G, Selinski S, Hengstler JG, Dietrich H, Fletcher T, Rudnai P, Gurzau E, Koppova K, Bolick SC, Godfrey A, Xu Z, Sanz-Velez JI, M DG-P, Sanchez M, Valdivia G, Porru S, Benhamou S, Hoover RN, Fraumeni JF, Jr., Silverman DT, Chanock SJ. A Multi-Stage Genome-Wide Association Study of Bladder Cancer Identifies Multiple Susceptibility Loci. Nature genetics. 2010;42(11):978-84.
[70] Li Q, Tang T, Zhang P, Liu C, Pu Y, Zhang Y, Song H, Wang Y, Song Y, Su M, Zhou B, Zhang L. Correlation of Il-31 Gene Polymorphisms with Susceptibility and Clinical Recurrence of Bladder Cancer. Familial cancer. 2018;17(4):577-85.
[71] Wu J, Huang Q, Meng D, Huang M, Li C, Qin T. A Functional Rs353293 Polymorphism in the Promoter of Mir-143/145 Is Associated with a Reduced Risk of Bladder Cancer. PloS one. 2016;11(7):e0159115.
[72] Timirci-Kahraman O, Ozkan NE, Turan S, Farooqi AA, Verim L, Ozturk T, Inal-Gultekin G, Isbir T, Ozturk O, Yaylim I. Genetic Variants in the Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand and Death Receptor Genes Contribute to Susceptibility to Bladder Cancer. Genetic testing and molecular biomarkers. 2015;19(6):309-15.
[73] Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, Kakehi Y, Kakinuma H, Sato K, Nakamura A, Ogawa O, Kato T. Cyclin D1 Gene Polymorphism Is Associated with an Increased Risk of Urinary Bladder Cancer. Carcinogenesis. 2002;23(2):257-64.
[74] Gautam KA, Muktanand T, Sankhwar SN, Goel A, Sankhwar PL, Rajender S. Functional Polymorphisms in the Il6 Gene Promoter and the Risk of Urinary Bladder Cancer in India. Cytokine. 2016;77:152-6.
[75] Jaiswal PK, Goel A, Mittal RD. Association of P53 Codon 248 (Exon7) with Urinary Bladder Cancer Risk in the North Indian Population. Bioscience trends. 2011;5(5):205-10.
[76] Ratanajaraya C, Nishiyama H, Takahashi M, Kawaguchi T, Saito R, Mikami Y, Suyama M, Lathrop M, Yamada R, Ogawa O, Matsuda F. A Polymorphism of the

Polg2 Gene Is Genetically Associated with the Invasiveness of Urinary Bladder Cancer in Japanese Males. Journal of human genetics. 2011;56(8):572-6.
[77] Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun Wee J, Kim WJ. Genotypes of Tnf-Alpha, Vegf, Hogg1, Gstm1, and Gstt1: Useful Determinants for Clinical Outcome of Bladder Cancer. Urology. 2005;65(1):70-5.
[78] Gu J, Zhao H, Dinney CP, Zhu Y, Leibovici D, Bermejo CE, Grossman HB, Wu X. Nucleotide Excision Repair Gene Polymorphisms and Recurrence after Treatment for Superficial Bladder Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(4):1408-15.
[79] Decobert M, Larue H, Bergeron A, Harel F, Pfister C, Rousseau F, Lacombe L, Fradet Y. Polymorphisms of the Human Nramp1 Gene Are Associated with Response to Bacillus Calmette-Guerin Immunotherapy for Superficial Bladder Cancer. The Journal of urology. 2006;175(4):1506-11.
[80] Chiong E, Kesavan A, Mahendran R, Chan YH, Sng JH, Lim YK, Kamaraj R, Tan TM, Esuvaranathan K. Nramp1 and Hgpx1 Gene Polymorphism and Response to Bacillus Calmette-Guerin Therapy for Bladder Cancer. Eur Urol. 2011;59(3):430-7.
[81] Gangawar R, Ahirwar D, Mandhani A, Mittal RD. Impact of Nucleotide Excision Repair Ercc2 and Base Excision Repair Apex1 Genes Polymorphism and Its Association with Recurrence after Adjuvant Bcg Immunotherapy in Bladder Cancer Patients of North India. Medical oncology (Northwood, London, England). 2010;27(2):159-66.
[82] Ahirwar DK, Agrahari A, Mandhani A, Mittal RD. Cytokine Gene Polymorphisms Are Associated with Risk of Urinary Bladder Cancer and Recurrence after Bcg Immunotherapy. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2009;14(4):213-8.
[83] Wang M, Wang M, Yuan L, Wu D, Zhang Z, Yin C, Fu G, Wei Q, Zhang Z. A Novel Xpf -357a>C Polymorphism Predicts Risk and Recurrence of Bladder Cancer. Oncogene. 2010;29(13):1920-8.
[84] Chen M, Hildebrandt MA, Clague J, Kamat AM, Picornell A, Chang J, Zhang X, Izzo J, Yang H, Lin J, Gu J, Chanock S, Kogevinas M, Rothman N, Silverman DT, Garcia-Closas M, Grossman HB, Dinney CP, Malats N, Wu X. Genetic Variations in the Sonic Hedgehog Pathway Affect Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Cancer prevention research (Philadelphia, Pa). 2010;3(10):1235-45.
[85] Srivastava P, Kapoor R, Mittal RD. Association of Single Nucleotide Polymorphisms in Promoter of Matrix Metalloproteinase-2, 8 Genes with Bladder Cancer Risk in Northern India. Urologic oncology. 2013;31(2):247-54.
[86] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.
[87] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.
[88] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.
[89] Andrew AS, Gui J, Hu T, Wyszynski A, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Ekstrom RM, Li Z, Zens MS, Borsuk M, Moore JH, Karagas MR. Genetic Polymorphisms Modify Bladder Cancer Recurrence and

Survival in a USA Population-Based Prognostic Study. BJU international. 2015;115(2):238-47.
[90] Lima L, Oliveira D, Ferreira JA, Tavares A, Cruz R, Medeiros R, Santos L. The Role of Functional Polymorphisms in Immune Response Genes as Biomarkers of Bacille Calmette-Guerin (Bcg) Immunotherapy Outcome in Bladder Cancer: Establishment of a Predictive Profile in a Southern Europe Population. BJU international. 2015;116(5):753-63.
[91] Ke HL, Lin J, Ye Y, Wu WJ, Lin HH, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Glutathione Pathway Genes Predict Cancer Recurrence in Patients Treated with Transurethral Resection and Bacillus Calmette-Guerin Instillation for Non-Muscle Invasive Bladder Cancer. Annals of surgical oncology. 2015;22(12):4104-10.
[92] Deng X, Yang X, Cheng Y, Liu X, Li X, Zhao R, Qin C, Lu Q, Yin C. Gstp1 and Gsto1 Single Nucleotide Polymorphisms and the Response of Bladder Cancer Patients to Intravesical Chemotherapy. Scientific reports. 2015;5:14000.
[93] Wang M, Li Z, Chu H, Lv Q, Ye D, Ding Q, Xu C, Guo J, Du M, Chen J, Song Z, Yin C, Qin C, Gu C, Zhu Y, Xia G, Liu F, Zhang Z, Yuan L, Fu G, Hu Z, Tong N, Shen J, Liu K, Sun J, Sun Y, Li J, Li X, Shen H, Xu J, Shi Y, Zhang Z. Genome-Wide Association Study of Bladder Cancer in a Chinese Cohort Reveals a New Susceptibility Locus at 5q12.3. Cancer research. 2016;76(11):3277-84.
[94] Williams SB, Kamat AM, Mmeje C, Ye Y, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variants in the Inflammation Pathway as Predictors of Recurrence and Progression in Non-Muscle Invasive Bladder Cancer Treated with Bacillus Calmette-Guerin. Oncotarget. 2017;8(51):88782-91.
[95] Wang Z, Lim YK, Lim HCC, Chan YH, Ngiam N, Raman Nee Mani L, Esuvaranathan K, Ng CF, Teoh J, Chan E, Mahendran R, Chiong E. The Role of Vitamin D Receptor Polymorphisms in Predicting the Response to Therapy for Nonmuscle Invasive Bladder Carcinoma. The Journal of urology. 2018;200(4):737-42.
[96] Yang X, Li P, Yang X, Qin C, Cao Q, Zhang Z, Wang M, Cai H, Gu J, Tao J, Gu M, Lu Q, Yin C. Tsp-1-1223 a/G Polymorphism as a Potential Predictor of the Recurrence Risk of Bladder Cancer in a Chinese Population. International Journal of Genomics. 2013;2013:9.
[97] Zhao H, Liang D, Grossman HB, Wu X. Glutathione Peroxidase 1 Gene Polymorphism and Risk of Recurrence in Patients with Superficial Bladder Cancer. Urology. 2005;66(4):769-74.
[98] Lin J, Dinney CP, Grossman HB, Jhamb M, Zhu Y, Spitz MR, Wu X. E-Cadherin Promoter Polymorphism (C-160a) and Risk of Recurrence in Patients with Superficial Bladder Cancer. Clinical genetics. 2006;70(3):240-5.
[99] Ahirwar D, Kesarwani P, Manchanda PK, Mandhani A, Mittal RD. Anti- and Proinflammatory Cytokine Gene Polymorphism and Genetic Predisposition: Association with Smoking, Tumor Stage and Grade, and Bacillus Calmette-Guerin Immunotherapy in Bladder Cancer. Cancer genetics and cytogenetics. 2008;184(1):1-8.
[100] Horikawa Y, Nadaoka J, Saito M, Kumazawa T, Inoue T, Yuasa T, Tsuchiya N, Nishiyama H, Ogawa O, Habuchi T. Clinical Implications of the Mdm2 Snp309 and P53 Arg72pro Polymorphisms in Transitional Cell Carcinoma of the Bladder. Oncology reports. 2008;20(1):49-55.
[101] Ahirwar DK, Mandhani A, Mittal RD. Il-8 -251 T > a Polymorphism Is Associated with Bladder Cancer Susceptibility and Outcome after Bcg

Immunotherapy in a Northern Indian Cohort. Archives of medical research. 2010;41(2):97-103.
[102] Jaiswal PK, Goel A, Mandhani A, Mittal RD. Functional Polymorphisms in Promoter Survivin Gene and Its Association with Susceptibility to Bladder Cancer in North Indian Cohort. Molecular biology reports. 2012;39(5):5615-21.
[103] Wang M, Chu H, Li P, Yuan L, Fu G, Ma L, Shi D, Zhong D, Tong N, Qin C, Yin C, Zhang Z. Genetic Variants in Mirnas Predict Bladder Cancer Risk and Recurrence. Cancer research. 2012;72(23):6173-82.
[104] Deng X, Zhang X, Cheng Y, Yang X, Zhao R, Liu X, Li X, Qin C, Lu Q, Yin C. Xrcc1 Polymorphisms Associated with Survival among Chinese Bladder Cancer Patients Receiving Epirubicin and Mitomycin C. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(6):4591-6.
[105] Li P, Zhang X, Deng X, Tao J, Qin C, Yang X, Cheng Y, Lu Q, Wang Z, Yin C. Pharmacogenetic Association between Xrcc1 Polymorphisms and Improved Outcomes in Bladder Cancer Patients Following Intravesical Instillation of Epirubicin. International journal of clinical and experimental medicine. 2015;8(7):11167-73.
[106] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.
[107] Ryk C, Wiklund NP, Nyberg T, De Verdier PJ. Ser608leu Polymorphisms in the Nitric Oxide Synthase-2 Gene May Influence Urinary Bladder Cancer Pathogenesis. Scandinavian journal of urology and nephrology. 2011;45(5):319-25.
[108] Sakano S, Berggren P, Kumar R, Steineck G, Adolfsson J, Onelov E, Hemminki K, Larsson P. Clinical Course of Bladder Neoplasms and Single Nucleotide Polymorphisms in the Cdkn2a Gene. International journal of cancer. 2003;104(1):98-103.
[109] Basturk B, Yavascaoglu I, Oral B, Goral G, Oktay B. Cytokine Gene Polymorphisms Can Alter the Effect of Bacillus Calmette-Guerin (Bcg) Immunotherapy. Cytokine. 2006;35(1-2):1-5.
[110] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).
[111] Hess J, Stelmach P, Eisenhardt A, Rubben H, Reis H, Schmid KW, Bachmann HS. Impact of Bcl2 Polymorphisms on Survival in Transitional Cell Carcinoma of the Bladder. Journal of cancer research and clinical oncology. 2017;143(9):1659-70.
[112] Castillejo A, Rothman N, Murta-Nascimento C, Malats N, Garcia-Closas M, Gomez-Martinez A, Lloreta J, Tardon A, Serra C, Garcia-Closas R, Chanock S, Silverman DT, Dosemeci M, Kogevinas M, Carrato A, Soto JL, Real FX. Tgfb1 and Tgfbr1 Polymorphic Variants in Relationship to Bladder Cancer Risk and Prognosis. International journal of cancer. 2009;124(3):608-13.
[113] Teo MT, Dyrskjot L, Nsengimana J, Buchwald C, Snowden H, Morgan J, Jensen JB, Knowles MA, Taylor G, Barrett JH, Borre M, Orntoft TF, Bishop DT, Kiltie AE. Next-Generation Sequencing Identifies Germline Mre11a Variants as Markers of Radiotherapy Outcomes in Muscle-Invasive Bladder Cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2014;25(4):877-83.

[114] Eisenhardt A, Siffert W, Rosskopf D, Musch M, Mosters M, Roggenbuck U, Jockel KH, Rubben H, Lummen G. Association Study of the G-Protein Beta3 Subunit C825t Polymorphism with Disease Progression in Patients with Bladder Cancer. World journal of urology. 2005;23(4):279-86.
[115] Teo MT, Landi D, Taylor CF, Elliott F, Vaslin L, Cox DG, Hall J, Landi S, Bishop DT, Kiltie AE. The Role of Microrna-Binding Site Polymorphisms in DNA Repair Genes as Risk Factors for Bladder Cancer and Breast Cancer and Their Impact on Radiotherapy Outcomes. Carcinogenesis. 2012;33(3):581-6.
[116] Shinohara A, Sakano S, Hinoda Y, Nishijima J, Kawai Y, Misumi T, Nagao K, Hara T, Matsuyama H. Association of Tp53 and Mdm2 Polymorphisms with Survival in Bladder Cancer Patients Treated with Chemoradiotherapy. Cancer science. 2009;100(12):2376-82.
[117] Chen M, Gu J, Delclos GL, Killary AM, Fan Z, Hildebrandt MA, Chamberlain RM, Grossman HB, Dinney CP, Wu X. Genetic Variations of the Pi3k-Akt-Mtor Pathway and Clinical Outcome in Muscle Invasive and Metastatic Bladder Cancer Patients. Carcinogenesis. 2010;31(8):1387-91.
[118] Andrew AS, Gui J, Sanderson AC, Mason RA, Morlock EV, Schned AR, Kelsey KT, Marsit CJ, Moore JH, Karagas MR. Bladder Cancer Snp Panel Predicts Susceptibility and Survival. Human genetics. 2009;125(5-6):527-39.
[119] Mason RA, Morlock EV, Karagas MR, Kelsey KT, Marsit CJ, Schned AR, Andrew AS. Egfr Pathway Polymorphisms and Bladder Cancer Susceptibility and Prognosis. Carcinogenesis. 2009;30(7):1155-60.
[120] Sacerdote C, Guarrera S, Ricceri F, Pardini B, Polidoro S, Allione A, Critelli R, Russo A, Andrew AS, Ye Y, Wu X, Kiemeney LA, Bosio A, Casetta G, Cucchiarale G, Destefanis P, Gontero P, Rolle L, Zitella A, Fontana D, Vineis P, Matullo G. Polymorphisms in the Xrcc1 Gene Modify Survival of Bladder Cancer Patients Treated with Chemotherapy. International journal of cancer. 2013;133(8):2004-9.
[121] Djukic TI, Savic-Radojevic AR, Pekmezovic TD, Matic MG, Pljesa-Ercegovac MS, Coric VM, Radic TM, Suvakov SR, Krivic BN, Dragicevic DP, Simic TP. Glutathione S-Transferase T1, O1 and O2 Polymorphisms Are Associated with Survival in Muscle Invasive Bladder Cancer Patients. PloS one. 2013;8(9):e74724.
[122] Wray NR, Wijmenga C, Sullivan PF, Yang J, Visscher PM. Common Disease Is More Complex Than Implied by the Core Gene Omnigenic Model. Cell. 2018;173(7):1573-80.
[123] Grotenhuis AJ, Dudek AM, Verhaegh GW, Witjes JA, Aben KK, van der Marel SL, Vermeulen SH, Kiemeney LA. Prognostic Relevance of Urinary Bladder Cancer Susceptibility Loci. PloS one. 2014;9(2):e89164.
[124] Ilic M, Ilic I. Epidemiology of Pancreatic Cancer. World journal of gastroenterology. 2016;22(44):9694-705.
[125] Ba X, Boldogh I. 8-Oxoguanine DNA Glycosylase 1: Beyond Repair of the Oxidatively Modified Base Lesions. Redox biology. 2018;14:669-78.
[126] Boiteux S, Radicella JP. The Human Ogg1 Gene: Structure, Functions, and Its Implication in the Process of Carcinogenesis. Archives of biochemistry and biophysics. 2000;377(1):1-8.
[127] Zou H, Li Q, Xia W, Liu Y, Wei X, Wang D. Association between the Ogg1 Ser326cys Polymorphism and Cancer Risk: Evidence from 152 Case-Control Studies. Journal of Cancer. 2016;7(10):1273-80.
[128] Zhou PT, Li B, Ji J, Wang MM, Gao CF. A Systematic Review and Meta-Analysis of the Association between Ogg1 Ser326cys Polymorphism and Cancers. Medical oncology (Northwood, London, England). 2015;32(2):472.

[129] Wenjuan C, Jianzhong L, Chong L, Yanjun G, Keqing L, Hanzhang W, Zhiping W. The Hogg1 Ser326cys Gene Polymorphism and Susceptibility for Bladder Cancer: A Meta-Analysis. International braz j urol : official journal of the Brazilian Society of Urology. 2016;42(5):883-96.
[130] Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The Mdm2-P53 Pathway Revisited. Journal of biomedical research. 2013;27(4):254-71.
[131] Hellwege JN, Keaton JM, Giri A, Gao X, Velez Edwards DR, Edwards TL. Population Stratification in Genetic Association Studies. Current protocols in human genetics. 2017;95:1.22.1-1..3.
[132] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.

CHAPTER 4.

GENOME-WIDE ASSOCIATION STUDY FOR TUMOUR STAGE, GRADE, SIZE, AND AGE AT THE TIME OF DIAGNOSIS OF NON-MUSCLE-INVASIVE BLADDER CANCER

Contents of this chapter have been published:

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Galesloot TE, Kiemeney L, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer. Eur Urol Oncol. 2019;2(4):381-9.

ABSTRACT

INTRODUCTION

Non-muscle-invasive bladder cancer (NMIBC) causes a considerable health burden due to the high recurrence and progression rates. Past studies have identified multiple candidate loci associated with NMIBC prognosis, albeit lacking validation. Moreover, scarce reports exist on genetic susceptibility to independent prognostic predictors of NMIBC, such as stage or grade. Our objective was to investigate genetic associations with NMIBC tumour and patient characteristics at the time of diagnosis.

METHODS

A sample of 653 NMIBC cases come from the Bladder Cancer Prognosis Programme (BCPP). Replication of the significant findings was conducted in the Nijmegen Bladder Cancer Study (NBCS) cohort (N=,1470). Genome-wide association study (GWAS) was carried out for outcomes of tumour size (as continuous variable in centimetres), stage (Tis and T1 vs Ta), grade (G3 vs G2 and G1), and age (as continuous (years) and dichotomous (70.2 years as a cut-off) variables).

RESULTS

Significant (P<5E-08) associations (N=61) with tumour size, stage, grade, and age were identified in the GWAS discovery stage. None of the variants were independently significantly associated in the replication cohort. A meta-analysis of both cohorts suggests rs180940944 (13q13.3 locus, *NBEA*) was associated with tumour size as a continuous variable (ß=0.9 cm, p=2.92E-09). However, other SNPs in this region did not show evidence of association in the meta-analysis.

CONCLUSIONS

Our study suggests rs180940944 (*NBEA*) is associated with an increased NMIBC tumour size at the time of diagnosis. Given study limitations, further replication is essential to validate the finding. Current study reports on a genome-wide association study on non-muscle-invasive bladder cancer tumour and patient characteristics. We suggest *NBEA* gene might be associated with increased tumour size at the time of diagnosis. The result must be replicated to establish validity.

INTRODUCTION

Urinary bladder cancer (UBC) accounts for 430,000 new cases worldwide annually, with 70-80% of new cases presenting as non-muscle-invasive bladder cancer (NMIBC) [1]. NMIBC causes significant burden on healthcare systems due to high recurrence and progression rates (5-year recurrence rate: 50-70%, 5-year progression rate: 10-30%) [1]. Considerable clinical improvements could be made by better, even personalised, prognostication and risk stratification [1]. There have been several attempts to apply different approaches for accurate disease prognostication, and although descriptive on a population-level, a substantial lack of precision of individual outcomes remains [2], requiring ongoing improvement.

Few candidate-gene studies of UBC prognosis exist, with limited successful replication [3-5]. A recent study reported that out of 114 reported loci for UBC progression and prognosis, only six single nucleotide polymorphisms (SNPs) showed significant associations in an independent cohort, namely: NMIBC progression (rs6678136 (*RGS4*), rs11585883 (*RGS5*)), recurrence among Bacillus Calmette–Guérin (BCG)-treated NMIBC patients (rs1799793 (*ERCC2*), rs187238 (*IL18*)), and muscle-invasive bladder cancer (MIBC) overall survival (rs12035879 (*RGS5*), rs2075786 (*TERT*)) [3]. Powerful GWAS studies on NMIBC prognosis show promise, but are still ongoing [6]. A previous attempt to include genetic variation failed to increase prognostic tool performance [7], suggesting the issue is more complex. However, latter study utilised a relatively small panel of SNPs (170,000), which has lower power of discovering significant loci in comparison to genotype-imputed sets harbouring millions of variants for analysis [8]. The inter-study lack of consensus might be due to several reasons: spurious findings, lack of statistical power, and variation in outcome definition.

Other studies also suggest significant genetic signals might be only present for tumours of certain grade or stage [9, 10]. However, reports on genetic associations for characteristics that directly influence NMIBC outcome are scarce, precluding further investigations on their relevance for NMIBC prognostication.

To provide more evidence on potential genetic associations, we have performed a GWAS on key NMIBC characteristics (stage, grade, size of the tumour, risk category assigned by The European Organization for Research and Treatment of Cancer (EORTC)), as well as age at the time of diagnosis within the West Midlands' Bladder Cancer Prognosis Programme (BCPP) cohort including replication in the Nijmegen Bladder Cancer Study (NBCS).

METHODS

Participants and genotyping

BCPP is a prospective cohort that initially recruited 1,544 eligible patients and is described in more detail elsewhere [11]. Clinical data on stage, grade, and size of tumours and demographic information (age, gender) were gathered with bespoke case report forms. Tumour size of the largest tumour was established visually whilst performing transurethral resection of the bladder tumour (TURBT). Blood samples of 888 participants with confirmed UBC were genotyped on the Illumina Infinium OmniExpress-24 BeadChip array at deCODE Genetics (Reykjavik, Iceland).

Tumours of stages pTa, pT1, or pTis were included to limit our analyses to NMIBC, resulting in a dataset of 712 cases.

Quality control (QC)

QC procedures were carried out using PLINK v1.90 [12]. The exact thresholds applied and number of exclusions per step are outlined in **Figure 4.1**.

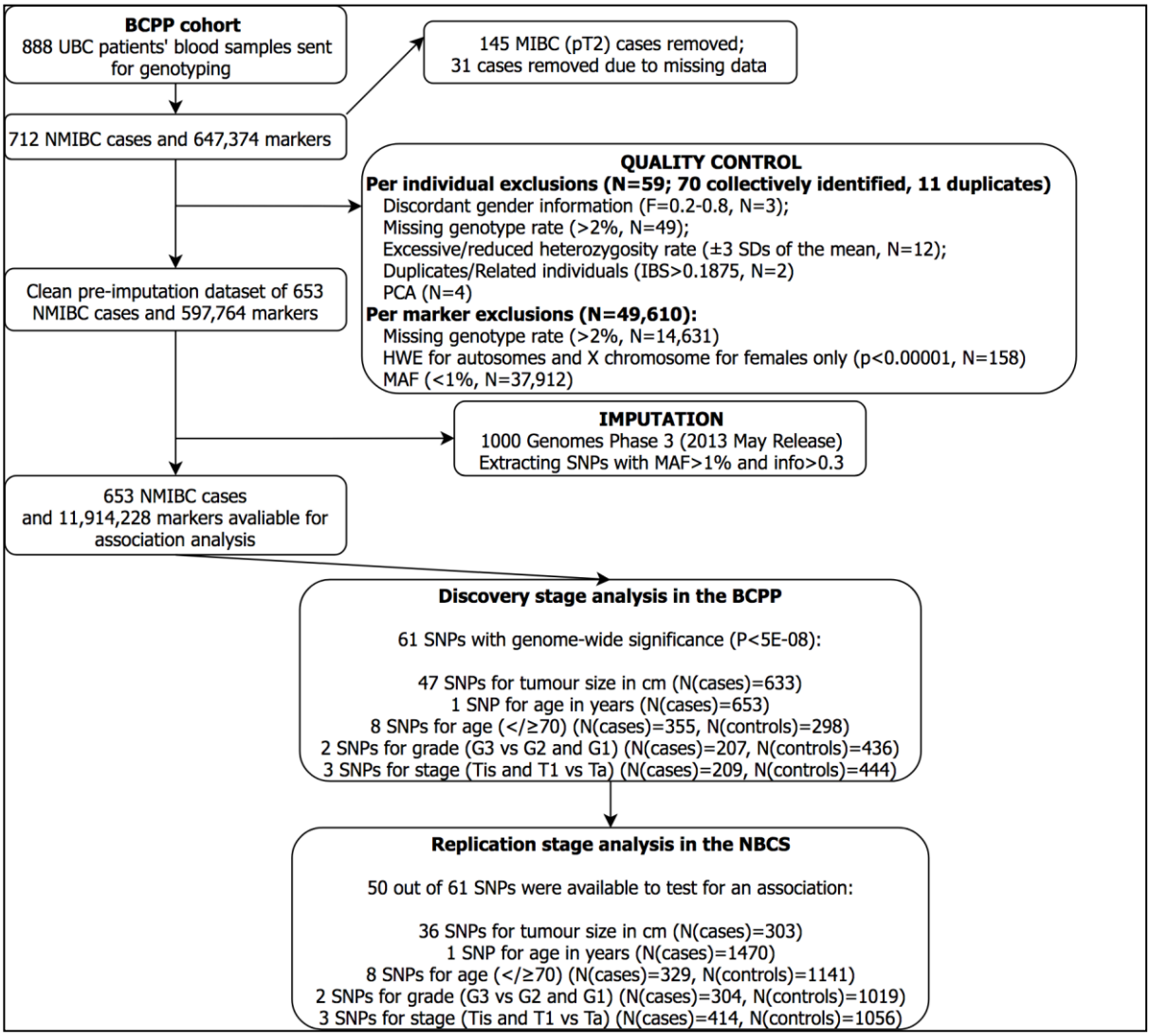


Figure 4.1. A flowchart of the main steps in the GWAS analysis.
BCPP-Bladder Cancer Prognosis Programme; HWE-Hardy-Weinberg equilibrium; MAF-minor allele frequency; MIBC-muscle-invasive bladder cancer; NBCS-Nijmegen Bladder Cancer Study; NMIBC-non-muscle-invasive bladder cancer; PCA-principal component analysis; QC-quality control; SD-standard deviation; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer**.**

Generic QC procedures per individual excluded those with an inconclusive gender call, excessive genotype missingness rate, increased or reduced genotype heterozygosity rate, duplicate samples, and related individuals.

To avoid any bias introduced by population stratification, a principal component analysis (PCA) was carried out. Investigation of PCA plots resulted in exclusion of

clear population outliers. Genomic inflation factor (λ) value was estimated for all outcomes of interest; none of the values exceeded 1.03.

Marker-specific QC procedures covered excluding SNPs deviating from the Hardy-Weinberg equilibrium, exceeding acceptable missing rate, and rare variants.

In total, a dataset consisting of 653 individuals and 597,764 markers remained for further analyses.

Imputation

Imputation utilised a two-step approach: haplotype phasing by Eagle v2.3.2 [13], followed by genotype imputation with IMPUTE2 [14], using 1000 Genomes Phase 3 [15] as a reference panel in the genome build 19 (GRCh37/hg19). Once imputed, the dataset was filtered for SNPs with info values (an imputation accuracy measure) of >0.3 and MAFs of >1%, resulting in a dataset containing 11,914,228 markers available for genetic association analyses.

Statistical analysis

Statistical analyses were performed using SNPtest v2.5.2 [8] and R statistical package (v3.3.2) [16].

To establish the relation between germline variation and tested outcomes, linear regression was used for continuous variables and logistic regression for all binary endpoints. Age was tested as a continuous (years) and binary variable (mean was considered as a cut-off value for categorisation (resulting in strata of </≥ 70 years)). Tumour size (cm) was tested as a continuous and categorical variable (</≥ 3cm [17]). Stage (Tis and T1 versus Ta) and grade (G3 versus G2 and G1) were treated as binary variables. In addition, low-, intermediate-, and high-risk EORTC categories were

assigned to each NMIBC case and were tested as a dichotomous variable of high- versus low- and intermediate-risk groups [17].

All analyses were adjusted for participant gender and first five genetic principal components to increase estimate precision and to adjust for any potential residual population stratification bias. An association was held significant if p-value <5E-08, and promising if below 5E-06.

Post-GWAS power calculations were carried out in web-based GAS Power Calculator [18].

Manhattan and Quantile-quantile (QQ) graphs were plotted for each tested outcome. For significant hits, regional association plots were constructed using LocusZOOM tool [19], except for hits that have not yet been assigned an ID (rsID).

Functional annotation

Identified significant SNPs were mapped using a web-based SNPnexus tool [20], with Ensembl [21] (Version 74) as a functional annotation system.

Replication

Genome-wide significant hits were attempted to replicate in a sample of 1,470 NMIBC cases from the NBCS [22] (**Figure 4.1**). Briefly, the NBCS recruited UBC patients via the population-based cancer registry in the Nijmegen region. Eligible cases were diagnosed during 1995-2006 and were under the age of 75; additional data was collected via linkage with hospital-patient records [22], including tumour size, which was reported after visual evaluation during cystoscopy. Details of genotype data cleaning and initial analysis is provided in detail elsewhere [22].

We used META [23] software to perform meta-analysis on association results of both cohorts and calculated a combined p-value per SNP. An inverse-variance method was used, assuming a random-effects model. I^2 index and p-value were calculated to evaluate potential heterogeneity between the estimates of the two cohorts [23].

RESULTS

Baseline clinical characteristics of the discovery and replication cohorts are shown in **Table 4.1.**

Majority of cases in BCPP were male (78.1%), with an average age of 70 years. Tumour size mean was 2.5 cm, and most of the participants were diagnosed with stage Ta (68%) and T1 (30.5%) tumours. More than a third of cases presented as G2 (37.5%), followed by G3 (31.7%) and G1 (29.2%) NMIBC. The distribution of variable categories and measures were similar between the BCPP and NBCS cohorts.

In the discovery-stage analysis, a total of 61 SNPs, corresponding to 29 different regions, showed genome-wide statistically significant associations with at least one of the outcomes. Out of those, 20 loci were mapped to genes (all intronic regions) (**Table 4.2**). Significant associations were observed for size and age as continuous variables, as well as for binary outcomes of stage, grade, and age.

Most of the SNPs (N=47) were found to be associated with tumour size, the effect sizes ranging from 0.65 (rs35225990 in *FAM194B*, p= 2.85E-08) to 2.6 (rs370572716 in 9p13.1, p=4.04E-09) centimetres (**Table 4.2).**

Table 4.1. Descriptive characteristics of the discovery (BCPP) and replication (NBCS) cohorts.

Variables	Discovery set (N=653)	Replication set (N=1470)
Age, years		
Mean (SD)	70.2 (10.5)	62.5 (9.7)
Median (range)	71.5 (34.3 - 91.5)	64 (25.0 - 91.0)
Age, years		
<70 (%)	298 (45.6)	329 (22.4)
≥70 (%)	355 (54.4)	1141 (77.6)
Sex		
Males (%)	510 (78.1)	1208 (82.2)
Females (%)	143 (21.9)	262 (17.8)
Tumour size (cm)		
Mean (SD)	2.5 (1.9)	2.4 (1.3)
Median (range)	2.0 (0.2-15.0)	2.0 (0.05 - 7.5)
Missing (%)	20 (3.1)	1168 (79.5)
Stage		
Ta (%)	444 (68.0)	1056 (71.8)
T1 (%)	199 (30.5)	349 (23.7)
Tis (%)	10 (1.5)	65 (4.4)
Grade		
G1 (%)	191 (29.2)	401 (27.3)
G2 (%)	245 (37.5)	618 (42.0)
G3 (%)	207 (31.7)	304 (20.7)
Missing (%)	10 (1.5)	147 (10.0)
EORTC risk category		
Low (%)	66 (10.1)	NA
Intermediate (%)	276 (42.3)	NA
High (%)	311 (47.6)	NA

BCPP-Bladder Cancer Prognosis Programme; EORTC-European Organisation for Research and Treatment of Cancer; SD-standard deviation; NA-Not available; NBCS-Nijmegen Bladder Cancer Study; SD-Standard deviation.

Table 4.2. Genetic associations with NMIBC tumour and patient characteristics at baseline in the discovery (BCPP) and replication (NBCS) stages and a joint analysis. Most promising SNP is marked in bold.

						Discovery cohort (BCPP)				Replication cohort (NBCS)					
Phenotype	**rsID**	**BP**	**Locus**	**REF**	**EFF**	**MAF**	**ß (SD)**	**OR (95% CI)**	**P value**	**MAF**	**ß (SD)**	**OR (95% CI)**	**P value**	**P (joint)**	**Annotation**
Size (cm)	**rs180940944**	**35950093**	**13q13.3**	**C**	**T**	**0.03**	**0.97 (0.16)**		**6.73E-09**	**0.004**	**0.71 (0.80)**		**0.38**	**2.92E-09**	***NBEA***
Size (cm)	rs113705641	5375733	3p26.1	A	G	0.02	1.38 (0.25)		2.99E-08	0.02	0.50 (0.34)		0.14	0.03	-
Size (cm)	rs74603364	79509518	6q14.1	C	T	0.02	1.38 (0.22)		6.54E-10	0.02	0.50 (0.31)		0.10	0.03	-
Size (cm)	rs143076258	136382230	4q28.3	G	A	0.02	1.18 (0.20)		9.21E-09	0.01	0.35 (0.38)		0.36	0.04	-
Size (cm)	rs4646911	34856662	6p21.31	G	A	0.01	1.67 (0.30)		3.76E-08	0.01	0.47 (0.53)		0.37	0.05	*TAF11*
Size (cm)	rs180910528	79821806	6q14.1	A	C	0.01	1.74 (0.28)		4.67E-10	0.01	0.43 (0.36)		0.23	0.09	-
Size (cm)	rs187040828	79802426	6q14.1	T	C	0.01	1.74 (0.28)		4.89E-10	0.02	0.36 (0.34)		0.29	0.12	-
Size (cm)	rs80026656	53756380	18q21.2	A	G	0.01	1.50 (0.26)		1.27E-08	0.02	0.29 (0.31)		0.34	0.13	*CTD-2008L17.2*
Size (cm)	rs35225990	46117489	13q14.13	C	T	0.07	0.65 (0.12)		2.85E-08	0.06	0.11 (0.17)		0.51	0.14	*FAM194B*
Size (cm)	rs144383242	79489625	6q14.1	G	T	0.01	1.66 (0.26)		1.88E-10	0.01	0.30 (0.34)		0.37	0.14	-
Size (cm)	rs117587674	79432536	6q14.1	G	A	0.01	1.67 (0.26)		1.70E-10	0.01	0.30 (0.34)		0.37	0.14	-
Size (cm)	rs180991319	36850863	19q13.12	T	A	0.01	1.87 (0.33)		2.35E-08	0.00	0.12 (0.98)		0.90	0.14	*ZFP14*
Size (cm)	rs117407537	35652859	13q13.3	G	A	0.03	0.98 (0.17)		2.16E-08	0.02	0.15 (0.30)		0.62	0.15	*NBEA*
Size (cm)	rs77827766	35808410	13q13.3	G	C	0.03	1.00 (0.18)		1.58E-08	0.02	0.15 (0.30)		0.61	0.15	*NBEA*

Size (cm)	rs117318492	35776449	13q13.3	T	C	0.03	1.00 (0.18)		1.58E-08	0.02	0.15 (0.30)		0.61	0.15	*NBEA*
Size (cm)	rs112579236	35742893	13q13.3	A	G	0.03	0.96 (0.17)		3.47E-08	0.02	0.14 (0.29)		0.62	0.15	*NBEA*
Size (cm)	rs117989790	35758974	13q13.3	G	C	0.03	1.01 (0.18)		1.47E-08	0.02	0.15 (0.30)		0.61	0.15	*SCAND3P1*
Size (cm)	rs117286929	35804780	13q13.3	A	G	0.03	1.01(0.18)		1.52E-08	0.02	0.15(0.30)		0.61	0.15	*NBEA*
Size (cm)	rs200899670	46170799	15q21.1	TCAAA	T	0.01	2.47 (0.34)		1.63E-12	0.03	0.42 (0.29)		0.16	0.16	*RP11-718O11.1*
Size (cm)	rs143664498	35919424	13q13.3	C	A	0.03	0.99 (0.17)		1.84E-08	0.02	0.12 (0.29)		0.69	0.18	*NBEA*
Size (cm)	rs117382849	35924241	13q13.3	A	G	0.03	0.99 (0.17)		1.90E-08	0.02	0.12 (0.29)		0.69	0.18	*NBEA*
Size (cm)	rs117576619	35887557	13q13.3	T	C	0.03	1.00 (0.17)		1.66E-08	0.02	0.12 (0.29)		0.69	0.18	*NBEA*
Size (cm)	rs144366722	35845426	13q13.3	A	G	0.03	1.00 (0.18)		1.57E-08	0.02	0.12 (0.29)		0.69	0.18	*NBEA*
Size (cm)	rs116854115	35865482	13q13.3	T	C	0.03	1.01 (0.18)		1.57E-08	0.02	0.12 (0.29)		0.69	0.18	*NBEA*
Size (cm)	rs151184057	5665859	2p25.2	C	T	0.01	1.51 (0.27)		3.88E-08	0.02	0.19 (0.35)		0.59	0.19	-
Size (cm)	rs78813710	3160739	7p22.2	T	G	0.01	1.57 (0.28)		3.12E-08	0.01	0.15 (0.41)		0.72	0.21	-
Size (cm)	rs117889651	35987813	13q13.3	A	G	0.03	0.92 (0.17)		3.91E-08	0.03	0.06 (0.26)		0.83	0.24	*NBEA*
Size (cm)	rs148373773	14919905	6p23	AC	A	0.03	0.96 (0.17)		3.17E-08	0.05	0.07 (0.20)		0.72	0.24	-
Size (cm)	rs75585701	2194093	3p26.3	C	G	0.02	1.60 (0.22)		2.66E-12	0.02	0.09 (0.33)		0.79	0.25	*CNTN4*
Grade (G3 vs G2 and G1)	rs150914897	22460455	14q11.2	C	T	0.06		3.42 (2.11-5.55)	5.13E-09	0.05		1.11 (0.74-1.65)	0.60	0.26	*TRAV16*

Size (cm)	rs75801131	70017072	18q22.3	C	A	0.02	1.53 (0.25)		2.08E-09	0.02	0.04 (0.32)		0.90	0.28	-
Age (years)	rs142492877	98482828	9q22.32	A	G	0.04	-0.95 (0.16)		1.05E-08	0.03	-0.03 (0.12)		0.79	0.29	-
Size (cm)	rs76779534	11737232	10p14	A	G	0.02	1.30 (0.22)		5.57E-09	0.01	-0.09 (0.50)		0.86	0.33	-
Size (cm)	rs73570873	11737713	10p14	T	A	0.02	1.30 (0.22)		4.97E-09	0.01	-0.10 (0.50)		0.84	0.34	-
Size (cm)	rs12265817	11738801	10p14	C	T	0.02	1.28 (0.22)		6.82E-09	0.01	-0.13 (0.50)		0.79	0.36	-
Grade (G3 vs G2 and G1)	rs116923391	22406144	14q11.2	C	T	0.06		3.86(2.38-6.26)	2.07E-10	0.06		0.93(0.64-1.37)	0.69	0.37	-
Age (</≥70.2)	rs41515546	125998959	7q31.33	T	C	0.16		2.49 (1.81-3.44)	1.96E-08	0.15		0.93 (0.72-1.19)	0.59	0.40	-
Age (</≥70.2)	rs17149636	126018952	7q31.33	A	G	0.17		2.51 (1.82-3.46)	1.62E-08	0.15		0.92 (0.72-1.18)	0.57	0.40	*AC000370.2*
Age (</≥70.2)	rs17149628	126006965	7q31.33	C	T	0.16		2.49 (1.81-3.44)	1.95E-08	0.15		0.92 (0.72-1.18)	0.56	0.41	-
Age (</≥70.2)	rs12666814	125979540	7q31.33	C	T	0.16		2.49 (1.80-3.44)	2.05E-08	0.15		0.92 (0.72-1.18)	0.55	0.41	-
Age (</≥70.2)	rs73223045	125992106	7q31.33	G	C	0.16		2.49 (1.81-3.44)	1.97E-08	0.15		0.92 (0.72-1.18)	0.53	0.41	-
Age (</≥70.2)	rs12673089	126006133	7q31.33	C	T	0.16		2.49 (1.81-3.44)	1.95E-08	0.15		0.92 (0.72-1.18)	0.53	0.41	-
Age (</≥70.2)	rs17149580	125978216	7q31.33	A	G	0.16		2.46 (1.78-3.39)	2.18E-08	0.15		0.91 (0.71-1.17)	0.50	0.42	-
Age (</≥70.2)	rs17149630	126006996	7q31.33	C	T	0.16		2.49 (1.81-3.44)	1.95E-08	0.15		0.91 (0.71-1.17)	0.49	0.42	-

Stage (Tis and T1 vs Ta)	rs76497895	21393419	12p12.1	G	T	0.02		0.03 (0.001-0.83)	4.18E-08	0.02		1.39 (0.84-2.32)	0.10	0.44	*SLCO1B1*
Stage (Tis and T1 vs Ta)	rs116946525	21391500	12p12.1	T	A	0.02		0.03 (0.001-0.83)	4.23E-08	0.02		1.39 (0.84-2.32)	0.10	0.44	*SLCO1B1*
Size (cm)	rs141965746	46544198	21q22.3	T	G	0.02	1.27 (0.23)		3.61E-08	0.02	-0.21 (0.28)		0.45	0.47	*ADARB1*
Stage (Tis and T1 vs Ta)	rs117248430	101506559	9q22.33	C	T	0.01		0.003 (1.71E-09-3895.6)	3.73E-08	0.01		1.13 (0.50-2.56)	0.76	0.48	*ANKS6*
Size (cm)	rs188958632	38266174	14q21.1	G	A	0.01	1.53 (0.27)		1.42E-08	0.03	-0.39 (0.22)		0.08	0.56	*TTC6*
Size (cm)	rs189352109	145555946	2q22.3	T	C	0.01	1.46 (0.26)		3.77E-08	0.01	-0.75 (0.45)		0.10	0.73	*TEX41*
Size (cm)	rs3752175	2516839	19p13.3	G	A	0.01	2.14 (0.38)		3.57E-08	NA	NA	NA	NA	NA	*GNG7*
Size (cm)	rs182792180	3164492	7p22.2	C	T	0.01	1.59 (0.28)		2.18E-08	NA	NA	NA	NA	NA	-
Size (cm)	rs117108730	35735418	13q13.3	T	C	0.02	1.10 (0.19)		5.83E-09	NA	NA	NA	NA	NA	*NBEA*
Size (cm)	rs117215187	35950090	13q13.3	C	T	0.03	0.97(0.16)		6.73E-09	NA	NA	NA	NA	NA	*NBEA*
Size (cm)	14	38247577	14q21.1	CTGG	C	0.01	2.21 (0.37)		2.46E-09	NA	NA	NA	NA	NA	*TTC6*
Size (cm)	rs183885923	38310637	19q13.13	G	A	0.01	1.96 (0.33)		5.64E-09	NA	NA	NA	NA	NA	*CTD-2554C21.2*
Size (cm)	rs370572716	38920614	9p13.1	T	A	0.01	2.59 (0.43)		4.04E-09	NA	NA	NA	NA	NA	-
Size (cm)	rs2937268	66553607	1p31.3	C	T	0.04	0.94 (0.16)		1.07E-08	NA	NA	NA	NA	NA	*PDE4B*
Size (cm)	X	117703032	23q24	C	T	0.01	1.05 (0.18)		7.93E-09	NA	NA	NA	NA	NA	*DOCK11*

Size (cm)	rs76670367	136254151	4q28.3	G	T	0.02	1.16 (0.21)		2.97E-08	NA	NA	NA	NA	NA	-
Size (cm)	rs151220146	180402493	2q31.2	CA	C	0.01	2.03 (0.31)		8.03E-11	NA	NA	NA	NA	NA	*ZNF385B*

BP-base-pair; BCPP-Bladder Cancer Prognosis Programme; CI-confidence interval; EFF-effect allele; MAF-minor allele frequency (corresponds to the alternative allele); NBCS-Netherlands Bladder Cancer Study; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; REF-reference allele; rsID-SNP ID; SE-standard error, SD-Standard deviation.

One SNP in 9q22.32, rs142492877, showed statistically significant association with decreased age at diagnosis of almost one year (ß=-0.95, SE=0.16, p=1.05E-08). Age as a binary trait showed associations in the same direction, although in a different genomic region (7q31.33) with an odds ratio (OR) ranging between 2.46 (rs17149580, p=2.18E-08) and 2.51 (rs17149636, p=1.62E-08) across eight SNPs.

The 14q11.2 locus showed strong associations with being diagnosed with a higher grade of NMIBC (rs15091489 in the *TRAV16* gene (OR=3.42, 95%CI: 2.11-5.55, p=5.13E-09) and rs116923391 (OR=3.86, 95% CI: 2.38-6.26, p=2.07E-10)).

Several protective variants for tumour stage were observed, namely: rs117248430 in *ANKS6* (OR=0.003, 95%CI=1.71E-09-3895.6, p=3.73E-08), and two markers in the *SLCO1B1* gene (rs76497895 (OR=0.03, 95%CI=0.001-0.83, p=4.18E-08); rs116946525 (OR=0.03, 95%CI=0.001-0.83, p=4.23E-08)). The strength of the effect and corresponding confidence intervals in ANKS6 might be explained by a very low MAF (<0.01%) among cases.

A Manhattan plot for tumour size as a continuous outcome (**Figure 4.2**) also shows there are several polymorphisms in linkage disequilibrium (LD) with the leading SNP (Manhattan plots for all other tested outcomes are available in **Figures 4.3-4.7**).

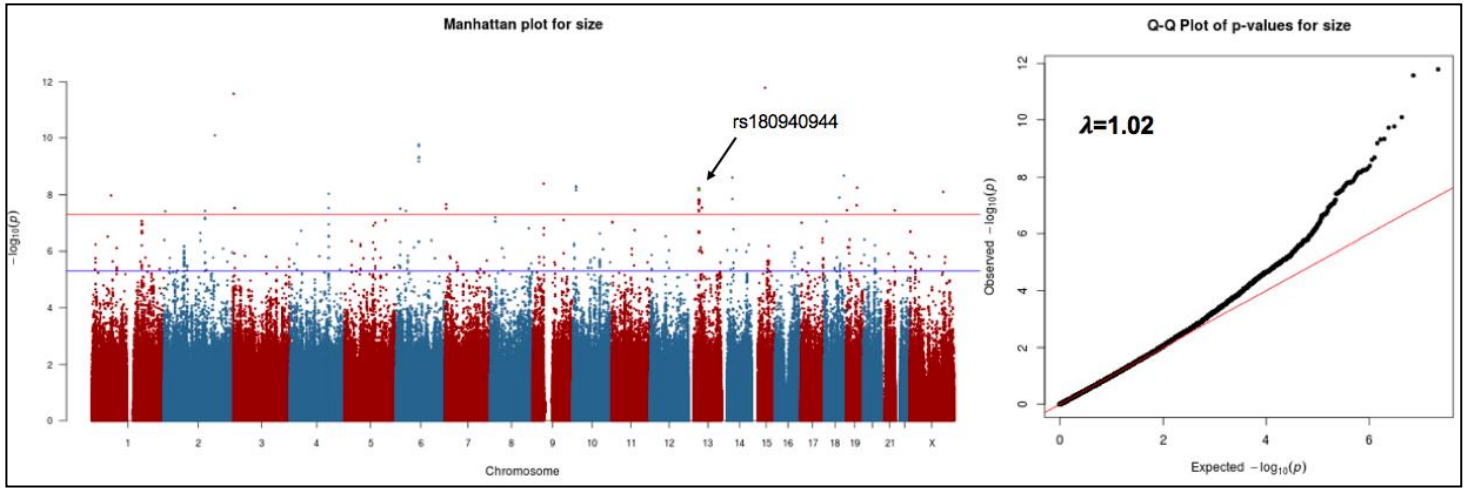


Figure 4.2. Manhattan and quantile-quantile plots for tumour size (cm) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively. Highlighted variant shows the SNP reaching statistical significance in the meta-analysis of BCPP and NBCS (independent association was observed in the BCPP, and no significant effect was detected among NBCS participants only).

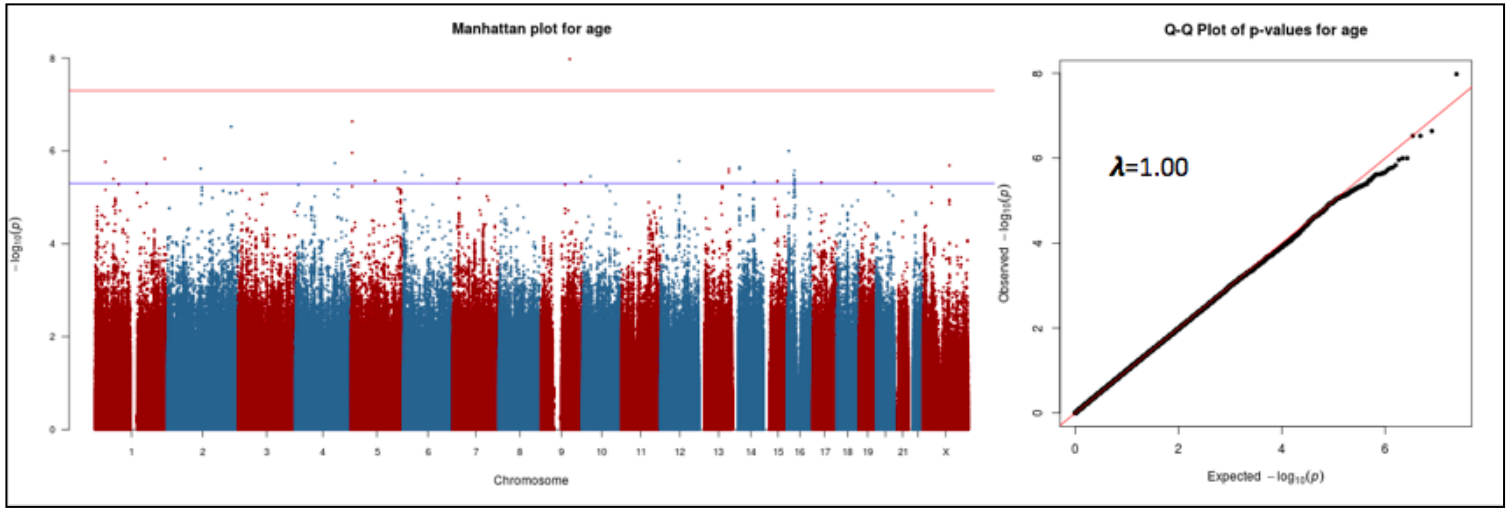


Figure 4.3. Manhattan and Quantile-quantile plots for age (years) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively.

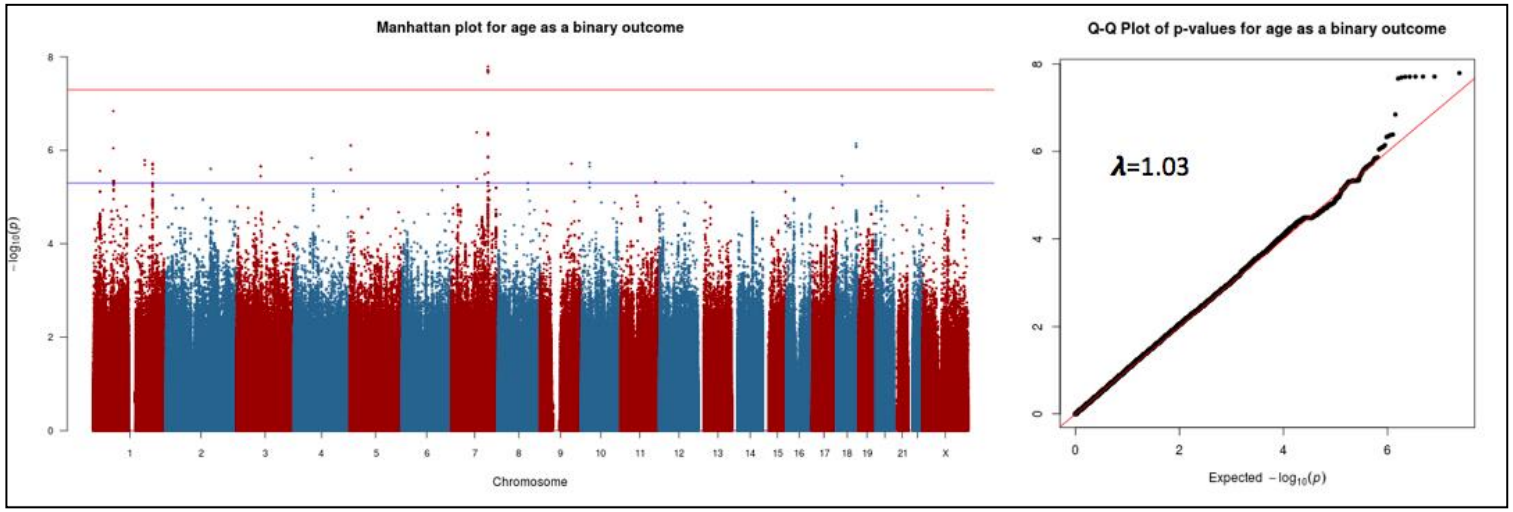


Figure 4.4. Manhattan and Quantile-quantile plots for age (<70.2/≥70.2 years) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively.

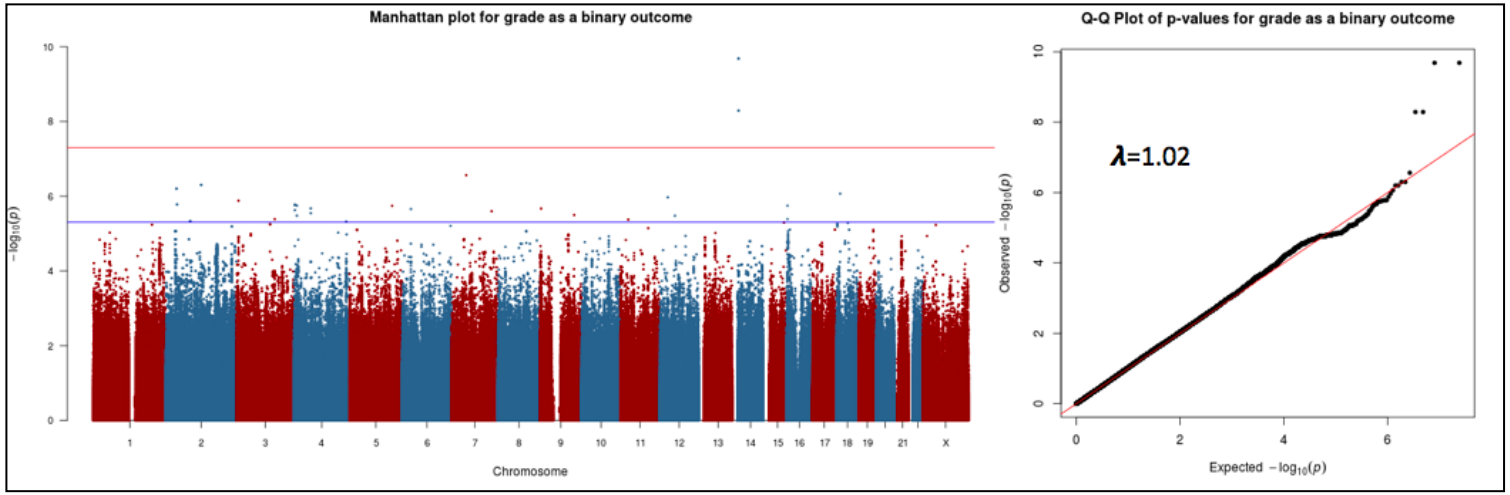


Figure 4.5. Manhattan and Quantile-quantile plots for tumour grade (G3 vs G2 and G1) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively.

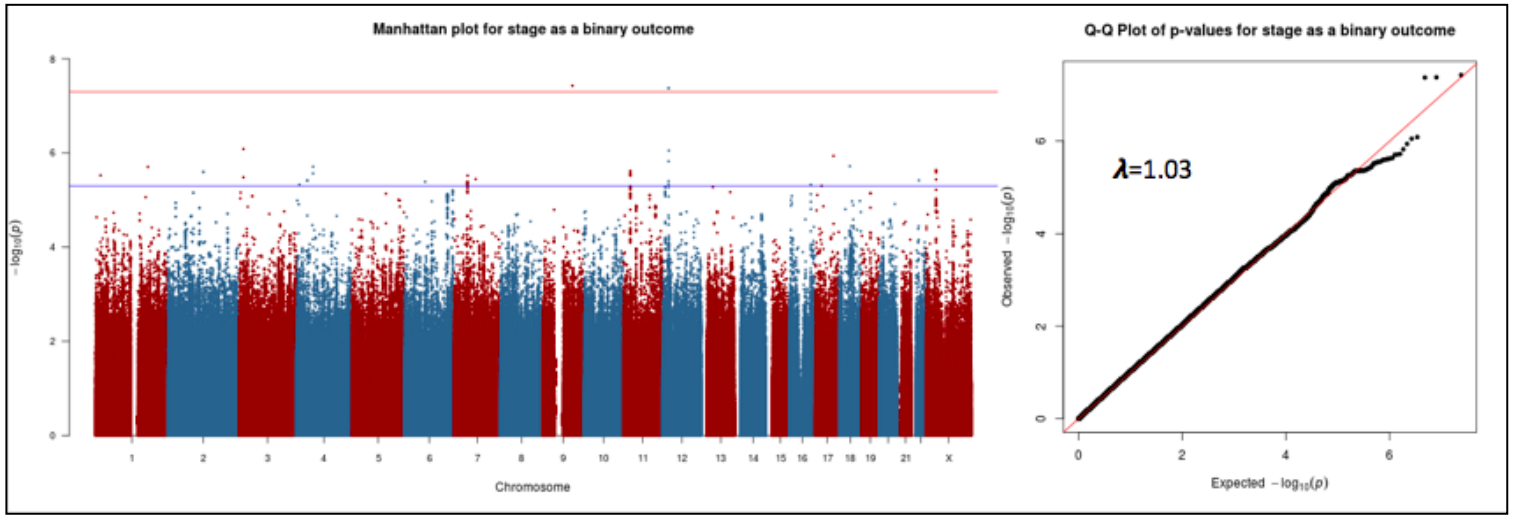


Figure 4.6 Manhattan and Quantile-quantile plots for tumour stage (Tis and T1 vs Ta) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively.

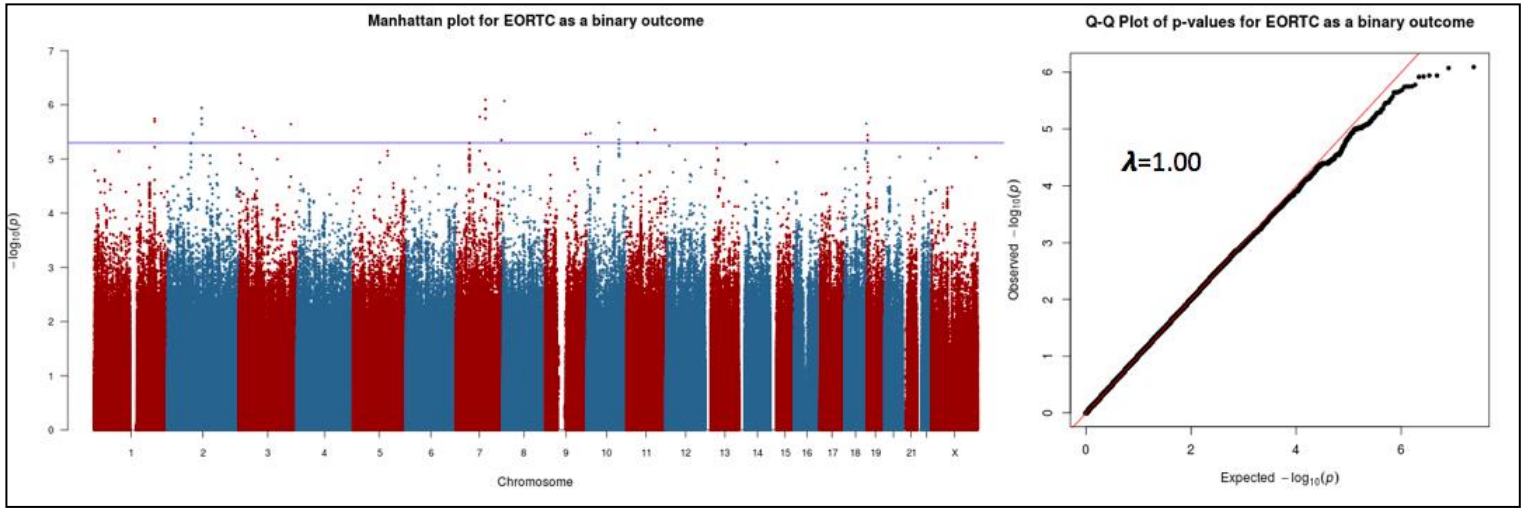


Figure 4.7. Manhattan and Quantile-quantile plots for EORTC risk category (High vs Low and Intermediate) in the BCPP cohort. Blue and red horizontal lines indicate p values of <5E-06 and <5E-08, respectively.

Regional association plot of 13q13.3 (**Figure 4.8**) in the BCPP confirms high LD with surrounding variants, all mapping to the *NBEA* gene (although they did not reach the statistical significance). Regional association plots for the remaining SNPs identified in the discovery stage are presented in **Figures 4.9-4.35**.

In the replication stage, 50 out of 61 SNPs were available to test in NBCS (**Table 4.2**). None of these SNPs were significantly associated with the same outcomes in NBCS. A meta-analysis of both cohorts showed variant rs180940944 in 13q13.3 locus to be associated with increased tumour size at diagnosis (ß=0.96, SE=0.16, p=2.92E-09), although the effect is likely driven by BCPP data. Nevertheless, low I^2 estimate (I^2=0%, p(heterogeneity)=0.75) indicated there was no significant heterogeneity.

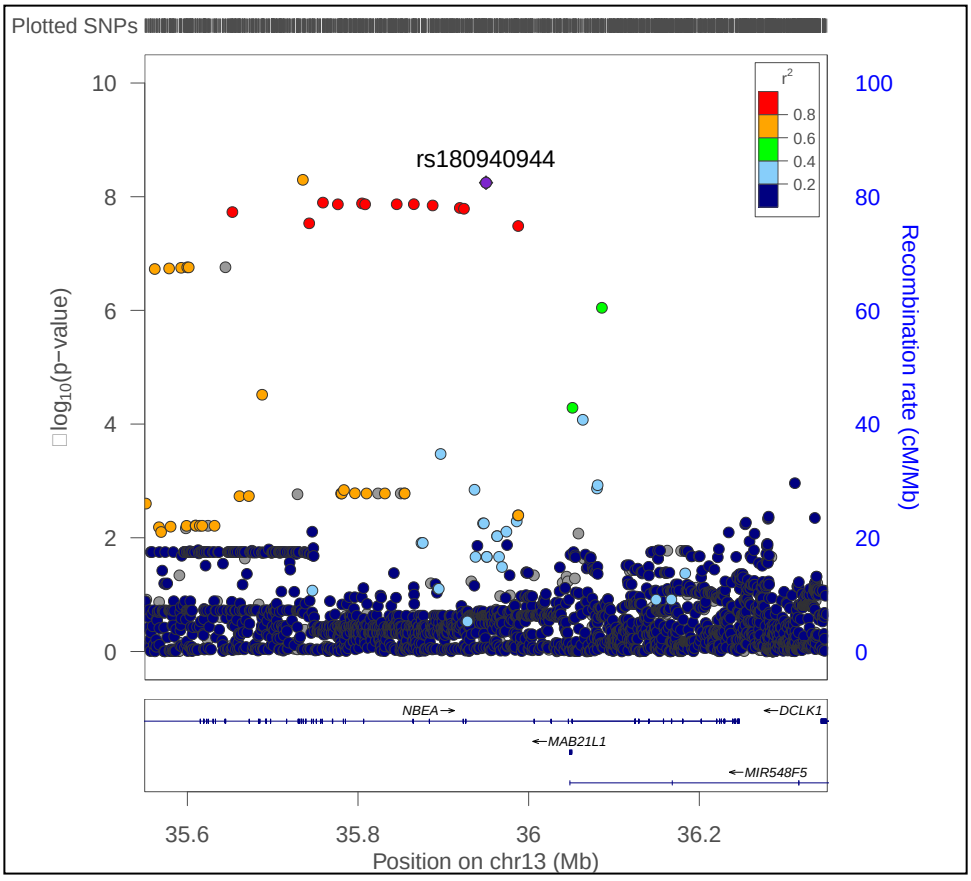


Figure 4.8. Regional association plot for 13q13.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort (annotated SNP has reached statistical significance in the meta-analysis of BCPP and NBCS cohorts).

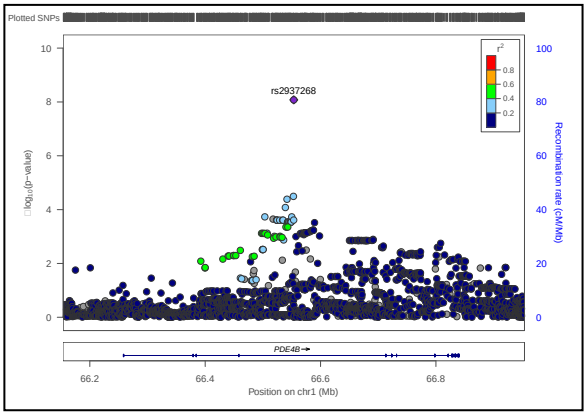


Figure 4.9. Regional association plot for 1p31.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

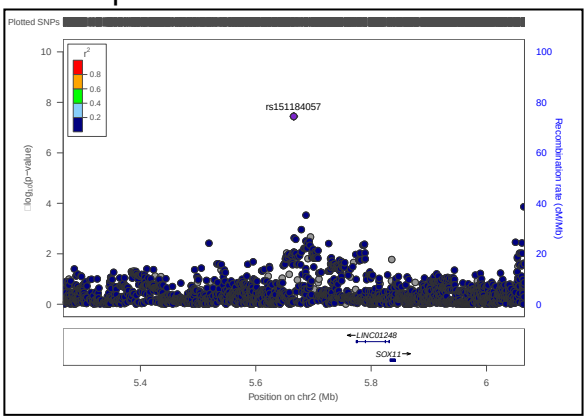


Figure 4.10. Regional association plot for 2p25.2 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

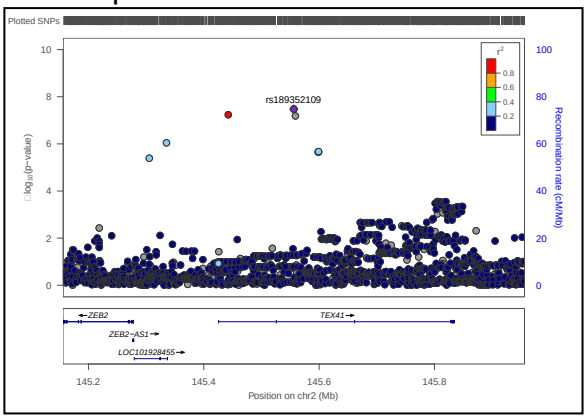


Figure 4.11. Regional association plot for 2q22.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

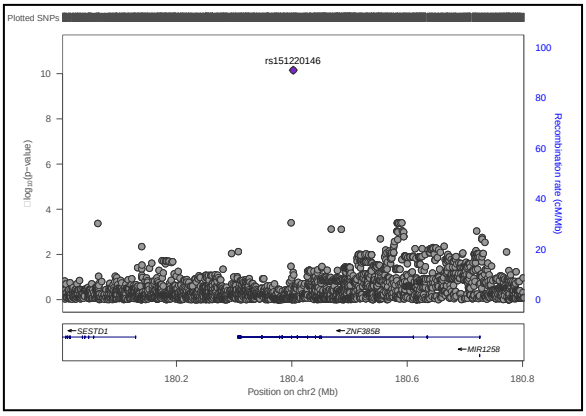


Figure 4.12. Regional association plot for 2q31.2 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

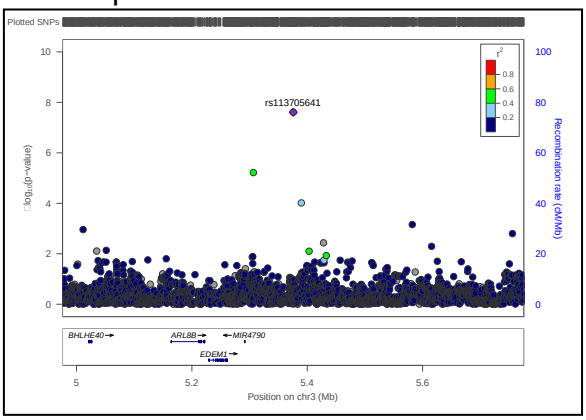


Figure 4.13. Regional association plot for 3p26.1 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

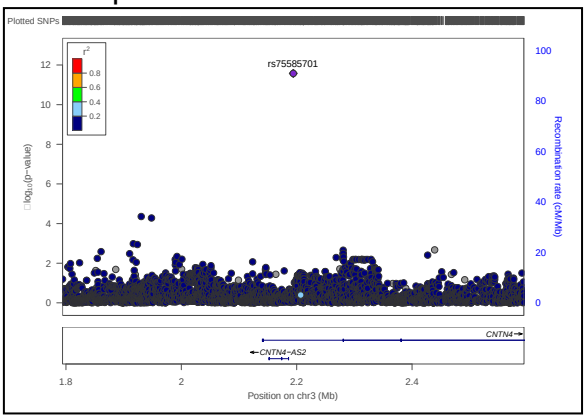


Figure 4.14. Regional association plot for 3p26.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

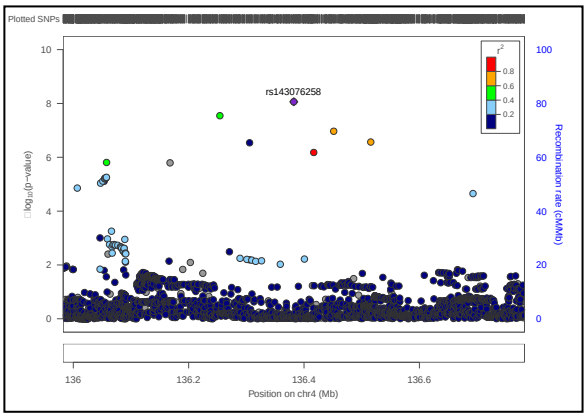


Figure 4.15. Regional association plot for 4q28.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

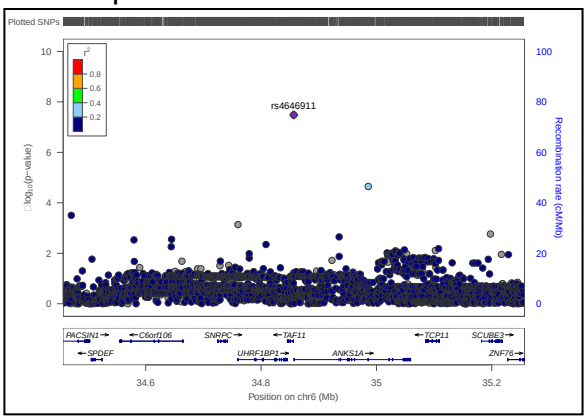


Figure 4.16. Regional association plot for 6p21.31 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

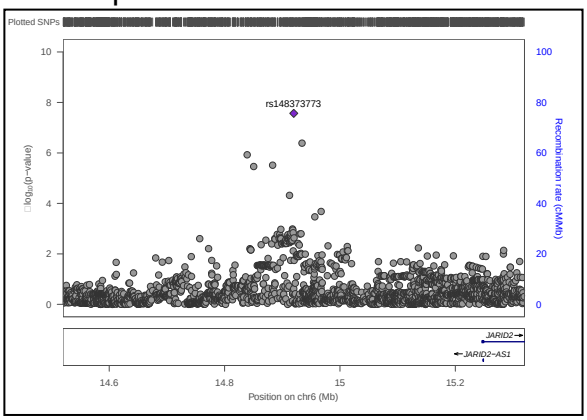


Figure 4.17. Regional association plot for 6p23 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

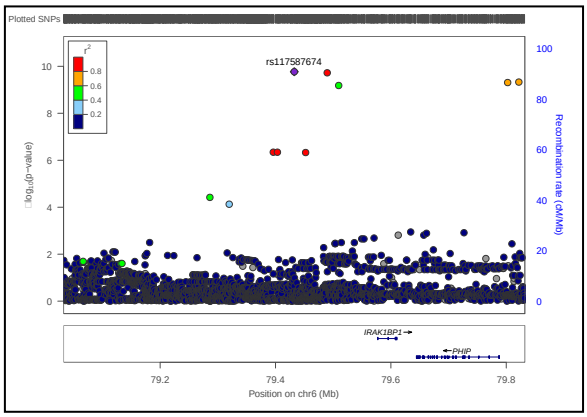


Figure 4.18. Regional association plot for 6q14.1 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

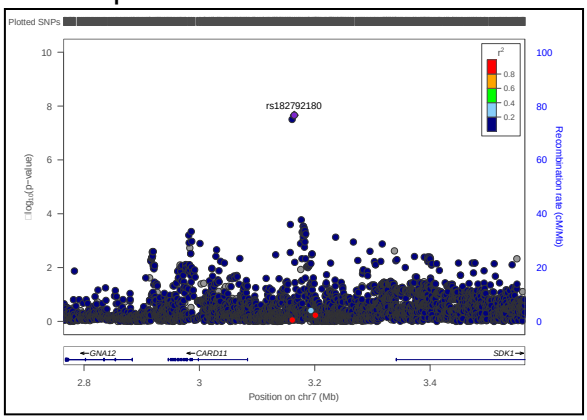


Figure 4.19. Regional association plot for 7p22.2 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

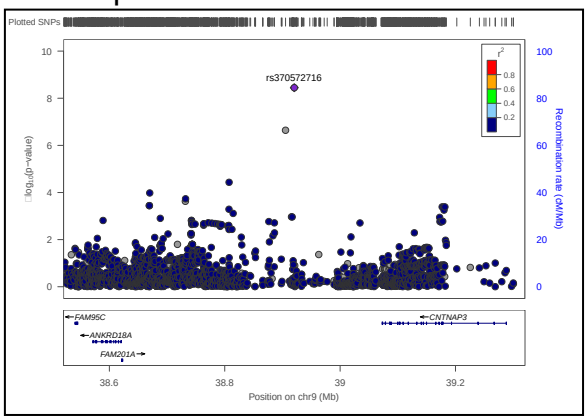


Figure 4.20. Regional association plot for 9p13.1 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

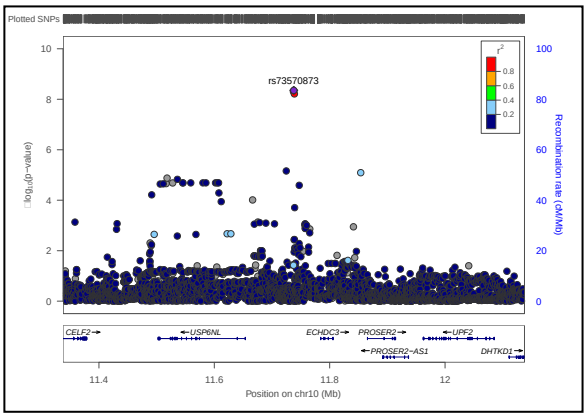


Figure 4.21. Regional association plot for 10p14 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

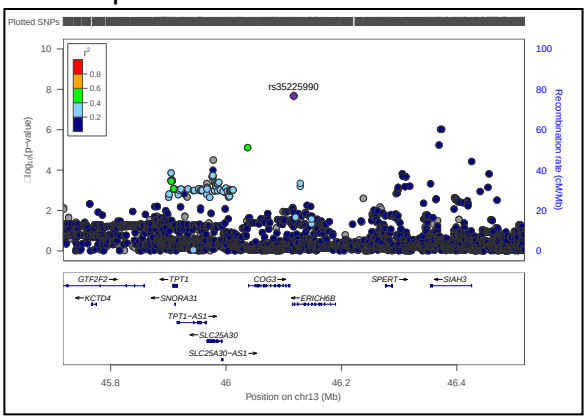


Figure 4.22. Regional association plot for 13q14.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

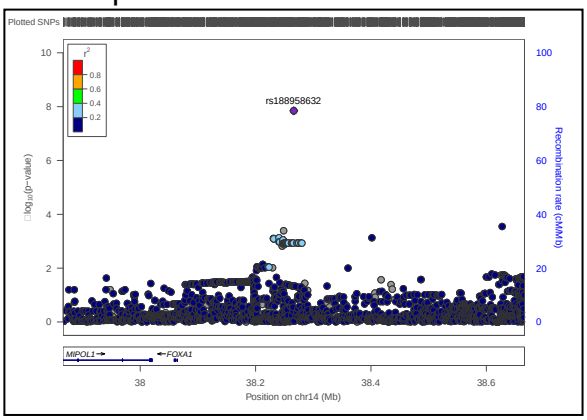


Figure 4.23. Regional association plot for 14q21.1 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

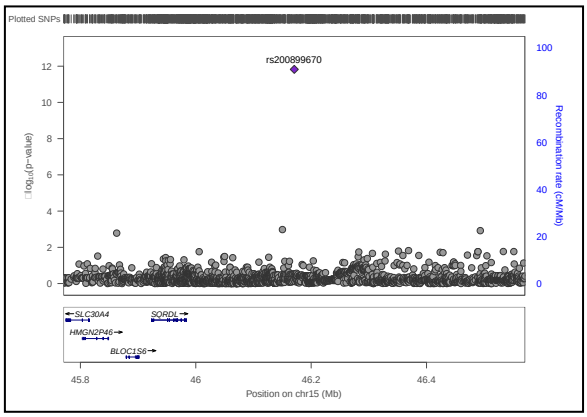


Figure 4.24. Regional association plot for 15q21.1 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

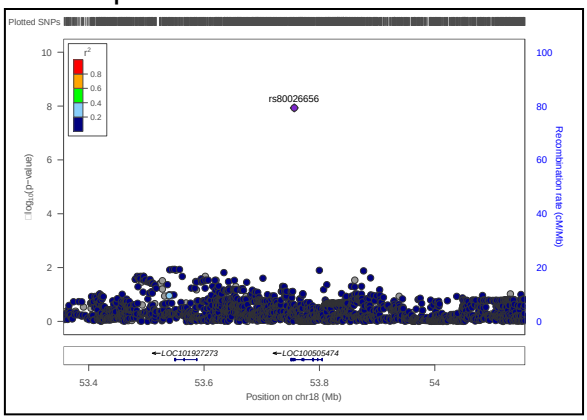


Figure 4.25. Regional association plot for 18q21.2 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

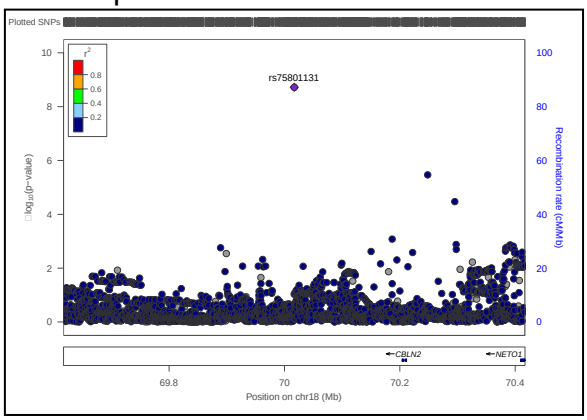


Figure 4.26. Regional association plot for 18q22.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

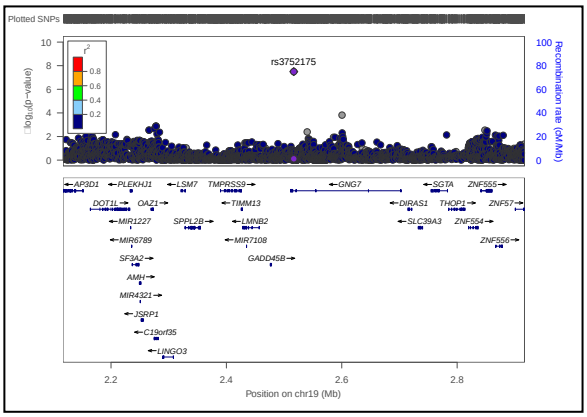


Figure 4.27. Regional association plot for 19p13.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

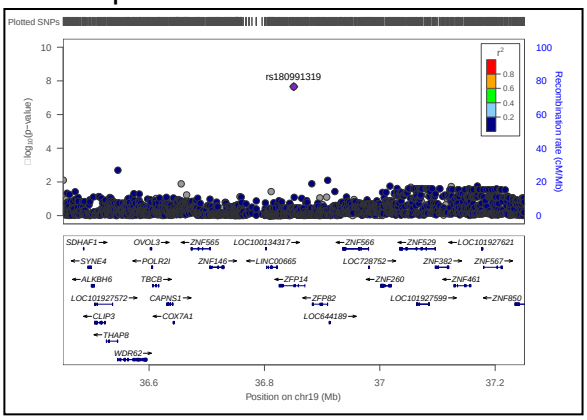


Figure 4.28. Regional association plot for 19q13.12 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

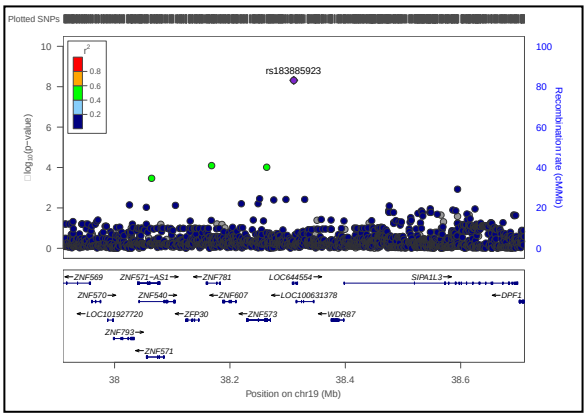


Figure 4.29. Regional association plot for 19q13.13 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

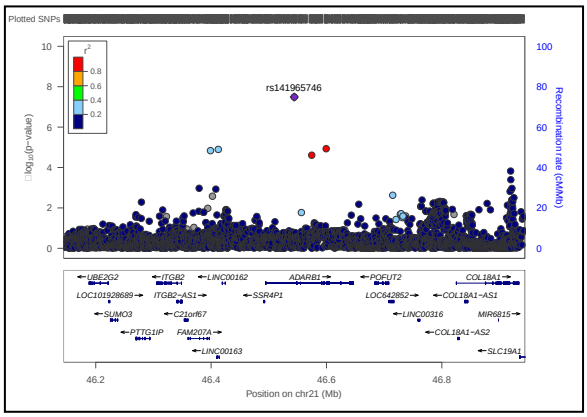


Figure 4.30. Regional association plot for 21q22.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort.

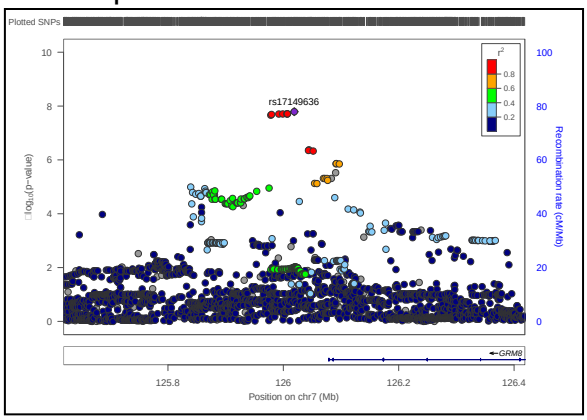


Figure 4.31. Regional association plot for 7q31.3 locus with age as a binary trait (</≥ 70.2 years) in NMIBC patients of the BCPP cohort.

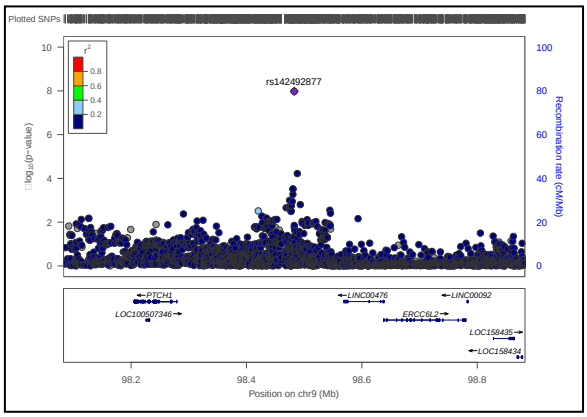


Figure 4.32. Regional association plot for 9q22.32 locus with age (years) in NMIBC patients of the BCPP cohort.

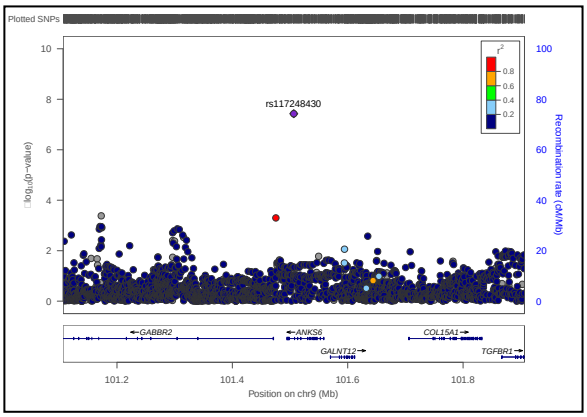


Figure 4.33. Regional association plot for 9q22.33 locus with stage as a binary trait (Tis and T1 vs Ta) in NMIBC patients of the BCPP cohort.

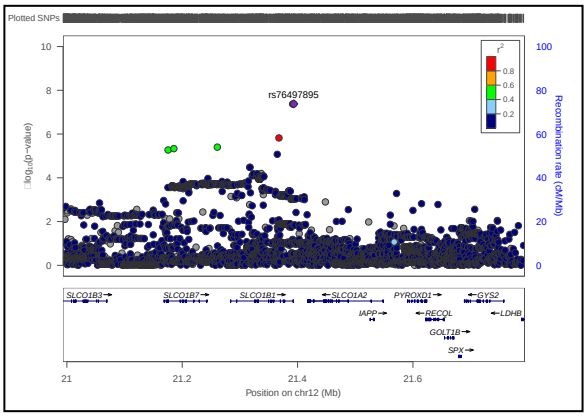


Figure 4.34. Regional association plot for 12p12.1 locus with stage as a binary trait

. (Tis and T1 vs Ta) in NMIBC patients of

the BCPP cohort.

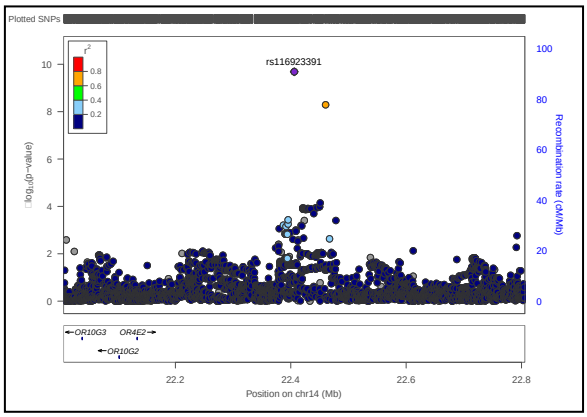


Figure 4.35. Regional association plot for 14q11.2 locus with grade as a binary trait (G3 vs G2 and G1) in NMIBC patients of the BCPP cohort

DISCUSSION

We have investigated genetic associations with NMIBC tumour (size, stage, grade) and patient (age, EORTC risk category) characteristics at the time of diagnosis within the BCPP cohort.

Multiple loci were identified in the discovery stage that are novel in the context of NMIBC. One SNP, rs180940944, has reached statistical significance in a meta-analysis of two NMIBC cohorts, mapping to the intronic region of the *NBEA* gene on 13q13.3. However, associations of other SNPs in the *NBEA* have failed to be reproduced.

NBEA proteins have been mostly observed to play a significant role in synapse development and function [24]. *NBEA* dysregulation does not affect the establishment of synapses *per se*, but rather their intra-cellular organisation [24]. An in-depth analysis revealed impaired synaptic ability was mostly due to the inappropriate distribution of actin, a protein essential for synapse cytoskeleton structure [24]. The effect is most likely present due to alterations in the Golgi-dependent processes of inter- and intra-cellular compound trafficking, including actin and neural receptors [24].

The synaptic alterations are likely to be the contributing cause of autism spectrum disorders [24]; however, the Golgi-related pathway may have a wider phenotypic manifestation [25], including cancer. The prognostic utility of *NBEA* has been investigated in gastric cancer [26] and oropharyngeal squamous cell carcinomas (OPSCC) [27], with promising results. Collectively, these observations implicate the pleiotropic nature of *NBEA* effect across a variety of traits.

In our study, we suggest there is an association between *NBEA* and increased NMIBC tumour size. The role of Golgi complex in cancer progression has been reported

independently, and disruptions in normal protein transportation can contribute to increased tumour size and, eventually, progression [25].

Our findings should be interpreted cautiously. Substantial sample sizes of specific phenotypes such as ours are rare, and suffer from limited power to capture true genetic associations, and spurious associations due to random effects cannot be ruled out. Our post-hoc power calculations [18], underscore the importance of current analysis being ran on bigger cohorts (e.g. association rs150914897 (14q11.2) of an OR=3.42 had power of 79%, but it drops to only 16% for an OR=2.5, hence we may have missed existing associations of more modest effect size). Moreover, observed statistically significant results may represent synthetic associations that are driven by rare alleles. In our analyses, all alleles that have a frequency of <1% were excluded to reduce the likelihood of false-positive results. However, investigation of the regional association plots shows many significant SNPs seem to be isolated hits in a genetic locus, which warrants their careful consideration. The associated SNPs that show to be in low correlation with surrounding variants may point towards them being spurious results or indicating an association with rare variants, which were not tested in the current study.

Furthermore, tumour size measurements are subject to variability, degree of which is difficult to establish. The lack of any genome-wide significant associations for categorised tumour size (</≥ 3 cm [17]) adds substantial caution in consideration of our main findings and study power. However, clinically-relevant tumour size categories may not be adequate in a genetic context, and different categorisation may be used in future analyses.

Our study only focused on NMIBC instead of a merged group of UBC, and we are unable to comment on whether these genetic loci are relevant for advanced UBC.

Given considered limitations, we see this study as true to the GWAS design of hypothesis-generating nature, instead of one offering conclusive findings. Hence, further replication is of essence to establish validity of described results.

The 13q13.3 locus has not been observed in prior studies on NMIBC. It might be due to us using an independent prognostic marker of NMIBC (i.e. tumour size) instead of recurrence and/or progression as an outcome. Larger tumour indicates a worse disease course [17], but there are other components that contribute to NMIBC prognosis. In a clinical setting, each tumour characteristic (e.g. size) carries a different weighting [17], collectively contributing to an endpoint (e.g. recurrence). Importantly, our sample had a low number of Carcinoma *in situ* (CIS) tumours (N=10), and could not be analysed as a separate category. However, it must be underscored these tumours show a high likelihood of progression (Table 1.2), and should be considered as an independent endpoint if sample size permits such investigations.

Importantly, powerful studies on UBC risk have already shown some signals to only be associated with MIBC (UBC of T2-T4) [10]. Furthermore, a genome-wide methylation investigation on high-grade NMIBC cases revealed epigenetic changes different from their low-grade counterparts [9]. Direct comparability of these reports is limited, but we see the unravelling genetic complexity within UBC being a connecting thread between all studies. We therefore believe it is likely separate genetic relationships are present for NMIBC determinants, rather than overall prognostic outcomes. Our study suggests variations in 13q13.3 locus may contribute to an increased NMIBC tumour size in a European population. Further studies are warranted to confirm the association.

REFERENCES

[1] Kamat AM, Hahn NM, Efstathiou JA, Lerner SP, Malmstrom PU, Choi W, Guo CC, Lotan Y, Kassouf W. Bladder Cancer. Lancet (London, England). 2016;388(10061):2796-810.

[2] Sylvester RJ. How Well Can You Actually Predict Which Non-Muscle-Invasive Bladder Cancer Patients Will Progress? Eur Urol. 2011;60(3):431-3; discussion 3-4.

[3] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.

[4] Chen M, Hildebrandt MA, Clague J, Kamat AM, Picornell A, Chang J, Zhang X, Izzo J, Yang H, Lin J, Gu J, Chanock S, Kogevinas M, Rothman N, Silverman DT, Garcia-Closas M, Grossman HB, Dinney CP, Malats N, Wu X. Genetic Variations in the Sonic Hedgehog Pathway Affect Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Cancer prevention research (Philadelphia, Pa). 2010;3(10):1235-45.

[5] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.

[6] Galesloot TE, Grotenhuis AJ, Fleshner NE, Bryan RT, Cheng KK, Zeegers MP, Kolev D, Vermeulen SH, Kiemeney LALM. The Role of Germline Genetic Variants in the Prognosis of Non-Muscle Invasive Bladder Cancer: A Meta-Gwas. Urologic Oncology: Seminars and Original Investigations. 2017;35(10):614.

[7] Lopez de Maturana E, Picornell A, Masson-Lecomte A, Kogevinas M, Marquez M, Carrato A, Tardon A, Lloreta J, Garcia-Closas M, Silverman D, Rothman N, Chanock S, Real FX, Goddard ME, Malats N. Prediction of Non-Muscle Invasive Bladder Cancer Outcomes Assessed by Innovative Multimarker Prognostic Models. BMC cancer. 2016;16:351.

[8] Marchini J, Howie B. Genotype Imputation for Genome-Wide Association Studies. Nature reviews Genetics. 2010;11(7):499-511.

[9] Kitchen MO, Bryan RT, Emes RD, Glossop JR, Luscombe C, Cheng KK, Zeegers MP, James ND, Devall AJ, Mein CA, Gommersall L, Fryer AA, Farrell WE. Quantitative Genome-Wide Methylation Analysis of High-Grade Non-Muscle Invasive Bladder Cancer. Epigenetics. 2016;11(3):237-46.

[10] Figueroa JD, Middlebrooks CD, Banday AR, Ye Y, Garcia-Closas M, Chatterjee N, Koutros S, Kiemeney LA, Rafnar T, Bishop T, Furberg H, Matullo G, Golka K, Gago-Dominguez M, Taylor JA, Fletcher T, Siddiq A, Cortessis VK, Kooperberg C, Cussenot O, Benhamou S, Prescott J, Porru S, Dinney CP, Malats N, Baris D, Purdue MP, Jacobs EJ, Albanes D, Wang Z, Chung CC, Vermeulen SH, Aben KK, Galesloot TE, Thorleifsson G, Sulem P, Stefansson K, Kiltie AE, Harland M, Teo M, Offit K, Vijai J, Bajorin D, Kopp R, Fiorito G, Guarrera S, Sacerdote C, Selinski S, Hengstler JG, Gerullis H, Ovsiannikov D, Blaszkewicz M, Castelao JE, Calaza M, Martinez ME, Cordeiro P, Xu Z, Panduri V, Kumar R, Gurzau E, Koppova K, Bueno-De-Mesquita HB, Ljungberg B, Clavel-Chapelon F, Weiderpass E, Krogh V, Dorronsoro M, Travis RC, Tjonneland A, Brennan P, Chang-Claude J, Riboli E, Conti D, Stern MC, Pike MC, Van Den Berg D, Yuan JM, Hohensee C, Jeppson RP, Cancel-Tassin G, Roupret M, Comperat E, Turman C, De Vivo I, Giovannucci E, Hunter DJ, Kraft P, Lindstrom S, Carta A, Pavanello S, Arici C, Mastrangelo G,

Kamat AM, Zhang L, Gong Y, Pu X, Hutchinson A, Burdett L, Wheeler WA, Karagas MR, Johnson A, Schned A, Monawar Hosain GM, Schwenn M, Kogevinas M, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Andriole G, Jr., Grubb R, 3rd, Black A, Diver WR, Gapstur SM, Weinstein S, Virtamo J, Haiman CA, Landi MT, Caporaso NE, Fraumeni JF, Jr., Vineis P, Wu X, Chanock SJ, Silverman DT, Prokunina-Olsson L, Rothman N. Identification of a Novel Susceptibility Locus at 13q34 and Refinement of the 20p12.2 Region as a Multi-Signal Locus Associated with Bladder Cancer Risk in Individuals of European Ancestry. Human Molecular Genetics. 2016;25(6):1203-14.
[11] Zeegers MP, Bryan RT, Langford C, Billingham L, Murray P, Deshmukh NS, Hussain S, James N, Wallace DM, Cheng KK. The West Midlands Bladder Cancer Prognosis Programme: Rationale and Design. BJU international. 2010;105(6):784-8.
[12] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. American journal of human genetics. 2007;81(3):559-75.
[13] Loh PR, Danecek P, Palamara PF, Fuchsberger C, Y AR, H KF, Schoenherr S, Forer L, McCarthy S, Abecasis GR, Durbin R, A LP. Reference-Based Phasing Using the Haplotype Reference Consortium Panel. Nature genetics. 2016;48(11):1443-8.
[14] Howie BN, Donnelly P, Marchini J. A Flexible and Accurate Genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. PLoS genetics. 2009;5(6):e1000529.
[15] Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, Tasse AM, Flicek P. The International Genome Sample Resource (Igsr): A Worldwide Collection of Genome Variation Incorporating the 1000 Genomes Project Data. Nucleic acids research. 2017;45(D1):D854-d9.
[16] Goossens ME, Zeegers MP, van Poppel H, Joniau S, Ackaert K, Ameye F, Billiet I, Braeckman J, Breugelmans A, Darras J, Dilen K, Goeman L, Tombal B, Van Bruwaene S, Van Cleyenbreugel B, Van der Aa F, Vekemans K, Buntinx F. Phase Iii Randomised Chemoprevention Study with Selenium on the Recurrence of Non-Invasive Urothelial Carcinoma. The Selenium and Bladder Cancer Trial. European journal of cancer (Oxford, England : 1990). 2016;69:9-18.
[17] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.
[18] Johnson JL, Abecasis GR. Gas Power Calculator: Web-Based Power Calculator for Genetic Association Studies. bioRxiv. 2017.
[19] Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. Locuszoom: Regional Visualization of Genome-Wide Association Scan Results. Bioinformatics (Oxford, England). 2010;26(18):2336-7.
[20] Dayem Ullah AZ, Lemoine NR, Chelala C. Snpnexus: A Web Server for Functional Annotation of Novel and Publicly Known Genetic Variants (2012 Update). Nucleic acids research. 2012;40(Web Server issue):W65-70.
[21] Aken BL, Ayling S, Barrell D, Clarke L, Curwen V, Fairley S, Fernandez Banet J, Billis K, García Girón C, Hourlier T, Howe K, Kähäri A, Kokocinski F, Martin FJ, Murphy DN, Nag R, Ruffier M, Schuster M, Tang YA, Vogel JH, White S, Zadissa A, Flicek P, Searle SMJ. The Ensembl Gene Annotation System. Database: The Journal of Biological Databases and Curation. 2016;2016.

[22] Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KK, Stacey SN, Gudmundsson J, Jakobsdottir M, Bergthorsson JT, Sigurdsson A, Blondal T, Witjes JA, Vermeulen SH, Hulsbergen-van de Kaa CA, Swinkels DW, Ploeg M, Cornel EB, Vergunst H, Thorgeirsson TE, Gudbjartsson D, Gudjonsson SA, Thorleifsson G, Kristinsson KT, Mouy M, Snorradottir S, Placidi D, Campagna M, Arici C, Koppova K, Gurzau E, Rudnai P, Kellen E, Polidoro S, Guarrera S, Sacerdote C, Sanchez M, Saez B, Valdivia G, Ryk C, de Verdier P, Lindblom A, Golka K, Bishop DT, Knowles MA, Nikulasson S, Petursdottir V, Jonsson E, Geirsson G, Kristjansson B, Mayordomo JI, Steineck G, Porru S, Buntinx F, Zeegers MP, Fletcher T, Kumar R, Matullo G, Vineis P, Kiltie AE, Gulcher JR, Thorsteinsdottir U, Kong A, Rafnar T, Stefansson K. Sequence Variant on 8q24 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2008;40(11):1307-12.
[23] Liu JZ, Tozzi F, Waterworth DM, Pillai SG, Muglia P, Middleton L, Berrettini W, Knouff CW, Yuan X, Waeber G, Vollenweider P, Preisig M, Wareham NJ, Zhao JH, Loos RJ, Barroso I, Khaw KT, Grundy S, Barter P, Mahley R, Kesaniemi A, McPherson R, Vincent JB, Strauss J, Kennedy JL, Farmer A, McGuffin P, Day R, Matthews K, Bakke P, Gulsvik A, Lucae S, Ising M, Brueckl T, Horstmann S, Wichmann HE, Rawal R, Dahmen N, Lamina C, Polasek O, Zgaga L, Huffman J, Campbell S, Kooner J, Chambers JC, Burnett MS, Devaney JM, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein S, Wilson JF, Wild SH, Campbell H, Vitart V, Reilly MP, Li M, Qu L, Wilensky R, Matthai W, Hakonarson HH, Rader DJ, Franke A, Wittig M, Schafer A, Uda M, Terracciano A, Xiao X, Busonero F, Scheet P, Schlessinger D, St Clair D, Rujescu D, Abecasis GR, Grabe HJ, Teumer A, Volzke H, Petersmann A, John U, Rudan I, Hayward C, Wright AF, Kolcic I, Wright BJ, Thompson JR, Balmforth AJ, Hall AS, Samani NJ, Anderson CA, Ahmad T, Mathew CG, Parkes M, Satsangi J, Caulfield M, Munroe PB, Farrall M, Dominiczak A, Worthington J, Thomson W, Eyre S, Barton A, Mooser V, Francks C, Marchini J. Meta-Analysis and Imputation Refines the Association of 15q25 with Smoking Quantity. Nature genetics. 2010;42(5):436-40.
[24] Niesmann K, Breuer D, Brockhaus J, Born G, Wolff I, Reissner C, Kilimann MW, Rohlmann A, Missler M. Dendritic Spine Formation and Synaptic Function Require Neurobeachin. Nature communications. 2011;2:557.
[25] Petrosyan A. Onco-Golgi: Is Fragmentation a Gate to Cancer Progression? Biochemistry & molecular biology journal. 2015;1(1).
[26] Li X, Wu WK, Xing R, Wong SH, Liu Y, Fang X, Zhang Y, Wang M, Wang J, Li L, Zhou Y, Tang S, Peng S, Qiu K, Chen L, Chen K, Yang H, Zhang W, Chan MT, Lu Y, Sung JJ, Yu J. Distinct Subtypes of Gastric Cancer Defined by Molecular Characterization Include Novel Mutational Signatures with Prognostic Capability. Cancer research. 2016;76(7):1724-32.
[27] Gao G, Kasperbauer JL, Tombers NM, Cornell MD, Smith DI. Prognostic Significance of Decreased Expression of Six Large Common Fragile Site Genes in Oropharyngeal Squamous Cell Carcinomas. Translational oncology. 2014;7(6):726-31.

CHAPTER 5.

GENE-ENVIRONMENT INTERACTION WITH SMOKING FOR INCREASED NON-MUSCLE-INVASIVE BLADDER CANCER TUMOUR SIZE

Contents of this chapter are in press:

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier, JB Zeegers MP. Gene-environment interaction with smoking for increased non-muscle-invasive bladder cancer tumour size. Transl Androl Urol. 2019.

aABSTRACT

INTRODUCTION

Multiple genetic loci have been identified as potentially conferring increased risk of urinary bladder cancer (UBC). Although evidence for gene-environment (GxE) interactions with smoking exist for overall UBC risk, few studies have investigated

outcomes that could indicate non-muscle-invasive bladder cancer (NMIBC) prognosis. In the current study, we have investigated if smoking status and/or smoking intensity interact with the effect of discovered variants on key NMIBC characteristics of tumour grade, stage, size, and age within the Bladder Cancer Prognosis Programme (BCPP) cohort.

METHODS

Analysed sample consisted of 546 NMIBC patients with valid smoking data from the BCPP.

In a previous genome-wide association study (GWAS), we have identified 61 single nucleotide polymorphism (SNPs) potentially associated with the NMIBC characteristics of tumour stage, grade, size, and patient's age. In the current analysis, we have tested those 61 associations for potential GxE interaction with smoking.

RESULTS

Ten out of 61 SNPs reached showed suggestion (statistical significance level of $p<0.05$) for GxE with NMIBC tumour size.

CONCLUSIONS

Our study suggests interaction between genetic variance and smoking behaviour for increased NMIBC tumour size at the time of diagnosis. Further replication is required to validate these findings.

INTRODUCTION

Multiple genetic loci have been identified as potentially conferring increased risk of urinary bladder cancer (UBC), and it is hypothesized that these associations might be altered by environmental exposures, such as smoking [1-4]. However, it remains unknown if any gene-environment interactions (GxE) are also present for UBC outcomes, which may permit improvements in the clinical management of UBC patients.

To date, studies on gene-tobacco (GxT) interactions for UBC have only considered the overall risk of developing UBC as an outcome of interest. These investigations have resulted in overlapping findings, collectively reporting multiple loci interacting with tobacco [2-4]. Some of the most commonly reported genes for GxT interaction on UBC risk (*NAT2, UGT1A6)* are regulatory of phase II detoxification, a pathway for metabolising tobacco-related carcinogens (i.e. aromatic amines) [2, 3, 5]. Alterations in smoking-related carcinogen clearance result in increased risk in tobacco users, whilst the effect is reduced [2] or virtually missing for never smokers [5]. In addition, the overall risk of developing UBC has been shown to increase with smoking intensity, revealing an important pattern of certain genotypes interacting with smoking in a dose-dependent manner [2, 4-7].

Other genotypes, namely *GSTM1-null* status [3], appear more important for non-smokers, although significant effects are present regardless of tobacco use. Expectedly, the effect of these genotypes on UBC risk does not vary within smoking intensity categories [6].

UBC is one of few cancers with an established gene-environment interaction [4], yet previous research has not distinguished between muscle-invasive (MIBC) and non-

muscle-invasive (NMIBC) groupings. Given our continually-evolving knowledge of the pathogenesis of MIBC and NMIBC [8], not stratifying for these categories in genetic analyses could result in overlooking important biological mechanisms. Furthermore, it is now known that genetic loci for UBC risk are not relevant for UBC prognosis [9], which continues to highlight the complexity of genetic associations. Finally, little evidence is present for genetic associations with specific characteristics such as tumour size, grade, stage or patient age [2, 5]. However, these characteristics are especially influential for NMIBC outcomes [10], and are more specific entities than the more broadly-defined prognostic outcomes such as recurrence or progression.

We have previously carried out a genome-wide association study (GWAS) on NMIBC tumour and patient characteristics in the Bladder Cancer Prognosis Programme (BCPP) cohort, identifying potentially novel genetic associations [11]. In the current study, we have investigated if smoking status and/or smoking intensity interact with the effect of discovered variants on key NMIBC characteristics of tumour grade/stage/size and age within the BCPP cohort.

METHODS

Participants and genotyping

BCPP

BCPP is a prospective cohort, recruiting 1,544 patients from December 2005 to April 2011 [12]. Baseline clinical tumour characteristics (stage, grade, size) were collected from medical records, whilst demographic and smoking data were retrieved from records of semi-structured interviews that were conducted at baseline. Blood samples of 888 participants were genotyped on the Illumina Infinium OmniExpress-24

BeadChip array (previously known as The HumanOmniExxpress-24 BeadChip) at deCODE genetics (Reykjavik, Iceland) [13].

Nijmegen Bladder Cancer Study (NBCS)

In NBCS, patients were recruited between years 1995-2006 via the population-based cancer registry operating in the Nijmegen area [14]. For confirmed UBC cases, clinical and demographic data were collected via self-administered questionnaires. Additional information was retrieved via linkage to the medical records. Genotyping on the Illumina HumanHapCNV370 BeadChip panel was performed for patients who have consented to donating a blood sample [9]. Quality control and analytic procedures on genetic data have been presented previously [14].

A total of 303 NMIBC patients with valid data on tumour size and smoking were included in the replication.

Quality control (QC)

Quality control for the initial GWAS was carried out in PLINK v1.90 (released 17th November 2016) [15, 16] and is described in more detail elsewhere [11]. In brief, genotype samples yielding inconclusive gender calls, excessive missingness, low or increased heterozygosity rate were excluded from the analysis. Related individuals and participants presenting as population outliers (as identified in the principal component analysis (PCA)) were additionally excluded from the study. Genetic markers deviating from the Hardy-Weinberg equilibrium (HWE), those with high missingness rate and low minor allele frequency (MAF) were excluded. All QC steps resulted in a pre-imputed dataset consisting of 653 individuals and 597,764 single nucleotide polymorphisms (SNPs).

Imputation

A two-step imputation was performed with Eagle v2.3.2 [17] for haplotype phasing and IMPUTE2 [18] for genotype imputation, using 1000 Genomes Phase 3 (released 2nd May 2013) [19] as a reference panel in the genome build 19 (GRCh37/hg19). Post-imputation QC consisted of deleting markers having info score of <0.3 and MAFs of <1%, resulting in a dataset containing 11,914,228 markers that were used for GWAS analysis with tumour and patient baseline characteristics. The exact thresholds applied and number of exclusions per step are outlined in detail in **Figure 5.1**.

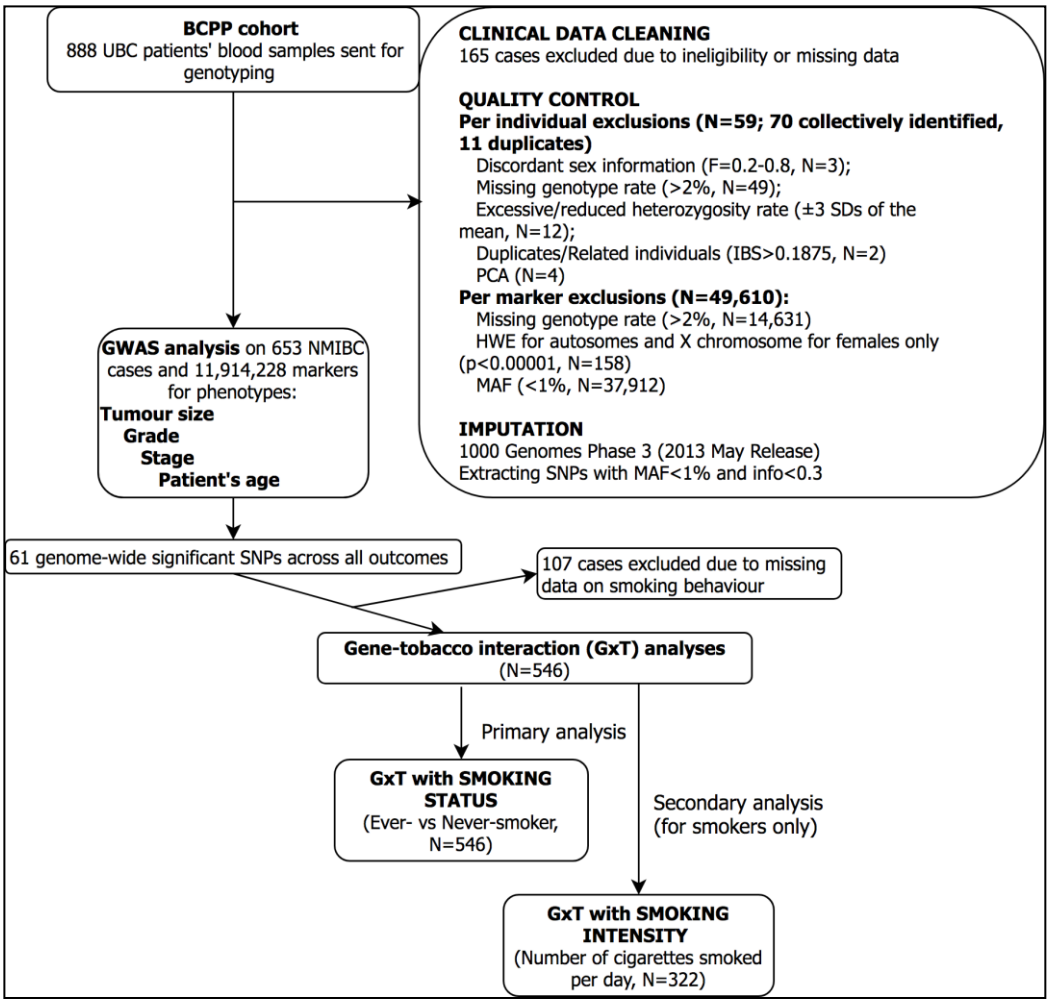


Figure 5.1. Flowchart for the data analysis on gene-tobacco (GxT) interactions in the BCPP cohort (BCPP-Bladder Cancer Prognosis Programme; HWE-Hardy-Weinberg equilibrium; SD-standard deviation; IBS-identity-by-state; PCA-principal component analysis; MAF-minor allele frequency; NMIBC-non-muscle invasive bladder cancer; PCA-principal component analysis; SD-standard deviation; SNP-single nucleotide polymorphism).

Outcomes and exposure variables

As studies on genetic associations with NMIBC baseline characteristics are scarce, we have used results of a previously-reported GWAS in the BCPP cohort [11]. Hence, a total of 61 discovered variants were used to test for GxT interaction. The outcomes were as follows: tumour size (centimetres), stage (T1 vs Ta/Tis), grade (G3/G2 vs G1), and patients' age (years as a continuous outcome and a binary variable with sample mean as a cut-off value (</≥69.9 years)). Our analyses were restricted to NMIBC cases only (corresponding to recorded stage at the time of diagnosis of Ta, T1, or Tis) who also had valid records on tobacco use, resulting in 546 patients.

Smoking exposure was modelled as two variables: smoking status (ever vs never smokers) and smoking intensity (number of cigarettes smoked daily for smokers only). GxT interaction with smoking status was considered as a primary analysis (including 546 subjects), whilst analysis on smoking intensity was considered secondary (consisting of 322 smokers with valid data on number of cigarettes smoked per day).

Statistical analysis

To test interaction terms that account for the uncertainty of imputed genotypes, QUICKTEST software was used [20]. All analyses were adjusted for gender and first five genetic principal components to increase estimate precision.

A total set of 61 SNPs that were discovered in the previous GWAS analysis of the BCPP cohort [11], were tested. GxT interaction term (depicted as SNPxSmoking or SNPxSmoking intensity) was modelled for each discovered association. An interaction term was deemed to be significant if p value<0.05.

Functional annotation and network analysis

All identified significant SNPs were annotated to an overlapping or closest gene using a web-based SNPnexus tool [21, 22], with Ensembl [23] (Version 74) as a functional annotation system. Gene-Tissue Expression database (GTEx) [24] was queried for all significant SNPs to identify if any expression quantitative loci (eQTL) were present.

RESULTS

BCPP and NBCS patient characteristics are presented in **Table 5.1**. Out of the total BCPP sample of 546 participants, 72 were never-smokers, whilst 474 had a history of tobacco use. In the NBCS, 246 participants have never smoked and 1061 were ever-smokers. Overall, The BCPP cohort was older (mean age=70 years) than the NBCS sample (mean age=62.5 years). No striking differences were observed between the BCPP and the NBCS with regard to tumour stage and grade, as well as number of cigarettes smoked per day. However, although NBCS is a much larger sample overall, the information on tumour size was only available for 303 participants.

Out of 61 tested SNPs tested for a GxT interaction in the BCPP cohort, 10 have reached $p<0.05$, all associated with tumour size (**Table 5.2**). Five of these SNPs were located in 6q14.1 (rs180910528, rs74603364, rs187040828, rs144383242, and rs117587674); two in 14q21.1 (rs188958632 and a SNP that has not yet been assigned an rsID (base-pair (BP): 38247577)); whilst the rest were mapped to 1p31.3 (rs2937268), 3p26.1 (rs113705641), and 13q14.13 (rs35225990) (**Table 5.2**).

Table 5.1. Descriptive characteristics of the BCPP and NBCS samples for gene-environment interaction with smoking analysis.

	BCPP				NBCS			
	Total number of participants	**Overall**	**Never smokers** (N=72)	**Ever smokers** (N=474)	**Total number of participants**	**Overall**	**Never smokers** (N=246)	**Ever smokers** (N=1061)
Tumour size (cm), mean (SD)	N=533	2.50 (1.90)	2.69 (2.14)	2.48 (1.86)	N=303	2.4 (1.3)	2.4 (1.1)	2.4 (1.3)
Age (years), mean (SD)	N=546	70.0 (10.3)	72.2 (10.9)	69.7 (10.2)	N=1307	62.5 (9.7)	60.8 (11.0)	62.5 (9.0)
Grade	N=539				N=1307			
G3 (%)			49 (9.1)	312 (57.9)			56 (4.28)	237 (18.1)
G2 and G1 (%)			22 (4.1)	156 (28.9)			190 (14.6)	824 (63.1)
Tumour stage	N=546				N=1267			
T1 and Tis (%)			47 (12.6)	326 (87.4)			72 (5.7)	288 (22.7)
Ta (%)			25 (14.5)	148 (85.5)			174 (13.7)	733 (57.9)
Cigarettes smoked/day, mean (SD)	N=322	N/A	N/A	15.8 (12.9)	N=1061	N/A	N/A	15.5 (8.1)

BCPP- Bladder Cancer Prognosis Programme; NBCS – Nijmegen Bladder Cancer Study; SD -Standard deviation.

The most significant interaction with smoking status was observed for a SNP in 14q21.1 locus (BP: 14:38247577, p=0.0008), that maps to the *TTC6* gene (**Table 5.3**). Another SNP in the same locus has also yielded statistical significance for interaction (rs188958632, p=0.008, *TTC6*), but the two variants show differential results for smokers and non-smokers. The SNP in 14:38247577 reaches significance among smokers only, with a large effect on tumour size (**β**=7.1 cm, p=1.93E-13), and is suggestive of an interaction with smoking intensity (p=0.07). On the contrary, rs188958632 SNP is significant among both tobacco use groups, with a larger effect size for never-smokers (**β** (never-smokers) =9.9 cm, p=9.84E-05; **β** (ever-smokers) =2.6 cm, p=6.98E-07), and no implications of interaction with smoking intensity (p=0.29) (**Table 5.2, Figure 5.2**).

Five variants on 6q14.1 were statistically significantly associated with NMIBC tumour size among both strata of smoking behaviours; however, **β** estimates for never-smokers were universally higher, more than doubling the effect sizes of ever-smokers (**Table 5.2**). All five SNPs (rs180910528, rs74603364, rs187040828, rs144383242, and rs117587674) have mapped to intergenic regions (**Table 5.3**).

Rs180910528 and rs187040828 are situated between *PHIP* and *HMGN3* protein-coding genes, whereas the remaining three are surrounded by long non-coding intergenic RNA (lincRNA) molecules. Interestingly, two of these SNPs (rs144383242 and rs117587674) are recorded in the GTEx database as having an effect on the expression of *HMGN3-AS1* in the tibial nerve tissue (**Supplementary Figures 5.1, 5.2**) [24]. None of the 6q14.1-located SNPs have shown significant interaction between tumour size and smoking intensity in our sample.

Table 5.2. SNPs attaining statistical significance (p (SNP x Smoking status) <0.05) for interaction with smoking status in the BCPP cohort.

Phenotype	rsID	BP	Locus	REF allele	EFF allele	EAF	β (SNP)[a]	p (SNP)[b]	p (SNP x Smoking status)[c]	p (SNP x Smoking intensity)[d]	β (never smokers)[e]	p (never smokers)[f]	β (smokers)[g]	p (smokers)[h]
Tumour size (cm)	rs113705641	5375733	3p26.1	A	G	0.02	9.5	2.68E-27	0.03	0.95	-3.1	1	2.4	2.39E-06
	Not yet assigned	38247577	14q21.1	CTGG	C	0.01	8.1	9.41E-14	0.0008	0.07	-1.6	1	7.1	1.93E-13
	rs188958632	38266174	14q21.1	G	A	0.01	2.8	1.02E-06	0.01	0.29	9.9	9.84E-05	2.6	6.98E-07
	rs35225990	46117489	13q14.13	C	T	0.07	1.5	5.17E-09	0.01	0.37	7.9	8.54E-14	1.0	4.52E-05
	rs2937268	66553607	1p31.3	C	T	0.04	2.6	3.28E-11	0.04	0.06	1.4	0.29	2.3	7.08E-11
	rs117587674	79432536	6q14.1	G	A	0.01	3.5	4.67E-11	0.03	0.24	8.1	0.0004	3.0	5.56E-09
	rs144383242	79489625	6q14.1	G	T	0.01	3.5	5.74E-11	0.03	0.24	8.1	0.0004	3.0	6.55E-09
	rs74603364	79509518	6q14.1	C	T	0.02	2.5	1.88E-08	0.02	0.20	7.1	5.25E-05	2.3	1.42E-07
	rs187040828	79802426	6q14.1	T	C	0.01	3.9	3.58E-11	0.02	0.33	9.3	0.0001	3.4	6.79E-09
	rs180910528	79821806	6q14.1	A	C	0.01	3.9	2.64E-11	0.02	0.33	9.9	8.44E-05	3.4	5.88E-09

[a] An estimate coefficient calculated in linear regression.
[b] Level of statistical significance achieved in linear regression between a SNP and tumour size in centimetres.
[c] Multiplicative interaction with smoking status (never- or ever-smokers).
[d] Multiplicative interaction with smoking intensity (number of cigarettes smoked per day). Restricted to smokers only.
[e] An estimate coefficient calculated in linear regression only among never smokers.
[f] Level of statistical significance achieved in linear regression between a SNP and tumour size in centimetres only among never smokers.
[g] An estimate coefficient calculated in linear regression only among smokers
[h] Level of statistical significance achieved in linear regression between a SNP and tumour size in centimetres only among smokers.
BP-base pair; EAF-effect allele frequency; EFF-effect; SNP-single nucleotide polymorphism; REF-reference.

Table 5.3. Functional annotation of the variants that reached statistical significance for gene-environment interaction with smoking status.

Phenotype	rsID	BP	Locus	REF allele	EFF allele	Overlapped gene	Nearest Upstream Gene	Distance (BP)	Nearest Downstream Gene	Distance (BP)
Tumour size (cm)	Not yet assigned	38247577	14q21.1	CTGG	C	TTC6 (intronic)	-	-	-	-
	rs35225990	46117489	13q14.13	C	T	FAM194B (intronic)	-	-	-	-
	rs188958632	38266174	14q21.1	G	A	TTC6 (intronic)	-	-	-	-
	rs180910528	79821806	6q14.1	A	C	-	PHIP (protein coding)	33853	HMGN3 (protein coding)	89156
	rs74603364	79509518	6q14.1	C	T	-	RP3-390M24.1 (lincRNA)	193765	RP11-173D14.3 (lincRNA)	9914
	rs187040828	79802426	6q14.1	T	C	-	PHIP (protein coding)	14473	HMGN3 (protein coding)	108536
	rs144383242	79489625	6q14.1	G	T	-	RP3-390M24.1 (lincRNA)	173872	RP11-173D14.3 (lincRNA)	29807
	rs117587674	79432536	6q14.1	G	A	-	RP3-390M24.1 (lincRNA)	116783	RP11-173D14.3 (lincRNA)	86896
	rs113705641	5375733	3p26.1	A	G	-	AC026202.5 (lincRNA)	77287	AC027119.1	628796
	rs2937268	66553607	1p31.3	C	T	PDE4B (intronic)	-	-	-	-

BP-Base pair; EFF-Effect; REF-reference; rsID-SNP ID.

Rs113705641 on 3p26.1 (intergenic region) has shown to only be statistically significantly associated with tumour size among ever-smokers (**β**=2.4 cm, p=2.39E-06), and no interaction with smoking intensity. Similarly, rs2937268 on 1p31.3, mapped to the *PDE4B* gene, has not only reached significance among ever-smokers (**β**=2.3 cm, p=7.08E-11), but has also shown suggestive interaction with smoking intensity (p=0.06) among tobacco users.

It is notable that variants associated with a stronger effect among never-smokers, had higher estimates overall (ranging from 7.1 to 9.9 cm). In contrast, rs2937268 (1p31.3, *PDE4B*) and rs113705641 (3p26.1) variants, shown to be significant only for smokers, have been observed to carry a more modest increase in tumour size. The exception would be the 14:38247577 variant in 14q21.1 that was not significant among never-smokers, but resulted in a very large tumour size increase of 7.1 cm in smokers (**Table 5.2, Figure 5.2**).

In the replication analyses, we were not able to test all of the SNPs of interest (Table 5.4). Three SNPs, namely rs187040828, rs2937268, and one located in 14q21.1 (BP: 38247577, no rsID assigned) were missing from the NBCS dataset. The remaining loci did not show any interaction with smoking.

DISCUSSION

We hereby report findings for gene-environment interaction with smoking status for SNPs previously found to be significant in a GWAS for NMIBC baseline characteristics in the BCPP cohort [11].

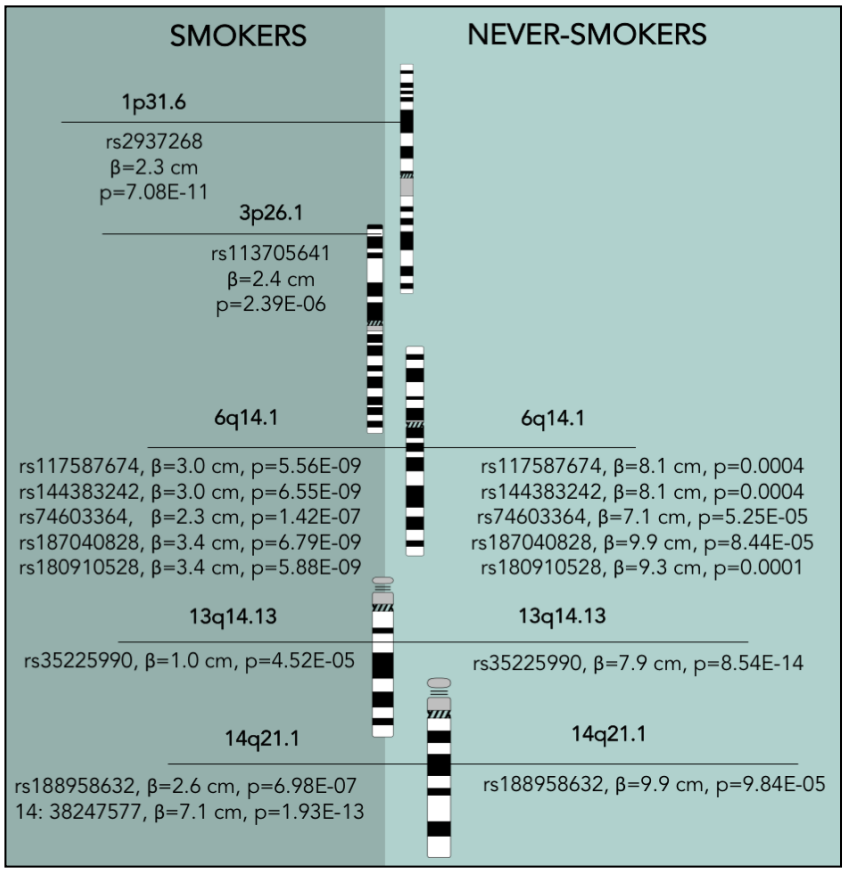


Figure 5.2. Group-specific patterns of the significant loci for gene-environment interaction for NMIBC tumour size in the BCPP cohort. ***Idiogram credit: David Adler,*** ***http://www.pathology.washington.edu/research/cytopages/idiograms/human/***

Table 5.4. Replication results of the gene-environment interaction with smoking on tumour size in the Netherlands Bladder Cancer Study.

Phenotype	rsID	BP	Locus	REF allele	EFF allele	β (joint)	p (joint)	P (Interaction, SNP x Smoking status)[a]	MAF
Tumour size (cm)	No yet assigned	38247577	14q21.1	CTGG	C	NA	NA	NA	NA
	rs35225990	46117489	13q14.13	C	T	0.20	0.37	0.78	0.05
	rs188958632	38266174	14q21.1	G	A	-0.50	0.09	0.39	0.04
	rs180910528	79821806	6q14.1	A	C	0.52	0.26	1.00	0.01
	rs74603364	79509518	6q14.1	C	T	0.60	0.12	0.84	0.02
	rs187040828	79802426	6q14.1	T	C	NA	NA	NA	NA
	rs144383242	79489625	6q14.1	G	T	0.36	0.41	0.32	0.02
	rs117587674	79432536	6q14.1	G	A	0.36	0.41	0.32	0.02
	rs113705641	5375733	3p26.1	A	G	0.75	0.13	0.15	0.02
	rs2937268	66553607	1p31.3	C	T	NA	NA	NA	NA

a Interaction with smoking status (Never or Ever smokers).

BP-base pair; EFF – effect; MAF-minor allele frequency; SNP-single nucleotide polymorphism; REF - reference.

The study provides indication for ten variants interacting with smoking status for tumour size at the time of NMIBC diagnosis, and two of those additionally having suggestive interactions with smoking intensity. To the best of our knowledge, all of these loci are novel reports for GxE interaction.

Previous studies on GxT interactions for UBC risk show repeating patterns that highlight potential biological mechanisms [2, 4, 25]. Smoker-specific UBC risk genes are associated with pathways of metabolite detoxification [25], offering a plausible explanation on why the effect is more penetrant among smokers. Alternatively, genes having more importance among never smokers are more often enriched in those regulating cell cycle and DNA integrity [25]. Additionally, results across multiple GxT studies on UBC demonstrate that variants significant for never-smokers carry a large effect (i.e. multiplicative interaction), whilst SNPs that are more important for smokers usually exhibit a milder effect (additive interaction) [5].

Among smokers, the strongest evidence for GxT interaction in our sample was observed for variants rs113705641 (3p26.1) and rs2937268 (1p31.3, *PDE4B*).

Rs113705641 maps to a long non-coding RNA molecule, and reports on its function are lacking, therefore we are unable to postulate on a potential biological mechanism of interaction.

On the other hand, Phosphodiesterase 4B (*PDE4B*) enzymes are well described to regulate cyclic adenosine 3',5'-monophosphate (cAMP) concentration in cells by breakdown to non-active molecules [26]. The PDE4 family is cAMP-specific, and four existing isoforms of PDE4B account for most cAMP-degradation in a cell [26].

The beneficial effect of PDE inhibitors is well-known and they are commonly used for a variety of disorders: inflammatory conditions (namely chronic obstructive pulmonary disease (COPD) [27, 28], asthma [27], and psoriasis [27, 28]), overactive bladder [29,

30], and various cancer types [31-36]. Importantly, studies also suggest tumour *PDE4* expression can serve as a prognostic marker in colorectal cancer [36]. As cAMP signalling is pleiotropic, the mechanisms behind the observed benefit have garnered more than one explanation. For example, elevated cAMP levels supress cell invasion and migration by disrupting the microtubule cytoskeleton in bladder cancer cells [37], increase phosphatidylinositol 3-kinase (PI3K/AKT)-dependent apoptosis in B-cell malignancies [38], and prohibit proliferation via protein kinase (PKA) and cAMP response element binding (CREB) protein pathways in ovarian cancer [39].

Interestingly, tobacco exposure also increases cAMP signalling in epithelial cells [40], specifically by polycyclic aromatic hydrocarbons (PAHs, found in tobacco smoke) binding to aryl hydrocarbon receptors (AhR) [40]. Stimulation of cAMP results in increased levels of amphiregulin (AR), which is hypothesized to eventually form a self-sustainable loop of survival for neoplastic cells [40-42].

In our analysis, cAMP seems to be the overlapping component between *PDE4B* and smoking, which results in a counter-effect of cAMP regulation (i.e. overexpressed PDE4B decreases cAMP, whilst smoking increases cAMP). Thus, we hypothesize that smoking interacts with *PDE4B* mutation status and results in a smaller tumour size increase than the *PDE4B* mutation itself. This pattern would explain the difference between a main effect exhibited by the SNP in *PDE4B* (resulting in 2.6 cm increase in tumour size) and a surprising reduced estimate of a joint effect of the SNP and smoking exposure (2.1 cm increase in tumour size).

Smoking, when considered as an independent factor, is the main external risk factor for developing UBC [43]. Our described interaction is conditional on the overexpression of PDE4B enzyme; thus, it should be viewed not as a case for the

benefits of smoking, but one describing a potential cAMP-related pathway of bladder cancer pathogenesis.

An additional variant in 14q21.1 (BP: 38247577) reached statistical significance among smokers only. However, a SNP in the same locus (rs188958632) showed contradicting results and had a significant effect regardless of smoking exposure. We suspect that the SNP on 14:38247577 is more likely to be a random result due to a lower imputation accuracy (info=0.59) than rs188958632 (info=0.95) (remaining SNPs have an info score of ≥0.90, with the exception for rs113705641, with info=0.62).

In contrast, variants on 13q14.13 and 6q14.1 have reached significant values for main effects among all participants. However, the clinical importance might be higher for never smokers as they showed substantially higher effect sizes than for smokers. The mapped *FAM194B* (*ERICH6B*) gene (13q14.13) is recorded in the GTEx database as excessively expressed in testis tissue [24], but other reports on gene function or phenotype associations are scarce, thus precluding a discussion on its potential biological pathway. SNPs in 6q14.1 were mapped to intergenic regions, with rs180910528 and rs187040828 located between protein coding genes of *PHIP* and *HMGN3*. Interestingly, four of the total five SNPs in 6q14 region (rs180910528, rs187040828, rs144383242, and rs117587674) are recorded in the GTEx database as having a cis-regulatory effect on an RNA gene of *HMGN3-AS1* [24]. Antisense RNAs bind to messenger RNA molecules, preventing them from being translated into protein [44]. Thus, lower expression of *HMGN3-AS1* may cause higher levels of proteins coded by *HMGN3* gene. HMGN is a family of nucleosome-binding proteins that alter chromatin structure and regulate essential cell functions, such as differentiation and development [45].

The current study has several limitations. Our analysis was carried out on a relatively small sample of individuals, and false-positives are naturally not to be excluded. The potential associations between genetic variation and NMIBC tumour size should be interpreted with caution, as they lack validation [11]. Besides tobacco, other variables, such as occupational-related exposures with aromatic amines might have independent gene-environment interaction effects and were not adjusted for in our analyses [46]. Most importantly, we attempted a replication of our results in an independent cohort of NMIBC in the NBCS, without any of the tested variants showing interaction with smoking. The sample size for replication was small, and some SNPs were unavailable to test for interaction terms altogether. Unfortunately, that includes rs2937268 in *PDE4B*, which is arguable the most important SNP to test. Larger samples with detailed clinical data are a next step to exploring and validating reported gene-environment interactions with smoking for NMIBC. Until robust evidence exists for including genetic variance into NMIBC prognostication tools, our study is complementary to other proof-of-principle reports [2]. In summary, our study suggests interaction between genetic variance and smoking behaviour for increased NMIBC tumour size at the time of diagnosis. These results may provide more evidence on the complexity of the joint influence of genetic background and external exposures on bladder cancer; however the failed replication also warrants careful consideration of our results being false positives.

REFERENCES

[1] Selinski S. The Post Gwas Era: Strategies to Identify Gene-Gene and Gene-Environment Interactions in Urinary Bladder Cancer. EXCLI journal. 2014;13:1198-203.

[2] Garcia-Closas M, Rothman N, Figueroa JD, Prokunina-Olsson L, Han SS, Baris D, Jacobs EJ, Malats N, De Vivo I, Albanes D, Purdue MP, Sharma S, Fu YP, Kogevinas M, Wang Z, Tang W, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Andriole G, Jr., Grubb R, 3rd, Black A, Gapstur SM, Thun M, Diver WR, Weinstein SJ, Virtamo J, Hunter DJ, Caporaso N, Landi MT, Hutchinson A, Burdett L, Jacobs KB, Yeager M, Fraumeni JF, Jr., Chanock SJ, Silverman DT, Chatterjee N. Common Genetic Polymorphisms Modify the Effect of Smoking on Absolute Risk of Bladder Cancer. Cancer research. 2013;73(7):2211-20.

[3] Rothman N, Garcia-Closas M, Chatterjee N, Malats N, Wu X, Figueroa JD, Real FX, Van Den Berg D, Matullo G, Baris D, Thun M, Kiemeney LA, Vineis P, De Vivo I, Albanes D, Purdue MP, Rafnar T, Hildebrandt MA, Kiltie AE, Cussenot O, Golka K, Kumar R, Taylor JA, Mayordomo JI, Jacobs KB, Kogevinas M, Hutchinson A, Wang Z, Fu YP, Prokunina-Olsson L, Burdett L, Yeager M, Wheeler W, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Andriole G, Jr., Grubb R, 3rd, Black A, Jacobs EJ, Diver WR, Gapstur SM, Weinstein SJ, Virtamo J, Cortessis VK, Gago-Dominguez M, Pike MC, Stern MC, Yuan JM, Hunter DJ, McGrath M, Dinney CP, Czerniak B, Chen M, Yang H, Vermeulen SH, Aben KK, Witjes JA, Makkinje RR, Sulem P, Besenbacher S, Stefansson K, Riboli E, Brennan P, Panico S, Navarro C, Allen NE, Bueno-de-Mesquita HB, Trichopoulos D, Caporaso N, Landi MT, Canzian F, Ljungberg B, Tjonneland A, Clavel-Chapelon F, Bishop DT, Teo MT, Knowles MA, Guarrera S, Polidoro S, Ricceri F, Sacerdote C, Allione A, Cancel-Tassin G, Selinski S, Hengstler JG, Dietrich H, Fletcher T, Rudnai P, Gurzau E, Koppova K, Bolick SC, Godfrey A, Xu Z, Sanz-Velez JI, M DG-P, Sanchez M, Valdivia G, Porru S, Benhamou S, Hoover RN, Fraumeni JF, Jr., Silverman DT, Chanock SJ. A Multi-Stage Genome-Wide Association Study of Bladder Cancer Identifies Multiple Susceptibility Loci. Nature genetics. 2010;42(11):978-84.

[4] Figueroa JD, Han SS, Garcia-Closas M, Baris D, Jacobs EJ, Kogevinas M, Schwenn M, Malats N, Johnson A, Purdue MP, Caporaso N, Landi MT, Prokunina-Olsson L, Wang Z, Hutchinson A, Burdette L, Wheeler W, Vineis P, Siddiq A, Cortessis VK, Kooperberg C, Cussenot O, Benhamou S, Prescott J, Porru S, Bueno-de-Mesquita HB, Trichopoulos D, Ljungberg B, Clavel-Chapelon F, Weiderpass E, Krogh V, Dorronsoro M, Travis R, Tjonneland A, Brenan P, Chang-Claude J, Riboli E, Conti D, Gago-Dominguez M, Stern MC, Pike MC, Van Den Berg D, Yuan JM, Hohensee C, Rodabough R, Cancel-Tassin G, Roupret M, Comperat E, Chen C, De Vivo I, Giovannucci E, Hunter DJ, Kraft P, Lindstrom S, Carta A, Pavanello S, Arici C, Mastrangelo G, Karagas MR, Schned A, Armenti KR, Hosain GM, Haiman CA, Fraumeni JF, Jr., Chanock SJ, Chatterjee N, Rothman N, Silverman DT. Genome-Wide Interaction Study of Smoking and Bladder Cancer Risk. Carcinogenesis. 2014;35(8):1737-44.

[5] Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Castano-Vinyals G, Yeager M, Welch R, Chanock S, Chatterjee N, Wacholder S, Samanic C, Tora M, Fernandez F, Real FX, Rothman N. Nat2 Slow Acetylation, Gstm1 Null Genotype,

and Risk of Bladder Cancer: Results from the Spanish Bladder Cancer Study and Meta-Analyses. Lancet (London, England). 2005;366(9486):649-59.
[6] Moore LE, Baris DR, Figueroa JD, Garcia-Closas M, Karagas MR, Schwenn MR, Johnson AT, Lubin JH, Hein DW, Dagnall CL, Colt JS, Kida M, Jones MA, Schned AR, Cherala SS, Chanock SJ, Cantor KP, Silverman DT, Rothman N. Gstm1 Null and Nat2 Slow Acetylation Genotypes, Smoking Intensity and Bladder Cancer Risk: Results from the New England Bladder Cancer Study and Nat2 Meta-Analysis. Carcinogenesis. 2011;32(2):182-9.
[7] Lubin JH, Kogevinas M, Silverman D, Malats N, Garcia-Closas M, Tardon A, Hein DW, Garcia-Closas R, Serra C, Dosemeci M, Carrato A, Rothman N. Evidence for an Intensity-Dependent Interaction of Nat2 Acetylation Genotype and Cigarette Smoking in the Spanish Bladder Cancer Study. International journal of epidemiology. 2007;36(1):236-41.
[8] Czerniak B, Dinney C, McConkey D. Origins of Bladder Cancer. Annual review of pathology. 2016;11:149-74.
[9] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.
[10] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.
[11] Lipunova N, Wesselius A, Cheng KK, Schooten F-Jv, Bryan RT, Cazier J-B, Galesloot TE, Kiemeney LALM, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer (Manuscript in Submission). 2018.
[12] Zeegers MP, Bryan RT, Langford C, Billingham L, Murray P, Deshmukh NS, Hussain S, James N, Wallace DM, Cheng KK. The West Midlands Bladder Cancer Prognosis Programme: Rationale and Design. BJU international. 2010;105(6):784-8.
[13] Hakonarson H, Gulcher JR, Stefansson K. Decode Genetics, Inc. Pharmacogenomics. 2003;4(2):209-15.
[14] Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KK, Stacey SN, Gudmundsson J, Jakobsdottir M, Bergthorsson JT, Sigurdsson A, Blondal T, Witjes JA, Vermeulen SH, Hulsbergen-van de Kaa CA, Swinkels DW, Ploeg M, Cornel EB, Vergunst H, Thorgeirsson TE, Gudbjartsson D, Gudjonsson SA, Thorleifsson G, Kristinsson KT, Mouy M, Snorradottir S, Placidi D, Campagna M, Arici C, Koppova K, Gurzau E, Rudnai P, Kellen E, Polidoro S, Guarrera S, Sacerdote C, Sanchez M, Saez B, Valdivia G, Ryk C, de Verdier P, Lindblom A, Golka K, Bishop DT, Knowles MA, Nikulasson S, Petursdottir V, Jonsson E, Geirsson G, Kristjansson B, Mayordomo JI, Steineck G, Porru S, Buntinx F, Zeegers MP, Fletcher T, Kumar R, Matullo G, Vineis P, Kiltie AE, Gulcher JR, Thorsteinsdottir U, Kong A, Rafnar T, Stefansson K. Sequence Variant on 8q24 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2008;40(11):1307-12.
[15] Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-Generation Plink: Rising to the Challenge of Larger and Richer Datasets. GigaScience. 2015;4:7.
[16] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A Tool Set for Whole-Genome

Association and Population-Based Linkage Analyses. American journal of human genetics. 2007;81(3):559-75.
[17] Loh PR, Danecek P, Palamara PF, Fuchsberger C, Y AR, H KF, Schoenherr S, Forer L, McCarthy S, Abecasis GR, Durbin R, A LP. Reference-Based Phasing Using the Haplotype Reference Consortium Panel. Nature genetics. 2016;48(11):1443-8.
[18] Howie BN, Donnelly P, Marchini J. A Flexible and Accurate Genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. PLoS genetics. 2009;5(6):e1000529.
[19] Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, Tasse AM, Flicek P. The International Genome Sample Resource (Igsr): A Worldwide Collection of Genome Variation Incorporating the 1000 Genomes Project Data. Nucleic acids research. 2017;45(D1):D854-d9.
[20] Johnson T, Kutalik Z. Quicktest User Guide V0.94. 2008.
[21] Dayem Ullah AZ, Lemoine NR, Chelala C. Snpnexus: A Web Server for Functional Annotation of Novel and Publicly Known Genetic Variants (2012 Update). Nucleic acids research. 2012;40(Web Server issue):W65-70.
[22] Dayem Ullah AZ, Lemoine NR, Chelala C. A Practical Guide for the Functional Annotation of Genetic Variations Using Snpnexus. Briefings in Bioinformatics. 2013;14(4):437-47.
[23] Aken BL, Ayling S, Barrell D, Clarke L, Curwen V, Fairley S, Fernandez Banet J, Billis K, García Girón C, Hourlier T, Howe K, Kähäri A, Kokocinski F, Martin FJ, Murphy DN, Nag R, Ruffier M, Schuster M, Tang YA, Vogel JH, White S, Zadissa A, Flicek P, Searle SMJ. The Ensembl Gene Annotation System. Database: The Journal of Biological Databases and Curation. 2016;2016.
[24] The Genotype-Tissue Expression (Gtex) Project. Nature genetics. 2013;45(6):580-5.
[25] Schwender H, Selinski S, Blaszkewicz M, Marchan R, Ickstadt K, Golka K, Hengstler JG. Distinct Snp Combinations Confer Susceptibility to Urinary Bladder Cancer in Smokers and Non-Smokers. PLoS ONE. 2012;7(12):e51880.
[26] Conti M, Richter W, Mehats C, Livera G, Park JY, Jin C. Cyclic Amp-Specific Pde4 Phosphodiesterases as Critical Components of Cyclic Amp Signaling. The Journal of biological chemistry. 2003;278(8):5493-6.
[27] Page CP, Spina D. Phosphodiesterase Inhibitors in the Treatment of Inflammatory Diseases. Handbook of experimental pharmacology. 2011(204):391-414.
[28] Abdel-Magid AF. Selective Inhibitors of Phosphodiesterase 4b (Pde-4b) May Provide a Better Treatment for Cns, Metabolic, Autoimmune, and Inflammatory Diseases. ACS medicinal chemistry letters. 2017;8(11):1132-3.
[29] Rahnama'i MS, Uckert S, Hohnen R, van Koeveringe GA. The Role of Phosphodiesterases in Bladder Pathophysiology. Nature reviews Urology. 2013;10(7):414-24.
[30] Agis-Torres A, Recio P, Lopez-Oliva ME, Martinez MP, Barahona MV, Benedito S, Bustamante S, Jimenez-Cidre MA, Garcia-Sacristan A, Prieto D, Fernandes VS, Hernandez M. Phosphodiesterase Type 4 Inhibition Enhances Nitric Oxide- and Hydrogen Sulfide-Mediated Bladder Neck Inhibitory Neurotransmission. Scientific reports. 2018;8(1):4711.
[31] Dong H, Claffey KP, Brocke S, Epstein PM. Inhibition of Breast Cancer Cell Migration by Activation of Camp Signaling. Breast cancer research and treatment. 2015;152(1):17-28.

[32] Powers GL, Hammer KDP, Domenech M, Frantskevich K, Malinowski RL, Bushman W, Beebe DJ, Marker PC. Phosphodiesterase 4d Inhibitors Limit Prostate Cancer Growth Potential. Molecular cancer research : MCR. 2015;13(1):149-60.
[33] Domvri K, Zarogoulidis K, Zogas N, Zarogoulidis P, Petanidis S, Porpodis K, Kioseoglou E, Hohenforst-Schmidt W. Potential Synergistic Effect of Phosphodiesterase Inhibitors with Chemotherapy in Lung Cancer. Journal of Cancer. 2017;8(18):3648-56.
[34] Cooney JD, Aguiar RC. Phosphodiesterase 4 Inhibitors Have Wide-Ranging Activity in B-Cell Malignancies. Blood. 2016;128(25):2886-90.
[35] Suhasini AN, Wang L, Holder KN, Lin AP, Bhatnagar H, Kim SW, Moritz AW, Aguiar RCT. A Phosphodiesterase 4b-Dependent Interplay between Tumor Cells and the Microenvironment Regulates Angiogenesis in B-Cell Lymphoma. Leukemia. 2016;30(3):617-26.
[36] Tsunoda T, Ota T, Fujimoto T, Doi K, Tanaka Y, Yoshida Y, Ogawa M, Matsuzaki H, Hamabashiri M, Tyson DR, Kuroki M, Miyamoto S, Shirasawa S. Inhibition of Phosphodiesterase-4 (Pde4) Activity Triggers Luminal Apoptosis and Akt Dephosphorylation in a 3-D Colonic-Crypt Model. Molecular Cancer. 2012;11(1):46.
[37] Ou Y, Zheng X, Gao Y, Shu M, Leng T, Li Y, Yin W, Zhu W, Huang Y, Zhou Y, Tang J, Qiu P, Yan G, Hu J, Ruan H, Hu H. Activation of Cyclic Amp/Pka Pathway Inhibits Bladder Cancer Cell Invasion by Targeting Map4-Dependent Microtubule Dynamics. Urologic oncology. 2014;32(1):47.e21-8.
[38] Smith PG, Wang F, Wilkinson KN, Savage KJ, Klein U, Neuberg DS, Bollag G, Shipp MA, Aguiar RC. The Phosphodiesterase Pde4b Limits Camp-Associated Pi3k/Akt-Dependent Apoptosis in Diffuse Large B-Cell Lymphoma. Blood. 2005;105(1):308-16.
[39] Gong S, Chen Y, Meng F, Zhang Y, Wu H, Wu F. Roflumilast Restores Camp/Pka/Creb Signaling Axis for Ftmt-Mediated Tumor Inhibition of Ovarian Cancer. Oncotarget. 2017;8(68):112341-53.
[40] Du B, Altorki NK, Kopelovich L, Subbaramaiah K, Dannenberg AJ. Tobacco Smoke Stimulates the Transcription of Amphiregulin in Human Oral Epithelial Cells: Evidence of a Cyclic Amp-Responsive Element Binding Protein-Dependent Mechanism. Cancer research. 2005;65(13):5982-8.
[41] Zuo WL, Yang J, Gomi K, Chao I, Crystal RG, Shaykhiev R. Egf-Amphiregulin Interplay in Airway Stem/Progenitor Cells Links the Pathogenesis of Smoking-Induced Lesions in the Human Airway Epithelium. Stem cells (Dayton, Ohio). 2017;35(3):824-37.
[42] Bryan RT, Hussain SA, James ND, Jankowski JA, Wallace DM. Molecular Pathways in Bladder Cancer: Part 1. BJU international. 2005;95(4):485-90.
[43] Burger M, Catto JWF, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and Risk Factors of Urothelial Bladder Cancer. European Urology. 2013;63(2):234-41.
[44] Park H, Yoon Y, Suk S, Lee JY, Lee Y. Effects of Different Target Sites on Antisense Rna-Mediated Regulation of Gene Expression. BMB reports. 2014;47(11):619-24.
[45] Furusawa T, Cherukuri S. Developmental Function of Hmgn Proteins. Biochimica et biophysica acta. 2010;1799(1-2):69.
[46] Murta-Nascimento C, Schmitz-Drager BJ, Zeegers MP, Steineck G, Kogevinas M, Real FX, Malats N. Epidemiology of Urinary Bladder Cancer: From Tumor Development to Patient's Death. World journal of urology. 2007;25(3):285-95.

CHAPTER 6.

EXTERNAL REPLICATION OF URINARY BLADDER CANCER PROGNOSTIC POLYMORPHISMS IN THE UK BIOBANK

Contents of this chapter have been published:

Lipunova N, Wesselius A, Cheng KK, van Schooten FJ**,** Cazier JB**,** Bryan RT, Zeegers MP. External replication of urinary bladder cancer prognostic polymorphisms in the UK Biobank. Front Oncol. 2019; 9:1082; doi: 10.3389/fonc.201901082

ABSTRACT

INTRODUCTION

Multiple studies have reported genetic associations with prognostic outcomes of urinary bladder cancer. However, the lack of replication of these associations prohibits establishing further evidence-based research directions. Moreover, there is a lack of independent bladder cancer patient samples that contain prognostic measures, making genetic replication analyses even more challenging.

MATERIALS AND METHODS

We have identified 1,534 eligible patients and used data on Hospital Episode Statistics in the UK Biobank to model variables of otherwise non-collected events on bladder cancer recurrence and progression. Data on survival was extracted from the Death Registry. We have used SNPTEST software to replicate previously reports genetic associations with bladder cancer recurrence (N=69), progression (N=23), survival (N=53), and age at the time of diagnosis (N=20).

RESULTS

Using our algorithm, we have identified 618 recurrence and 58 UBC progression events. In total, there were 209 deaths (106 UBC-specific). In replication analyses, eight single nucleotide polymorphisms (SNPs) have reached nominal statistical significance ($p<0.05$). Rs2042329 (*CWC27*) for UBC recurrence; rs804256, rs4639, and rs804276 (in/close to *NEIL2*) for NMIBC recurrence; rs2293347 (*EGFR*) for UBC OS; rs3756712 (*PDCD6)* for NMIBC OS; rs2344673 (*RGS5)* for MIBC OS, and rs2297518 (*NOS2*) for UBC progression. However, none have remained significant after adjustments for multiple comparisons.

CONCLUSION

External replication in genetic epidemiology is an essential step to identify credible findings. In our study, we identify potential genetic targets of higher interest for UBC prognosis. In addition, we propose an algorithm for identifying UBC recurrence and progression using routinely-collected data on patient interventions.

INTRODUCTION

Urinary bladder cancer (UBC) is a disease of great burden; yet the diagnosis, clinical management, and patient survivorship has changed little over the last few decades [1, 2]. Genetic studies may provide important clues on biological pathways underlying the development of UBC. Importantly, advances in understanding what drives a favourable UBC prognosis could aid in predicting patient outcomes. As a result, and informed and timely patient stratification would allow an individually-tailored cancer management plan, which is likely to better reflect patient needs than current group-level recommendations [3].

Multiple genetic associations with UBC prognostic outcomes (e.g. survival, recurrence) have been reported in the literature (*Manuscript in submission,* [4]). However, the number of potential genetic clues far exceeds the available resources for clinical and functional investigation. As such, the scientific community must take an approach of targeting most-promising associations first.

There are multiple ways to define clinical relevance of a genetic variant, including external replication to reduce the chance of false-positives [5, 6]. However, replication of genetic associations includes many hurdles, such as a lack of independent participant cohorts with adequate sample sizes. Moreover, focus on a sub-phenotype (e.g. recurrence) makes it even more difficult due to required additional sources of data (e.g. hospital records).

Increased availability of population-based electronic health records can help to alleviate the burden of investigating diseases for which adequate sample sizes are difficult to acquire. UK Biobank is the largest population-based cohort in the United Kingdom and serves as a powerful resource for investigating genetic associations [7]

and has not yet been widely used for investigating UBC. The presence of Hospital Episode Statistics (HES) in the UK Biobank offers an unprecedented opportunity to use these data to identify UBC recurrence and progression events, that are not a part of the usually-collected information.

In the current study, we have aimed to identify UBC patients in the UK Biobank and use HES statistics to construct prognostic events. We have further used this data to externally replicate previously reported genetic associations on UBC survival, recurrence, and age at the time of diagnosis.

METHODS

Single nucleotide polymorphism (SNP) selection

We have aimed to replicate all SNPs that have been previously associated with UBC recurrence, progression, death (overall or cancer-specific), and age at the time of diagnosis. The polymorphisms were extracted from a recent review on prognostic UBC outcomes (*Manuscript in submission* [4]). To capture any associations reported since the review, we have updated the list of SNPs by querying PubMed database for new articles using identical search terms to those used in the review (**Figure 6.1**). The search was limited to articles published in English language between 13th November 2018 and 19th February 2019. Eleven papers were identified in total, with one study being eligible for inclusion [8]. Additionally, we have included associations for age at the time of diagnosis from a genome-wide association study (GWAS) previously carried out in the Bladder Cancer Prognosis Programme (BCPP) [9].

After removing duplicate entries, there were 69 SNPs to test for recurrence, 53 for survival, 20 for age, and 23 for progression (**Supplementary Tables 6.1-4**).

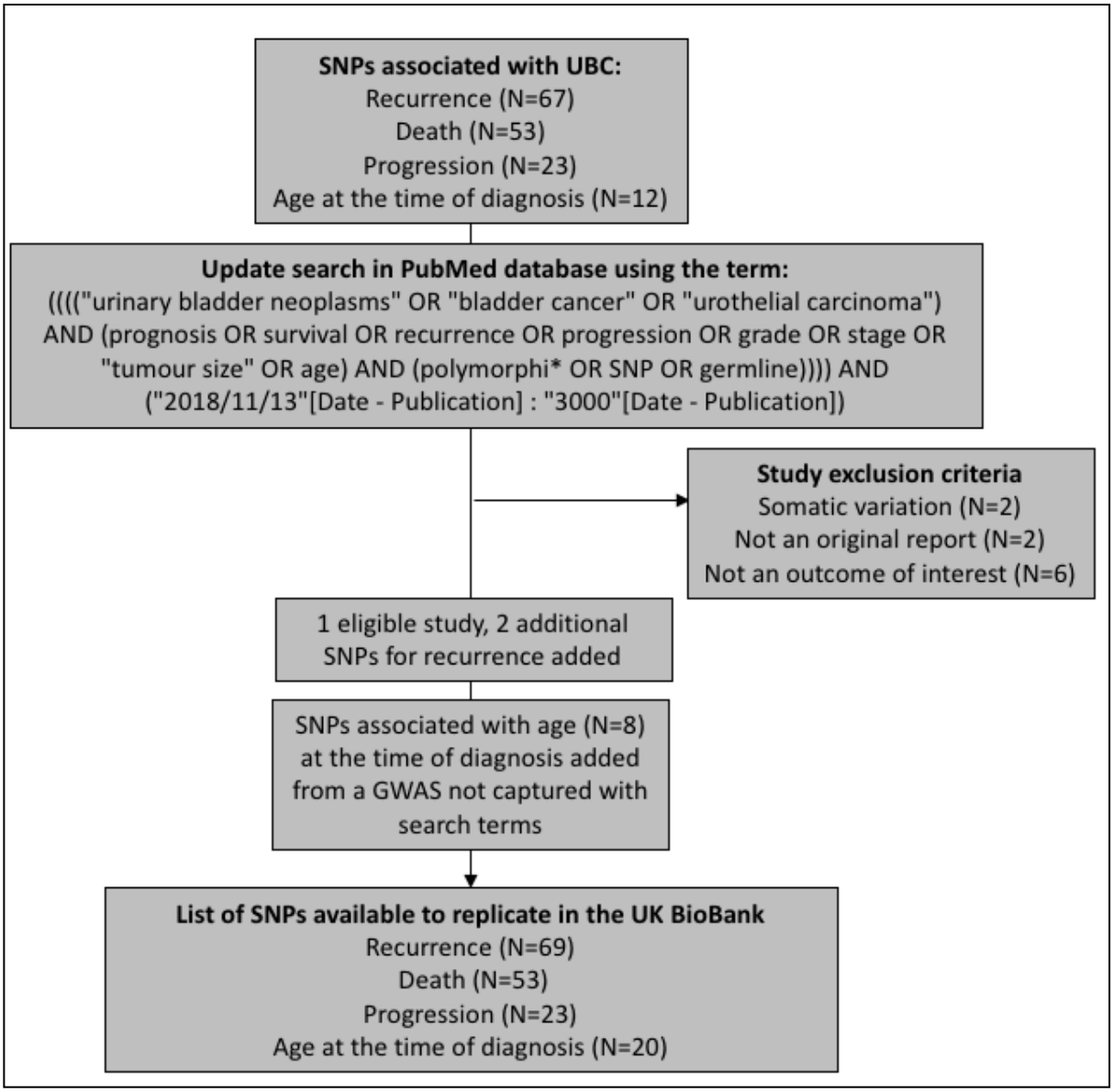


Figure 6.1. Selection process of the SNPs used in replication analyses. GWAS-genome-wide association study; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer.

Study population

UK Biobank is a population-based cohort in the UK, having collected genetic and clinical data on over 500,000 participants, aged 40-69 at the time of recruitment in 2006-2010. The design, data collection and processing are described in detail elsewhere [7, 10].

Our analysis was restricted to UBC patients (corresponding International Classification of Diseases (ICD) codes of C67.0, C67.1, C67.2, C67.3, C67.4, C67.5, C67.6, C67.7, C67.8, C67.9, D09.0 (ICD10) and 1880, 1882, 1884, 1886, 1888, 1889, 2337 (ICD9). To prevent bias from analysing heterogeneous molecular UBC subtypes, histology was limited to the following ICD-O (ICD Oncology) codes: 8000 (Neoplasm), 8001 (Tumour cells), 8010 (Carcinoma), 8020 (Carcinoma, undifferentiated), 8050 (Papillary carcinoma), 8120 (Transitional cell carcinoma), and 8130 (Papillary transitional cell carcinoma).

In total, there were 1,534 UBC patients with clinical and genetic data available for analysis.

Outcomes

Age

Age at the time of diagnosis was modelled both as a continuous and categorical variable.

To replicate previous associations as accurately as possible, we have dichotomised age variables using the cut-off points reported in the original research articles (≥/< 50, 55, 60, 65, and 70 years, Supplementary Table 6.3).

Death

Death was modelled as an overall (death vs no death) or a UBC-specific event (death vs no death, when primary cause of death was assigned C67- (ICD10) or 188-related (ICD9) codes).

Recurrence

The events of bladder recurrence and progression are not part of the routinely collected data in the Cancer Registry, or other national/regional datasets. However,

the HES in the UK Biobank make it possible to identify a fraction of these events using proxy data.

HES contains admitted in-patient data starting with 1997 [11] and includes data on patients both under national health service (NHS) and private care. HES data is provided to the UK Biobank on an annual basis, covering the past financial year (starting 1st April of each year). In our analyses, the follow-up covers all in-hospital interventions registered until March 31st, 2017. Operative procedures use OPCS4 (Office of Population, Censuses and Surveys: Classification of Interventions and Procedures, Version 4) coding system.

For recurrence, we have considered three conditions to be representative of an event (**Figure 2**). First, a transurethral resection of a bladder tumour (TURBT) (OPCS4 code M42) is regarded to be enough to signify a UBC event. Secondly, a time gap of longer than 4 months between chemotherapeutic treatments into urinary bladder (OPCS4 codes M494/M495) was considered to be substantial to correspond to two different events. Thirdly, we have assumed a UBC diagnosis if an examination of the urinary bladder (OPCS4 code M45) was led by an intervention within 6 months. Relevant interventions were chemotherapeutic treatments into urinary bladder, cystectomy, radiotherapy, and chemotherapy (corresponding to OPCS4 codes of M494/M495; M34; X65; X72; X292, X298, X308, X352, respectively). Currently presented list of chemotherapy-related OPCS4 is not exhaustive, but rather based on interventions observed in our data. Further development of the algorithm is likely to adjust the list as needed.

Progression

In our framework, all events of progression are recurrences by default. However, we have considered adding additional criteria would allow distinguishing which

recurrences were also representative of UBC progression. We have considered an event of UBC progression to have taken place if either a TURBT (OPCS4 code M42) or examination of the urinary bladder (OPCS4 code M45) was followed by interventions of cystectomy (OPCS4 code M34) and/or radiotherapy (OPCS4 code X65) within 6 months (**Figure 6.2**).

To prevent registration duplicates, two recurrence and/or progression events were considered independent of one another if time in between the records was greater than 3 months.

Invasiveness at the time of diagnosis

Finally, UBC clinical management is heavily dependent on its' invasiveness at the initial diagnosis. A UBC diagnosis that was followed by either cystectomy or radiotherapy was considered to represent a muscle-invasive bladder cancer (MIBC), whilst the remaining diagnoses are held to be non-muscle-invasive bladder cancer (NMIBC) cases (**Figure 6.2**).

Ethics and consent

All UK Biobank participants have provided informed consent. Current research has been conducted using the UK Biobank Resource under Application Number 42772.

Genotype data quality control (QC) and imputation

Detailed procedures on QC and imputation in the UK Biobank are described elsewhere [10]. To verify the high quality of all tested SNPs, we have extracted imputation accuracy measures (INFO scores) and MAF (minor allele frequencies) (**Supplementary Table 6.5**). To avoid population stratification bias, we have restricted

our sample to a homogenous group of White British participants, as previously identified by the UK Biobank team [10].

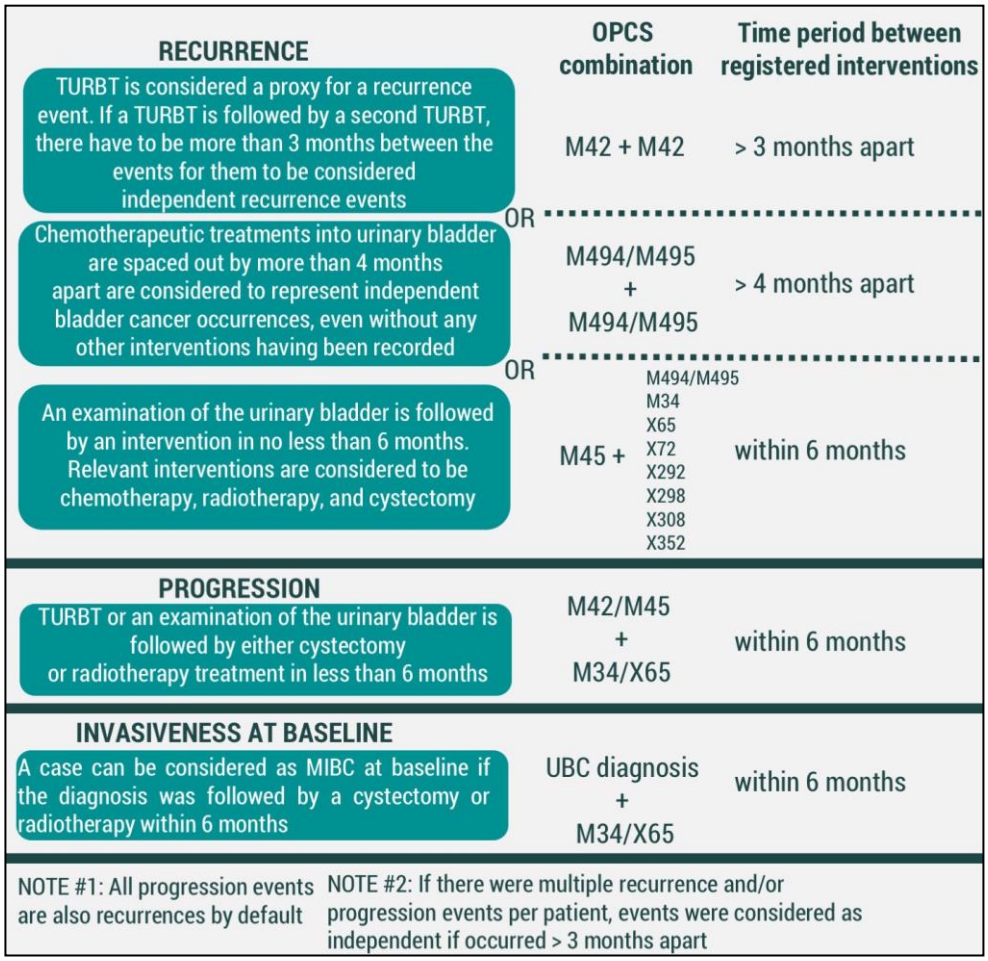


Figure 6.2. Conditions for modelled events of UBC recurrence, progression, and invasiveness at baseline (MIBC-muscle-invasive bladder cancer, TURBT transurethral resection of bladder tumour). All codes correspond to OPCS4 classification.

Statistical analysis

To test for an association between selected SNPs and UBC recurrence, progression, death, and age, we have utilized SNPTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html). To estimate Linkage disequilibrium (LD), an online tool was used (https://ldlink.nci.nih.gov/). LD defines the correlation between alleles in a given population. Due to some SNPs being in high LD, it might be difficult to establish which allele is representing the cause, as

they are often inherited together. At the same time, linkage equilibrium suggests alleles are inherited independent of one another. Logistic regression using allele dosages was applied to estimate odds ratios (OR) and corresponding confidence intervals (CI) for death, recurrence, progression, and categorical age events; whilst linear regression was used to estimate the effect of age as a continuous variable. All associations were tested under additive model of inheritance and adjusted for participant sex. To reduce multiple testing, analyses were ran for the outcome that resembled the originally-reported association most closely (e.g. if a variant has been associated with NMIBC recurrence, we have only tested NMIBC patients instead of the whole UBC sample). To better estimate the strength of evidence for replication results, we additionally included calculation of the Bayes Factor (BF). In simple terms, BF can be considered as a ratio of probabilities for two competing hypotheses (for example, the probability of a SNP being associated with an outcome *versus* the SNP not influencing the outcome). The ratio provides an estimate that shows the extent on of one hypothesis being more (or less) likely than the alternative one. In contrast, the generically-used frequentist approach (resulting in a p value) evaluates the probability of data under a specific hypothesis, which alone does not provide indication of the association strength.

Variants in the replication were considered promising if the nominal statistical significance (p value) has reached <0.05. Bonferroni adjustment per each outcome for multiple comparisons resulted in statistical significance level (p value) to be 0.0007 for recurrence (α=0.05/69), 0.002 for progression (α=0.05/23), 0.0009 for survival (α= 0.05/53), and 0.0025 for age (α =0.05/20).

RESULTS

In total, 1,534 UBC patients were available for replication analyses of prognostic events (**Table 6.1**).

Mean age of UBC patients was 61 years, and most were males (78%). Using our algorithm on HES data, we could identify UBC invasiveness at baseline, recurrent, and progressive events for UBC patients in the UK Biobank cohort. Majority of UBC cases were NMIBC (93%). Death was recorded for 209 (13.6%) patients, out of which 106 were UBC-specific. In addition, we estimate 618 patients (40%) have experienced a recurrence, and 58 (3.8%) have had a UBC progression.

Table 6.1. Descriptive characteristics of the UBC patients in the UK Biobank.

	N	**p value***
Sex	**1534**	**<0.001**
Males (%)	1197 (78.0)	
Females (%)	337 (22.0)	
Age (Mean (SD))	61.3 (9.0)	
Death	**1534**	**<0.001**
No (%)	1325 (86.4)	
Yes (%)	209 (13.6)	
UBC-specific death	**1534**	**<0.001**
No (%)	1428 (93.1)	
Yes (%)	106 (6.9)	
Recurrence	**1534**	**<0.001**
No (%)	916 (59.7)	
Yes (%)	618 (40.3)	
Progression	**1534**	**<0.001**
No (%)	1476 (96.2)	
Yes (%)	58 (3.8)	
NMIBC at baseline	**1534**	**<0.001**
No (%)	114 (7.4)	
Yes (%)	1420 (92.6)	

*Chi-square test for group independence.
NMIBC-non-muscle-invasive bladder cancer; SD-standard deviation; UBC-urinary bladder cancer.

In the replication analyses, eight SNPs have reached a p-value of <0.05 (**Table 6.2**). However, none of the variants remained significant after applying Bonferroni-corrections for multiple comparisons (corrected for each tested outcome).

Recurrence

Four of these SNPs were associated with bladder cancer recurrence. Rs2042329 (*CWC27*) was linked to an increased risk of UBC recurrence (OR=1.26, 95% CI: 1.10; 1.48); whilst rs804256, rs4639, and rs804276, all located in/close to *NEIL2* were associated with NMIBC-only recurrence (OR=1.23, 95% CI: 1.05-1.43; OR=1.20, 95%CI: 1.03-1.39; OR=1.17, 95% CI: 1.01-1.36, respectively). All SNPs that were associated with recurrence showed consistent direction but were universally more modest in comparison to the original studies (HR=1.54 (1.10-2.16) for rs2042329 [12], HR=4.58 (2.61-8.02) for rs804256 [13], HR= 2.60 (1.68-4.03) for rs4639[13], and HR= 2.71 (1.75-4.20) for rs804276 [13]).

Although SNPs rs804256, rs4639, and rs804276 all map to the same locus, LD values imply they are independent results (R^2 for rs804276 and rs804256 =0.09; R^2 for rs804276 and rs4639 =0.43; R^2 for rs4639 and rs804256=0.38).

Death

Three SNPs (rs2344673 (*RGS5)*, rs3756712 (*PDCD6)*, and rs2293347 (*EGFR*)) were associated with events of bladder cancer death, albeit in different subgroups. Rs2293347 (*EGFR*) was associated with lower death rates among all UBC patients (OR=0.69, 95% CI: 0.47-0.99), rs3756712 (*PDCD6)* was significant for NMIBC patients (OR=1.29, 95% CI: 1.02-1.63), and rs2344673 (*RGS5)* showed reduced rate of death among MIBC cases (OR=0.22, 95% CI: 0.05-0.98).

In comparison to the original study, replicated SNPs in PDCD6 showed effect in the same direction, but had a reduced estimate (HR=5.11 (1.43-18.22) [14].

However, inconsistency in direction of the effect was observed for SNPs in EGFR and RGS5 (HR=1.5 (1.0-2.3) for rs2293347 [15] and HR=1.55 (1.15-2.11) for rs2344673 [16].

Progression

Carrying a minor allele of rs2297518 in *NOS2* corresponded to a lower chance of UBC progression (OR=0.56, 95% CI: 0.32-0.99). In the original study, rs2297518 was also associated with a lower risk of progression (HR=0.21 (0.05-0.87) [17]**.**

Bayes factor was highest for the variant associated with NMIBC recurrence in *CWC27*, reaching log10(BF)=1.56. For all remaining SNPs, Bayes statistic indicates replication sample was low-powered [18], with log10(BF) ranging between 0 and 1.

DISCUSSION

In the current study, we describe an external replication of previously reported genetic associations for UBC recurrence, progression, death, and age at the time of diagnosis using HES data available the UK Biobank.

The aim of our study is twofold. Firstly, mining routinely-collected data for identifying complex phenotypes is inevitable to become a common practice. In the light of current needs, we propose an algorithm that identifies UBC recurrences and progression events via recorded interventions in a hospital setting.

Table 6.2. Replication results that have reached nominal significance (p<0.05) in the UK Biobank cohort.

Outcome	rsID	Locus	REF	EFF	info value	MAF, % (all)	N(total) (AA/AB/BB)	MAF, % (cases)	N (cases) (AA/AB/BB)	MAF, % (controls)	N (controls) (AA/AB/BB)	OR	(95% CI)	P-value	log10(BF)	Annotated Gene
UBC Recurrence	rs2042329	5q12.3	T	G	1	41	1534 (255/739/540)	37	618 (88/284/246)	43	916 (167/455/294)	1.26	(1.10; 1.48)	0.001	1.56	CWC27
NMIBC Recurrence	rs804256	8p23.1	T	C	0.99	34	1420 (634.7/604.96/180.4)	37	607 (254.1/261.2/91.7	32	813 (380.6/343.8/88.6)	1.23	(1.05; 1.43)	0.012	0.74	NEIL2
MIBC Overall Survival	rs2344673	1q23.3	G	A	1	12	123 (94/29/0)	4	26 (24/2/0)	14	97 (70/27/0)	0.22	(0.05; 0.98)	0.019	0.10	RGS5
NMIBC Overall Survival	rs3756712	5p15.33	A	C	0.99	38	1420 (200.4/667.6/552.0)	33	184 (16.9/85.9/81.2)	38	1236 (183.5/581.7/470.8)	1.29	(1.02; 1.63)	0.03	0.49	PDCD6
NMIBC Recurrence	rs4639	8p23.1	A	G	0.99	43	1420 (472.7/672.9/274.4)	46	607 (182.2/297.0/127.8)	41	813 (290.5/375.8/146.6)	1.20	(1.03; 1.39)	0.02	0.56	NEIL2
NMIBC Recurrence	rs804276	8p23.1	G	A	0.99	41	1420 (496.9/670.1/253.0)	44	607 (200.0/284.5/122.5)	40	813 (296.9/385.6/130.5)	1.17	(1.01; 1.36)	0.04	0.34	-
UBC Overall Survival	rs2293347	7p11.2	C	T	0.99	11	1534 (1215.6/303.5/14.9)	8	209 (176.4/31.6/1.0)	11	1325 (1039.2/271.9/13.9	0.69	(0.47; 0.99)	0.04	0.35	EGFR
UBC Progression	rs2297518	17q11.2	G	A	1	19	1534 (999/475/60)	12	58 (45/12/1)	20	1476 (954/463/59)	0.56	(0.32; 0.99)	0.03	0.26	NOS2

BF-Bayes' factor; CI-confidence interval; EFF-effect allele; MAF-minor allele frequency NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; REF-reference allele; UBC-urinary bladder cancer.

Current approach uses OPCS4 classification system, but we are confident applied assumptions can be translated to other globally-used systems (e.g. International Classification of Health Interventions, ICHI). We acknowledge identified prognostic events make up only a fraction of the true event volume, and are likely to be an underestimate. The extent of the underestimation requires testing the algorithm in an external cohort and is a necessary subsequent step of refining the currently-described approach. The level of underestimation is likely to vary for differed outcomes, as some events are arguably easier to identify (e.g. recurrence), whilst progression requires more detailed data and is subject to a higher level of underrepresentation. However, we saw an overestimation resulting in a greater rate of error and data misrepresentation. Moreover, inclusion of other clinically-relevant characteristics (tumour stage, grade) would increase the accuracy of modelled prognostic events. The provisioned release of such data in the UK Biobank (https://biobank.ctsu.ox.ac.uk/crystal/exinfo.cgi?src=future_timelines) will provide further opportunities of updating the algorithm. Naturally, our proposed approach and used assumptions are subjective by nature and we encourage the expert field to contribute ideas to make the assumptions more accurate.

Secondly, an external replication of genetic associations is a rare endeavour. Unfortunately, as simply put by Kraft et al. [5], "*Genetic epidemiology learned the importance of replication the hard way*". External validation studies perform at much lower rates, which underscores the significance of such efforts [6]. Most genetic studies are still exploratory in nature, and false-positive results are inevitable. By prioritising evidence-based targets, more resources can be allocated towards investigating variants with better promise of true impact on human health.

For UBC recurrence, the strongest result was mapped to *CWC27*. Previous study reported rs2042329 to correspond to higher expression of *CWC27* in bladder cancer cells [12]. Additional functional analyses showed *CWC27* might affect bladder carcinogenesis via apoptosis. Interestingly, the original finding was made for Chinese patients, and authors failed to replicate the significance of rs2042329 on bladder cancer risk among Europeans [12]. However, it is unknown if the lack of effect was also present for recurrence.

Additionally, it is surprising to see three SNPs in *NEIL2* being significant for NMIBC recurrence, especially keeping in mind the low likelihood of successful replication. Despite the high number of SNPs, strength of evidence for these associations is low, as reflected in Bayes Factor. Nonetheless, they might be promising targets in future replications. *NEIL2* is involved in DNA repair mechanisms, and research suggest it influences malignancies beyond bladder cancer. Alterations in normal *NEIL2* activity most likely result in accumulated oxidative damage, as elegantly presented by Benitez-Buelga et al. [19].

For UBC progression, the replicated variant maps to *NOS2*. The gene has been specifically linked to progression of various cancers [20, 21]. It seems *NOS2* affects multiple oncogenic pathways, that simultaneously affect tumour proliferation, angiogenesis, chemoresistance, and cell migration [20, 21].

As for UBC survival, three replicated SNPs are located in *RGS5*, *PDCD6*, and *EGFR*. Interestingly, a previous independent replication of SNPs associated with UBC prognosis has also successfully validated a variant in *RGS5* (rs12035879) for overall survival (OS) of MIBC cases [22]. Comparison of two external replications offers potential insights – for example, the rs11585883 did not replicate in our study; however, another SNP in *RGS5* was successful, and associated with the same

outcome (MIBC OS). These findings may be seen as cumulative towards the involvement of *RGS5* in cancer survival, even if specific SNPs are yet to be identified. We have checked if previously and current replicated *RGS5* SNPs are in LD, and they seem to represent independent signals in the gene (R^2=0.03 for rs12035879 and rs2344673 among Europeans). One major weakness of the replicated rs2344673 in our study is small sample size (29 cases and 109 controls). A post-hoc analysis on the overall survival of the whole sample, regardless of UBC invasiveness (209 cases and 1,325 controls) was not significant. *RGS5* may not be relevant for all UBC patients, or might reflect power issues, which highlights further investigation being essential.

Remaining two genes implicated in UBC and NMIBC survival, namely *EGFR* and *PDCD6,* are both well-known cancer genes [14, 23]. *PDCD6* seems to be heavily involved in apoptosis [14]; however, the exact role of *PDCD6* is contrasting between various cancers [24], and further molecular research will help making evidence-based interpretations.

A replicated SNP (rs2293347) in *EGFR* has also previously corresponded to a protective effect on survival of lung cancer patients [25]. The effect may be due to higher responsiveness to chemotherapy [26], which is a worthwhile investigation in future analyses.

Or study is subject to limitations, with one of the largest drawbacks being the difference between founders' and replication cohorts. A lot of studies have investigated populations of non-European ancestry, and it is possible we are not able to observe a true effect due to differences in LD of candidate SNPs in different samples. At the same time, the most reliable replication in our study was rs2042329, first reported in a Chinese population [12].

None of our replicated SNPs have passed the Bonferroni-corrected statistical significance level, suggesting some promising SNPs may have been identified by chance.

Furthermore, current analyses have only focused on estimating the overall risk of a prognostic event, without considering the relevance of elapsed time to event. We see such and other more sophisticated analyses as a further direction in utilising the described approach.

We were also unable to reliably estimate assigned treatment for UBC patients in the UK Biobank cohort, which would unquestionably confer to a more precise replication analysis. However, as the detail of released HES is increasing, we do not see this data out of reach and likely to include in future algorithm updates.

Finally, some replicated SNPs showed conflicting direction of the effect when compared to the original studies. These issues are likely to be clarified once more studies are ran to first of all, confirm the overall association and, secondly, to establish the effect.

To summarise, we have carried out an external replication of previously reported SNPs for UBC recurrence, progression, death and age using a novel approach of identifying clinically-relevant outcomes using HES data. Our analysis suggests specific targets, namely *CWC27*, *NEIL2*, *PDCD6*, *EGFR*, and *NOS2*, might be prioritised in efforts to further study the role of genetics in UBC prognosis. We are cautious about our findings, as there is no one metric or design to provide unquestionable evidence; instead, it should be viewed as one of the studies in a long line of accelerating research on UBC.

Acknowledgments: This research has been conducted using the UK Biobank Resource under Application Number 42772.

REFERENCES

[1] Park JC, Hahn NM. Bladder Cancer: A Disease Ripe for Major Advances. Clinical advances in hematology & oncology : H&O. 2014;12(12):838-45.
[2] Berdik C. Unlocking Bladder Cancer. Nature. 2017;551(7679):S34-s5.
[3] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.
[4] Lipunova N, Wesselius A, Cheng KK, Schooten FJv, Cazier J-B, Bryan RT, Zeegers MP. Genetic Associations for Prognostic Factors of Urinary Bladder Cancer: A Systematic Review (Article in Submission). 2019.
[5] Kraft P, Zeggini E, Ioannidis JP. Replication in Genome-Wide Association Studies. Statistical science : a review journal of the Institute of Mathematical Statistics. 2009;24(4):561-73.
[6] Palmer C, Pe'er I. Statistical Correction of the Winner's Curse Explains Replication Variability in Quantitative Trait Genome-Wide Association Studies. PLoS genetics. 2017;13(7):e1006916.
[7] Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. Uk Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS medicine. 2015;12(3):e1001779.
[8] Deng S, He SY, Zhao P, Zhang P. The Role of Oncostatin M Receptor Gene Polymorphisms in Bladder Cancer. World journal of surgical oncology. 2019;17(1):30.
[9] Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Galesloot TE, Kiemeney L, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer. Eur Urol Oncol. 2019;2(4):381-9.
[10] Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The Uk Biobank Resource with Deep Phenotyping and Genomic Data. Nature. 2018;562(7726):203-9.
[11] Uk Biobank. Hospital Episode Statistics Data in Showcase, Version 1.0 2013 [Available from: http://biobank.ctsu.ox.ac.uk/showcase/docs/HospitalEpisodeStatistics.pdf
[12] Wang M, Li Z, Chu H, Lv Q, Ye D, Ding Q, Xu C, Guo J, Du M, Chen J, Song Z, Yin C, Qin C, Gu C, Zhu Y, Xia G, Liu F, Zhang Z, Yuan L, Fu G, Hu Z, Tong N, Shen J, Liu K, Sun J, Sun Y, Li J, Li X, Shen H, Xu J, Shi Y, Zhang Z. Genome-Wide Association Study of Bladder Cancer in a Chinese Cohort Reveals a New Susceptibility Locus at 5q12.3. Cancer research. 2016;76(11):3277-84.
[13] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.
[14] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.

[15] Mason RA, Morlock EV, Karagas MR, Kelsey KT, Marsit CJ, Schned AR, Andrew AS. Egfr Pathway Polymorphisms and Bladder Cancer Susceptibility and Prognosis. Carcinogenesis. 2009;30(7):1155-60.
[16] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.
[17] Ryk C, Wiklund NP, Nyberg T, De Verdier PJ. Ser608leu Polymorphisms in the Nitric Oxide Synthase-2 Gene May Influence Urinary Bladder Cancer Pathogenesis. Scandinavian journal of urology and nephrology. 2011;45(5):319-25.
[18] Sawcer S. Bayes Factors in Complex Genetics. European journal of human genetics : EJHG. 2010;18(7):746-50.
[19] Benítez-Buelga C, Baquero JM, Vaclova T, Fernández V, Martín P, Inglada-Perez L, Urioste M, Osorio A, Benítez J. Genetic Variation in the Neil2 DNA Glycosylase Gene Is Associated with Oxidative DNA Damage in Brca2 Mutation Carriers. Oncotarget. 2017;8(70):114626-36.
[20] Thomas DD, Wink DA. Nos2 as an Emergent Player in Progression of Cancer. Antioxidants & redox signaling. 2017;26(17):963-5.
[21] Palumbo P, Lombardi F, Siragusa G, Dehcordi SR, Luzzi S, Cimini A, Cifone MG, Cinque B. Involvement of Nos2 Activity on Human Glioma Cell Growth, Clonogenic Potential, and Neurosphere Generation. International journal of molecular sciences. 2018;19(9).
[22] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.
[23] Sigismund S, Avanzato D, Lanzetti L. Emerging Functions of the Egfr in Cancer. Molecular oncology. 2018;12(1):3-20.
[24] Hashemi M, Bahari G, Markowski J, Małecki A, Łos MJ, Ghavami S. Association of Pdcd6 Polymorphisms with the Risk of Cancer: Evidence from a Meta-Analysis. Oncotarget. 2018;9(37):24857-68.
[25] Winther-Larsen A, Fynboe Ebert EB, Meldgaard P, Sorensen BS. Egfr Gene Polymorphism Predicts Improved Outcome in Patients with Egfr Mutation-Positive Non-Small Cell Lung Cancer Treated with Erlotinib. Clinical lung cancer. 2019.
[26] Takahashi H, Kaniwa N, Saito Y, Sai K, Hamaguchi T, Shirao K, Shimada Y, Matsumura Y, Ohtsu A, Yoshino T, Doi T, Takahashi A, Odaka Y, Okuyama M, Sawada J, Sakamoto H, Yoshida T. Construction of Possible Integrated Predictive Index Based on Egfr and Anxa3 Polymorphisms for Chemotherapy Response in Fluoropyrimidine-Treated Japanese Gastric Cancer Patients Using a Bioinformatic Method. BMC cancer. 2015;15:718

CHAPTER 7.

GENERAL DISCUSSION

The current thesis intended to investigate the possibility of established factors contributing to the prognosis in non-muscle-invasive bladder cancer (NMIBC). Broadly, the thesis is split into two parts; firstly, part one focuses on discovering loci that may be associated with NMIBC baseline characteristics, as well investigating if any of the discovered single nucleotide polymorphisms (SNPs) are implicated in a phenomenon of gene-environment (GxE) interaction with smoking. The second part of the thesis describes the attempted replication of both – findings of our own and those published previously.

SUMMARY OF THE MAIN FINDINGS

In **Chapter 3**, we have attempted to review all previous evidence on genetic polymorphisms and urinary bladder cancer (UBC) prognostic measures. In total, 112 studies were summarised that reported 316 SNPs. Our review considered various outcomes, namely UBC recurrence, progression, and death, as well as key UBC characteristics of tumour stage, grade, size, risk group, and patients' age. We have discovered extensive heterogeneity in the literature with regard to practiced methodologies and principal findings. There were few genes (*OGG1*, *TP53*, and *MDM2*) that were commonly reported for most outcomes, which may indicate overarching pathogenic pathways. However, the remaining substantial differences in reported associations across endpoints suggest that different prognostic outcomes (recurrence, progression, death) may have different mechanisms and represent distinct phenotypes.

Chapter 4 describes explorations of new genetic variants that may influence specific characteristics of NMIBC patients and their tumours at the time of diagnosis. A genome-wide association study (GWAS) on 653 patients in the West Midlands

Bladder Cancer Prognosis Programme (BCPP) yielded 61 statistically significant SNPs for the characteristics of tumour size, stage, grade, and patient's age. A replication study in the Nijmegen Bladder Cancer Study (NBCS, [1]) suggested that most of these associations might be spurious.

Out of all significant hits in our discovery GWAS, ten have also demonstrated GxE interactions with smoking behaviour and tumour size in the BCCP sample (**Chapter 5**). **Chapter 5** additionally reports on an attempt to replicate variants showing the GxE interaction in an independent NMIBC cohort (NBCS), albeit with no success. However, there are important limitations in the replication analysis that suggest an equivocal rather than a conclusive result.

Chapter 6 continues to address the reproducibility issue in the field of UBC genetics, using UK Biobank [2] as the main data source. In this chapter, we present a newly-developed algorithm for inferring UBC prognostic outcomes from hospital episode statistics (HES), Cancer and Death Registries, alongside demographic data. Subsequently, we tested all previously reported genetic loci for UBC recurrence, progression, and death (identified in **Chapter 3**) for their significance in a newly-developed UBC patient cohort in the UK Biobank. Out of 165 SNPs, eight have reached nominal statistical significance ($p<0.05$) for various outcomes.

DISCOVERY

In the first part of the discovery phase, we performed a GWAS to look for SNPs that might be associated with tumour stage, grade, size, risk group, and patient's age among NMIBC cases. A total of 653 patients were available for the analysis, resulting in 61 loci reaching a statistical significance level of $p<5E-08$ [3]. Most of the SNPs (N=47) were associated with tumour size, whilst the rest were scattered across the

outcomes of age at diagnosis, tumour grade, and stage. NMIBC risk-group, however, did not yield any associations [3].

Most of our discovered loci have not been previously reported in the context of UBC and we did not replicate any previous associations [4]. This finding alone cannot be held as an argument for their validity or lack thereof, but it does prompt some considerations, presented below.

Many of the previous genetic analyses in UBC have targeted specific candidates [4, 5], and were not designed to test beyond a specific hypothesis. Previously investigated hypotheses are the product of a variety of rationales: evidence for involvement in UBC whilst implying a specific pathway (e.g. *NAT2* [6-9]), genes of general interest (e.g. *TP53* [5, 10-15]), or potential significance of the locus to a wide range of phenotypes (e.g. rs1052133/Ser326Cys variant in *OGG1* [16-22]). Regardless of the exact reasoning, all other genetic variation in a candidate-gene (CG) design remains *terra incognita* and can only be addressed in either an almost-infinite amount of CG studies, or in an agnostic design of GWAS [23]. Since the number of GWASs investigating specific UBC characteristics and subsequent prognosis is severely lacking [3, 24, 25], and most information comes from CG studies [4], they cannot be held as a single reference point. Moreover, the studied populations differ, as do exact methodologies and endpoints [4]. Given these considerations, explorations of the genetics of UBC are still in their infancy, where emphasis should be placed on collecting as much evidence as possible, instead of providing conclusions.

In addition, GWASs have a well-recognised issue of multiple hypothesis testing [26-28] which warrants applying stringent thresholds for defining statistical significance. The commonly-used p value of <5E-08 is usually too low to validate any associations

from CG studies, as they often regard the p value of <0.05 being sufficient for statistical significance [29, 30].

Importantly, our study addressed phenotypes that have rarely been targeted directly. Firstly, we limited our research to NMIBC, instead of a merged group of UBC. A mounting evidence shows NMIBC and MIBC are heterogeneous in their development [31, 32] and optimally require separate investigations.

Moreover, we shifted the focus from overarching and potentially nebulous outcomes of cancer susceptibility and prognosis to specific characteristics at the time of NMIBC diagnosis. Identifying genetic associations for baseline characteristics might reveal subtle differences among NMIBC patients and point to potential pathological mechanisms. It is known that NMIBC prognosis is dependent on many factors which, when combined, define a risk category [33, 34] (**Figure 1.4**). In practice, baseline NMIBC characteristics are known to differ in their relative weight; for example, high grade is more predictive for NMIBC prognosis than tumour stage [33] (**Table 1.2**). Our analyses point to an abundance of potential associations with NMIBC tumour size at the time of diagnosis. If continuously replicated, these associations may be used in clinical practice to provide a better understanding of individual course of disease; for example, patients who are at a higher risk of developing large tumours may require a more intensive monitoring schedule even if the tumour at diagnosis is of low grade and/or stage.

As such, when broad outcomes of recurrence and/or progression are studied, the independent roles of each characteristic might be overlooked. Consequently, it remains unknown whether a genetic locus contributes to the prognosis overall or, rather, is associated with a specific characteristic that is predictive of disease course. These considerations do not encourage abandoning the phenotypes of UBC

recurrence and progression, as they are important clinical outcomes for NMIBC patients [33]. However, other constituents of these outcomes should also be investigated to gather more knowledge on the genetic landscape of bladder cancer prognosis.

Taking account of the above differences between this and previous studies in terms of design and outcomes, it is not surprising that our analysis yielded novel associations.

Nonetheless, a few SNPs associated with NMIBC stage (Tis+T1 vs Ta) have been mapped to the *SLCO1B1* gene, located on 12p12.1 [35]. *SLCO1B1* has already been reported to increase UBC susceptibility in a Japanese population [36]. The SNP reported before (rs2306283) and those observed in our study are in weak LD (R^2=0.003 for rs76497895 and rs116946525 in a global population), showing that they are most likely independent associations in a shared gene. *SLCO1B1* codes a membrane transporter protein in hepatic cells, responsible for the clearance of multiple chemical compounds [37-40]. These proteins are involved in detoxification of various substances [37], and potentially includes carcinogens introduced by tobacco smoking [41]. Malfunctions in smoking-related metabolite clearance due to genetic variation have long been implicated to have a role in NMIBC development [7], with our study contributing additional findings in the same direction.

Importantly, one study, performed by Bui et al. [36], showed a variant in *SLCO1B1* increased the risk of NMIBC only, without an association for MIBC. It is known that the UBC patient population largely comprises of NMIBC (75-80% [33, 42, 43]), and those mostly develop via the papillary pathway [31, 32]. The papillary pathway is linked with a better disease course [44]; however, some papillary cancers are genomically unstable and can develop into invasive cancers (**Figure 1.1**) [32, 45]. The two studies

may complement each other in suggesting that *SLCO1B1* has implications for NMIBC. Whereas Bui *et al*. [36] show *SLCO1B1* is associated with the development of NMIBC overall, our study potentially provides an in-depth investigation into the relevance of *SLCO1B1* for subgroups of NMIBC. Variations in *SLCO1B1* might pose a risk for NMIBCs that progress beyond the Ta stage and, hence, would require a more rapid intervention instead of a passive tumour surveillance. Importantly, our sample has merged Tis and Ta staged tumours due to low number is Tis (N=10) (Table 4.1), but given that large samples are available, Tis likely represents yet another entity in the context of bladder cancer. Such patterns could be particularly helpful in distinguishing between papillary NMIBCs with different progression risks at baseline and, therefore, may pose an attractive topic for future research, primarily to identify the role of specific SNPs and comparability of the findings across different populations.

Although other identified loci in our GWAS [3] have not been previously reported for UBC, they have nonetheless received attention in the context of other phenotypes (**Figure 7.1**) [46]. To date, there are a variety of traits associated with each gene, and the lack of any clear pattern is indicative of the highly pleiotropic nature of genetic variation [47].

With well-described evidence of UBC susceptibility loci being involved in a GxE interaction with smoking [48, 49], our investigations extended to testing our newly discovered loci for their involvement in GxE with tobacco use.

Analyses on all identified SNPs in the discovery stage identified ten variants that were also suggestive of an interaction with smoking status (defined as ever- or never-smokers). Importantly, we observed patterns for SNPs being significant either irrespective of tobacco use, or in smokers only (**Figure 5.2**). We considered that the most promising locus was in *PDE4B*, and hypothesize that it may affect NMIBC tumour

size via altering the levels of intracellular cAMP, whilst cAMP concentration is concurrently modified by tobacco exposure (**Figure 7.2**).

It is worth noting that *PDE4B* has been reported to be associated with smoking behaviour (amongst other genes, namely *SLCO1B1*, *GNG7*, *ADARB1*, and *CNTN4)*. (**Figure 7.1**), and thus presents an interesting target for further investigation of its role in bladder cancer, smoking, and both taken together.

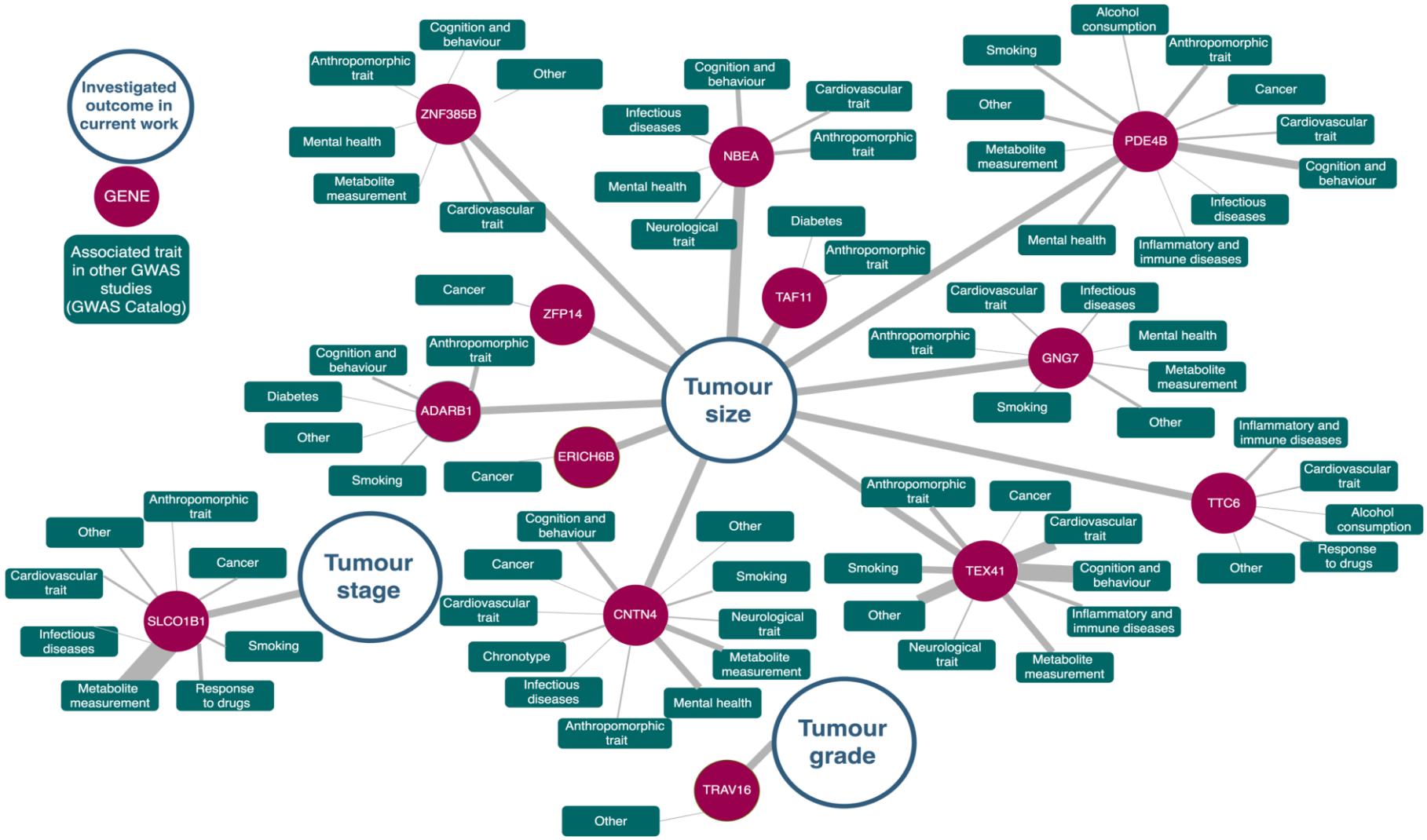


Figure 7.1. A map of genes identified in our study and previously associated phenotypes (GWASCatalog [46]).

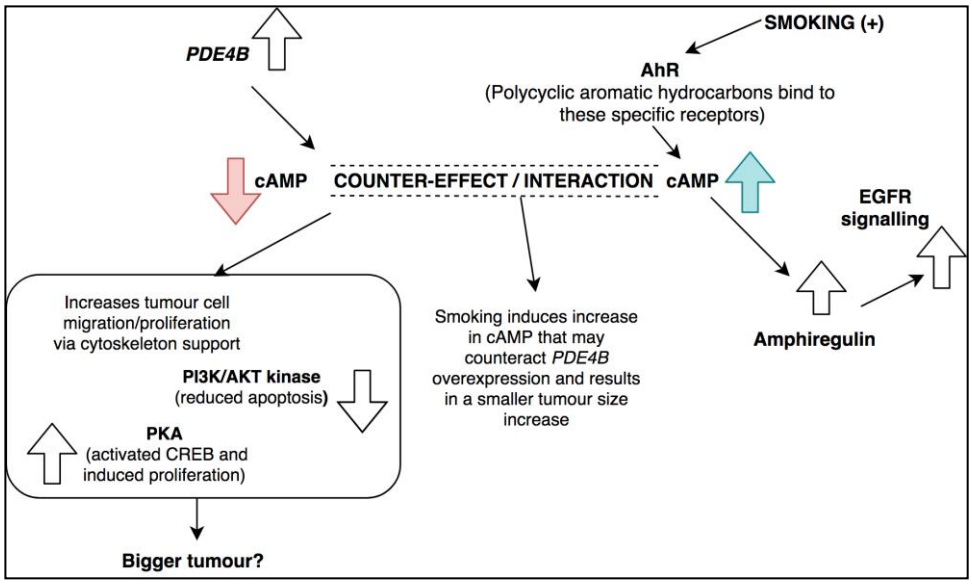


Figure 7.2. Proposed interaction mechanism between *PDE4B* and smoking on NMIBC tumour size (AhR – aryl hydrocarbon receptor; cAMP – cyclic adenosine monophosphate; CREB – cAMP response element binding protein; EGFR – epidermal growth factor receptor; PDE4B – phosphodiesterase 4B; PKA – protein kinase A).

Furthermore, it is intriguing not to see variants in *SLCO1B1* having significant interaction effects with smoking, especially due to the gene's proposed role in clearing carcinogens [36]. In fact, in our stratified analyses by smoking status, SNPs in *SLCO1B1* have only reached significance among smokers, but the interaction term itself was not significant. This discrepancy is likely due to the lack of power, and we would encourage other research groups to test variants in *SLCO1B1* for interaction with smoking in larger samples of NMIBC. There need for investigations into solute-carrier family genes is also strengthened by having another study reporting borderline significance for GxE interaction with smoking for a SNP in *SLC14A1* (p=0.053) [48].

Overall, the current thesis has explored the potential of genetic variation having an impact on NMIBC baseline characteristics, both independently and combined with an external exposure of smoking. These investigations have produced novel and intriguing findings. However, the use and true utility of our results is highly conditional on their validity and existing limitations. The remaining, and arguably the most

important part of the General Discussion focuses on various attempts of result replication and essential considerations when interpreting the current work.

REPLICATION, REPLICATION, REPLICATION

Our first attempt at replicating discovery results concerned the significant hits from our GWAS on NMIBC tumour size, stage, grade, and patent's age.

A sample of 1,470 bladder patients from the NBCS was available to use as an independent cohort. However, not a single discovery SNP was successfully replicated. One variant, namely rs180940944 in the *NBEA* gene, has reached statistical significance in the meta-analysis of the two cohorts; however, the effect is mostly driven by the BCPP samples. As a result, the lack of validation puts our initial results at a very high risk of falsity. Not surprisingly, the replication of SNPs significant for GxE interaction with smoking have also failed to produce promising results in the NBCS cohort.

The differences in tested variant significance between the two cohorts are unlikely to be due to ethnicity. All UBC samples are of European origin, thus the distribution of alleles is expected to have similar frequencies. In addition, NBCS contains a higher number of UBC patients, resulting in a higher statistical power, especially for testing only pre-selected candidate variants. The high comparability of phenotypes also prevents outcome misclassifications. Sources of data used to define endpoints in all cohorts are considered to be reliable (e.g. Cancer Registries, hospital records, or information gathered during cystoscopy, as in the case of tumour size). Naturally, all measurements are subject to error, but we would not expect the degree of error to differ between BCPP and NCBS.

However, validation analyses also have their own drawbacks that make the overall interpretation more complex. Firstly, we were unable to test a fraction of our discovered SNPs in the NBCS cohort, as only 50 variants were accessible in the NBCS cohort. Unfortunately, one of the most promising variant in our analyses (rs2937268, mapped to the *PDE4B*) was unavailable in the NBCS. Consequently, replication of its involvement in GxE interaction with smoking has also not taken place. Secondly, although NBCS in total consists of 1,470 participants, the sample has dropped to 302 cases for the outcome of tumour size. Of note, tumour size gathered an overwhelming majority of our significant associations (47 out of 61). Given that only 50 SNPs were available for replication overall, it resulted in virtually all of our variants (94%) being tested in a sample half the size of ours.

Regardless of the existing limitations of the replication analyses, the biggest problems with conducting GWAS have been well-described before, and apply extensively to our discovery analyses, as discussed below.

Firstly, the burden of testing multiple hypotheses in each GWAS can result in detecting significant results just by chance alone. Moreover, synthetic associations, defined as a link between non-causative SNP and the phenotype, may be a by-product of either a SNP being in linkage to a rare causative variant or an unmeasured source of error [30, 50]**.** Current empirical evidence suggests synthetic associations due to the causative SNP being rare may occur, but are unlikely to be the norm in genetic association studies [30]. To reduce the amount of unmeasured error in the current work, we have applied rigorous quality control (QC) procedures and adjusted the p value to detect only highly significant variants; however, the likelihood of false positives remains high [50].

Moreover, even considering that we have observed multiple significant hits, it is almost certain there are many more genetic variants that our study was severely underpowered to detect [30, 51, 52]. Penalising GWAS with very low significance threshold contributes to overlooking many variants of modest effect [30, 51], which has been described as one of the largest problem of GWAS, known as “missing heritability” [53].

In the GWAS field, no panacea to these problems has been found. However, there are two main options for reducing the impact of false-positives and low study power.

Firstly, increasing the sample size has been demonstrated as very effective for increasing the GWAS quality and result interpretation [30, 51, 52]. International consortia on various phenotypes, such as diabetes [54], schizophrenia [55], height and BMI [56] have validated multiple associations and substantially increased the explained proportion of heritability [52]. However, dramatic increases in sample sizes are not possible for all traits, including bladder cancer. The relatively low prevalence of UBC makes it virtually impossible to assemble cohorts exceeding a few thousand patients. Moreover, maintenance of such samples is financially challenging, as is measuring clinically-important bladder cancer traits may require very long-term follow-up and repeated access to medical records to establish phenotypic variation over time (i.e. recurrence, progression, and death) [57].

However, the increasing availability of large, deeply-phenotyped population-based cohorts may provide an unprecedented opportunity to boost UBC sample sizes without recruiting new patients. As a first known attempt, the current work provides an algorithm for inferring clinical phenotypes based on recorded interventions in the UK Biobank (**Chapter 7**). Merging multiple sources of data, namely Cancer and Death Registries, HES, clinical records, and genotyping, allows the formation of an entirely

new cohort of relevant outcomes, using only publicly-available population-level data. Our developed framework may be tested and applied by any research group, and is not limited to the OPCS4 (Office of Population, Censuses and Surveys: Classification of Interventions and Procedures, Version 4) coding system, as our defined rules can be translated to other intervention classification systems (e.g. International Classification of Health Interventions, ICHI). Naturally, our approach and algorithm assumptions must be calibrated according to further input by a wider net of professionals (both urologists and genetic epidemiologists), but it nonetheless marks the beginning of efforts to increase UBC sample sizes with meaningful phenotypes in observational research.

Aside from increasing sample sizes, a second option for reducing the limitations of multiple testing in genetic studies is to reduce the number of comparisons [51]. CG studies have been widely regarded as inferior to GWAS [58], but investigation of well-defined hypotheses cannot be undermined. For example, even though replication studies target only a subset of associations, they are of very high value for identifying the most–promising genetic loci [26]. Potentially, the lack of replication studies in genetic epidemiology is detrimental to setting appropriate guidance for future research. As most of the findings from the investigations of genetic factors for UBC prognosis have limited validation (**Chapter 3**), we have also addressed this issue by attempting to replicate all previously reported genetic loci for UBC prognosis in the UK Biobank. Eight SNPs have reached nominal statistical significance, located in *CWC27* (UBC recurrence), *NEIL2* (NMIBC recurrence), *EGFR* (UBC overall survival), *PDCD6* (NMIBC overall survival), *RGS5* (MIBC overall survival), and *NOS2* (UBC progression). However, as discussed in our own effort to replicate genetic associations, one external validation study cannot be seen as providing definitive

results. There are many differences between discovery and replication cohorts for most SNPs, especially in terms of ethnicity. In fact, ancestral divergence in discovery and replication samples has been recognised as one of the main culprits for failure to replicate SNP signals [26].

Our tested phenotypes were modelled as closely as possible to the originals, but complete identity is unlikely to have been achieved. Nonetheless, the most promising finding of a variant in *CWC27* could be a target of interest in future studies on UBC recurrence.

To summarise, replication of associations is an essential part of genetic epidemiology. These efforts are laborious and time-consuming, yet essential for prioritising loci of interest. The current dissertation contributes meaningful evidence to the understanding of genetic variation in bladder cancer. Arguably, another valuable contribution of this thesis is describing a first attempt for using large population-level datasets to advance bladder cancer research.

FUTURE DIRECTIONS

Based on the currently described work, it is inevitable that bladder cancer research will expand in its complexity, and there are several directions that can be expected within the field in the upcoming years.

Firstly, the era of genomics overlaps with the era of big data, where the need for merging multiple sources of information is well recognised and is in a constant process of implementation. As such, the potential for advanced analyses grows significantly. The use of conventional linear and logistic regression will likely be replaced by machine learning and artificial intelligence methods that are better equipped to handle large datasets [51]. Moreover, applying machine learning allows the data to “speak for

itself", as there are fewer assumptions imposed by the researcher. Importantly, most statistical testing will also shift from a purely frequentist approach to Bayesian, which accounts for all possible hypotheses in a given dataset. Optimally, both tests are used simultaneously to identify most-promising results. For example, Bayes factor statistic was calculated in **Chapter 6** for the validation of previously reported prognostic loci, and has provided meaningful information of the strength of each replicated SNP.

Secondly, increasing UBC sample sizes for discovery and replication analyses is of major importance. The collaborations between different research groups are understandably difficult, but the success stories of other phenotypes may prove inspirational for accumulating data to provide high quality investigation into bladder cancer genetics [30, 51].

Finally, with advances in statistical analyses and increasing sample sizes, the field will be able to move from only detecting SNPs of interest to analysing more complex notions: epistatic and gene-environment interactions, biological pathways and their utility in a clinical setting, the level of pleiotropy of discovered loci, as well as aspects of polygenicity of bladder cancer diagnosis and prognostic outcomes [30, 47, 51].

CONCLUSION

Genetic association studies for a complex and heterogeneous disease such as UBC have inherent limitations and demand careful interpretation. However, success likely follows resilient efforts, trial and error, as well as rumination. The current thesis should be viewed not as an isolated piece of work offering conclusive findings, but as a contribution into the field that can only be evaluated properly in a wider context of bladder cancer genetics. This thesis suggests bladder cancer baseline characteristics and prognosis are influenced by a variety of single nucleotide polymorphisms, albeit

requiring further validation. Moreover, genetic associations with bladder cancer phenotypes are expected to interact with tobacco exposure, and should be explored further. Data mining of multiple data sources may prove very useful to continue investigations into new discoveries and replications.

Finally, the mixture of collaborative efforts in the era of big data offers endless opportunities, where methodology is equally driven by experience, creativity, and technology. The future of bladder cancer genetics is as exciting as we are ready to make it.

REFERENCES

[1] Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KK, Stacey SN, Gudmundsson J, Jakobsdottir M, Bergthorsson JT, Sigurdsson A, Blondal T, Witjes JA, Vermeulen SH, Hulsbergen-van de Kaa CA, Swinkels DW, Ploeg M, Cornel EB, Vergunst H, Thorgeirsson TE, Gudbjartsson D, Gudjonsson SA, Thorleifsson G, Kristinsson KT, Mouy M, Snorradottir S, Placidi D, Campagna M, Arici C, Koppova K, Gurzau E, Rudnai P, Kellen E, Polidoro S, Guarrera S, Sacerdote C, Sanchez M, Saez B, Valdivia G, Ryk C, de Verdier P, Lindblom A, Golka K, Bishop DT, Knowles MA, Nikulasson S, Petursdottir V, Jonsson E, Geirsson G, Kristjansson B, Mayordomo JI, Steineck G, Porru S, Buntinx F, Zeegers MP, Fletcher T, Kumar R, Matullo G, Vineis P, Kiltie AE, Gulcher JR, Thorsteinsdottir U, Kong A, Rafnar T, Stefansson K. Sequence Variant on 8q24 Confers Susceptibility to Urinary Bladder Cancer. Nat Genet. 2008;40(11):1307-12.

[2] Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The Uk Biobank Resource with Deep Phenotyping and Genomic Data. Nature. 2018;562(7726):203-9.

[3] Lipunova N, Wesselius A, Cheng KK, van Schooten FJ, Bryan RT, Cazier JB, Galesloot TE, Kiemeney L, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer. Eur Urol Oncol. 2019;2(4):381-9.

[4] Lipunova N, Wesselius A, Cheng KK, Schooten FJv, Cazier J-B, Bryan RT, Zeegers MP. Genetic Associations for Prognostic Factors of Urinary Bladder Cancer: A Systematic Review (Article in Submission). 2019.

[5] de Maturana EL, Rava M, Anumudu C, Sáez O, Alonso D, Malats N. Bladder Cancer Genetic Susceptibility. A Systematic Review. Bladder cancer (Amsterdam, Netherlands). 2018;4(2):215-26.

[6] An Y, Li H, Wang KJ, Liu XH, Qiu MX, Liao Y, Huang JL, Wang XS. Meta-Analysis of the Relationship between Slow Acetylation of N-Acetyl Transferase 2 and the Risk of Bladder Cancer. Genetics and molecular research : GMR. 2015;14(4):16896-904.

[7] Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Castano-Vinyals G, Yeager M, Welch R, Chanock S, Chatterjee N, Wacholder S, Samanic C, Tora M, Fernandez F, Real FX, Rothman N. Nat2 Slow Acetylation, Gstm1 Null Genotype, and Risk of Bladder Cancer: Results from the Spanish Bladder Cancer Study and Meta-Analyses. Lancet (London, England). 2005;366(9486):649-59.

[8] Kellen E, Zeegers M, Paulussen A, Vlietinck R, Vlem EV, Veulemans H, Buntinx F. Does Occupational Exposure to Pahs, Diesel and Aromatic Amines Interact with Smoking and Metabolic Genetic Polymorphisms to Increase the Risk on Bladder Cancer?; the Belgian Case Control Study on Bladder Cancer Risk. Cancer letters. 2007;245(1-2):51-60.

[9] Lubin JH, Kogevinas M, Silverman D, Malats N, Garcia-Closas M, Tardon A, Hein DW, Garcia-Closas R, Serra C, Dosemeci M, Carrato A, Rothman N. Evidence for an Intensity-Dependent Interaction of Nat2 Acetylation Genotype and Cigarette Smoking in the Spanish Bladder Cancer Study. Int J Epidemiol. 2007;36(1):236-41.

[10] Mantovani F, Collavin L, Del Sal G. Mutant P53 as a Guardian of the Cancer Cell. Cell death and differentiation. 2019;26(2):199-212.

[11] Shinohara A, Sakano S, Hinoda Y, Nishijima J, Kawai Y, Misumi T, Nagao K, Hara T, Matsuyama H. Association of Tp53 and Mdm2 Polymorphisms with Survival in Bladder Cancer Patients Treated with Chemoradiotherapy. Cancer science. 2009;100(12):2376-82.
[12] Pandith AA, Shah ZA, Khan NP, Rasool R, Afroze D, Yousuf A, Wani S, Siddiqi M. Role of Tp53 Arg72pro Polymorphism in Urinary Bladder Cancer Predisposition and Predictive Impact of Proline Related Genotype in Advanced Tumors in an Ethnic Kashmiri Population. Cancer genetics and cytogenetics. 2010;203(2):263-8.
[13] Jiang DK, Ren WH, Yao L, Wang WZ, Peng B, Yu L. Meta-Analysis of Association between Tp53 Arg72pro Polymorphism and Bladder Cancer Risk. Urology. 2010;76(3):765.e1-7.
[14] Liu ZH, Bao ED. Quantitative Assessment of the Association between Tp53 Arg72pro Polymorphism and Bladder Cancer Risk. Mol Biol Rep. 2013;40(3):2389-95.
[15] Moore LE, Baris DR, Figueroa JD, Garcia-Closas M, Karagas MR, Schwenn MR, Johnson AT, Lubin JH, Hein DW, Dagnall CL, Colt JS, Kida M, Jones MA, Schned AR, Cherala SS, Chanock SJ, Cantor KP, Silverman DT, Rothman N. Gstm1 Null and Nat2 Slow Acetylation Genotypes, Smoking Intensity and Bladder Cancer Risk: Results from the New England Bladder Cancer Study and Nat2 Meta-Analysis. Carcinogenesis. 2011;32(2):182-9.
[16] Corella D, Ramirez-Sabio JB, Coltell O, Ortega-Azorin C, Estruch R, Martinez-Gonzalez MA, Salas-Salvado J, Sorli JV, Castaner O, Aros F, Garcia-Corte FJ, Serra-Majem L, Gomez-Gracia E, Fiol M, Pinto X, Saez GT, Toledo E, Basora J, Fito M, Cofan M, Ros E, Ordovas JM. Effects of the Ser326cys Polymorphism in the DNA Repair Ogg1 Gene on Cancer, Cardiovascular, and All-Cause Mortality in the Predimed Study: Modulation by Diet. Journal of the Academy of Nutrition and Dietetics. 2018;118(4):589-605.
[17] Boiteux S, Radicella JP. The Human Ogg1 Gene: Structure, Functions, and Its Implication in the Process of Carcinogenesis. Archives of biochemistry and biophysics. 2000;377(1):1-8.
[18] Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun Wee J, Kim WJ. Genotypes of Tnf-Alpha, Vegf, Hogg1, Gstm1, and Gstt1: Useful Determinants for Clinical Outcome of Bladder Cancer. Urology. 2005;65(1):70-5.
[19] Zhou PT, Li B, Ji J, Wang MM, Gao CF. A Systematic Review and Meta-Analysis of the Association between Ogg1 Ser326cys Polymorphism and Cancers. Medical oncology (Northwood, London, England). 2015;32(2):472.
[20] Zou H, Li Q, Xia W, Liu Y, Wei X, Wang D. Association between the Ogg1 Ser326cys Polymorphism and Cancer Risk: Evidence from 152 Case-Control Studies. Journal of Cancer. 2016;7(10):1273-80.
[21] Ba X, Boldogh I. 8-Oxoguanine DNA Glycosylase 1: Beyond Repair of the Oxidatively Modified Base Lesions. Redox biology. 2018;14:669-78.
[22] Wenjuan C, Jianzhong L, Chong L, Yanjun G, Keqing L, Hanzhang W, Zhiping W. The Hogg1 Ser326cys Gene Polymorphism and Susceptibility for Bladder Cancer: A Meta-Analysis. International braz j urol : official journal of the Brazilian Society of Urology. 2016;42(5):883-96.
[23] Qu HQ, Tien M, Polychronakos C. Statistical Significance in Genetic Association Studies. Clinical and investigative medicine Medecine clinique et experimentale. 2010;33(5):E266-70.

[24] Fu YP, Kohaar I, Moore LE, Lenz P, Figueroa JD, Tang W, Porter-Gill P, Chatterjee N, Scott-Johnson A, Garcia-Closas M, Muchmore B, Baris D, Paquin A, Ylaya K, Schwenn M, Apolo AB, Karagas MR, Tarway M, Johnson A, Mumy A, Schned A, Guedez L, Jones MA, Kida M, Hosain GM, Malats N, Kogevinas M, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Wu X, Purdue M, Andriole GL, Jr., Grubb RL, 3rd, Black A, Landi MT, Caporaso NE, Vineis P, Siddiq A, Bueno-de-Mesquita HB, Trichopoulos D, Ljungberg B, Severi G, Weiderpass E, Krogh V, Dorronsoro M, Travis RC, Tjønneland A, Brennan P, Chang-Claude J, Riboli E, Prescott J, Chen C, De Vivo I, Govannucci E, Hunter D, Kraft P, Lindstrom S, Gapstur SM, Jacobs EJ, Diver WR, Albanes D, Weinstein SJ, Virtamo J, Kooperberg C, Hohensee C, Rodabough RJ, Cortessis VK, Conti DV, Gago-Dominguez M, Stern MC, Pike MC, Van Den Berg D, Yuan JM, Haiman CA, Cussenot O, Cancel-Tassin G, Roupret M, Comperat E, Porru S, Carta A, Pavanello S, Arici C, Mastrangelo G, Grossman HB, Wang Z, Deng X, Chung CC, Hutchinson A, Burdette L, Wheeler W, Fraumeni J, Jr., Chanock SJ, Hewitt SM, Silverman DT, Rothman N, Prokunina-Olsson L. The 19q12 Bladder Cancer Gwas Signal: Association with Cyclin E Function and Aggressive Disease. Cancer research. 2014;74(20):5808-18.
[25] Grotenhuis AJ, Dudek AM, Verhaegh GW, Aben KK, Witjes JA, Kiemeney LA, Vermeulen SH. Independent Replication of Published Germline Polymorphisms Associated with Urinary Bladder Cancer Prognosis and Treatment Response. Bladder Cancer (Amsterdam, Netherlands). 2016;2(1):77-89.
[26] Palmer C, Pe'er I. Statistical Correction of the Winner's Curse Explains Replication Variability in Quantitative Trait Genome-Wide Association Studies. PLoS genetics. 2017;13(7):e1006916.
[27] Sham PC, Purcell SM. Statistical Power and Significance Testing in Large-Scale Genetic Studies. Nature reviews Genetics. 2014;15(5):335-46.
[28] Korthauer K, Kimes PK, Duvallet C, Reyes A, Subramanian A, Teng M, Shukla C, Alm EJ, Hicks SC. A Practical Guide to Methods Controlling False Discoveries in Computational Biology. Genome biology. 2019;20(1):118.
[29] Rohde PD, Østergaard S, Kristensen TN, Sørensen P, Loeschcke V, Mackay TFC, Sarup P. Functional Validation of Candidate Genes Detected by Genomic Feature Models. G3 (Bethesda, Md). 2018;8(5):1659-68.
[30] Visscher PM, Brown MA, McCarthy MI, Yang J. Five Years of Gwas Discovery. Am J Hum Genet. 2012;90(1):7-24.
[31] Czerniak B, Dinney C, McConkey D. Origins of Bladder Cancer. Annual review of pathology. 2016;11:149-74.
[32] Guo CC, Czerniak B. Bladder Cancer in the Genomic Era. Arch Pathol Lab Med. 2019;143(6):695-704.
[33] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. Eau Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder: Update 2016. Eur Urol. 2016.
[34] Zamboni S, Moschini M, Simeone C, Antonelli A, Mattei A, Baumeister P, Xylinas E, Hakenberg OW, Aziz A. Prediction Tools in Non-Muscle Invasive Bladder Cancer. Translational andrology and urology. 2019;8(1):39-45.
[35] Lipunova N, Wesselius A, Cheng KK, Schooten F-Jv, Bryan RT, Cazier J-B, Galesloot TE, Kiemeney LALM, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non-Muscle-Invasive Bladder Cancer (Manuscript in Submission). 2018.

[36] Bui HT, Fujimoto N, Kubo T, Inatomi H, Matsumoto T. Slco1b1, Slco2b1, and Slco1b3 Polymorphisms and Susceptibility to Bladder Cancer Risk. Cancer investigation. 2014;32(6):256-61.
[37] Niemi M, Pasanen MK, Neuvonen PJ. Organic Anion Transporting Polypeptide 1b1: A Genetically Polymorphic Transporter of Major Importance for Hepatic Drug Uptake. Pharmacological reviews. 2011;63(1):157-81.
[38] Kalliokoski A, Niemi M. Impact of Oatp Transporters on Pharmacokinetics. British journal of pharmacology. 2009;158(3):693-705.
[39] Niemi M. Role of Oatp Transporters in the Disposition of Drugs. Pharmacogenomics. 2007;8(7):787-802.
[40] Murata H, Ito S, Kusuhara H, Nomura Y, Taniguchi T. Proposal of a Parameter for Oatp1b1 Inhibition Screening at the Early Drug Discovery Stage. Journal of pharmaceutical sciences. 2019.
[41] Sayyed K, Vee ML, Abdel-Razzak Z, Jouan E, Stieger B, Denizot C, Parmentier Y, Fardel O. Alteration of Human Hepatic Drug Transporter Activity and Expression by Cigarette Smoke Condensate. Toxicology. 2016;363-364:58-71.
[42] Bryan RT, Zeegers MP, van Roekel EH, Bird D, Grant MR, Dunn JA, Bathers S, Iqbal G, Khan HS, Collins SI, Howman A, Deshmukh NS, James ND, Cheng KK, Wallace DM. A Comparison of Patient and Tumour Characteristics in Two Uk Bladder Cancer Cohorts Separated by 20 Years. BJU international. 2013;112(2):169-75.
[43] Cumberbatch MGK, Jubber I, Black PC, Esperto F, Figueroa JD, Kamat AM, Kiemeney L, Lotan Y, Pang K, Silverman DT, Znaor A, Catto JWF. Epidemiology of Bladder Cancer: A Systematic Review and Contemporary Update of Risk Factors in 2018. Eur Urol. 2018;74(6):784-95.
[44] Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, Castro MAA, Gibb EA, Kanchi RS, Gordenin DA, Shukla SA, Sanchez-Vega F, Hansel DE, Czerniak BA, Reuter VE, Su X, de Sa Carvalho B, Chagas VS, Mungall KL, Sadeghi S, Pedamallu CS, Lu Y, Klimczak LJ, Zhang J, Choo C, Ojesina AI, Bullman S, Leraas KM, Lichtenberg TM, Wu CJ, Schultz N, Getz G, Meyerson M, Mills GB, McConkey DJ, Weinstein JN, Kwiatkowski DJ, Lerner SP. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell. 2017;171(3):540-56.e25.
[45] Choi W, Ochoa A, McConkey DJ, Aine M, Höglund M, Kim WY, Real FX, Kiltie AE, Milsom I, Dyrskjøt L, Lerner SP. Genetic Alterations in the Molecular Subtypes of Bladder Cancer: Illustration in the Cancer Genome Atlas Dataset. Eur Urol. 2017;72(3):354-65.
[46] The Nhgri-Ebi Catalog of Published Genome-Wide Association Studies. [Internet]. [cited 2017-01-03]. Available from: http://www.ebi.ac.uk/gwas. .
[47] Watanabe K, Stringer S, Frei O, Umicevic Mirkov M, de Leeuw C, Polderman TJC, van der Sluis S, Andreassen OA, Neale BM, Posthuma D. A Global Overview of Pleiotropy and Genetic Architecture in Complex Traits. Nature genetics. 2019;51(9):1339-48.
[48] Garcia-Closas M, Rothman N, Figueroa JD, Prokunina-Olsson L, Han SS, Baris D, Jacobs EJ, Malats N, De Vivo I, Albanes D, Purdue MP, Sharma S, Fu YP, Kogevinas M, Wang Z, Tang W, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Andriole G, Jr., Grubb R, 3rd, Black A, Gapstur SM, Thun M, Diver WR, Weinstein SJ, Virtamo J, Hunter DJ, Caporaso N, Landi MT, Hutchinson A, Burdett L, Jacobs KB, Yeager M, Fraumeni JF, Jr., Chanock SJ, Silverman DT, Chatterjee N. Common Genetic Polymorphisms

Modify the Effect of Smoking on Absolute Risk of Bladder Cancer. Cancer research. 2013;73(7):2211-20.
[49] Figueroa JD, Han SS, Garcia-Closas M, Baris D, Jacobs EJ, Kogevinas M, Schwenn M, Malats N, Johnson A, Purdue MP, Caporaso N, Landi MT, Prokunina-Olsson L, Wang Z, Hutchinson A, Burdette L, Wheeler W, Vineis P, Siddiq A, Cortessis VK, Kooperberg C, Cussenot O, Benhamou S, Prescott J, Porru S, Bueno-de-Mesquita HB, Trichopoulos D, Ljungberg B, Clavel-Chapelon F, Weiderpass E, Krogh V, Dorronsoro M, Travis R, Tjonneland A, Brenan P, Chang-Claude J, Riboli E, Conti D, Gago-Dominguez M, Stern MC, Pike MC, Van Den Berg D, Yuan JM, Hohensee C, Rodabough R, Cancel-Tassin G, Roupret M, Comperat E, Chen C, De Vivo I, Giovannucci E, Hunter DJ, Kraft P, Lindstrom S, Carta A, Pavanello S, Arici C, Mastrangelo G, Karagas MR, Schned A, Armenti KR, Hosain GM, Haiman CA, Fraumeni JF, Jr., Chanock SJ, Chatterjee N, Rothman N, Silverman DT. Genome-Wide Interaction Study of Smoking and Bladder Cancer Risk. Carcinogenesis. 2014;35(8):1737-44.
[50] Korte A, Farlow A. The Advantages and Limitations of Trait Analysis with Gwas: A Review. Plant methods. 2013;9:29.
[51] Tam V, Patel N, Turcotte M, Bosse Y, Pare G, Meyre D. Benefits and Limitations of Genome-Wide Association Studies. Nat Rev Genet. 2019;20(8):467-84.
[52] Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, Yang J. 10 Years of Gwas Discovery: Biology, Function, and Translation. American journal of human genetics. 2017;101(1):5-22.
[53] Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA, Visscher PM. Finding the Missing Heritability of Complex Diseases. Nature. 2009;461:747.
[54] Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y, McRae AF, Visscher PM, Zeng J, Yang J. Genome-Wide Association Analyses Identify 143 Risk Variants and Putative Regulatory Mechanisms for Type 2 Diabetes. Nature communications. 2018;9(1):2941.
[55] Biological Insights from 108 Schizophrenia-Associated Genetic Loci. Nature. 2014;511(7510):421-7.
[56] Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, Frayling TM, Hirschhorn J, Yang J, Visscher PM. Meta-Analysis of Genome-Wide Association Studies for Height and Body Mass Index in Approximately 700000 Individuals of European Ancestry. Human Molecular Genetics. 2018;27(20):3641-9.
[57] Bryan RT, Evans T, Dunn JA, Iqbal G, Bathers S, Collins SI, James ND, Catto JWF, Wallace DMA. A Comparative Analysis of the Influence of Gender, Pathway Delays, and Risk Factor Exposures on the Long-Term Outcomes of Bladder Cancer. European urology focus. 2015;1(1):82-9.
[58] Duncan LE, Ostacher M, Ballon J. How Genome-Wide Association Studies (Gwas) Made Traditional Candidate Gene Studies Obsolete. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2019;44(9):1518-23.

APPENDIX

Supplementary Table 3.1. A checklist for the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA).

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	64
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	65
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	66
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	66, 67
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	67- 69
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	67
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	67
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	67, Figure 3.1

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	-
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	69
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	69
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	-
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	69

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	69
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	69, 70
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 3.1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	69, Supplementary Tables 3.2-3.8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	69
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Supplementary Tables 3.2-3.8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	-
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	69
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see	75, 76

		Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	76-79
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	79
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	79
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	-

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Supplementary Table 3.2. Summary associations for previously reported SNPs on age at the time of diagnosis for urinary bladder cancer.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
An increase in age at the time of diagnosis:															
>56 years	2009	Stern et al. [1]	Low-risk NMIBC (Ta+G1/G2)	UC	Caucasian (Northern American)	297 (>55 years) / 201 (≤55 years)	rs710521	3q28	TP63	A	G	0.80 (1KG)	OR (A/G)=1.77	1.22-2.56	<0.05
≥65 years / Age of healthy controls	2010	Yuan et al. [2]	UBC	UC	Chinese	214 (UBC cases) / 212 (Healthy controls)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.41 (1KG)	OR (AG+AA/GG)=1.74	1.06-2.88	0.029
>65 years	2012	Ma et al. [3]	UBC	UC	Chinese	450 (GG) / 808 (CG+CC)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	OR (GG/CG+CC)=1.31	1.04-1.66	p<0.05
>65 years	2013	Chu et al. [4]	UBC	UC	Chinese	475 (CC) / 632 (CT+TT)	rs884225	7p11.2	EGFR	C	T	0.19 (1KG)	OR (CC/CT+TT)=1.87	1.39–2.53	<0.05
>65 years	2008	Wang et al. [5]	UBC	UC	Chinese	103 (Age >65 years) / 113 (Healthy controls)	rs7003908	8q11.21	XRCC7 (PRKDC)	T	G	0.33 (1KG)	OR (TT/GT+GG)=2.27	1.25-4.10	0.007
>60 years	2018	Wang et al. [6]	UBC	Not reported	Chinese	690 (AG+AA) / 1015 (GG)	rs874945	12q13.13	HOTAIR	A	G	0.36 (1KG)	OR (AA+AG/GG)=1.35	1.10-1.65	0.004
A decrease in age at the time of diagnosis:															
≤60 years	2016	Hua et al. [7]	UBC	UC	Chinese	60 (AA) / 296 (GG+GA)	rs217727	11p15.5	H19 (lncRNA)	A	G	0.36 / 0.20 (1KG)	OR (AA / GG+GA)=1.80	1.16–2.81	0.009
≤65 years / Healthy controls	2009	Wang et al. [8]	UBC	UC	Chinese	203 (≤65 years) / 238 (Healthy controls)	rs9642880	8q24.21	CASC11	T	G	0.54 (1KG)	OR (GT+TT/GG)=2.31	1.56–3.43	<0.0001
≥50 years	2004	Kelsey et al. [9]	UBC	UC + Other	Caucasian (Northern American)	29 (AA) / 294 (AG+GG)	Gln399Arg (rs25487)	19q13.2	XRCC1	A	G	0.26 (1KG)	OR (AA / AG+GG)=0.6	0.3-0.9	<0.05

Age, years	2010	Kiemeney et al. [10]	UBC	UC	European (Multiple)	4211	rs798766	4p16.3	TACC3/FGFR3	T	C	0.23 / 0.24 (1KG)	β=-0.81	-1.35; -0.26	0.0036
>65 years	2015	Xiao et al. [11]	UBC	Not reported	Chinese	548 (UBC) / 709 (Healthy controls)	Ala503Val (rs1057868)	7q11.23	POR	T	C	0.29 (1KG)	OR (TT/CT+CC)=0.586	0.417-0.823	0.002
>60 years	2017	Lin et al. [12]	UBC	UC	Chinese	673 (AG+GG) / 1015 (AA)	rs710886	8q24.21	PCAT1	G	A	0.47 (1KG)	OR (AG+GG/AA)=0.77	0.67-0.88	<0.001

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Stern MC, Van Den Berg D, Yuan JM, Conti DV, Gago-Dominguez M, Pike MC, Xiang YB, Gao YT, Cortessis VK. Sequence Variant on 3q28 and Urinary Bladder Cancer Risk: Findings from the Los Angeles-Shanghai Bladder Case-Control Study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(11):3057-61.

[2] Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, Zhang Z. Cyclin D1 G870a Polymorphism Is Associated with Risk and Clinicopathologic Characteristics of Bladder Cancer. DNA and cell biology. 2010;29(10):611-7.

[3] Ma L, Chu H, Wang M, Shi D, Zhong D, Li P, Tong N, Yin C, Zhang Z. Hogg1 Ser326cys Polymorphism Is Associated with Risk of Bladder Cancer in a Chinese Population: A Case-Control Study. Cancer science. 2012;103(7):1215-20.

[4] Chu H, Wang M, Jin H, Lv Q, Wu D, Tong N, Ma L, Shi D, Zhong D, Fu G, Yuan L, Qin C, Yin C, Zhang Z. Egfr 3'utr 774t>C Polymorphism Contributes to Bladder Cancer Risk. Mutagenesis. 2013;28(1):49-55.

[5] Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD, Liu QZ. Genetic Variants of the Xrcc7 Gene Involved in DNA Repair and Risk of Human Bladder Cancer. International journal of urology : official journal of the Japanese Urological Association. 2008;15(6):534-9.

[6] Wang X, Wang W, Zhang Q, Gu D, Zhang K, Ge Y, Chu H, Du M, Xu B, Wang M, Lv X, Zhang Z, Yuan L, Gong W. Tagging Snps in the Hotair Gene Are Associated with Bladder Cancer Risk in a Chinese Population. Gene. 2018;664:22-6.

[7] Hua Q, Lv X, Gu X, Chen Y, Chu H, Du M, Gong W, Wang M, Zhang Z. Genetic Variants in Lncrna H19 Are Associated with the Risk of Bladder Cancer in a Chinese Population. Mutagenesis. 2016;31(5):531-8.

[8] Wang M, Wang M, Zhang W, Yuan L, Fu G, Wei Q, Zhang Z. Common Genetic Variants on 8q24 Contribute to Susceptibility to Bladder Cancer in a Chinese Population. Carcinogenesis. 2009;30(6):991-6.

[9] Kelsey KT, Park S, Nelson HH, Karagas MR. A Population-Based Case-Control Study of the Xrcc1 Arg399gln Polymorphism and Susceptibility to Bladder Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(8):1337-41.

[10] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.
[11] Xiao X, Ma G, Li S, Wang M, Liu N, Ma L, Zhang Z, Chu H, Zhang Z, Wang SL. Functional Por A503v Is Associated with the Risk of Bladder Cancer in a Chinese Population. Scientific reports. 2015;5:11751.
[12] Lin Y, Ge Y, Wang Y, Ma G, Wang X, Liu H, Wang M, Zhang Z, Chu H. The Association of Rs710886 in Lncrna Pcat1 with Bladder Cancer Risk in a Chinese Population. Gene. 2017;627:226-32.

Supplementary Table 3.3. Summary associations for previously reported SNPs on urinary bladder cancer tumour size at the time of diagnosis.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations for an increase in tumour size:															
Tumour size (≥3 cm)	2014	Gu et al. [1]	UBC	Not reported	Chinese	218 (G3) / 670 (Healthy subjects)	rs2664139	15q14	TSP-1 (THBS1)	C	T	0.43 (1KG)	OR (CC/TT+CT)=1.94	1.22-3.10	0.006
Associations for a decrease in tumour size:															
Tumour size (T1-T4)	2018	Lee et al. [2]	UBC	UC	Taiwanese	279 (T1-T4) / 90 (Ta)	rs2929973	8q24.22	WISP1 (CCN4)	G	T	0.17 (1KG)	OR (TG+GG/TT)=0.61	0.371-0.990	0.044

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Gu J, Tao J, Yang X, Li P, Yang X, Qin C, Cao Q, Cai H, Zhang Z, Wang M, Gu M, Lu Q, Yin C. Effects of Tsp-1-696 C/T Polymorphism on Bladder Cancer Susceptibility and Clinicopathologic Features. Cancer genetics. 2014;207(6):247-52.

[2] Lee HL, Chiou HL, Wang SS, Hung SC, Chou MC, Yang SF, Hsieh MJ, Chou YE. Wisp1 Genetic Variants as Predictors of Tumor Development with Urothelial Cell Carcinoma. Urologic oncology. 2018;36(4):160.e15-.e21.

Supplementary Table 3.4. Summary associations for previously reported SNPs on stage at the time of diagnosis for urinary bladder cancer.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations for stages of Ta/T1/Tis (or combinations of these):															
Associations reducing the risk of stages of Ta/T1/Tis (or combinations of these):															
Ta+T1	2004	Kelsey et al. [1]	UBC	UC + Other	Caucasian (Northern American)	22 (AA) / 234 (AG+GG)	Gln399Arg (rs25487)	19q13.2	XRCC1	A	G	0.26 (1KG)	OR (AA / AG+GG)=0.5	0.3-0.9	<0.05
Ta+T1	2011	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	102 (Ta+T1)	Arg594Gln (rs2274976)	1p36.22	MTHFR	A	G	0.07 (1KG)	OR (A/G)=0.57	0.41-0.75	0.012
Ta+T1	2011	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	102 (Ta+T1)	Ala222Val (rs1801133)	1p36.22	MTHFR	T	C	0.25 (1KG)	OR (T/C)=0.47	0.36–0.68	0.001
Ta+T1	2011	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	102 (Ta+T1)	Glu470Ala (rs1801131)	1p36.22	MTHFR	C	A	0.25 (1KG)	OR (C/A)=0.46	0.28-0.71	0.001
Ta+T1	2011	Sobti et al. [3]	UBC	Not reported	Indian	18 (CC) / 127 (GG)	rs1801320	15q15.1	RAD51	C	G	0.26 (Cases) / 0.31 (Controls) / 0.14 (1KG)	OR (CC/GG)= 0.51	0.27–0.99	0.031
Ta+T1+Tis / Healthy controls	2008	Verhaegh et al. [4]	NMIBC	Not reported	European (The Netherlands)	83 (NMIBC) / 152 (Healthy controls)	rs2839698	11p15.5	H19	C	T	0.71 (1KG)	OR (TC/TT)=0.52	0.28-0.94	<0.05

Ta+T1+Tis / Healthy controls	2015	Zhao et al. [5]	UBC	Not reported	Chinese	76 (Tis+Ta+T1) / 210 (Healthy controls)	rs2227485	12q15	IL22	T	C	0.48 (all UBC) / 0.49 (1KG)	OR (TT / CT+CC)=0.48	0.23-0.9	0.04
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	248 (NMIBC) / 400 (Controls) (Allele count instead of individuals)	Thr241Met (rs861539)	14q32.33	XRCC3	T	C	0.22 (1KG)	OR (T/C)=0.63	0.43-0.94	<0.05
Associations increasing the risk for stages of Ta/T1/Tis (or combinations of these):															
Ta+T1	2010	Yuan et al. [7]	UBC	UC	Chinese	255 (Ta+T1 / 402 (Healthy controls)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.41 (1KG)	OR (AG+AA/GG)=1.86	1.10-3.16	0.021
Ta+T1 / Healthy controls	2002	Chen et al. [8]	UBC	UC	Taiwanese	33 (Ta+T1) / 119 (Healthy controls)	Ser31Arg (rs1801270)	6p21.2	P21 (CDKN1A)	A	C	0.74 (Cases) / 0.57 (Controls) / 0.26 (1KG)	OR (A/C)=2.55	1.54-4.79	0.012
Ta+T1 / Healthy controls	2008	Gangwar et al. [9]	NMIBC	UC	Indian	76 (Ta+T1) / 146 (Healthy controls)	Asp312Asn (rs1799793)	19q13.3	ERCC2	A	G	0.19 (1KG)	OR(AA/GG)=4.62	2.29-9.29	0.003
Ta+T1 / Healthy controls	2009	Wen et al. [10]	NMIBC	Not reported	Chinese	304 (Cases) / 90 (Healthy controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC+CC/AA)=1.89	1.21-3.24	0.02
Ta+T1 / T2+	2004	Ichimura et al. [11]	UBC	UC	Japanese	152 (Ta+T1) / 42 (T2+)	Pro198Leu (rs1050450)	3p21.31	GPX1	T	C	0.22 (1KG)	OR (CT/CC)=2.58	1.07-6.18	0.034
Ta+T1+Tis / Healthy controls	2017	Ali et al. [12]	UBC	UC	Pakistani	124 (Ta+T1) / 200 (Healthy controls)	Gln223Arg (rs1137101)	1p31.3	LEPR	A	G	0.52 (Cases) / 0.42 (Controls) /	OR (G/A)=1.4	1.04–2	<0.05

												0.42 (1KG)			
Tis (or multiple tumours)	2011	Lima et al. [13]	NMIBC	Not reported	European (Portugal)	65 (Multiple/CIS) / 52 (Single)	rs2243250	5q31.1	IL4	T	C	0.47 (1KG)	OR (T/C) =2.80	1.08-7.27	0.031
Tis / Ta+T1	2004	Ito et al. [14]	NMIBC	UC	Japanese	14 (Tis)/ 47 (no Tis)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.41 (1KG)	OR (AG/GG)=3.67 OR (AA/AG+GG)=3.94	1.00-13.45 1.40-11.10	0.049 0.009
T1	2013	Pandith et al. [15]	UBC	UC	Indian	18 (TC+CC) / 32 (TT)	His27His (rs12628)	11p15.5	HRAS	C	T	0.30 (1KG)	OR (TC+CC/TT)=3.0	1.50–5.97	0.004
Ta+T1 (patients ≤71 yrs)	2006	Sakano et al. [16]	NMIBC	UC	European (Sweden)	30 (T1) / 83 (Ta)	Asp1104His (rs17655)	13q33.1	XPG (ERCC5)	C	G	0.36 (1KG)	OR (GC+CC/GG)=4.9	2.0-12.9	<0.001
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	65 (NMIBC) / 99 (Controls)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (GG/AA)=4.03	1.87–8.67	<0.05
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	248 (NMIBC) / 400 (Controls) (Allele count instead of individuals)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (G/A)=1.78	1.25-2.53	<0.05
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	107 (NMIBC) / 184 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC/AA)=2.14	1.20–3.81	<0.05
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	92 (NMIBC) / 167 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (CC/AA)=2.52	1.17-5.41	<0.05
Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	248 (NMIBC) / 400 (Controls) (Allele count instead of individuals)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (C/A)=1.72	1.21-2.43	<0.05

Ta+T1	2018	Ahmed et al. [6]	NMIBC	UC	Pakistani	248 (NMIBC) / 400 (Controls) (Allele count instead of individuals)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	OR (G/C)=1.57	1.03-2.37	<0.05
Associations for stages of T2+:															
Associations reducing the risk of stages of T2+:															
T2+	2009	Ahirwar et al. [17]	UBC	UC	Indian	82 (T2+) / 132 (Ta+T1)	rs1799724	6p21.33	TNFA	T	C	0.09 (1KG)	OR (CT/CC)=0.52	0.27-0.99	0.049
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	Ser260Ser (rs6463524, merged into rs1805319)	7p22.1	PMS2	G?	A/C/T?	G=0.17 (1KG)	OR (G?/A?C?T?)=0.56	0.41-0.77	0.0002
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs3213427	12p13.31	CD4	C?	T?	C=0.26 (1KG)	OR (C?/T?)=0.71	0.57-0.88	0.001
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs828702	2q35	XRCC5	G?	A?	G=0.45 (1KG)	OR (G?/A?)=0.80	0.65-0.99	0.037
T2+	2010	Gangwar et al. [19]	UBC	UC	Indian	34 (T2+) / 25 (TaG1)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (GG/TT)=0.30	0.09-0.99	0.049
T2+ / Ta+T1	2015	Deng et al. [20]	UBC	Not reported	Chinese	84 (Invasive) / (75 (Superficial)	rs2910164	5q33.3	MIR146A	G	C	0.39 (all UBC) / 0.32 (TOPMED)	OR (GG / CG+CC)=0.20	0.05–0.72	0.012

T2+ / Ta+T1	2011	Safarinejad et al. [21]	UBC	UC	Iranian	56 (T2+) / 84 (Ta+T1) 37 (T2+) / 40 (Ta+T1)	rs2854744	7p12.3	IGFBP3	A	C	0.39 (UBC) / 0.49 (Healthy controls) / 0.47 (1KG)	OR (AC/CC)=0.32 OR (AA/CC)= 0.17	0.24–0.52 0.11–0.31	0.0001 0.00001
T2+ / Ta+T1	2011	Lin et al. [22]	UBC	UC	Taiwanese	34 (T2+) / 58 (Ta+T1)	Pro870Pro (rs9344)	11q13.3	CCND1	G	A	0.59 (1KG)	OR (AG/AA)=0.29	0.12-0.70	0.009
T2+ / Ta+T1	2013	Safarinejad et al. [23]	UBC	UC	Iranian	59 (T2+) / 107 (Ta+T1)	Ile105Val (rs1695)	11q13.2	GSTP1	G	A	0.35 (1KG)	OR (AG+GG/AA)= 0.72	0.51-0.87	0.002
T2+ / TaG1	2009	Gangwar et al. [24]	UBC	UC	Indian	59 (T2+) / 34 (TaG1)	rs4645978	1p36.21	CASP9	G	A	0.42 (1KG)	OR (AG/AA)=0.28	0.10-0.76	0.013
T2+ / TaG1	2013	Jaiswal et al. [25]	UBC	UC	Indian	50 (MIBC) / 36 (NMIBC)	rs187238	11q23.1	IL18	C	G	0.79 (1KG)	OR (GC/GG)=0.36	0.15–0.89	0.027
T2+ / Healthy controls	2017	Ali et al. [12]	UBC	UC	Pakistani	76 (T2+) / 200 (Healthy controls)	Pro12Ala (rs1801282)	3p25.2	PPARG	G	C	0.16 (Cases) / 0.12 (Controls) / 0.07 (1KG)	OR (GG/CC)=5.4	1.2–24	<0.05
Associations increasing the risk for stages of T2+:															
T1+T2+ / Ta	2007	Sanyal et al. [26]	UBC	Not reported	European (Sweden)	106 (T1+T2+) / 146 (Ta)	Gly39Glu (rs1042821)	2p16.3	MSH6	A	G	0.20 (1KG)	RR (AG+AA/GG)=1.9	1.1-3.2	0.03
T1+T2+ / Ta	2007	Sanyal et al. [27]	UBC	Not reported	European (Sweden)	103 (T1+T2+) / 145 (Ta)	Pro187Ser (rs1800566)	16q22.1	NQO1	T	C	0.29 (1KG)	RR (CT+TT/CC)=1.8	1.0-3.1	0.04
T2+	2007	Sanyal et al. [27]	UBC	Not reported	European (Sweden)	40 (AG+GG) / 25 (AA)	Ile105Val (rs1695)	11q13.2	GSTP1	G	A	0.35 (1KG)	RR (AG+GG/AA)=2.7	1.3-5.6	0.008

T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs11738738	5q31.2	MATR3	A?	C/T	A=0.39 (1KG)	OR (A?/C?T?)=1.26	1.02-1.56	0.032
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	Ala473Ala (rs2274700)	1q31.3	CFH	A?	C/G/T?	A=0.48 (1KG)	OR (A?/C?G?T?)=1.24	1.00-1.52	0.046
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs6596471	5q31.2	SLC23A1	A?	C/G/T?	A=0.48 (1KG)	OR (A?/C?G?T?)=1.29	1.05-1.60	0.019
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs10063949	5q31.2	SLC23A1	T?	C?	T=0.42 (1KG)	OR (T?/C?)=1.25	1.01-1.54	0.039
T2+	2009	Guey et al. [18]	UBC	UC	European (Spain)	246 (Only reported overall)	rs4315920	5q31.2	DNAJC18	A?	G?	A=0.46 (1KG)	OR (A?/G?)=1.31	1.06-1.62	0.013
T2+	2010	Wang et al. [28]	UBC	UC	Chinese	115 (CT/TT) / 86 (CC)	rs2294008	8q24.3	PSCA	T	C	0.60 (1KG)	OR (CT+TT/CC)=1.65	1.18-2.31	0.003
T2+	2010	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	56 (T2+)	Arg594Gln (rs2274976)	1p36.22	MTHFR	A	G	0.07 (1KG)	OR (A/G)=3.55	2.42–5.71	0.001
T2+	2010	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	56 (T2+)	Ala222Val (rs1801133)	1p36.22	MTHFR	T	C	0.25 (1KG)	OR (T/C)=3.52	2.25–5.37	0.001
T2+	2010	Safarinejad et al. [2]	UBC (men only)	UC	Iranian	56 (T2+)	Glu470Ala (rs1801131)	1p36.22	MTHFR	C	A	0.25 (1KG)	OR (C/A)=3.62	2.35–5.67	0.001
T2+	2013	Pandith et al. [15]	UBC	UC	Indian	25 (TC+CC) / 40 (TT)	His27His (rs12628)	11p15.5	HRAS	C	T	0.30 (1KG)	OR (TC+CC/TT)=3.3	1.71–6.30	<0.001
T2+	2012	Zhou et al. [29]	UBC	UC	Chinese	115 (AG+AA) / 29 (GG)	rs2275913	6p12.2	IL17	A	G	0.29 (1KG)	OR (AG+AA/GG)=1.79	1.04-3.03	0.032
T2+	2013	Chu et al. [30]	MIBC	UC	Chinese	81 (CC) / 238 (CT+TT)	rs884225	7p11.2	EGFR	C	T	0.19 (1KG)	OR (CC/CT+TT)=1.40	1.05–1.89	<0.05
T2+	2016	Hua et al. [31]	UBC	UC	Chinese	56 (AA) / 3015 (GG+GA)	rs217727	11p15.5	H19 (lncRNA)	A	G	0.36 / 0.20 (1KG)	OR (AA/GG+GA)=1.48	1.06–2.06	0.022

T2+ / Healthy controls	2002	Chen et al. [8]	UBC	UC	Taiwanese	20 (T2+) / 119 (Healthy controls)	Ser31Arg (rs1801270)	6p21.2	P21 (CDKN1A)	A	C	0.74 (Cases) / 0.57 (Controls) / 0.26 (1KG)	OR (A/C)= 1.55	0.77-3.12	0.01
T2+ / Healthy controls	2009	Gangwar et al. [32]	UBC	UC	Indian	43 (T2+) / 134 (Healthy controls)	rs7003908	8q11.21	XRCC7 (PRKDC)	G	T	0.33 (1KG)	OR (GG/TT)=6.80	2.30-15.75	<0.001
T2+ / Healthy controls	2012	Lin et al. [33]	MIBC	Not reported	Taiwanese	42 (CC) / 151 (CT+TT)	Arg72Pro (rs1042522)	17p13.1	TP53	C	G	0.63 (Cases) / 0.67 (Controls) / 0.54 (1KG)	OR (CC/CT+TT)=3.36	1.58–7.15	0.002
T2+ / Ta+T1	2005	Leibovici et al. [34]	UBC	Not reported	Caucasian (White)	203 (T2+) / 238 (Ta+T1)	rs1800629	6p21.33	TNFA	A	G	0.09 (1KG)	OR (AG+AA/GG)=1.91	1.27-2.89	<0.05
T2+ / Ta+T1	2005	Leibovici et al. [34]	UBC	Not reported	Caucasian (White)	187 (T2+) / 232 (Ta+T1)	Pro12Ala (rs1801282)	3p25.2	PPARG	G	C	0.07 (1KG)	OR (CG+GG/CC)=1.61	1.03-2.53	<0.05
T2+ / Ta+T1	2007	Kader et al. [35]	UBC	Not reported	Caucasian (Northern American)	241 (MIBC) / 314 (NMIBC)	rs2276109	11q22.2	MMP12	G	A	0.06 (1KG)	OR (AG+GG/AA)=1.50	1.00-2.28	<0.05
T2+ / Ta+T1	2012	Kucukgergin et al. [36]	UBC	UC	Turkish	20 (T2+) / 71 (Ta+T1)	Pro198Leu (rs1050450)	3p21.31	GPX1	T	C	0.22 (1KG)	OR (TT/CC)=4.13	1.98–8.58	<0.001
T2+ / Ta+T1	2012	Guirado et al. [37]	UBC	UC	European (Spain)	92 (T2+) / 136 (Ta+T1)	Ile254Val (rs3764147)	13q14.11	C13ORF31 (LACC1)	T	C	0.7 (1KG)	OR (TT/CT)=1.87	1.05–3.32	0.033
T2+ / Ta+T1	2012	Guirado et al. [37]	UBC	UC	European (Spain)	98 (T2+) / 148 (Ta+T1)	Ile775Val (rs4129009)	4p14	TLR10	T	C	0.85 (1KG)	OR (TT/CT+CC)=1.75	1.04-2.94	0.033
T2+ / Ta+T1	2012	Kucukgergin et al. [38]	UBC	UC	Turkish	27 (T2+) / 105 (Ta+T1)	Ile142Ile (rs2228014)	2q2	CXCR4	T	C	0.06 (1KG	OR (CT+TT/CC)=1.61	1.03-2.52	0.035

T2+ / Ta+T1	2012	Kucukgergin et al. [38]	UBC	UC	Turkish	20 (T2+) / 85 (Ta+T1) 27 (T2+) / 115 (Ta+T1)	Val64Ile (rs1799864)	3p21.31	CCR2	A	G	0.15 (1KG)	OR (AA/GG)=6.56 OR (AG+AA/GG)=1.66	2.18–19.7 1.06–2.60	0.001 0.026
T2+ / Ta+T1	2012	Kucukgergin et al. [38]	UBC	UC	Turkish	20 (T2+) / 64 (Ta+T1)	rs1801157	10q11.21	SDF1 (CXCL12)	A	G	0.19 (1KG)	OR (AA/GG)=1.93	1.11–3.38	0.02
T2+ / Ta+T1	2013	Wen et al. [39]	UBC	UC	Chinese	36 (MIBC) / 94 (NMIBC)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	Chi-square = 18.89	N/A	<0.001
T2+ / Ta+T1	2014	Zhang et al. [40]	UBC	UC	Chinese	151 (MIBC) / 174 (NMIBC)	rs61330082	7q22.3	NAMPT	C	T	0.73 (1KG)	OR (CT/CC+TT)=1.70	1.01–2.87	<0.05
T2+ / Ta+T1	2014	Zhou et al. [41]	UBC (excluding Tis)	UC	Chinese	156 (T2+) / 176 (Ta+T1)	rs3756712	5p15.33	PDCD6	G	T	0.40 (1KG)	OR (GG/GT+TT)=3.33	1.23–10.00	<0.05
T2+ / Ta+T1	2015	Weng et al. [42]	UBC	UC	Taiwanese	108 (T2+) / 167 (Ta+T1)	rs187115	11p13	CD44	G	A	0.20 (all UBC) / 0.35 (1KG)	OR (AA/AG+GG)=1.69	1.020–2.793	0.041
T2+ / TaG1	2008	Mittal et al. [43]	NMIBC	UC	Indian	32 (T2+) / 25 (TaG1)	Arg194Trp (rs1799782)	19q13.2	XRCC1	T	C	0.12 (1KG)	OR (CT+TT/CC)=11.00	1.29-92.30	0.03
T2+ / TaG1	2010	Gangwar et al. [44]	UBC	UC	Indian	63 (T2+) / 35 (TaG1)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC+CC/AA)=2.52	1.03-6.14	0.041
T2+ / TaG1	2011	Gangwar et al. [45]	MIBC	UC	Indian	63 (T2+) / 33 (TaG1)	rs20417	1q31.1	COX2 (PTGS2)	C	G	0.80 (1KG)	OR (GC/GG)=2.73	1.08-6.88	0.033
T2+/Ta, T1	2015	Zhou et al. [46]	UBC	UC	Chinese	156 (MIBC) / 176 (NMIBC)	Ser61Ala (rs17855750)	16p12.1-p11.2	IL27	G	T	0.07 (1KG)	OR (TG+GG/TT)=2.04	1.02–4.17	0.042
T2+ / Ta+T1	2010	Pandith et al. [47]	UBC	UC + Other	Indian	59 (T2+) / 49 (T1+T1)	Arg72Pro (rs1042522)	17p13.1	TP53	C	G	0.54 (1KG)	OR (CC+CG/GG)=4.2	1.5-11.3	0.004

T2+ / Ta+G1	2012	Mittal et al. [48]	UBC	Not reported	Indian	29 (T2+) / 16 (Ta+G1)	Asp312Asn (rs1799793)	19q13.32	XPD (ERCC2)	A	G	0.19 (1KG)	OR (AA/GG)=4.53	1.05-19.4	0.042
T2+	2018	Ahmed et al. [6]	MIBC	UC	Pakistani	50 (MIBC) / 99 (Controls)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (GG/AA)=3.06	1.31–7.13	<0.05
T2+	2018	Ahmed et al. [6]	MIBC	UC	Pakistani	65 (MIBC) / 184 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC/AA)=2.95	1.51–5.75	<0.05
T2+	2018	Ahmed et al. [6]	MIBC	UC	Pakistani	52 (MIBC) / 167 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (CC/AA)=3.18	1.32-7.66	<0.05
T2+	2018	Ahmed et al. [6]	MIBC	UC	Pakistani	152 (MIBC) / 400 (Controls) (Allele count instead of individuals)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (C/A)=2.02	1.36-3.01	<0.05
T2+	2018	Ahmed et al. [6]	MIBC	UC	Pakistani	57 (MIBC) / 136 (Controls)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	OR (GG/CC)=5.95	1.37-25.7	<0.05

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Kelsey KT, Park S, Nelson HH, Karagas MR. A Population-Based Case-Control Study of the Xrcc1 Arg399gln Polymorphism and Susceptibility to Bladder Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(8):1337-41.

[2] Safarinejad MR, Shafiei N, Safarinejad S. Genetic Susceptibility of Methylenetetrahydrofolate Reductase (Mthfr) Gene C677t, A1298c, and G1793a Polymorphisms with Risk for Bladder Transitional Cell Carcinoma in Men. Medical oncology (Northwood, London, England). 2011;28 Suppl 1:S398-412.

[3] Sobti RC, Kaur S, Sharma VL, Singh SK, Hosseini SA, Kler R. Susceptibility of Xpd and Rad51 Genetic Variants to Carcinoma of Urinary Bladder in North Indian Population. DNA and cell biology. 2012;31(2):199-210.

[4] Verhaegh GW, Verkleij L, Vermeulen SH, den Heijer M, Witjes JA, Kiemeney LA. Polymorphisms in the H19 Gene and the Risk of Bladder Cancer. Eur Urol. 2008;54(5):1118-26.

[5] Zhao T, Wu X, Liu J. Association between Interleukin-22 Genetic Polymorphisms and Bladder Cancer Risk. Clinics (Sao Paulo, Brazil). 2015;70(10):686-90.

[6] Ahmed T, Nawaz S, Noreen R, Bangash KS, Rauf A, Younis M, Anwar K, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R, Ali SHB. A 3' Untranslated Region Polymorphism Rs2304277 in the DNA Repair Pathway Gene Ogg1 Is a Novel Risk Modulator for Urothelial Bladder Carcinoma. Annals of human genetics. 2018;82(2):74-87.
[7] Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, Zhang Z. Cyclin D1 G870a Polymorphism Is Associated with Risk and Clinicopathologic Characteristics of Bladder Cancer. DNA and cell biology. 2010;29(10):611-7.
[8] Chen WC, Wu HC, Hsu CD, Chen HY, Tsai FJ. P21 Gene Codon 31 Polymorphism Is Associated with Bladder Cancer. Urologic oncology. 2002;7(2):63-6.
[9] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Influence of Xpd and Ape1 DNA Repair Gene Polymorphism on Bladder Cancer Susceptibility in North India. Urology. 2009;73(3):675-80.
[10] Wen H, Ding Q, Fang ZJ, Xia GW, Fang J. Population Study of Genetic Polymorphisms and Superficial Bladder Cancer Risk in Han-Chinese Smokers in Shanghai. International urology and nephrology. 2009;41(4):855-64.
[11] Ichimura Y, Habuchi T, Tsuchiya N, Wang L, Oyama C, Sato K, Nishiyama H, Ogawa O, Kato T. Increased Risk of Bladder Cancer Associated with a Glutathione Peroxidase 1 Codon 198 Variant. The Journal of urology. 2004;172(2):728-32.
[12] Ali SHB, Bangash KS, Rauf A, Younis M, Anwar K, Khurram R, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R. Identification of Novel Potential Genetic Predictors of Urothelial Bladder Carcinoma Susceptibility in Pakistani Population. Familial cancer. 2017;16(4):577-94.
[13] Lima L, Silva J, Amaro T, Morais A, Lopes C, Medeiros R, Videira PA, Santos L. Il-4 and Tnf-Alpha Polymorphisms Are Associated with Risk of Multiple Superficial Tumors or Carcinoma in Situ Development. Urologia internationalis. 2011;87(4):457-63.
[14] Ito M, Habuchi T, Watanabe J, Higashi S, Nishiyama H, Wang L, Tsuchiya N, Kamoto T, Ogawa O. Polymorphism within the Cyclin D1 Gene Is Associated with an Increased Risk of Carcinoma in Situ in Patients with Superficial Bladder Cancer. Urology. 2004;64(1):74-8.
[15] Pandith AA, Shah ZA, Khan NP, Baba KM, Wani MS, Siddiqi MA. Hras T81c Polymorphism Modulates Risk of Urinary Bladder Cancer and Predicts Advanced Tumors in Ethnic Kashmiri Population. Urologic oncology. 2013;31(4):487-92.
[16] Sakano S, Kumar R, Larsson P, Onelov E, Adolfsson J, Steineck G, Hemminki K. A Single-Nucleotide Polymorphism in the Xpg Gene, and Tumour Stage, Grade, and Clinical Course in Patients with Nonmuscle-Invasive Neoplasms of the Urinary Bladder. BJU international. 2006;97(4):847-51.
[17] Ahirwar DK, Mandhani A, Dharaskar A, Kesarwani P, Mittal RD. Association of Tumour Necrosis Factor-Alpha Gene (T-1031c, C-863a, and C-857t) Polymorphisms with Bladder Cancer Susceptibility and Outcome after Bacille Calmette-Guerin Immunotherapy. BJU international. 2009;104(6):867-73.
[18] Guey LT, Garcia-Closas M, Murta-Nascimento C, Lloreta J, Palencia L, Kogevinas M, Rothman N, Vellalta G, Calle ML, Marenne G, Tardon A, Carrato A, Garcia-Closas R, Serra C, Silverman DT, Chanock S, Real FX, Malats N. Genetic Susceptibility to Distinct Bladder Cancer Subphenotypes. Eur Urol. 2010;57(2):283-92.

[19] Gangwar R, Mittal RD. Association of Selected Variants in Genes Involved in Cell Cycle and Apoptosis with Bladder Cancer Risk in North Indian Population. DNA and cell biology. 2010;29(7):349-56.
[20] Deng S, Wang W, Li X, Zhang P. Common Genetic Polymorphisms in Pre-Micrornas and Risk of Bladder Cancer. World journal of surgical oncology. 2015;13:297.
[21] Safarinejad MR, Shafiei N, Safarinejad SH. The Association between Bladder Cancer and a Single Nucleotide Polymorphism (Rs2854744) in the Insulin-Like Growth Factor (Igf)-Binding Protein-3 (Igfbp-3) Gene. Archives of toxicology. 2011;85(10):1209-18.
[22] Lin HH, Ke HL, Hsiao KH, Tsai CW, Wu WJ, Bau DT, Chang LL. Potential Role of Ccnd1 G870a Genotype as a Predictor for Urothelial Carcinoma Susceptibility and Muscle-Invasiveness in Taiwan. The Chinese journal of physiology. 2011;54(3):196-202.
[23] Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Association of Genetic Polymorphism of Glutathione S-Transferase (Gstm1, Gstt1, Gstp1) with Bladder Cancer Susceptibility. Urologic oncology. 2013;31(7):1193-203.
[24] Gangwar R, Mandhani A, Mittal RD. Caspase 9 and Caspase 8 Gene Polymorphisms and Susceptibility to Bladder Cancer in North Indian Population. Annals of surgical oncology. 2009;16(7):2028-34.
[25] Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of Il-12, Il-18 Variants and Serum Il-18 with Bladder Cancer Susceptibility in North Indian Population. Gene. 2013;519(1):128-34.
[26] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.
[27] Sanyal S, Ryk C, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Nqo1 and the Clinical Course of Urinary Bladder Neoplasms. Scandinavian journal of urology and nephrology. 2007;41(3):182-90.
[28] Wang S, Tang J, Wang M, Yuan L, Zhang Z. Genetic Variation in Psca and Bladder Cancer Susceptibility in a Chinese Population. Carcinogenesis. 2010;31(4):621-4.
[29] Zhou B, Zhang P, Wang Y, Shi S, Zhang K, Liao H, Zhang L. Interleukin-17 Gene Polymorphisms Are Associated with Bladder Cancer in a Chinese Han Population. Molecular carcinogenesis. 2013;52(11):871-8.
[30] Chu H, Wang M, Jin H, Lv Q, Wu D, Tong N, Ma L, Shi D, Zhong D, Fu G, Yuan L, Qin C, Yin C, Zhang Z. Egfr 3'utr 774t>C Polymorphism Contributes to Bladder Cancer Risk. Mutagenesis. 2013;28(1):49-55.
[31] Hua Q, Lv X, Gu X, Chen Y, Chu H, Du M, Gong W, Wang M, Zhang Z. Genetic Variants in Lncrna H19 Are Associated with the Risk of Bladder Cancer in a Chinese Population. Mutagenesis. 2016;31(5):531-8.
[32] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Do DNA Repair Genes Ogg1, Xrcc3 and Xrcc7 Have an Impact on Susceptibility to Bladder Cancer in the North Indian Population? Mutation research. 2009;680(1-2):56-63.
[33] Lin HY, Huang CH, Yu TJ, Wu WJ, Yang MC, Lung FW. P53 Codon 72 Polymorphism as a Progression Index for Bladder Cancer. Oncology reports. 2012;27(4):1193-9.

[34] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.
[35] Kader AK, Liu J, Shao L, Dinney CP, Lin J, Wang Y, Gu J, Grossman HB, Wu X. Matrix Metalloproteinase Polymorphisms Are Associated with Bladder Cancer Invasiveness. Clinical cancer research : an official journal of the American Association for Cancer Research. 2007;13(9):2614-20.
[36] Kucukgergin C, Sanli O, Amasyali AS, Tefik T, Seckin S. Genetic Variants of Mnsod and Gpx1 and Susceptibility to Bladder Cancer in a Turkish Population. Medical oncology (Northwood, London, England). 2012;29(3):1928-34.
[37] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.
[38] Kucukgergin C, Isman FK, Dasdemir S, Cakmakoglu B, Sanli O, Gokkusu C, Seckin S. The Role of Chemokine and Chemokine Receptor Gene Variants on the Susceptibility and Clinicopathological Characteristics of Bladder Cancer. Gene. 2012;511(1):7-11.
[39] Wen H, Feng CC, Fang ZJ, Xia GW, Jiang HW, Xu G, Huang XD, Ding Q. Study on Bladder Cancer Susceptibility and Genetic Polymorphisms of Xpc, Xpg, and Cyp in Smokers and Non-Smokers. Actas urologicas espanolas. 2013;37(5):259-65.
[40] Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y, Song Y, Zhang L. Genetic Variants in Nampt Predict Bladder Cancer Risk and Prognosis in Individuals from Southwest Chinese Han Group. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4031-40.
[41] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[42] Weng WC, Huang YH, Yang SF, Wang SS, Kuo WH, Hsueh CW, Huang CH, Chou YE. Effect of Cd44 Gene Polymorphisms on Risk of Transitional Cell Carcinoma of the Urinary Bladder in Taiwan. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;37(5):6971-7.
[43] Mittal RD, Singh R, Manchanda PK, Ahirwar D, Gangwar R, Kesarwani P, Mandhani A. Xrcc1 Codon 399 Mutant Allele: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Cancer biology & therapy. 2008;7(5):645-50.
[44] Gangwar R, Mandhani A, Mittal RD. Xpc Gene Variants: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Journal of cancer research and clinical oncology. 2010;136(5):779-86.
[45] Gangwar R, Mandhani A, Mittal RD. Functional Polymorphisms of Cyclooxygenase-2 (Cox-2) Gene and Risk for Urinary Bladder Cancer in North India. Surgery. 2011;149(1):126-34.

[46] Zhou B, Zhang P, Tang T, Liao H, Zhang K, Pu Y, Chen P, Song Y, Zhang L. Polymorphisms and Plasma Levels of Il-27: Impact on Genetic Susceptibility and Clinical Outcome of Bladder Cancer. BMC cancer. 2015;15:433.
[47] Pandith AA, Shah ZA, Khan NP, Rasool R, Afroze D, Yousuf A, Wani S, Siddiqi M. Role of Tp53 Arg72pro Polymorphism in Urinary Bladder Cancer Predisposition and Predictive Impact of Proline Related Genotype in Advanced Tumors in an Ethnic Kashmiri Population. Cancer genetics and cytogenetics. 2010;203(2):263-8.
[48] Mittal RD, Mandal RK. Genetic Variation in Nucleotide Excision Repair Pathway Genes Influence Prostate and Bladder Cancer Susceptibility in North Indian Population. Indian journal of human genetics. 2012;18(1):47-55.

Supplementary Table 3.5. Summary associations for previously reported SNPs on tumour grade at the time of diagnosis for urinary bladder cancer.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations for G1 and G2 (or combinations of these):															
Associations reducing the risk of G1 and G2 (or combinations of these):															
G1+G2	2011	Safarinejad et al. [1]	UBC (men only)	UC	Iranian	115 (G1+G2)	Ala222Val (rs1801133)	1p36.22	MTHFR	T	C	0.25 (1KG)	OR (T/C)=0.46	0.35–0.66	0.001
G1+G2	2011	Safarinejad et al. [1]	UBC (men only)	UC	Iranian	115 (G1+G2)	Glu470Ala (rs1801131)	1p36.22	MTHFR	C	A	0.25 (1KG)	OR (C/A)=0.45	0.26-0.70	0.001
G2	2011	Sobti et al. [2]	UBC	Not reported	Indian	6 (CC) / 92 (GG)	rs1801320	15q15.1	RAD51	C	G	0.26 (Cases) / 0.31 (Controls) / 0.14 (1KG)	OR (CC/GG)=0.24	0.09–0.62	0.0008
Low grade UBC	2015	Xie et al. [3]	UBC	UC	Chinese	211 (Low grade) / 649 (Controls)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (TG+GG/TT)=0.613	0.427-0.881	0.008
Associations increasing the risk of G1 and G2 (or combinations of these):															
G1	2010	Yuan et al. [4]	UBC	UC	Chinese	164 (G1) / 402 (Healthy controls)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.41 (1KG)	OR (AG+AA/GG)=1.81	1.10-2.97	0.019
G1	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	827 (G1) / 5,117 (Healthy controls)	rs798766	4p16.3	TMEM129 TACC3-FGFR3	T	C	0.24 (1KG)	OR (T/C)=1.27	1.11-1.44	3.00E-04
G1	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	826 (G1) / 5,117 (Healthy controls)	rs401681	5p15.33	CLPTM1L	C	T	0.59 (1KG)	OR (C/T)=1.22	1.09-1.36	3.20E-04

G1	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	825 (G1) / 5,108 (Healthy controls)	rs9642880	8q24.21	MYC	T	G	0.54 (1KG)	OR (T/G)=1.20	1.08-1.34	7.50E-04
G1 / G2+Ta	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	1,147 (G2) / 2,539 (Healthy controls)	rs798766	4p16.3	TMEM129 TACC3-FGFR3	T	C	0.24 (1KG)	OR (T/C)=1.28	1.14-1.45	4.80E-05
G1 / G2+Ta	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	1,145 (Low-risk) / 2,532 (Healthy controls)	rs9642880	8q24.21	MYC	T	G	0.54 (1KG)	OR (T/G)=1.25	1.13-1.38	8.60E-06
G1 / Healthy controls	2008	Gangwar et al. [6]	UBC	UC	Indian	64 (G1) / 232 (Healthy controls) 36 (G1) / 146 (Healthy controls)	Asp312Asn (rs1799793)	19q13.3	ERCC2	A	G	0.19 (1KG)	OR (AG/GG)=2.51 OR (AA/GG)=5.21	1.39-4.54 2.23-12.1	0.006 0.003
G1+G2	2011	Safarinejad et al. [1]	UBC (men only)	UC	Iranian	115 (G1+G2)	Arg594Gln (rs2274976)	1p36.22	MTHFR	A	G	0.07 (1KG)	OR (G/A)=3.32	2.25-5.46	0.001
G2	2010	Wang et al. [7]	UBC	UC	Chinese	123 (Invasive) / 106 (Superficial)	rs2294008	8q24.3	PSCA	T	C	0.60 (1KG)	OR (CT+TT/CC)=1.43	1.05-1.96	0.024
G2	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	856 (G2) / 5,117 (Healthy controls)	rs798766	4p16.3	TMEM129 TACC3-FGFR3	T	C	0.24 (1KG)	OR (T/C)=1.21	1.07-1.37	2.80E-03
G2	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	825 (G2) / 5,108 (Healthy controls)	rs9642880	8q24.21	MYC	T	G	0.54 (1KG)	OR (T/G)=1.22	1.10-1.36	2.10E-04
G2 / Healthy controls	2008	Gangwar et al. [6]	UBC	UC	Indian	45 (G2) / 146 (Healthy controls)	Asp312Asn (rs1799793)	19q13.3	ERCC2	A	G	0.19 (1KG)	OR (AA/GG)=4.67	1.72-12.6	0.002
G1+G2+papilloma / Healthy controls	2017	Ali et al. [8]	UBC	UC	Pakistani	133 (G2+G1) / 200 (Healthy controls)	Gln223Arg (rs1137101)	1p31.3	LEPR	A	G	0.52 (Cases) / 0.42 (Controls) / 0.42 (1KG)	OR (G/A)=1.4	1.1–2	<0.05

G1	2018	Ahmed et al. [9]	UBC	UC	Pakistani	61 (Low grade UBC) / 99 (Controls)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (GG/AA) = 3.73	1.72–8.09	<0.05
G1	2018	Ahmed et al. [9]	UBC	UC	Pakistani	234 (Low grade UBC) / 400 (Controls) (Allele count instead of individuals)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (G/A)=1.72	1.20-2.46	<0.05
G1	2018	Ahmed et al. [9]	UBC	UC	Pakistani	100 (Low grade UBC) / 184 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC/AA)=2.18	1.21–3.92	<0.05
G1	2018	Ahmed et al. [9]	UBC	UC	Pakistani	87 (Low grade UBC) / 167 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (CC/AA)=2.55	1.20-5.44	<0.05
G1	2018	Ahmed et al. [9]	UBC	UC	Pakistani	234 (Low grade UBC) / 400 (Controls) (allele count instead of individuals)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (C/A)=1.72	1.22-2.44	<0.05
Low grade UBC	2018	Li et al. [10]	UBC	Not reported	Chinese	127 (Low grade UBC) / 167 (High grade UBC)	rs4758680	12q24.31	IL31	A	C	0.29 (1KG)	OR (CA+AA/CC)=2.34	1.25-4.40	0.007
Associations for G3 (or other definitions of High grade):															
Associations reducing the risk of G3 (or High grade):															
G3	2011	Safarinejad et al. [1]	UBC (men only)	UC	Iranian	43 (G3)	Arg594Gln (rs2274976)	1p36.22	MTHFR	A	G	0.07 (1KG)	OR (G/A)=0.55	0.44–0.72	0.014
G3 / G2, G1? (Not explicitly stated)	2016	Wu et al. [11]	UBC	Not reported	Chinese	180 (G3) / 153 (G2+G1)	rs353293	5q32	MIR143 (microRNA 143) / CARMN	A	G	10.1 (all UBC cases) / 0.26 (1KG)	OR (A/G)=0.54	0.32–0.92	0.02

G3 / G1+G2	2011	Safarineja d et al. [12]	UBC	UC	Iranian	46 (G3) / 94 (G1+G2) 31 (G3) / 44 (G1+G2)	rs2854744	7p12.3	IGFBP3	A	C	0.39 (UBC) / 0.49 (Healthy controls) / 0.47 (1KG)	OR (AC/CC)= 0.34 OR (AA/CC)= 0.21	0.24–0.68 0.12–0.42	0.0001 0.00001
G3 / G1+G2	2013	Safarineja d et al. [13]	UBC	UC	Iranian	47 (G3) / 119 (G1+G2))	Ile105Val (rs1695)	11q13.2	GSTP1	G	A	0.35 (1KG)	OR (AG+GG/AA) = 0.62	0.48-0.79	0.002
G3 / Healthy controls	2017	Ali et al. [8]	UBC	UC	Pakistani	67 (High grade) / 200 (Healthy controls)	rs2854744	7p12.3	IGFBP3	A	C	0.44 (Cases) / 0.51 (Controls) / 0.53 (1KG)	OR (CA/CC)=0.5	0.3–0.98	<0.05
High grade (G2B+G3) NMIBC / Low grade (G1+G2A) NMIBC (>71 year-old group)	2006	Sakano et al. [14]	NMIBC	UC	European (Sweden)	48 (High grade) / 64 (Low-group)	Asp1104His (rs17655)	13q33.1	XPG (ERCC5)	C	G	0.36 (1KG)	OR (GC+CC/GG) =0.3	0.1-0.7	0.004
Associations increasing the risk of G3 (or High grade):															
G3	2010	Rothman et al. [5]	UBC	Not reported	Multi-ethnic	1.338 (G3) / 6,170 (Healthy controls)	rs8102137	19q12	CCNE1	C	T	0.16 (1KG)	OR (C/T)=1.23	1.13-1.35	4.60E-06
G3	2011	Safarineja d et al. [1]	UBC (men only)	UC	Iranian	43 (G3)	Ala222Val (rs1801133)	1p36.22	MTHFR	T	C	0.25 (1KG)	OR (T/C)=3.55	2.32–5.41	0.001
G3	2011	Safarineja d et al. [1]	UBC (men only)	UC	Iranian	43 (G3)	Glu470Ala (rs1801131)	1p36.22	MTHFR	C	A	0.25 (1KG)	OR (C/A)=3.67	2.37–5.71	0.001

G3	2013	Pandith et al. [15]	UBC	UC	Indian	31 (TC+CC) / 31 (TT)	His27His (rs12628)	11p15.5	HRAS	C	T	0.30 (1KG)	OR (TC+CC/TT)=5.4	2.8–10.2	<0.001
G3	2016	Hua et al. [16]	UBC	UC	Chinese	31 (AA) / 132 (GG+GA)	rs217727	11p15.5	H19 (lncRNA)	A	G	0.36 / 0.20 (1KG)	OR (AA / GG+GA)=1.89	1.23–2.91	0.004
G3 / G1+G2	2015	Timirci-Kahraman et al. [17]	UBC	UC	Turkish	28 (G3) / 52 (G1+G2)	Arg209Thr (rs4871857, has merged into rs20575)	8p21.3	DR4 (TNFRSF10A)	G	C	0.58 (1KG)	OR (GG/CG+CC)=2.13	1.031–4.397	0.036
G3 / G1, G2	2002	Wang et al. [18]	UBC	UC	Japanese	80 (G3) /138 (G2+G1)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.6 (Cases) / 0.45 (Controls) / 0.41 (1KG)	OR (A/G)=1.77	1.16-2.69	0.008
G3 / G1+G2	2012	Kucukgergin et al. [19]	UBC	UC	Turkish	70 (G3) / 72 (G1+G2)	Val64Ile (rs1799864)	3p21.31	CCR2	A	G	0.15 (1KG)	OR (AA/GG)=3.09 OR (AG+AA/GG)=1.58	1.02–9.34 1.07–2.32	0.045 0.020
G3 / G1+G2	2014	Zhou et al. [20]	UBC (excluding Tis)	UC	Chinese	188 (G3) / 104 (G1+G2)	rs3756712	5p15.33	PDCD6	G	T	0.40 (1KG)	OR (GT/TT+GG)=2.38 OR (GG/GT+TT)=3.57 OR (TT+GG/GG)=1.89	1.35–4.17 1.03–12.50 1.05–3.33	<0.05
G3 / G1+G2	2014	Zhou et al. [20]	UBC (excluding Tis)	UC	Chinese	188 (G3) / 104 (G1+G2)	rs4957014	5p15.33	PDCD6	G	T	0.65 (1KG)	OR (GT+GG/TT)=1.92	1.14–3.33	<0.05
G3 / G1+G2	2016	Gautam et al. [21]	UBC	UC	Indo-European	47 (G3) / 185 (G1+G2)	rs1800795	7p15.3	IL6	C	G	0.14 (1KG)	Chi-square = 10.59	N/A	0.032

					(Caucasian)										
G3 / Healthy controls	2014	Gu et al. [22]	UBC	Not reported	Chinese	117 (G3) / 670 (Healthy subjects)	rs2664139	15q14	TSP-1 (THBS1)	C	T	0.43 (1KG)	OR (CC/TT+CT)= 1.84	1.00-3.36	0.049
G3 / Healthy controls	2017	Ali et al. [8]	UBC	UC	Pakistani	67 (High grade) / 200 (Healthy controls)	Pro12Ala (rs1801282)	3p25.2	PPARG	G	C	0.19 (Cases) / 0.12 (Controls) / 0.07 (1KG)	OR (GG/CC)=5.97	1.3–26	<0.05
High grade (G2+G3+G4) / Low grade (G1+G2A)	2007	Sanyal et al. [23]	UBC	Not reported	European (Sweden)	150 (High grade) / 110 (Low grade)	Gly39Glu (rs1042821)	2p16.3	MSH6	A	G	0.20 (1KG)	RR (AG+AA/GG) =1.7	1.0-3.0	0.05
High grade (G2B+G3) NMIBC / Low grade (G1+G2A) NMIBC (patients ≤71 years)	2006	Sakano et al. [14]	NMIBC	UC	European (Sweden)	56 (High grade) / 60 (Low grade)	Asp1104His (rs17655)	13q33.1	XPG (ERCC5)	C	G	0.36 (1KG)	OR (GC+CC/GG) =3.3	1.5-7.3	0.003
G2+G3	2018	Ahmed et al. [9]	UBC	UC	Pakistani	54 (High grade UBC) / 99 (Controls)	rs2304277	3p25.3	OGG1	G	A	0.65 (1KG)	OR (GG/AA)=3.45	1.52–7.80	<0.05
G2+G3	2018	Ahmed et al. [9]	UBC	UC	Pakistani	72 (High grade UBC) / 184 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (AC/AA)=2.81	1.48–5.33	<0.05

G2+G3	2018	Ahmed et al. [9]	UBC	UC	Pakistani	57 (High grade UBC) / 167 (Controls)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (CC/AA)=2.58	1.09-6.11	<0.05
G2+G3	2018	Ahmed et al. [9]	UBC	UC	Pakistani	166 (High grade UBC) / 400 (Controls) (Allele count instead of individuals)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	OR (C/A)=1.86	1.27-2.73	<0.05
G3+G4 / G1+G2	2010	Pandith et al. [24]	UBC	UC + 1 adenocarcinoma	Indian	56 (G3+G4)/ 52 (G1+G2)	Arg72Pro (rs1042522)	17p13.1	TP53	C	G	0.54 (1KG)	OR (CC+CG/GG) =4.6	1.4-15.6	0.005

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Safarinejad MR, Shafiei N, Safarinejad S. Genetic Susceptibility of Methylenetetrahydrofolate Reductase (Mthfr) Gene C677t, A1298c, and G1793a Polymorphisms with Risk for Bladder Transitional Cell Carcinoma in Men. Medical oncology (Northwood, London, England). 2011;28 Suppl 1:S398-412.

[2] Sobti RC, Kaur S, Sharma VL, Singh SK, Hosseini SA, Kler R. Susceptibility of Xpd and Rad51 Genetic Variants to Carcinoma of Urinary Bladder in North Indian Population. DNA and cell biology. 2012;31(2):199-210.

[3] Xie L, Sun Y, Chen T, Tian D, Li Y, Zhang Y, Ding N, Shen Z, Xu H, Nian X, Sha N, Han R, Hu H, Wu C. Association between Mdm2 Snp309 T>G Polymorphism and the Risk of Bladder Cancer: New Data in a Chinese Population and an Updated Meta-Analysis. OncoTargets and therapy. 2015;8:3679-90.

[4] Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, Zhang Z. Cyclin D1 G870a Polymorphism Is Associated with Risk and Clinicopathologic Characteristics of Bladder Cancer. DNA and cell biology. 2010;29(10):611-7.

[5] Rothman N, Garcia-Closas M, Chatterjee N, Malats N, Wu X, Figueroa JD, Real FX, Van Den Berg D, Matullo G, Baris D, Thun M, Kiemeney LA, Vineis P, De Vivo I, Albanes D, Purdue MP, Rafnar T, Hildebrandt MA, Kiltie AE, Cussenot O, Golka K, Kumar R, Taylor JA, Mayordomo JI, Jacobs KB, Kogevinas M, Hutchinson A, Wang Z, Fu YP, Prokunina-Olsson L, Burdett L, Yeager M, Wheeler W, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Johnson A, Schwenn M, Karagas MR, Schned A, Andriole G, Jr., Grubb R, 3rd, Black A, Jacobs EJ, Diver WR, Gapstur SM, Weinstein SJ, Virtamo J, Cortessis VK, Gago-Dominguez M, Pike MC, Stern MC, Yuan JM, Hunter DJ, McGrath M, Dinney CP, Czerniak B, Chen M, Yang H, Vermeulen SH, Aben KK, Witjes JA, Makkinje RR, Sulem P, Besenbacher S, Stefansson K, Riboli E, Brennan P, Panico S, Navarro C, Allen NE, Bueno-de-Mesquita HB, Trichopoulos D, Caporaso N, Landi MT, Canzian F, Ljungberg B, Tjonneland A, Clavel-Chapelon F, Bishop DT, Teo MT, Knowles MA, Guarrera S, Polidoro S, Ricceri F, Sacerdote C, Allione A, Cancel-Tassin G, Selinski S,

Hengstler JG, Dietrich H, Fletcher T, Rudnai P, Gurzau E, Koppova K, Bolick SC, Godfrey A, Xu Z, Sanz-Velez JI, M DG-P, Sanchez M, Valdivia G, Porru S, Benhamou S, Hoover RN, Fraumeni JF, Jr., Silverman DT, Chanock SJ. A Multi-Stage Genome-Wide Association Study of Bladder Cancer Identifies Multiple Susceptibility Loci. Nature genetics. 2010;42(11):978-84.
[6] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Influence of Xpd and Ape1 DNA Repair Gene Polymorphism on Bladder Cancer Susceptibility in North India. Urology. 2009;73(3):675-80.
[7] Wang S, Tang J, Wang M, Yuan L, Zhang Z. Genetic Variation in Psca and Bladder Cancer Susceptibility in a Chinese Population. Carcinogenesis. 2010;31(4):621-4.
[8] Ali SHB, Bangash KS, Rauf A, Younis M, Anwar K, Khurram R, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R. Identification of Novel Potential Genetic Predictors of Urothelial Bladder Carcinoma Susceptibility in Pakistani Population. Familial cancer. 2017;16(4):577-94.
[9] Ahmed T, Nawaz S, Noreen R, Bangash KS, Rauf A, Younis M, Anwar K, Khawaja MA, Azam M, Qureshi AA, Akhter S, Kiemeney LA, Qamar R, Ali SHB. A 3' Untranslated Region Polymorphism Rs2304277 in the DNA Repair Pathway Gene Ogg1 Is a Novel Risk Modulator for Urothelial Bladder Carcinoma. Annals of human genetics. 2018;82(2):74-87.
[10] Li Q, Tang T, Zhang P, Liu C, Pu Y, Zhang Y, Song H, Wang Y, Song Y, Su M, Zhou B, Zhang L. Correlation of Il-31 Gene Polymorphisms with Susceptibility and Clinical Recurrence of Bladder Cancer. Familial cancer. 2018;17(4):577-85.
[11] Wu J, Huang Q, Meng D, Huang M, Li C, Qin T. A Functional Rs353293 Polymorphism in the Promoter of Mir-143/145 Is Associated with a Reduced Risk of Bladder Cancer. PloS one. 2016;11(7):e0159115.
[12] Safarinejad MR, Shafiei N, Safarinejad SH. The Association between Bladder Cancer and a Single Nucleotide Polymorphism (Rs2854744) in the Insulin-Like Growth Factor (Igf)-Binding Protein-3 (Igfbp-3) Gene. Archives of toxicology. 2011;85(10):1209-18.
[13] Safarinejad MR, Safarinejad S, Shafiei N, Safarinejad S. Association of Genetic Polymorphism of Glutathione S-Transferase (Gstm1, Gstt1, Gstp1) with Bladder Cancer Susceptibility. Urologic oncology. 2013;31(7):1193-203.
[14] Sakano S, Kumar R, Larsson P, Onelov E, Adolfsson J, Steineck G, Hemminki K. A Single-Nucleotide Polymorphism in the Xpg Gene, and Tumour Stage, Grade, and Clinical Course in Patients with Nonmuscle-Invasive Neoplasms of the Urinary Bladder. BJU international. 2006;97(4):847-51.
[15] Pandith AA, Shah ZA, Khan NP, Baba KM, Wani MS, Siddiqi MA. Hras T81c Polymorphism Modulates Risk of Urinary Bladder Cancer and Predicts Advanced Tumors in Ethnic Kashmiri Population. Urologic oncology. 2013;31(4):487-92.
[16] Hua Q, Lv X, Gu X, Chen Y, Chu H, Du M, Gong W, Wang M, Zhang Z. Genetic Variants in Lncrna H19 Are Associated with the Risk of Bladder Cancer in a Chinese Population. Mutagenesis. 2016;31(5):531-8.
[17] Timirci-Kahraman O, Ozkan NE, Turan S, Farooqi AA, Verim L, Ozturk T, Inal-Gultekin G, Isbir T, Ozturk O, Yaylim I. Genetic Variants in the Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand and Death Receptor Genes Contribute to Susceptibility to Bladder Cancer. Genetic testing and molecular biomarkers. 2015;19(6):309-15.

[18] Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, Kakehi Y, Kakinuma H, Sato K, Nakamura A, Ogawa O, Kato T. Cyclin D1 Gene Polymorphism Is Associated with an Increased Risk of Urinary Bladder Cancer. Carcinogenesis. 2002;23(2):257-64.
[19] Kucukgergin C, Isman FK, Dasdemir S, Cakmakoglu B, Sanli O, Gokkusu C, Seckin S. The Role of Chemokine and Chemokine Receptor Gene Variants on the Susceptibility and Clinicopathological Characteristics of Bladder Cancer. Gene. 2012;511(1):7-11.
[20] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[21] Gautam KA, Muktanand T, Sankhwar SN, Goel A, Sankhwar PL, Rajender S. Functional Polymorphisms in the Il6 Gene Promoter and the Risk of Urinary Bladder Cancer in India. Cytokine. 2016;77:152-6.
[22] Gu J, Tao J, Yang X, Li P, Yang X, Qin C, Cao Q, Cai H, Zhang Z, Wang M, Gu M, Lu Q, Yin C. Effects of Tsp-1-696 C/T Polymorphism on Bladder Cancer Susceptibility and Clinicopathologic Features. Cancer genetics. 2014;207(6):247-52.
[23] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.
[24] Pandith AA, Shah ZA, Khan NP, Rasool R, Afroze D, Yousuf A, Wani S, Siddiqi M. Role of Tp53 Arg72pro Polymorphism in Urinary Bladder Cancer Predisposition and Predictive Impact of Proline Related Genotype in Advanced Tumors in an Ethnic Kashmiri Population. Cancer genetics and cytogenetics. 2010;203(2):263-8.

Supplementary Table 3.6. Summary associations for previously reported SNPs on urinary bladder cancer risk group at the time of diagnosis.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations for low-risk bladder cancer:															
Associations reducing the risk of low-risk bladder cancer:															
Low-risk (TaG2)	2007	Sanyal et al. [1]	UBC	Not reported	European (Sweden)	31 (AG+AA) / 95 (GG)	Gly39Glu (rs1042821)	2p16.3	MSH6	A	G	0.20 (1KG)	RR (AG+AA/GG)=0.7	0.5-0.9	0.009
Low-risk (TaG2)	2007	Sanyal et al. [2]	UBC	Not reported	European (Sweden)	28 (CT+TT) / 98 (CC)	Pro187Ser (rs1800566)	16q22.1	NQO1	T	C	0.29 (1KG)	RR (CT+TT/CC)=0.7	0.5-0.9	0.005
Associations increasing the risk of low-risk bladder cancer:															
Low-risk (TaG1)	2007	Sanyal et al. [1]	UBC	Not reported	European (Sweden)	9 (TC+CC) / 2 (TT)	His27His (rs17350793, merged into rs12628)	11p15.5	HRAS	C	T	0.30 (1KG)	RR (TC+CC/TT)=5.0	1.1-22.2	0.02
Low-risk NMIBC (Ta+G1/G2)	2009	Wang et al. [3]	NMIBC	UC	Chinese	345 (Low-risk) / 465 (Healthy controls)	rs9642880	8q24.21	CASC11	T	G	0.54 (1KG)	OR(TG+TT/GG)=1.71	1.28-2.29	0.0002
Low-risk NMIBC (Ta+G1/G2)	2009	Stern et al. [4]	NMIBC (Ta+G1-2)	UC	Caucasian (Northern American)	274 (Low-risk) / 578 (Healthy controls)	rs710521	3q28	TP63	A	G	0.80 (1KG)	OR (A/G)=1.49	1.17-1.91	0.002
Low-risk NMIBC (Ta+G1/G2) / High-risk (Tis/T1/G3)	2010	Kiemeney et al. [5]	UBC	UC	European (multiple)	1559 (Low-risk) / 1875 (High-risk)	rs798766	4p16.3	TACC3/FGFR3	T	C	0.25 (Low-risk) / 0.22 (High-risk) / 0.24 (1KG)	OR (T)=1.17	1.04-1.31	0.009
Associations for high-risk bladder cancer:															
Associations reducing the risk of high-risk bladder cancer:															

High-risk NMIBC (TaG2-3+T1G1-3/ TaG1)	2010	Gangwar et al. [6]	UBC	UC	Indian	64 (High-risk NMIBC) / 25 (TaG1)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (GG/TT)=0.22	0.07-0.65	0.006
TaG2-G3+T1G1-G3 / TaG1	2013	Jaiswal et al. [7]	NMIBC	UC	Indian	106 (High-risk) / 33 (Low-risk)	rs1946518	11q23.1	IL18	A	C	0.41 (1KG)	OR (CA/CC)=0.44	0.19-0.97	0.042
TaG2-G3+T1G1-G3 / TaG1	2013	Jaiswal et al. [7]	NMIBC	UC	Indian	109 (High-risk) / 36 (Low-risk)	rs187238	11q23.1	IL18	C	G	0.79 (1KG)	OR (GC/GG)=0.50	0.22-1.12	0.042
High-risk NMIBC (TaG2-3+T1G1-3/ TaG1)	2009	Gangwar et al. [8]	NMIBC	UC	Indian	91 (High-risk NMIBC) / 34 (TaG1)	rs4645978	1p36.21	CASP9	G	A	0.42 (1KG)	OR (AG/AA)=0.39	0.15-0.96	0.042
High-risk NMIBC (TaG2-G3 + T1G1-3) / Low-risk NMIBC (TaG1)	2011	Jaiswal et al. [9]	NMIBC	Not reported	Indian	112 (High-risk) / 37 (Low-risk)	Arg248Leu (rs11540652)	17p13.1	TP53	A/C/T	G	T=0.00003 (TOPMED)	OR ((Arg/Trp, Arg/Gln) + (Trp/Trp, Arg/Arg))=0.32 ARG-G/GLN-A	0.15-0.69	0.003
G2+G3 with T1-T4 / G1+G2 with Ta	2011	Ratanajaraya et al. [10]	UBC	Not reported	Japanese	207 (Invasive) / 171 (Non-invasive)	rs17650301	17q23.3	POLG2	C	A	0.39 (Cases) / 0.29 (Controls) / 0.14 (1KG)	OR (A/C)=1.53	1.13–2.08	9.50E-03
High grade NMIBC (Ta+G3/T1+G2/T1+G3)	2009	Guey et al. [11]	NMIBC	UC	European (Spain)	219 (Only reported overall)	Lys1132Asn (rs1801406)	13q13.1	BRCA2	G?	A/C?	G=0.27 (1KG)	OR (G?/A?C?)=1.36	1.08-1.72	0.01
High grade NMIBC (TaG3+T1G2-3)	2009	Guey et al. [11]	NMIBC	UC	European (Spain)	219 (Only reported overall)	rs828702	2q35	XRCC5	G	A	0.45 (1KG)	OR (G/A)=1.28	1.03-1.58	0.026

High-risk (TaG3+T1)	2007	Sanyal et al. [1]	UBC	Not reported	European (Sweden)	25 (AG+AA) / 31 (GG)	Gly39Glu (rs1042821)	2p16.3	MSH6	A	G	0.20 (1KG)	RR (AG+AA/GG)=1.8	1.1-2.9	0.02
High-risk NMIBC (TaG2-3+T1G1-3) / Healthy controls	2009	Gangwar et al. [12]	UBC	UC	Indian	60 (High-risk NMIBC) / 139 (Heathy controls)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	OR (GG/TT)=2.46	1.1.0-5.48	0.027
High-risk NMIBC (TaG2-3+T1G1-3) / Healthy controls	2009	Gangwar et al. [12]	NMIBC	UC	Indian	59 (High-risk NMIBC) / 196 (Heathy controls) 63 (High-risk NMIBC) / 134 (Healthy controls) 59 (High-risk NMIBC) / 196 (Healthy controls)	rs7003908	8q11.21	XRCC7 (PRKDC)	G	T	0.33 (1KG)	OR (GT/TT)=3.38 OR (GG/TT)=8.00 OR (GG/GT+TT)=3.16	1.61-7.06 3.74-17.10 1.94-5.13	0.001 <0.001 <0.001
High-risk NMIBC (TaG2-3+T1G1-3/ TaG1)	2010	Gangwar et al. [6]	UBC	UC	Indian	73 (High-risk NMIBC) / 16 (TaG1)	Pro870Pro (rs9344)	11q13.3	CCND1	A	G	0.41 (1KG)	OR (AA/GG)=4.55	1.34-15.4	0.015

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.

[2] Sanyal S, Ryk C, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Nqo1 and the Clinical Course of Urinary Bladder Neoplasms. Scandinavian journal of urology and nephrology. 2007;41(3):182-90.

[3] Wang M, Wang M, Zhang W, Yuan L, Fu G, Wei Q, Zhang Z. Common Genetic Variants on 8q24 Contribute to Susceptibility to Bladder Cancer in a Chinese Population. Carcinogenesis. 2009;30(6):991-6.

[4] Stern MC, Van Den Berg D, Yuan JM, Conti DV, Gago-Dominguez M, Pike MC, Xiang YB, Gao YT, Cortessis VK. Sequence Variant on 3q28 and Urinary Bladder Cancer Risk: Findings from the Los Angeles-Shanghai Bladder Case-Control Study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(11):3057-61.
[5] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.
[6] Gangwar R, Mittal RD. Association of Selected Variants in Genes Involved in Cell Cycle and Apoptosis with Bladder Cancer Risk in North Indian Population. DNA and cell biology. 2010;29(7):349-56.
[7] Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of Il-12, Il-18 Variants and Serum Il-18 with Bladder Cancer Susceptibility in North Indian Population. Gene. 2013;519(1):128-34.
[8] Gangwar R, Mandhani A, Mittal RD. Caspase 9 and Caspase 8 Gene Polymorphisms and Susceptibility to Bladder Cancer in North Indian Population. Annals of surgical oncology. 2009;16(7):2028-34.
[9] Jaiswal PK, Goel A, Mittal RD. Association of P53 Codon 248 (Exon7) with Urinary Bladder Cancer Risk in the North Indian Population. Bioscience trends. 2011;5(5):205-10.
[10] Ratanajaraya C, Nishiyama H, Takahashi M, Kawaguchi T, Saito R, Mikami Y, Suyama M, Lathrop M, Yamada R, Ogawa O, Matsuda F. A Polymorphism of the Polg2 Gene Is Genetically Associated with the Invasiveness of Urinary Bladder Cancer in Japanese Males. Journal of human genetics. 2011;56(8):572-6.
[11] Guey LT, Garcia-Closas M, Murta-Nascimento C, Lloreta J, Palencia L, Kogevinas M, Rothman N, Vellalta G, Calle ML, Marenne G, Tardon A, Carrato A, Garcia-Closas R, Serra C, Silverman DT, Chanock S, Real FX, Malats N. Genetic Susceptibility to Distinct Bladder Cancer Subphenotypes. Eur Urol. 2010;57(2):283-92.
[12] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Do DNA Repair Genes Ogg1, Xrcc3 and Xrcc7 Have an Impact on Susceptibility to Bladder Cancer in the North Indian Population? Mutation research. 2009;680(1-2):56-63.

Supplementary Table 3.7. Summary associations for previously reported SNPs on urinary bladder cancer recurrence.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations reducing the risk of bladder cancer recurrence:															
NMIBC recurrence (non-BCG-treated)	2005	Leibovici et al. [1]	NMIBC	Not reported	Caucasian (White)	60 (Recurrence) / 29 (No recurrence)	Pro12Ala (rs1801282)	3p25.2	PPARG	G	C	0.07 (1KG)	HR (CG+GG/CC) =0.41	0.20-0.86	<0.05
NMIBC recurrence (<64 year-old group)	2006	Zhao et al. [2]	NMIBC	UC + Other	Caucasian (Northern American)	48 (Recurrence) / 51 (No recurrence)	Pro198Leu (rs1050450)	3p21.31	GPX1	T	C	0.22 (1KG)	HR (CT+TT/CC)= 0.37	0.20-0.70	<0.05
NMIBC recurrence (BCG-treated)	2006	Lin et al. [3]	NMIBC (BCG-treated)	UC + Other	Caucasian (Northern American)	18 (Recurrence) / 30 (No recurrence)	rs16260	16q22.1	CDH1	A	C	0.24 (1KG)	HR (AC+AA/CC)= 0.21	0.07-0.63	<0.05
NMIBC recurrence (BCG-treated)	2008	Ahirwar et al. [4]	NMIBC (BCG-treated)	Not reported	Indian	22 (Recurrence) / 33 (No recurrence)	rs1800795	7p15.3	IL6	C	G	0.14 (1KG)	HR (CC/GG)=0.29 8	0.09-0.91	0.03
NMIBC Recurrence (TUR-treated)	2009	Horikawa et al. [5]	NMIBC	UC	Japanese	49 (Recurrence) / 38 (No recurrence)	Arg72Pro (rs1042522)	17p13.1	TP53	C	G	0.54 (1KG)	HR (CC/CG+GG) =0.36	0.14-0.93	0.035
NMIBC recurrence (BCG-treated)	2009	Ahirwar et al. [6]	NMIBC	UC	Indian	19 (Recurrence) / 32 (No recurrence)	rs1799964	6p21.33	TNFA	C	T	0.22 (1KG)	HR (CC/TT)=0.38	0.14-0.98	0.024
NMIBC recurrence (BCG-treated)	2009	Gangwar et al. [7]	NMIBC	UC	Indian	20 (Recurrence) / 32 (No recurrence)	rs4645978	1p36.21	CASP9	G	A	0.42 (1KG)	HR (GG/AA)=0.22	0.62-0.75	0.005

NMIBC Recurrence (BCG-treated)	2009	Gangwar et al. [8]	UBC	UC	Indian	20 (Recurrence) / 27 (No recurrence)	rs7003908	8q11.21	XRCC7 (PRKDC)	G	T	0.33 (1KG)	HR (GG/TT)=0.25	0.09-0.65	0.004
NMIBC Recurrence (BCG-treated)	2010	Gangwar et al. [9]	NMIBC	UC	Indian	21 (Recurrence) / 26 (No recurrence)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	HR (GG/TT)=0.25	0.08-0.80	0.019
UBC Recurrence (BCG-treated)	2010	Ahirwar et al. [10]	UBC	Not reported	Indian	22 (Recurrence) / 32 (No recurrence)	rs4073	4q13.3	IL8 (CXCL8)	A	T	0.52 (1KG)	HR (AA/TT)=0.12	0.04-0.41	<0.001
NMIBC Recurrence (BCG-treated)	2012	Jaiswal et al. [11]	NMIBC	Not reported	Indian	32 (Recurrence) / 31 (No recurrence) 25 (Recurrence) / 26 (No recurrence)	rs9904341	17q25.3	Survivin (BIRC5)	C	G	0.39 (1KG)	HR (CG/GG)=0.35 HR (CC/GG)=0.22	0.16-0.77 0.05-0.95	0.009 0.043
NMIBC Recurrence (BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs804267	8p23.1	NEIL2	C	T	0.31 (1KG)	HR (CT/TT)=0.53	0.36–0.78	0.001
NMIBC Recurrence (BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs8191604	8p23.1	NEIL2	C	A	0.18 (1KG)	HR (CA/AA)=0.54	0.36–0.81	0.003
NMIBC Recurrence	2012	Wang et al. [13]	NMIBC	UC	Chinese	74 (Recurrence) / 125 (No recurrence)	rs2910164	5q33.3	MIR146A	C	G	0.28 (ExAC)	(HR (GC+CC/GG) =0.58	0.36–0.94	0.016
NMIBC Recurrence (TUR-treated)	2013	Ke et al. [14]	NMIBC (TUR-treated)	Not reported	Caucasian (Northern American)	91 (Recurrence) / 45 (No recurrence)	Ile636Thr (rs197412)	1p13.2	DDX20	T	C	0.53 (1KG)	HR (TT+TC/CC)= 0.58	0.40–0.82	0.002

NMIBC Recurrence	2013	Lee et al. [15]	NMIBC	UC	Caucasian (Northern American)	232 (Recurrence) / 189 (No recurrence)	rs511918	1q25.3	RGS16	T	G	0.47 (1KG)	HR (T/G)=0.81	0.66-0.99	0.0381
NMIBC Recurrence	2013	Lee et al. [15]	NMIBC	UC	Caucasian (Northern American)	232 (Recurrence) / 189 (No recurrence)	rs16829458	1q31.2	RGS2	A	G	0.12 (1KG)	HR (AG/GG)=0.63	0.41-0.95	0.0268
NMIBC Recurrence	2013	Lee et al. [15]	NMIBC	UC	Caucasian (Northern American)	232 (Recurrence) / 189 (No recurrence)	rs3795617	1q31.2	RGS13	A	G	0.38 (1KG)	HR (A/G)=0.79	0.65-0.96	0.0187
NMIBC Recurrence	2014	Zhang et al. [16]	NMIBC	UC	Chinese	28 (AT) / 297 (TT)	rs2505568	7q22.3	NAMPT	A	T	0.57 (1KG)	HR (AT/TT)=0.30	0.09–0.97	0.03 (log-rank)
UBC Recurrence	2014	Zhang et al. [16]	UBC	UC	Chinese	95 (Recurrence) / 230 (No recurrence)	rs2505568	7q22.3	NAMPT	A	T	0.57 (1KG)	OR (AT/TT)=0.25	0.07–0.86	<0.05
NMIBC Recurrence (TUR- and BCG-treated)	2015	Lima et al. [17]	NMIBC after TUR+BCG	Not reported	European (Southern Portugal)	70 (Recurrence) / 134 (No recurrence)	rs391835	3p21.31	CCR2	A	G	0.41 (1KG)	HR (AA/GG)=0.410 HR (GA+AA/GG)=0.455	0.191–0.879 0.232–0.893	0.022 0.022
NMIBC Recurrence (TUR-treated + Epirubicin)	2015	Deng et al. [18]	UBC (TUR- and Epirubicin-treated)	Not reported	Chinese	49 (Recurrence) / 75 (No recurrence) 50 (Recurrence) / 80 (No recurrence)	rs2854509	19q13.31	XRCC1	A	C	0.82 (1KG)	HR (AC/CC)=0.24 HR (AC+AA/CC)=0.23	0.10–0.57 0.10–0.53	0.036 (log-rank) 0.010 (log-rank)
NMIBC Recurrence (TUR-treated + Epirubicin)	2015	Deng et al. [18]	UBC (TUR- and Epirubici	Not reported	Chinese	48 (Recurrence) / 77 (No recurrence) 50	rs3213255	19q13.31	XRCC1	C	T	0.32 (1KG)	HR (CT/TT=0.17 HR (CT+CC/TT)=0.17	0.58–0.50 0.06–0.46	0.001 (log-rank) 0.001

			n-treated)			(Recurrence) / 80 (No recurrence)									(log-rank)
High-risk NMIBC Recurrence (BCG-treated)	2015	Ryk et al. [19]	High-risk (either TaG3, T1, TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (treated with BCG)	UC	European (Sweden)	5 (TT, BCG-treated) / 12 (TT, not BCG-treated)	rs2070744	7q36.1	NOS3	T	C	0.77 (1KG)	HR (BCG-reated TT/Not BCG-treated TT)=0.23	0.08–0.70	0.009
High-risk NMIBC Recurrence (BCG-treated)	2015	Ryk et al. [19]	High-risk (either TaG3, T1, TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (treated with BCG)	UC	European (Sweden)	17 (TT, BCG-treated) / 15 (TT, not BCG-treated)	rs2070744	7q36.1	NOS3	T	C	0.77 (1KG)	HR (BCG-reated CT+CC/Not BCG-treated CT+CC)=0.25	0.11–0.54	<0.001
High-risk NMIBC Recurrence (BCG-treated)	2015	Ryk et al. [19]	High-risk (either TaG3, T1,	UC	European (Sweden)	7 (GG, BCG-treated) / 9 (GG, not BCG-treated)	Asp298Glu (rs1799983)	7q36.1	NOS3	G	T	0.82 (1KG)	HR (BCG-reated GG/Not BCG-treated GG)=0.29	0.10–00.87	0.028

			TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (treated with BCG)												
NMIBC Recurrence	2015	Xie et al. [20]	NMIBC	UC	Chinese	62 (Recurrence) / 259 (No recurrence)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (TG/TT)=0.562	0.338-0.933	0.026
NMIBC Recurrence	2015	Xie et al. [20]	NMIBC	UC	Chinese	49 (Recurrence) / 178 (No recurrence)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (GG/TT)=0.501	0.279-0.900	0.021
NMIBC Recurrence	2015	Xie et al. [20]	NMIBC	UC	Chinese	81 (Recurrence) / 362 (No recurrence)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	OR (TG+GG/TT)=0.531	0.336-0.839	0.007
NMIBC Recurrence (Epirubicin-treated)	2015	Li et al. [21]	NMIBC (Epirubicin-treated)	UC	Chinese	47 (Recurrence) / 78 (No recurrence)	Pro206Pro (rs915927)	19q13.2	XRCC1	G	A	0.32 (1KG)	HR (AG/AA)=0.21	0.08-0.53	0.02
NMIBC Recurrence (Epirubicin-treated)	2015	Li et al. [21]	NMIBC (Epirubicin-treated)	UC	Chinese	48 (Recurrence) / 81 (No recurrence)	Pro206Pro (rs915927)	19q13.2	XRCC1	G	A	0.32 (1KG)	HR (AG+GG/AA)=0.24	0.10-0.59	0.009
NMIBC Recurrence	2015	Li et al. [21]	NMIBC (Epirubicin-treated)	UC	Chinese	46 (Recurrence) / 78 (No recurrence)	rs2854501	19q13.2	XRCC1	T	C	0.18 (1KG)	HR (CT/CC)=0.10	0.03-0.35	0.002

(Epirubicin-treated)															
NMIBC Recurrence (Epirubicin-treated)	2015	Li et al. [21]	NMIBC (Epirubicin-treated)	UC	Chinese	48 (Recurrence) / 81 (No recurrence)	rs2854501	19q13.2	XRCC1	T	C	0.18 (1KG)	HR (CT+TT/CC)= 0.16	0.06-0.43	0.001
Associations increasing the risk of bladder cancer recurrence:															
NMIBC Recurrence	2005	Kim et al. [22]	NMIBC	UC	Korean	38 (Recurrence) / 55 (No recurrence)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	OR (CG+GG/CC) =6.49	1.25-33.3	0.026
NMIBC recurrence (BCG-treated)	2005	Gu et al. [23]	NMIBC (BCG-treated)	Not reported	Caucasian (Northern American)	121 (Recurrence) / 77 (No recurrence)	Met1097Val (rs2228526)	10q11.23	ERCC6	C	G	0.18 (1KG)	HR (CG+CC/GG) =1.54	1.02-2.33	<0.05
NMIBC recurrence (maintenance BCG-treated)	2005	Leibovici et al. [1]	NMIBC (BCG-treated)	Not reported	Caucasian (White)	12 (Recurrence) / 16 (No recurrence) 8 (Recurrence) / 10 (No recurrence) 16 (Recurrence) / 17 (No recurrence)	rs1800795	7p15.3	IL6	C	G	0.14 (1KG)	HR (GC/GG)=4.31 HR (CC/GG)=5.47 HR (CG+CC/GG) =4.60	1.09-17.09 1.05-28.44 1.24-17.09	<0.05 <0.05 <0.05
NMIBC recurrence (BCG-treated)	2006	Decobert et al. [24]	NMIBC (BCG-treated)	Not reported	Caucasian (Canadian)	51 (Recurrence) / 16 (No recurrence)	Asp543Asn (rs17235409)	2q35	NRAMP1 (SLC11A1)	A	G	0.07 (1KG)	HR (AG/GG)=5.74	2.4-13.8	<0.001
NMIBC recurrence (BCG-treated)	2008	Mittal et al. [25]	NMIBC	UC	Indian	19 (Recurrence) / 16 (No recurrence)	Arg194Trp (rs1799782)	19q13.2	XRCC1	T	C	0.12 (1KG)	OR (CT/CC)=4.57	1.10-18.97	0.03
NMIBC recurrence	2008	Mittal et al. [25]	NMIBC (BCG-treated)	UC	Indian	10 (recurrence) / 15 (No recurrence)	Gln399Arg (rs25487)	19q13.2	XRCC1	A	G	0.26 (1KG)	HR (AA/GG)=5.05	1.34-19.01	0.01

(BCG-treated)															
NMIBC recurrence (BCG-treated)	2009	Gangawar et al. [26]	NMIBC	Not reported	Indian	27 (Recurrence) / 19 (No recurrence)	Asp312Asn (rs1799793)	19q13.3	ERCC2	A	G	0.19 (1KG)	HR(AA/GG)=3.07	1.22-7.68	0.016
NMIBC recurrence (BCG-treated)	2009	Ahirwar et al. [27]	NMIBC	UC	Indian	21 (Recurrence) / 29 (No recurrence) 23 (Recurrence) / 23 (No Recurrence)	rs2430561	12q15	IFN-G	A	T	0.28 (1KG)	HR (TA/TT)=2.80 HR (A/T)=2.24	1.13-6.97 1.06-5.80	0.024 0.036
NMIBC Recurrence (non-BCG-treated)	2009	Gangwar et al. [8]	UBC	UC	Indian	20 (Recurrence) / 23 (No recurrence)	Ser326Cys (rs1052133)	3p25.3	OGG1	G	C	0.30 (1KG)	HR (GG/TT)=4.04	1.33-12.1	0.013
NMIBC Recurrence (BCG-treated)	2010	Gangwar et al. [28]	NMIBC	UC	Indian	14 (Recurrence) / 27 (No recurrence) 28 (recurrence) / 49 (No recurrence)	Lys939Gln (rs2228001)	3p25.1	XPC	C	A	0.32 (1KG)	HR (CC/AA)=3.21 HR (AC+CC/AA)=3.98	1.07-9.61 1.02-10.7	0.036 <0.05
Low-risk NMIBC recurrence	2010	Wang et al. [29]	NMIBC	UC	Chinese	24 (Recurrence) / 24 (No recurrence)	rs744154	16p13.12	XPF (ERCC4)	C	A	0.22 (1KG)	HR (AC+CC/AA)=3.62	1.42-9.28	<0.05
Low-risk NMIBC recurrence	2010	Kiemeney et al. [30]	UBC	UC	European (multiple)	305 (Recurrence) / 358 (No recurrence) 322 (Recurrence) / 375 (No recurrence)	rs798766	4p16.3	TACC3/FGFR3	T	C	0.25 (All low-risk NMIBC) / 0.24 (1KG)	HR (CT/CC)=1.31 HR (T/C)=1.23	1.04-1.64 1.04-1.47	0.020 0.019

NMIBC Recurrence (BCG-treated)	2010	Chen et al. [31]	NMIBC	UC + Other	Caucasians (Northern American) + European (Spain) for replication	118 (Recurrence) / 83 (No recurrence)	rs6463089	7p14.1	GLI3	A	G	0.09 (1KG)	HR (AG+AA/GG)= 2.40	1.50-3.84	2.00E-04
NMIBC Recurrence (BCG-treated)	2010	Chen et al. [31]	NMIBC	UC + Other	Caucasians (Northern American) + European (Spain) for replication	117 (Recurrence) / 83 (No recurrence)	rs3801192	7p14.1	GLI3	A	G	0.07 (1KG)	HR (AG+AA/GG)= 2.54	1.47-4.39	9.00E-04
NMIBC Recurrence (TUR-treated)	2010	Chen et al. [31]	NMIBC	UC + Other	Caucasians (Northern American) + European (Spain) for replication	230 (Recurrence) / 272 (No recurrence)	rs1233560	7q36.3	SSH	G	A	0.46 (1KG)	HR (G/A)=1.39	1.14-1.70	0.001
NMIBC Recurrence (TUR-treated)	2010	Chen et al. [31]	NMIBC	UC + Other	Caucasians (Northern American) + European (Spain) for replication	230 (Recurrence) / 272 (No recurrence)	rs11685068	2q14.2	GLI2	A	G	0.06 (1KG	HR (AG+AA/GG)= 2.07	1.33-3.21	0.0013
NMIBC Recurrence (BCG-treated)	2010	Chiong et al. [32]	NMIBC (BCG-treated)	UC	Chinese	19 (Recurrence) / 4 (No recurrence)	Asp543Asn (rs17235409)	2q35	NRAMP1 (SLC11A1)	G	A	0.93 (1KG)	HR (GG/AG)=3.0	1.03–8.9	0.033
NMIBC Recurrence (BCG-treated)	2011	Srivastava et al. [33]	NMIBC (BCG-treated)	UC	Indian	31 (Recurrence) / 41 (No recurrence) 34 (Recurrence) / 44 (No recurrence)	rs243865	16q12.2	MMP2	C	T	0.86 (1KG)	HR (CT/CC)=4.32 HR (CT+TT/CC)= 2.06	1.51–12.39 1.01–4.18	0.006 0.047

NMIBC Recurrence-(BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs804256	8p23.1	NEIL2	C	T	0.26 (1KG)	HR (CC/CT+TT)= 4.58	2.61–8.02	1.00E-07
NMIBC Recurrence (BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs804276	8p23.1	NEIL2	G	A	0.62 (1KG)	HR (GG/AG+AA)= 2.71	1.75–4.20	9.00E-06
NMIBC Recurrence (BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs4639	8p23.1	NEIL2	G	A	0.47 (1KG)	HR (GG/AG+AA)= 2.60	1.68–4.03	2.00E-05
NMIBC Recurrence (BCG-treated)	2012	Wei et al. [12]	NMIBC	UC	Caucasian (Northern American)	110 (Recurrence) / 82 (No recurrence)	rs2173962	21q22.11	SOD1	G	A	0.06 (1KG	HR (GA/AA)=2.45	1.42–4.23	0.001
NMIBC Recurrence (TUR-treated)	2013	Ke et al. [14]	NMIBC (TUR-treated)	Not reported	Caucasian (Northern American)	91 (Recurrence) / 45 (No recurrence)	rs12186785	5p13.3	RNASEN (DROSHA)	C	T	0.05 (1KG)	HR (CT/TT)= 2.15	1.25–3.68	0.005
NMIBC Recurrence (BCG-treated)	2013	Jaiswal et al. [34]	NMIBC (BCG-treated)	UC	Indian	32 (Recurrence) / 42 (No recurrence)	rs187238	11q23.1	IL18	C	G	0.79 (1KG)	HR (GC/GG)=2.35	1.09-5.10	0.030
NMIBC Recurrence	2013	Lee et al. [15]	NMIBC	UC	Caucasian (Northern American)	232 (Recurrence) / 189 (No recurrence)	rs11199005	10q26.11	RGS10	A	G	0.35 (1KG)	HR (AG/GG)=1.47	1.02-2.12	0.041
NMIBC Recurrence	2013	Lee et al. [15]	NMIBC	UC	Caucasian (Northern American)	232 (Recurrence) / 189 (No recurrence)	rs1323291	1q31.2	RGS1	C	A	0.15 (1KG)	HR (CA/AA)=1.60	1.13-2.28	0.0084
NMIBC Recurrence	2014	Andrew et al. [35]	NMIBC	UC + Other	Caucasian (Northern American)	279 (Recurrence) / 248 (No recurrence) (but not	rs5742714	12q23.2	IGF1	C	G	0.9 (1KG)	HR (GC/GG)=1.61	1.19-2.17	0.002 (log-rank)

						presented for NMIBC group)									
NMIBC Recurrence	2014	Andrew et al. [35]	NMIBC	UC + Other	Caucasian (Northern American)	279 (Recurrence) / 248 (No recurrence) (but not presented for NMIBC group)	rs2238151	12q24.12	ALDH2	C	T	0.7 (1KG)	HR (CC/TT)=1.94	1.32-2.85	<0.001 (log-rank)
MIBC Recurrence	2014	Zhou et al. [36]	MIBC	UC	Chinese	47 (Recurrence) / 156 (No recurrence)	rs4957014	5p15.33	PDCD6	G	T	0.65 (1KG)	HR (GT/TT+GG)= 1.93	1.08-3.45	0.03
NMIBC Recurrence (TUR- and BCG-treated)	2015	Lima et al. [17]	NMIBC after TUR+BCG	Not reported	European (Southern Portugal)	70 (Recurrence) / 134 (No recurrence)	Lys469Glu (rs5498)	19p13.2	ICAM1	G	A	0.36 (1KG)	HR (GG/AA+AG)= 1.76	1.050–2.949	0.032
NMIBC Recurrence (TUR- and BCG-treated)	2015	Lima et al. [17]	NMIBC after TUR+BCG	Not reported	European (Southern Portugal)	70 (Recurrence) / 134 (No recurrence)	rs2275913	6p12.2	IL17A	A	G	0.29 (1KG)	HR (AA/GG+AG)= 2.097	1.118–3.933	0.021
NMIBC Recurrence (TUR- and BCG-treated)	2015	Lima et al. [17]	NMIBC after TUR+BCG	Not reported	European (Southern Portugal)	70 (Recurrence) / 134 (No recurrence)	rs1799964	6p21.33	TNFA	C	T	0.22 (1KG)	HR (CC/TT+TC)= 2.427	1.144–5.149	0.021
NMIBC Recurrence (TUR- and BCG-treated)	2015	Lima et al. [17]	NMIBC after TUR+BCG	Not reported	European (Southern Portugal)	70 (Recurrence) / 134 (No recurrence)	rs13278062	8p21.3	TRAILR1 (TNFRSF10A)	G	T	0.6 (1KG)	HR (TG/TT)=3.546 HR (GG/TT)=3.078 HR (TG+GG/TT)= 3.195	1.477–8.513 1.251–7.573 1.373–7.433	0.005 0.014 0.007

NMIBC Recurrence (TUR- and BCG-treated)	2015	Ke et al. [37]	NMIBC (treated with TUR)	UC	Caucasians (European decent)	88 (Recurrence) / 45 (No recurrence)	rs3746162	19p13.3	GPX4	A	G	0.2 (Cases) / 0.21 (Controls) / 0.16 (1KG)	HR (AA/AG+GG)= 5.43	2.19–13.46	0.0003
NMIBC Recurrence (TUR- and BCG-treated)	2015	Ke et al. [37]	NMIBC (TUR+BCG-treated)	UC	Caucasians (European decent)	110 (Recurrence) / 81 (No recurrence)	rs7265992	20q11.22	GSS	A	G	0.60 (Cases) / 0.62 (Controls) / 0.19 (1KG)	HR (AA/AG+GG)= 3.43	1.56–7.56	0.002
NMIBC Recurrence (TUR- and BCG-treated)	2015	Ke et al. [37]	NMIBC (TUR+BCG-treated)	UC	Caucasians (European decent)	110 (Recurrence) / 81 (No recurrence)	rs6060124	20q11.22	GSS	A	C	0.61 (Cases) / 0.69 (Controls) / 0.26 (1KG)	HR (AA/AG+GG)= 2.80	1.44–5.47	0.003
NMIBC Recurrence (TUR- and BCG-treated)	2015	Ke et al. [37]	NMIBC (TUR+BCG-treated)	UC	Caucasians (European decent)	110 (Recurrence) / 81 (No recurrence)	rs7260770	20q11.22	GSS	A	G	0.63 (Cases) / 0.69 (Controls) / 0.22 (1KG)	HR (AA/AG+GG)= 2.56	1.34–4.90	0.005
NMIBC Recurrence (TUR- and BCG-treated)	2015	Ke et al. [37]	NMIBC (TUR+BCG-treated)	UC	Caucasians (European decent)	110 (Recurrence) / 81 (No recurrence)	rs4911455	20q11.22	GSS	C	A	0.62 (Cases) / 0.69 (Controls) / 0.28 (1KG)	HR (AA/AG+GG)= 2.54	1.32–4.87	0.005
NMIBC Recurrenc	2015	Deng et al. [38]	NMIBC (treated	Not reported	Chinese	48 (Recurrence) /	Ile105Val (rs1695)	11q13.2	GSTP1	G	A	0.35 (1KG)	HR (AG/AA)=3.29	1.63–6.63	0.002 (log-

e (BCG-treated)			with intravesicular chemotherapy)			76 (No recurrence) 24 (Recurrence) / 60 (No recurrence) 50 (recurrence) / 80 (No recurrence)						HR (GG/AA)=5.18 HR (GG+AG/AA)=3.47	(AG/AA) 1.05–25.62 (GG/AA) 1.75–6.89 (GG+AG/AA)	rank) 0.001 (log-rank, for AG+GG)
NMIBC Recurrence (BCG-treated)	2015	Deng et al. [38]	NMIBC (treated with intravesicular chemotherapy)	Not reported	Chinese	35 (Recurrence) / 55 (No recurrence) 60 (Recurrence) / 70 (No recurrence)	Ala140Asp (rs4925)	10q25.1	GSTO1	A	C	0.1 (1KG)	HR (AA/CC)=3.23 HR (AC+AA/CC)=1.96	1.16-8.94 (AA/CC) 1.05–3.70 (AC+AA/CC)	0.019 (log-rank) 0.042 (log-rank, for AC+AA)
Recurrence	2016	Wang et al. [39]	UBC (not reported whether NMIBC/UBC)	Not reported	Chinese	74 (Recurrence) / 125 (No recurrence)	rs2042329	5q12.3	CWC27	T	G	0.18 / 0.34 (1KG)	HR (T/G)=1.54	1.10-2.16	0.012
MIBC recurrence	2018	Li et al. [40]	MIBC	Not reported	Chinese	42 (Recurrence) / 96 (No recurrence)	rs4758680	12q24.31	IL31	A	C	0.29 (1KG)	HR (CA+AA/CC)=2.02	1.06-3.85	0.03
MIBC recurrence	2018	Li et al. [40]	MIBC	Not reported	Chinese	42 (Recurrence) / 96 (No recurrence)	rs4758680	12q24.31	IL31	A	C	0.29 (1KG)	HR (CA/CC)=1.90	1.15-3.16	0.01
NMIBC Recurrence (BCG-treated)	2017	Williams et al. [41]	NMIBC	UC	Northern American (although n restriction on ethnicity	123 (Recurrence) / 82 (No recurrence)	rs3138056	14q13.2	NFKBIA	T?	C?	0.32 (Reported in the study) /	HR(TT/CC)=3.26	1.83-5.8	6.20x10-5

					were applied)							T=0.38 (1KG)			
NMIBC Recurrence (BCG-treated)	2018	Wang et al. [42]	NMIBC (BCG-treated)	UC	Chinese	63 (Recurrence) / 127 (No recurrence)	rs1544410	12q13.11	VDR	A	G	0.30 (1KG)	HR (AA+AG/GG)= 3.95	Not reported	0.037
NMIBC Recurrence	2013	Yang et al. [43]	NMIBC	Not reported	Chinese	45 (Recurrence) / 100 (No recurrence)	rs2169830	15q14	TSP-1 (THBS1)	G	A	0.42 (1KG)	HR (GG/AA)=2.63	1.43-4.83	0.002
NMIBC Recurrence	2013	Yang et al. [43]	NMIBC	Not reported	Chinese	45 (Recurrence) / 100 (No recurrence)	rs2169830	15q14	TSP-1 (THBS1)	G	A	0.42 (1KG)	HR (AG+GG/AA)= 1.95	1.20-3.19	0.007
NMIBC Recurrence	2013	Yang et al. [43]	NMIBC	Not reported	Chinese	45 (Recurrence) / 100 (No recurrence)	rs2169830	15q14	TSP-1 (THBS1)	G	A	0.42 (1KG)	HR (GG/AG+AA)= 2.07	1.23-3.49	0.006

BCG-Bacillus Calmette-Guérin; CI-confidence interval; CIS-carcinoma in situ; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; TUR-transurethral resection; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.

[2] Zhao H, Liang D, Grossman HB, Wu X. Glutathione Peroxidase 1 Gene Polymorphism and Risk of Recurrence in Patients with Superficial Bladder Cancer. Urology. 2005;66(4):769-74.

[3] Lin J, Dinney CP, Grossman HB, Jhamb M, Zhu Y, Spitz MR, Wu X. E-Cadherin Promoter Polymorphism (C-160a) and Risk of Recurrence in Patients with Superficial Bladder Cancer. Clinical genetics. 2006;70(3):240-5.

[4] Ahirwar D, Kesarwani P, Manchanda PK, Mandhani A, Mittal RD. Anti- and Proinflammatory Cytokine Gene Polymorphism and Genetic Predisposition: Association with Smoking, Tumor Stage and Grade, and Bacillus Calmette-Guerin Immunotherapy in Bladder Cancer. Cancer genetics and cytogenetics. 2008;184(1):1-8.

[5] Horikawa Y, Nadaoka J, Saito M, Kumazawa T, Inoue T, Yuasa T, Tsuchiya N, Nishiyama H, Ogawa O, Habuchi T. Clinical Implications of the Mdm2 Snp309 and P53 Arg72pro Polymorphisms in Transitional Cell Carcinoma of the Bladder. Oncology reports. 2008;20(1):49-55.
[6] Ahirwar DK, Mandhani A, Dharaskar A, Kesarwani P, Mittal RD. Association of Tumour Necrosis Factor-Alpha Gene (T-1031c, C-863a, and C-857t) Polymorphisms with Bladder Cancer Susceptibility and Outcome after Bacille Calmette-Guerin Immunotherapy. BJU international. 2009;104(6):867-73.
[7] Gangwar R, Mandhani A, Mittal RD. Caspase 9 and Caspase 8 Gene Polymorphisms and Susceptibility to Bladder Cancer in North Indian Population. Annals of surgical oncology. 2009;16(7):2028-34.
[8] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Do DNA Repair Genes Ogg1, Xrcc3 and Xrcc7 Have an Impact on Susceptibility to Bladder Cancer in the North Indian Population? Mutation research. 2009;680(1-2):56-63.
[9] Gangwar R, Mittal RD. Association of Selected Variants in Genes Involved in Cell Cycle and Apoptosis with Bladder Cancer Risk in North Indian Population. DNA and cell biology. 2010;29(7):349-56.
[10] Ahirwar DK, Mandhani A, Mittal RD. Il-8 -251 T > a Polymorphism Is Associated with Bladder Cancer Susceptibility and Outcome after Bcg Immunotherapy in a Northern Indian Cohort. Archives of medical research. 2010;41(2):97-103.
[11] Jaiswal PK, Goel A, Mandhani A, Mittal RD. Functional Polymorphisms in Promoter Survivin Gene and Its Association with Susceptibility to Bladder Cancer in North Indian Cohort. Molecular biology reports. 2012;39(5):5615-21.
[12] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.
[13] Wang M, Chu H, Li P, Yuan L, Fu G, Ma L, Shi D, Zhong D, Tong N, Qin C, Yin C, Zhang Z. Genetic Variants in Mirnas Predict Bladder Cancer Risk and Recurrence. Cancer research. 2012;72(23):6173-82.
[14] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.
[15] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.
[16] Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y, Song Y, Zhang L. Genetic Variants in Nampt Predict Bladder Cancer Risk and Prognosis in Individuals from Southwest Chinese Han Group. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4031-40.
[17] Lima L, Oliveira D, Ferreira JA, Tavares A, Cruz R, Medeiros R, Santos L. The Role of Functional Polymorphisms in Immune Response Genes as Biomarkers of Bacille Calmette-Guerin (Bcg) Immunotherapy Outcome in Bladder Cancer: Establishment of a Predictive Profile in a Southern Europe Population. BJU international. 2015;116(5):753-63.

[18] Deng X, Zhang X, Cheng Y, Yang X, Zhao R, Liu X, Li X, Qin C, Lu Q, Yin C. Xrcc1 Polymorphisms Associated with Survival among Chinese Bladder Cancer Patients Receiving Epirubicin and Mitomycin C. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(6):4591-6.
[19] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.
[20] Xie L, Sun Y, Chen T, Tian D, Li Y, Zhang Y, Ding N, Shen Z, Xu H, Nian X, Sha N, Han R, Hu H, Wu C. Association between Mdm2 Snp309 T>G Polymorphism and the Risk of Bladder Cancer: New Data in a Chinese Population and an Updated Meta-Analysis. OncoTargets and therapy. 2015;8:3679-90.
[21] Li P, Zhang X, Deng X, Tao J, Qin C, Yang X, Cheng Y, Lu Q, Wang Z, Yin C. Pharmacogenetic Association between Xrcc1 Polymorphisms and Improved Outcomes in Bladder Cancer Patients Following Intravesical Instillation of Epirubicin. International journal of clinical and experimental medicine. 2015;8(7):11167-73.
[22] Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun Wee J, Kim WJ. Genotypes of Tnf-Alpha, Vegf, Hogg1, Gstm1, and Gstt1: Useful Determinants for Clinical Outcome of Bladder Cancer. Urology. 2005;65(1):70-5.
[23] Gu J, Zhao H, Dinney CP, Zhu Y, Leibovici D, Bermejo CE, Grossman HB, Wu X. Nucleotide Excision Repair Gene Polymorphisms and Recurrence after Treatment for Superficial Bladder Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(4):1408-15.
[24] Decobert M, Larue H, Bergeron A, Harel F, Pfister C, Rousseau F, Lacombe L, Fradet Y. Polymorphisms of the Human Nramp1 Gene Are Associated with Response to Bacillus Calmette-Guerin Immunotherapy for Superficial Bladder Cancer. The Journal of urology. 2006;175(4):1506-11.
[25] Mittal RD, Singh R, Manchanda PK, Ahirwar D, Gangwar R, Kesarwani P, Mandhani A. Xrcc1 Codon 399 Mutant Allele: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Cancer biology & therapy. 2008;7(5):645-50.
[26] Gangawar R, Ahirwar D, Mandhani A, Mittal RD. Impact of Nucleotide Excision Repair Ercc2 and Base Excision Repair Apex1 Genes Polymorphism and Its Association with Recurrence after Adjuvant Bcg Immunotherapy in Bladder Cancer Patients of North India. Medical oncology (Northwood, London, England). 2010;27(2):159-66.
[27] Ahirwar DK, Agrahari A, Mandhani A, Mittal RD. Cytokine Gene Polymorphisms Are Associated with Risk of Urinary Bladder Cancer and Recurrence after Bcg Immunotherapy. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2009;14(4):213-8.
[28] Gangwar R, Mandhani A, Mittal RD. Xpc Gene Variants: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Journal of cancer research and clinical oncology. 2010;136(5):779-86.
[29] Wang M, Wang M, Yuan L, Wu D, Zhang Z, Yin C, Fu G, Wei Q, Zhang Z. A Novel Xpf -357a>C Polymorphism Predicts Risk and Recurrence of Bladder Cancer. Oncogene. 2010;29(13):1920-8.

[30] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.
[31] Chen M, Hildebrandt MA, Clague J, Kamat AM, Picornell A, Chang J, Zhang X, Izzo J, Yang H, Lin J, Gu J, Chanock S, Kogevinas M, Rothman N, Silverman DT, Garcia-Closas M, Grossman HB, Dinney CP, Malats N, Wu X. Genetic Variations in the Sonic Hedgehog Pathway Affect Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Cancer prevention research (Philadelphia, Pa). 2010;3(10):1235-45.
[32] Chiong E, Kesavan A, Mahendran R, Chan YH, Sng JH, Lim YK, Kamaraj R, Tan TM, Esuvaranathan K. Nramp1 and Hgpx1 Gene Polymorphism and Response to Bacillus Calmette-Guerin Therapy for Bladder Cancer. Eur Urol. 2011;59(3):430-7.
[33] Srivastava P, Kapoor R, Mittal RD. Association of Single Nucleotide Polymorphisms in Promoter of Matrix Metalloproteinase-2, 8 Genes with Bladder Cancer Risk in Northern India. Urologic oncology. 2013;31(2):247-54.
[34] Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of Il-12, Il-18 Variants and Serum Il-18 with Bladder Cancer Susceptibility in North Indian Population. Gene. 2013;519(1):128-34.
[35] Andrew AS, Gui J, Hu T, Wyszynski A, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Ekstrom RM, Li Z, Zens MS, Borsuk M, Moore JH, Karagas MR. Genetic Polymorphisms Modify Bladder Cancer Recurrence and Survival in a USA Population-Based Prognostic Study. BJU international. 2015;115(2):238-47.
[36] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[37] Ke HL, Lin J, Ye Y, Wu WJ, Lin HH, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Glutathione Pathway Genes Predict Cancer Recurrence in Patients Treated with Transurethral Resection and Bacillus Calmette-Guerin Instillation for Non-Muscle Invasive Bladder Cancer. Annals of surgical oncology. 2015;22(12):4104-10.
[38] Deng X, Yang X, Cheng Y, Liu X, Li X, Zhao R, Qin C, Lu Q, Yin C. Gstp1 and Gsto1 Single Nucleotide Polymorphisms and the Response of Bladder Cancer Patients to Intravesical Chemotherapy. Scientific reports. 2015;5:14000.
[39] Wang M, Li Z, Chu H, Lv Q, Ye D, Ding Q, Xu C, Guo J, Du M, Chen J, Song Z, Yin C, Qin C, Gu C, Zhu Y, Xia G, Liu F, Zhang Z, Yuan L, Fu G, Hu Z, Tong N, Shen J, Liu K, Sun J, Sun Y, Li J, Li X, Shen H, Xu J, Shi Y, Zhang Z. Genome-Wide

Association Study of Bladder Cancer in a Chinese Cohort Reveals a New Susceptibility Locus at 5q12.3. Cancer research. 2016;76(11):3277-84.
[40] Li Q, Tang T, Zhang P, Liu C, Pu Y, Zhang Y, Song H, Wang Y, Song Y, Su M, Zhou B, Zhang L. Correlation of Il-31 Gene Polymorphisms with Susceptibility and Clinical Recurrence of Bladder Cancer. Familial cancer. 2018;17(4):577-85.
[41] Williams SB, Kamat AM, Mmeje C, Ye Y, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variants in the Inflammation Pathway as Predictors of Recurrence and Progression in Non-Muscle Invasive Bladder Cancer Treated with Bacillus Calmette-Guerin. Oncotarget. 2017;8(51):88782-91.
[42] Wang Z, Lim YK, Lim HCC, Chan YH, Ngiam N, Raman Nee Mani L, Esuvaranathan K, Ng CF, Teoh J, Chan E, Mahendran R, Chiong E. The Role of Vitamin D Receptor Polymorphisms in Predicting the Response to Therapy for Nonmuscle Invasive Bladder Carcinoma. The Journal of urology. 2018;200(4):737-42.
[43] Yang X, Li P, Yang X, Qin C, Cao Q, Zhang Z, Wang M, Cai H, Gu J, Tao J, Gu M, Lu Q, Yin C. Tsp-1-1223 a/G Polymorphism as a Potential Predictor of the Recurrence Risk of Bladder Cancer in a Chinese Population. International Journal of Genomics. 2013;2013:9.

Supplementary Table 3.8. Summary associations for previously reported SNPs on urinary bladder cancer progression.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations reducing the risk of bladder cancer progression:															
NMIBC Progression (increase in stage)	2007	Sanyal et al. [1]	UBC	Not reported	European (Sweden)	94 (TC+CC) / 102 (TT)	His27His (rs17350793, merged into rs12628)	11p15.5	HRAS	C	T	0.30 (1KG)	HR (TC+CC/TT)= 0.3	0.1-0.9	0.03
UBC Progression (increase in stage)	2011	Ryk et al. [2]	UBC	Not reported	European (Sweden)	67 (Progression) / 215 (No progression)	Ser608Leu (rs2297518)	17q11.2	NOS2	T	C	0.17 (1KG)	HR (TT/CC)=0.21	0.05–0.87	0.031
NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs10917690	1q23.3	RGS5	G	A	0.29 (1KG)	HR (G/A)=0.58	(0.39-0.86	0.0065
NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs4075958	5q35.3	RGS14	A	G	0.18 (1KG)	HR (A/G)=0.65	0.44-0.97	0.0332
NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs10926466	1q23.1	RGS7	T	C	0.63 (1KG)	HR (TC/CC)=0.60	0.36-0.98	0.0414

NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs12038803	1q23.1	RGS7	C/G/T? (Not reported)	A	0.66 (1KG)	HR (?A/AA)=0.60	0.36-0.98	0.0431
High-risk NMIBC Progression (BCG-treated) (transition from NMIBC to MIBC, metastatic cancer, or cancer-specific death)	2015	Ryk et al. [4]	High-risk (either TaG3, T1, TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (treated with BCG)	UC	European (Sweden)	1 (TT, BCG-treated) / 11 (TT, not BCG-treated)	rs2070744	7q36.1	NOS3	T	C	0.77 (1KG)	HR (BCG-reated TT/Not BCG-treated TT)=0.05	0.01–0.42	0.005
High-risk NMIBC Progression (BCG-treated) (transition from NMIBC to MIBC, metastatic cancer, or cancer-specific death)	2015	Ryk et al. [4]	High-risk (either TaG3, T1, TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (treated with BCG)	UC	European (Sweden)	3 (GG, BCG-treated) / 9 (GG, not BCG-treated)	Asp298Glu (rs1799983)	7q36.1	NOS3	G	T	0.82 (1KG)	HR (BCG-reated GG/Not BCG-treated GG)=0.10	0.02–0.46	0.003

UBC Progression (metastases present)	2015	Deng et al. [5]	UBC	Not reported	Chinese	132 (metastasis) / 27 (no metastasis)	rs2910164	5q33.3	MIR146A	G	C	0.39 (all UBC) / 0.32 (TOPMED)	OR=0.45 (log-additive model)	0.21-0.93	0.025
Associations reducing the risk of bladder cancer progression:															
NMIBC Progression (increase in stage)	2003	Sakano et al. [6]	UBC	UC	European (Sweden)	33 (CT+TT) / 178 (CC)	rs3088440	9p21	CDKN2A	T	C	0.08 / 0.17 (1KG)	HR (CT+TT/CC)= 2.5	1.0-6.1	0.043
NMIBC progression (BCG-treated) (increase in stage)	2006	Basturk et al. [7]	NMIBC (BCG-treated)	UC	Turkish	59 (Progression) / 14 (No progression)	rs1800896	1q32.1	IL10	G	A	0.27 (1KG)	OR (GG)=5.47	0.84-38.3	0.05
NMIBC progression (BCG-treated) (increase in stage)	2006	Basturk et al. [7]	NMIBC (BCG-treated)	UC	Turkish	59 (Progression) / 14 (No progression)	rs2243248	5q31.1	IL4	G	T	0.11 (1KG)	OR (GG)=18.33	0.8-412.2	0.05
NMIBC progression (BCG-treated) (increase in stage)	2006	Basturk et al. [7]	NMIBC (BCG-treated)	UC	Turkish	59 (Progression) / 14 (No progression)	Pro10Leu/Arg (rs1800470)	19q13.2	TGFB1	T	C	0.55 (1KG)	OR (T)=7.5	1.33-55.1	0.006
NMIBC progression (BCG-treated) (increase in stage)	2006	Basturk et al. [7]	NMIBC (BCG-treated)	UC	Turkish	59 (Progression) / 14 (No progression)	Arg25Gln (rs1800471)	19q13.2	TGFB1	G	C	0.05 (1KG)	OR (G)=7.17	0.83-160.2	0.04

Progression (metastases present)	2012	Guirado et al. [8]	UBC	UC	European (Spain)	92 (Yes) / 136 (No)	rs9302752	16q12.1	NOD2	G	A	0.50 (1KG)	OR (GG/AG+AA)= 3.23 OR (GG/AG)=3.16	1.25-8.33 1.12-8.33	0.011 0.022
NMIBC Progression (BCG-treated) (transition from NMIBC to MIBC or metastatic cancer)	2012	Wei et al. [9]	NMIBC	UC	Caucasian (Northern American)	75 (Progression) / 327 (No progression)	rs3890995	12q24.11	UNG	C	T	0.22 (1KG)	HR (CC+CT/TT,= 1.92	1.33–2.77	0.0005
NMIBC Progression (TUR-treated) (transition from NMIBC to MIBC or metastatic cancer)	2013	Ke et al. [10]	NMIBC	Not reported	Caucasian (Northern American)	75 (Progression) / 324 (No progression)	rs720012	22q11.21	DGCR8	A	G	0.22 (1KG)	HR (AA/AG+GG) = 3.97	1.52–10.36	0.005
NMIBC Progression (TUR-treated) (transition from NMIBC to MIBC or metastatic cancer)	2013	Ke et al. [10]	NMIBC	Not reported	Caucasian (Northern American)	75 (Progression) / 326 (No progression)	rs2073778	22q11.21	DGCR8	T	C	0.22 (1KG)	HR (TT/CT+CC= 4.00	1.53–10.46	0.005
NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs1323291	1q31.2	RGS1	C	A	0.15 (1KG)	HR (CA/AA)=2.14	1.25-3.66	0.0059

NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs6678136	1q23.3	RGS4	A	G	0.46 (1KG)	HR (AG/GG=2.07	1.20-3.57	0.0094
NMIBC Progression (transition from NMIBC to MIBC or metastatic cancer)	2013	Lee et al. [3]	NMIBC	UC	Caucasian (Northern American)	85 (Progression) / 336 (No progression)	rs11585883	1q23.3	RGS5	C	T	0.03 (1KG)	HR (CT/TT)=1.93	1.12-3.32	0.018
UBC Progression (metastases present)	2015	Deng et al. [5]	UBC	Not reported	Chinese	132 (metastasis) / 27 (no metastasis)	rs2910164	5q33.3	MIR146A	G	C	0.39 (all UBC) / 0.32 (TOPMED)	OR (CG+GG / CC)= 2.63	1.03–6.67	0.04
MIBC Progression (confirmed disease relapse)	2016	Xu et al. [11]	Stage 4 UBC (treated with platinum-based chemotherapy)	UC	Chinese	41 (Overall; death and progression count not reported)	Asn118Asn (rs11615)	19q13.32	ERCC1	T	C	0.27 / 0.33 (1KG)	HR (CC/CT+TT)= 1.83	1.12-2.99	0.016
UBC Progression (transition from NMIBC to MIBC, metastatic cancer, or cancer-specific death)	2017	Hess et al. [12]	UBC	UC	European (Germany)	179 (Overall, death and progression count not reported)	Thr7Thr (rs1801018)	18q21.33	BCL2	A	G	0.53 / 0.76 (1KG)	HR (AA/GG)=3.08	1.16–8.16	0.024

BCG-Bacillus Calmette-Guérin; CI-confidence interval; CIS-carcinoma in situ; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; TUR-transurethral resection; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.

[2] Ryk C, Wiklund NP, Nyberg T, De Verdier PJ. Ser608leu Polymorphisms in the Nitric Oxide Synthase-2 Gene May Influence Urinary Bladder Cancer Pathogenesis. Scandinavian journal of urology and nephrology. 2011;45(5):319-25.

[3] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.

[4] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.

[5] Deng S, Wang W, Li X, Zhang P. Common Genetic Polymorphisms in Pre-Micrornas and Risk of Bladder Cancer. World journal of surgical oncology. 2015;13:297.

[6] Sakano S, Berggren P, Kumar R, Steineck G, Adolfsson J, Onelov E, Hemminki K, Larsson P. Clinical Course of Bladder Neoplasms and Single Nucleotide Polymorphisms in the Cdkn2a Gene. International journal of cancer. 2003;104(1):98-103.

[7] Basturk B, Yavascaoglu I, Oral B, Goral G, Oktay B. Cytokine Gene Polymorphisms Can Alter the Effect of Bacillus Calmette-Guerin (Bcg) Immunotherapy. Cytokine. 2006;35(1-2):1-5.

[8] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.

[9] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.

[10] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.

[11] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).

[12] Hess J, Stelmach P, Eisenhardt A, Rubben H, Reis H, Schmid KW, Bachmann HS. Impact of Bcl2 Polymorphisms on Survival in Transitional Cell Carcinoma of the Bladder. Journal of cancer research and clinical oncology. 2017;143(9):1659-70.

Supplementary Table 3.9. Summary associations for previously reported SNPs on overall and cancer-specific survival for urinary bladder cancer.

Outcome	Year	Study	Patient subgroup	Cancer subtype	Ethnic background	Sample size (Cases/Controls)	SNP	Locus	Gene	EA	RA	EAF	Effect Size	95% CI	p value
Associations for cancer-specific death:															
Associations reducing the risk of cancer-specific death:															
High-risk NMIBC Cancer-specific death (BCG-treated)	2015	Ryk et al. [1]	High-risk (either TaG3, T1, TaG1+conCIS,TaG2+conCIS or primary CIS) NMIBC (BCG-treated)	UC	European (Sweden)	2 (GG, BCG-treated) / 6 (GG, not BCG-treated)	Asp298Glu (rs1799983)	7q36.1	NOS3	G	T	0.82 (1KG)	HR (BCG-treated GG/Not BCG-treated GG)=0.16	0.03–0.84	0.030
MIBC Cancer-specific death	2005	Leibovici et al. [2]	MIBC	Not reported	Caucasian (White)	105 (CG+CC) / 44 (GG)	rs1800795	7p15.3	IL6	C	G	0.14 (1KG)	HR (CG+CC/CC) =0.39	0.15-1.00	<0.05
MIBC Cancer-specific death (Radiotherapy-treated)	2012	Teo et al. [3]	MIBC (Radiotherapy-treated)	Not reported	European (United Kingdom)	139 (AG+GG) / 50 (AA)	rs7180135	15q15.1	RAD51	G	A	0.27 (1KG)	HR (AG+GG/AA) =0.52	0.31-0.87	0.01
UBC Cancer-specific death	2009	Shinohara et al. [4]	UBC	UC + Other	Japanese	77 (GT+GG) / 18 (TT)	rs2279744	12q15	MDM2	G	T	0.37 (1KG)	RR (GT+GG/TT) =0.57	0.36-0.95	0.031
UBC Cancer-specific death	2012	Guirado et al. [5]	UBC	UC	European (Spain)	Not reported	Ile775Val (rs4129009)	4p14	TLR10	T	C	0.85 (1KG)	HR (TT/CT+CC) =0.49	Not reported	0.022
UBC Cancer-specific death	2007	Sanyal et al. [6]	UBC	Not reported	European (Sweden)	68 (Dead) / 198 (Alive)	Ala222Val (rs1801133)	1p36.22	MTHFR	T	C	0.25 (1KG)	HR (CT+TT/CC) =0.5	0.3-0.9	0.03
Associations increasing the risk of cancer-specific death															

MIBC Cancer-specific death	2009	Castillejo et al. [7]	MIBC	UC	European (Spain)	35 (GT) / 54 (GG) 5 (TT) / 54 (GG)	rs334358	9q22.33	TGFBR1	T	G	0.12 (1KG)	HR (GT/GG)=1.67 HR (TT/GG)=2.83	1.05-2.68 1.09-7.34	p-trend=0.009
MIBC Cancer-specific death	2009	Castillejo et al. [7]	MIBC	UC	European (Spain)	35 (AG) / 52 (AA) 5 (GG) / 52 (AA)	rs868	9q22.33	TGFBR1	G	A	0.12 (1KG)	HR (AG/AA)=1.85 HR (GG/AA)=3.00	1.15-2.97 1.15-7.82	p-trend=0.003
MIBC Cancer-specific death (Cystectomy-treated)	2009	Horikawa et al. [8]	MIBC	UC	Japanese	26 (Dead) / 60 (Alive)	Arg72Pro (rs1042522)	17p13.1	TP53	C	G	0.54 (1KG)	HR (CC/CG+GG)=2.76	1.11-6.84	0.028
MIBC Cancer-specific death (Radiotherapy-treated)	2014	Teo et al. [9]	MIBC (radiotherapy-treated)	Not reported	European (Denmark)	70 (Dead) / 116 (Alive)	rs1805363	11q21	MRE11	A	G	0.11 / 0.03 (1KG)	HR (A/G)=2.10	1.34–3.28	0.001 (trend)
UBC Cancer-specific death	2005	Eisenhardt et al. [10]	UBC	UC	European (Germany)	65 (CT+TT) / 15 (TT)	Ser825Ser (rs5443)	12p13.31	GNB3	T	C	0.49 (1KG)	HR (CT+TT/CC)=1.928	1.038-3.203	0.037 (log-rank)
UBC Cancer-specific death	2012	Guirado et al. [5]	UBC	UC	European (Spain)	Not reported	rs9302752	16q12.1	NOD2	A	G	0.50 (1KG)	HR (TT/CT+CC)=3.19	Not reported	0.006
Associations for overall death:															
Associations reducing the risk of overall death:															
MIBC Overall survival	2005	Leibovici et al. [2]	MIBC	Not reported	Caucasian (White)	105 (CG+CC) / 44 (GG)	rs1800795	7p15.3	IL6	C	G	0.14 (1KG)	HR (CG+CC/CC)=0.43	0.19-0.94	<0.05
MIBC Overall survival	2010	Chen et al. [11]	MIBC	UC	Caucasian (Northern American)	96 (Dead) / 128 (Alive) (AG/GG) 75 (Dead) / 91 (Alive)	rs9906827	17q25.3	RPTOR	A	G	0.42 (1KG)	HR (AG/GG)=0.55 HR (AA/GG)=0.5	0.36–0.84 0.34–0.88	0.006 0.01 0.002

						(AA/GG) 125 (Dead) / 174 (Alive) (AA+AG/GG)							4 HR (AG+AA/GG) =0.55	0.37–0.81	
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs1051013	9q32	RGS3	A/C (not reported)	T	0.84 (1KG)	HR (??/TT,)=0.44	0.20-0.95	0.0362
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs1395960	1q23.3	RGS5	A	G	0.24 (1KG)	HR (AG/GG)=0.53	0.29-0.96	0.0377
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs762861	4p16.3	RGS12	C	G	0.36 (1KG)	HR (CG/GG)=0.69	0.48-0.98	0.0389
MIBC Overall survival	2015	Zhou et al. [13]	MIBC	UC	Chinese	38 (Dead) / 118 (Alive)	Ser61Ala (rs17855750)	16p12.1-p11.2	IL27	G	T	0.07 (1KG)	OR (TG+GG / TT)= 0.2 OR (TG/TT)= 0.12	0.05–0.90 0.02–0.88	0.035 0.037
NMIBC Overall survival	2014	Andrew et al. [14]	NMIBC	UC + Other	Caucasian (Northern American)	230 (Dead) / 333 (Alive), but not presented for NMIBC group	rs2662238	5q14.2	XRCC4	A	G	0.36 (1KG)	HR (GA/GG)=0.52	0.37-0.72	0.016 (log-rank)
UBC Overall survival	2007	Sanyal et al. [15]	UBC	Not reported	European (Sweden)	163 (AC+CC) / 97 (AA)	Lys751Ter (rs13181)	19q13.3	XPD (ERCC2)	C	A	0.24 (1KG)	HR (AC+CC/AA) =0.6	0.4-0.9	0.008
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 319 (Alive)	rs2854461	1q42.12	EPHX1	C	A	0.64 (1KG)	HR (C/A)=0.7	0.5-1.0	<0.05 and 0.02 (log-rank)

UBC Overall survival	2017	Hess et al. [17]	UBC	UC	European (Germany)	179 (Overall, death and progression count not reported)	rs2279115	18q21.33	BCL2	A	C	0.61 / 0.39 (1KG)	HR (CC/AA)=0.24	0.07-0.83	0.024
UBC Overall survival	2009	Mason et al. [18]	UBC	Not reported	Caucasian (Northern American)	Not reported	Arg521Lys (rs11543848, merged into rs2227983)	7p11.2	EGFR	A	G	0.29 (1KG)	HR (AG+AA/GG)=0.3	0.1-0.9	<0.05
UBC Overall survival	2009	Mason et al. [18]	UBC	Not reported	Caucasian (Northern American)	Not reported	rs2017000	7p11.2	EGFR	G	A	0.33 (1KG)	HR (GA+GG/AA)=0.6	0.3-1.00	<0.05
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	114 (Only reported overall)	Pro206Pro (rs915927)	19q13.2	XRCC1	G	A	0.32 (1KG)	HR (G/A)=0.55	0.32–0.94	0.03
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	123 (Only reported overall)	rs762507	19q13.2	XRCC1	A	G	0.29 (1KG)	HR (A/G)=0.48	0.27–0.84	0.01
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	120 (Only reported overall)	rs2854501	19q13.2	XRCC1	T	C	0.82 (1KG)	HR (T/C)=0.25	0.12–0.52	0.001
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	205 (Only reported overall)	rs2854509	19q13.2	XRCC1	A	C	0.18 (1KG)	HR (A/C)=0.21	0.09–0.46	0.001
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	122 (Only reported overall)	rs3213255	19q13.2	XRCC1	C	T	0.32 (1KG)	HR (C/T)=0.46	0.26–0.80	0.01
Associations increasing the risk of overall death:															

MIBC Overall survival	2010	Chen et al. [11]	MIBC	UC	Caucasian (Northern American)	121 (Dead) / 169 (Alive) (GG/AA) 125 (Dead) / 175 (Alive) (GG+AG/AA)	rs10515074	5q13.1	PIK3R1	G	A	0.20 (1KG)	HR (GA/AA)=1.88 HR (AG+AA/GG)=1.83	1.27–2.78 1.24–2.69	0.002 0.002
MIBC Overall survival	2010	Chen et al. [11]	MIBC	UC	Caucasian (Northern American)	109 (Dead) / 166 (Alive) (AG/GG) 68 (Dead) / 108 (Alive) (AA/GG) 125 (Dead) / 177 (Alive) (AA+AG/GG)	rs3730050	19q13.2	AKT2	A	G	0.25 (1KG)	HR (AG/GG)=1.51 HR (AA/GG)=2.99 HR (AG+AA/GG)=1.68	1.02–2.23 1.65–5.42 1.16–2.44	0.05 0.0002 0.006
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs2344673	1q23.3	RGS5	A	G	0.05 (1KG)	HR (A/G)=1.55	1.15-2.11	0.0045
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs10917690	1q23.3	RGS5	G	A	0.29 (1KG)	HR (GG/AA)=1.88	1.19-2.96	0.0066
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs1890398	1q31.2	RGS2	C	T	0.56 (1KG)	HR (CT/TT)=1.46	1.03-2.08	0.0353
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs12035879	1q23.3	RGS5	G	A	0.68 (1KG)	HR (GG/AA)=1.65	1.02-2.66	0.0387
MIBC Overall survival	2013	Lee et al. [12]	MIBC	UC	Caucasian (Northern American)	144 (Dead) / 181 (Alive)	rs10753605	1q23.3	RGS5	C	T	0.35 (1KG)	HR (CC/TT)=1.88	1.03-3.46	0.0395

MIBC Overall survival	2013	Djukic et al. [20]	MIBC	UC	European (Serbia)	62 (Died) / 27 (Alive)	Asn142Asp (rs156697)	10q25.1	GSTO2	G	A	0.56 (1KG)	HR (GG/AG+AA) =3.97	1.760–8.939	0.001
MIBC Overall survival	2013	Djukic et al. [20]	MIBC	UC	European (Serbia)	62 (Died) / 27 (Alive)	Ala140Asp (rs4925)	10q25.1	GSTO1	A	C	0.18 (1KG)	HR (AA/AC+CC) =2.94	1.164–7.430	0.022
MIBC Overall survival (Platinum-based chemotherapy-treated)	2016	Xu et al. [21]	Stage 4 UBC (treated with platinum-based chemotherapy)	UC	Chinese	41 (Overall; death and progression count not reported)	Asn118Asn (rs11615)	19q13.32	ERCC1	T	C	0.27 / 0.33 (1KG)	HR (CC / CT+TT)=1.94	1.17-3.27	0.01
NMIBC Overall survival	2014	Andrew et al. [14]	NMIBC	UC + Other	Caucasian (Northern American)	230 (Dead) / 333 (Alive), but not presented for NMIBC group	rs4987059	11p15.5	DRD4	A	G	0.04 (1KG)	HR (GA/GG)=1.83	1.18-2.85	0.024 (log-rank)
NMIBC Overall survival	2014	Zhou et al. [22]	NMIBC	UC	Chinese	13 (Recurrence) / 176 (No recurrence)	rs3756712	5p15.33	PDCD6	G	T	0.40 (1KG)	HR (GG/TT+GT) =5.11	1.43-18.22	0.01
UBC Overall survival	2007	Sanyal et al. [6]	UBC	Not reported	European (Sweden)	113 (Dead) / 198 (Alive)	Arg139Trp (rs4986998, merged into rs1131341)	16q22.1	NQO1	T	C	0.02 (1KG)	HR (CT+TT/CC) =1.8	1.0-3.3	0.05
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 212 (Alive)	rs6024840	20q13.2	AURKA	C	T	0.45 (1KG)	HR (C/T)=1.4	1.0-2.0	<0.05
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 325 (Alive)	rs1042640	2q37.1	UGT1A1	C	G	0.82 (1KG)	HR (C/G)=1.4	1.0-2.0	<0.05

UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	101 (Died) / 326 (Alive)	rs1126579	2q35	IL8RB (CXCR2)	C	T	0.60 (1KG)	HR (A/G)=1.7	1.2-2.5	<0.05 and 0.003 (log-rank)
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 319 (Alive)	rs528778	10p14	GATA3	T	C	0.14 (1KG)	HR (T/C)=1.1	0.8-1.5	<0.05 and <0.001 (log-rank)
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 319 (Alive)	Arg415Gln (rs1800067)	16p13.12	ERCC4	A	G	0.03 (1KG)	HR (A/G)=1.8	1.2-2.9	<0.05
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	100 (Died) / 319 (Alive)	rs1994251	20q11.21	BCL2L1	C	A	0.24 (1KG)	HR (C/A)=1.5	1.1-2.1	<0.05 and 0.01 (log-rank)
UBC Overall survival	2009	Andrew et al. [16]	UBC	Not reported	Caucasian (Northern American)	171 (Dead) / 300 (Alive)	rs9282638	3q13.33	CD80	A	G	o.87 (1KG)	HR (A/G)=1.9	1.4-2.7	<0.05 and 0.008 (log-rank)
UBC Overall survival	2014	Andrew et al. [16]	UBC	UC + Other	Caucasian (Northern American)	230 (Dead) / 333 (Alive)	rs35402311	8q11.23	RB1CC1	T	C	0.02 (1KG)	HR (CT/CC)=2.29	1.54-3.41	0.001 (log-rank)
UBC Overall survival	2017	Hess et al. [17]	UBC	UC	European (Germany)	179 (Overall, death and progression count not reported)	Thr7Thr (rs1801018)	18q21.33	BCL2	A	G	0.53 / 0.76 (1KG)	HR (AA/GG)=3.31	1.10–9.94	0.033
UBC Overall survival	2009	Mason et al. [18]	UBC	Not reported	Caucasian (Northern American)	Not reported	Asp994Asp (rs2293347)	7p11.2	EGFR	T	C	0.14 (1KG)	HR (TC+TT/CC) =1.5	1.0-2.3	<0.05
UBC Overall survival (Chemotherapy-treated)	2013	Sacerdote et al. [19]	UBC	Not reported	European (Italy)	121 (Only reported overall)	rs171140	19q13.3	ERCC2	C	A	0.37 (1KG)	HR (C/A)=2.07	1.06-4.28	0.03

CI-confidence interval; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; OR-odds ratio; RA-reference allele; SNP-single nucleotide polymorphism; UBC-urinary bladder cancer; UC-urothelial carcinoma; 1KG-1000 Genomes Project.

REFERENCES

[1] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.

[2] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.

[3] Teo MT, Landi D, Taylor CF, Elliott F, Vaslin L, Cox DG, Hall J, Landi S, Bishop DT, Kiltie AE. The Role of Microrna-Binding Site Polymorphisms in DNA Repair Genes as Risk Factors for Bladder Cancer and Breast Cancer and Their Impact on Radiotherapy Outcomes. Carcinogenesis. 2012;33(3):581-6.

[4] Shinohara A, Sakano S, Hinoda Y, Nishijima J, Kawai Y, Misumi T, Nagao K, Hara T, Matsuyama H. Association of Tp53 and Mdm2 Polymorphisms with Survival in Bladder Cancer Patients Treated with Chemoradiotherapy. Cancer science. 2009;100(12):2376-82.

[5] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.

[6] Sanyal S, Ryk C, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Nqo1 and the Clinical Course of Urinary Bladder Neoplasms. Scandinavian journal of urology and nephrology. 2007;41(3):182-90.

[7] Castillejo A, Rothman N, Murta-Nascimento C, Malats N, Garcia-Closas M, Gomez-Martinez A, Lloreta J, Tardon A, Serra C, Garcia-Closas R, Chanock S, Silverman DT, Dosemeci M, Kogevinas M, Carrato A, Soto JL, Real FX. Tgfb1 and Tgfbr1 Polymorphic Variants in Relationship to Bladder Cancer Risk and Prognosis. International journal of cancer. 2009;124(3):608-13.

[8] Horikawa Y, Nadaoka J, Saito M, Kumazawa T, Inoue T, Yuasa T, Tsuchiya N, Nishiyama H, Ogawa O, Habuchi T. Clinical Implications of the Mdm2 Snp309 and P53 Arg72pro Polymorphisms in Transitional Cell Carcinoma of the Bladder. Oncology reports. 2008;20(1):49-55.

[9] Teo MT, Dyrskjot L, Nsengimana J, Buchwald C, Snowden H, Morgan J, Jensen JB, Knowles MA, Taylor G, Barrett JH, Borre M, Orntoft TF, Bishop DT, Kiltie AE. Next-Generation Sequencing Identifies Germline Mre11a Variants as Markers of Radiotherapy Outcomes in Muscle-Invasive Bladder Cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2014;25(4):877-83.

[10] Eisenhardt A, Siffert W, Rosskopf D, Musch M, Mosters M, Roggenbuck U, Jockel KH, Rubben H, Lummen G. Association Study of the G-Protein Beta3 Subunit C825t Polymorphism with Disease Progression in Patients with Bladder Cancer. World journal of urology. 2005;23(4):279-86.
[11] Chen M, Gu J, Delclos GL, Killary AM, Fan Z, Hildebrandt MA, Chamberlain RM, Grossman HB, Dinney CP, Wu X. Genetic Variations of the Pi3k-Akt-Mtor Pathway and Clinical Outcome in Muscle Invasive and Metastatic Bladder Cancer Patients. Carcinogenesis. 2010;31(8):1387-91.
[12] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.
[13] Zhou B, Zhang P, Tang T, Liao H, Zhang K, Pu Y, Chen P, Song Y, Zhang L. Polymorphisms and Plasma Levels of Il-27: Impact on Genetic Susceptibility and Clinical Outcome of Bladder Cancer. BMC cancer. 2015;15:433.
[14] Andrew AS, Gui J, Hu T, Wyszynski A, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Ekstrom RM, Li Z, Zens MS, Borsuk M, Moore JH, Karagas MR. Genetic Polymorphisms Modify Bladder Cancer Recurrence and Survival in a USA Population-Based Prognostic Study. BJU international. 2015;115(2):238-47.
[15] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.
[16] Andrew AS, Gui J, Sanderson AC, Mason RA, Morlock EV, Schned AR, Kelsey KT, Marsit CJ, Moore JH, Karagas MR. Bladder Cancer Snp Panel Predicts Susceptibility and Survival. Human genetics. 2009;125(5-6):527-39.
[17] Hess J, Stelmach P, Eisenhardt A, Rubben H, Reis H, Schmid KW, Bachmann HS. Impact of Bcl2 Polymorphisms on Survival in Transitional Cell Carcinoma of the Bladder. Journal of cancer research and clinical oncology. 2017;143(9):1659-70.
[18] Mason RA, Morlock EV, Karagas MR, Kelsey KT, Marsit CJ, Schned AR, Andrew AS. Egfr Pathway Polymorphisms and Bladder Cancer Susceptibility and Prognosis. Carcinogenesis. 2009;30(7):1155-60.
[19] Sacerdote C, Guarrera S, Ricceri F, Pardini B, Polidoro S, Allione A, Critelli R, Russo A, Andrew AS, Ye Y, Wu X, Kiemeney LA, Bosio A, Casetta G, Cucchiarale G, Destefanis P, Gontero P, Rolle L, Zitella A, Fontana D, Vineis P, Matullo G. Polymorphisms in the Xrcc1 Gene Modify Survival of Bladder Cancer Patients Treated with Chemotherapy. International journal of cancer. 2013;133(8):2004-9.
[20] Djukic TI, Savic-Radojevic AR, Pekmezovic TD, Matic MG, Pljesa-Ercegovac MS, Coric VM, Radic TM, Suvakov SR, Krivic BN, Dragicevic DP, Simic TP. Glutathione S-Transferase T1, O1 and O2 Polymorphisms Are Associated with Survival in Muscle Invasive Bladder Cancer Patients. PloS one. 2013;8(9):e74724.
[21] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).

[22] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.

Supplementary Table 3.10. Gene clusters by functional annotation for urinary bladder cancer recurrence (DAVID tool).

	Term*	Genes	Bonferroni-adjusted p-value	FDR, %
Annotation Cluster 1 Enrichment Score: 2.72	IPR024066:Regulator of G-protein signaling domain 1	RGS10, RGS16, RGS13	0.04	0.43
	domain:RGS	RGS10, RGS16, RGS13	0.12	0.93
	SM00315:RGS	RGS10, RGS16, RGS13	0.03	1.19
	IPR016137:Regulator of G protein signalling superfamily	RGS10, RGS16, RGS13	0.11	1.35
	Signal transduction inhibitor	RGS10, RGS16, RGS13	0.15	1.55
	GO:0005096~GTPase activator activity	RGS10, RGS16, RGS13	1.00	52.55
Annotation Cluster 2 Enrichment Score: 2.42	GO:0007442~hindgut morphogenesis	GLI2, GLI3, SHH	0.01	0.02
	hsa04340:Hedgehog signaling pathway	GLI2, GLI3, SHH	0.29	3.34
	GO:0042475~odontogenesis of dentin-containing tooth	GLI2, GLI3, SHH	0.85	4.38
	GO:0042733~embryonic digit morphogenesis	GLI2, GLI3, SHH	0.86	4.54
	GO:0007224~smoothened signaling pathway	GLI2, GLI3, SHH	0.95	6.76
	GO:0030324~lung development	GLI2, GLI3, SHH	0.97	8.11
	h_shhPathway:Sonic Hedgehog (Shh) Pathway	GLI2, GLI3, SHH	0.45	8.75
	hsa05217:Basal cell carcinoma	GLI2, GLI3, SHH	0.74	12.39
	GO:0007411~axon guidance	GLI2, GLI3, SHH	1.00	29.44
	GO:0007507~heart development	GLI2, GLI3, SHH	1.00	36.49

* As listed in DAVID
FDR-false discovery rate

Supplementary Table 3.11. Enrichment in functional pathways for genes reported for bladder cancer recurrence (DAVID tool).

Term*	Genes list	Bonferroni-adjusted p value	FDR, %
hsa05200:Pathways in cancer	CXCL8, NFKBIA, IGF1, CDH1, BIRC5, GLI2, GLI3, MMP2, SHH	0.001	0.01
GO:0007442~hindgut morphogenesis	GLI2, GLI3, SHH	0.008	0.02
GO:0045944~positive regulation of transcription from RNA polymerase II promoter	VDR, SLC11A1, IL17A, IFNG, NFKBIA, IGF1, GLI2, GLI3, SHH	0.04	0.09
GO:0048566~embryonic digestive tract development	CXCL8, GLI2, GLI3	0.15	0.37
IPR024066:Regulator of G-protein signaling domain 1	RGS10, RGS16, RGS13	0.04	0.43
GO:0043066~negative regulation of apoptotic process	NFKBIA, IGF1, BIRC5, GLI2, GLI3, SHH	0.26	0.72
domain:RGS	RGS10, RGS16, RGS13	0.12	0.93
GO:0000060~protein import into nucleus translocation	SLC11A1, IFNG, NFKBIA	0.39	1.16
sequence variant	ICAM1, CWC27, NEIL2, NFKBIA, IGF1, CDH1, BIRC5, SOD1, RGS16, GLI2, GLI3, MMP2, SHH, RGS13, GSS, DROSHA, SLC11A1, RGS10, VDR, ERCC6, GPX4, IFNG, ALDH2, DDX20	0.13	1.01
mutagenesis site	DROSHA, VDR, GPX4, NEIL2, NFKBIA, CDH1, BIRC5, RGS16, SOD1, SHH	0.13	1.05
IPR016137:Regulator of G protein signalling superfamily	RGS10, RGS16, RGS13	0.11	1.35
Signal transduction inhibitor	RGS10, RGS16, RGS14	0.15	1.55
SM00315:RGS	RGS10, RGS16, RGS15	0.03	1.19
GO:0005615~extracellular space	ICAM1, IL17A, IFNG, CXCL8, IGF1, SOD1, MMP2, SHH	0.15	1.90
Disease mutation	GSS, VDR, ERCC6, NFKBIA, CDH1, GLI2, SOD1, GLI3, MMP2, SHH	0.23	2.45
hsa05323:Rheumatoid arthritis	ICAM1, IL17A, IFNG, CXCL8	0.23	2.56
hsa04340:Hedgehog signaling pathway	GLI2, GLI3, SHH	0.29	3.34
GO:0042475~odontogenesis of dentin-containing tooth	GLI2, GLI3, SHH	0.85	4.38
GO:0042733~embryonic digit morphogenesis	GLI2, GLI3, SHH	0.86	4.54
h_freePathway:Free Radical Induced Apoptosis	GSS, CXCL8, SOD1	0.21	3.47

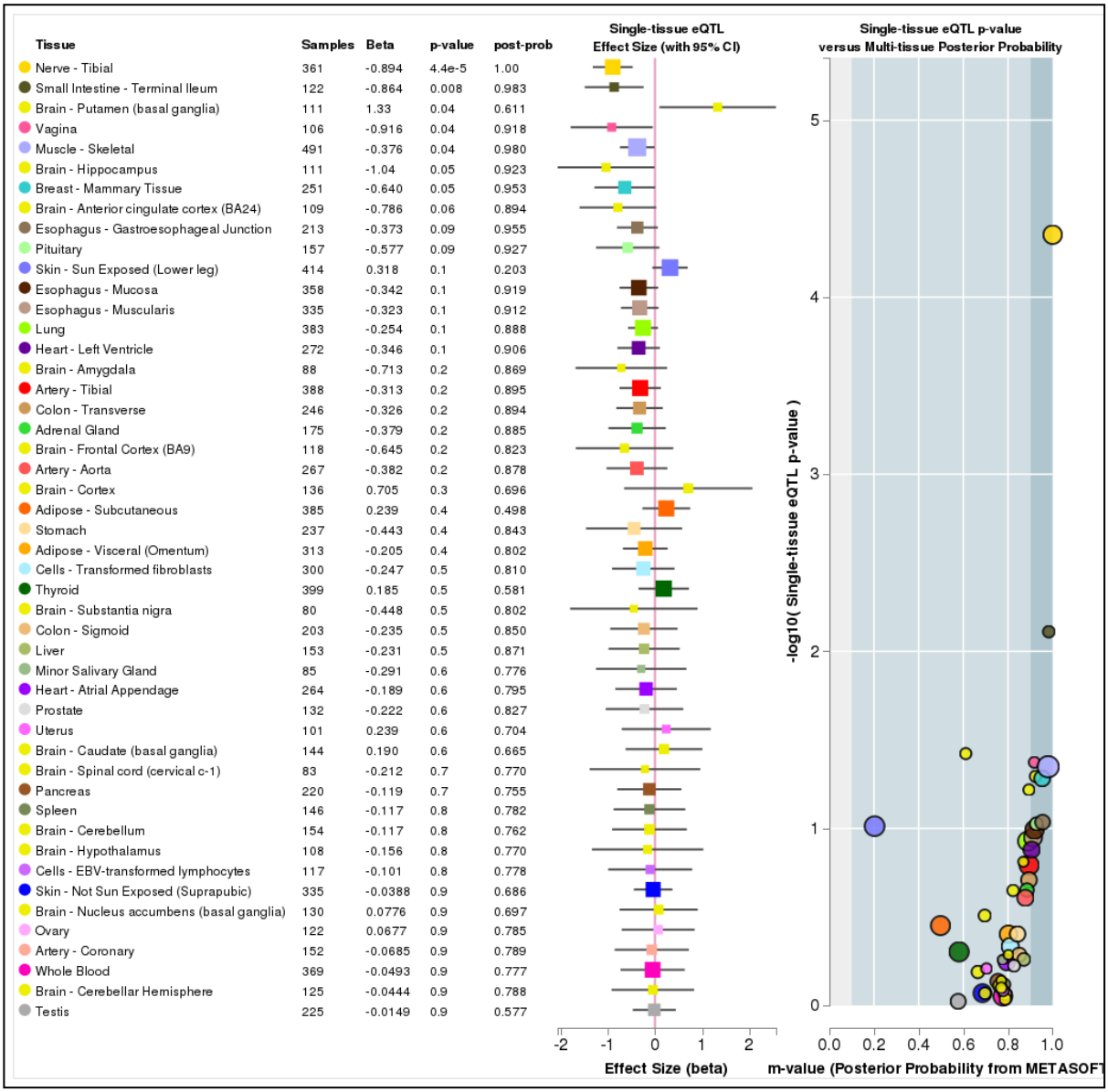
*As listed in DAVID.
FDR-false discovery rate

Supplementary Table 3.12. Enrichment in functional pathways for genes reported for bladder cancer death (DAVID tool).

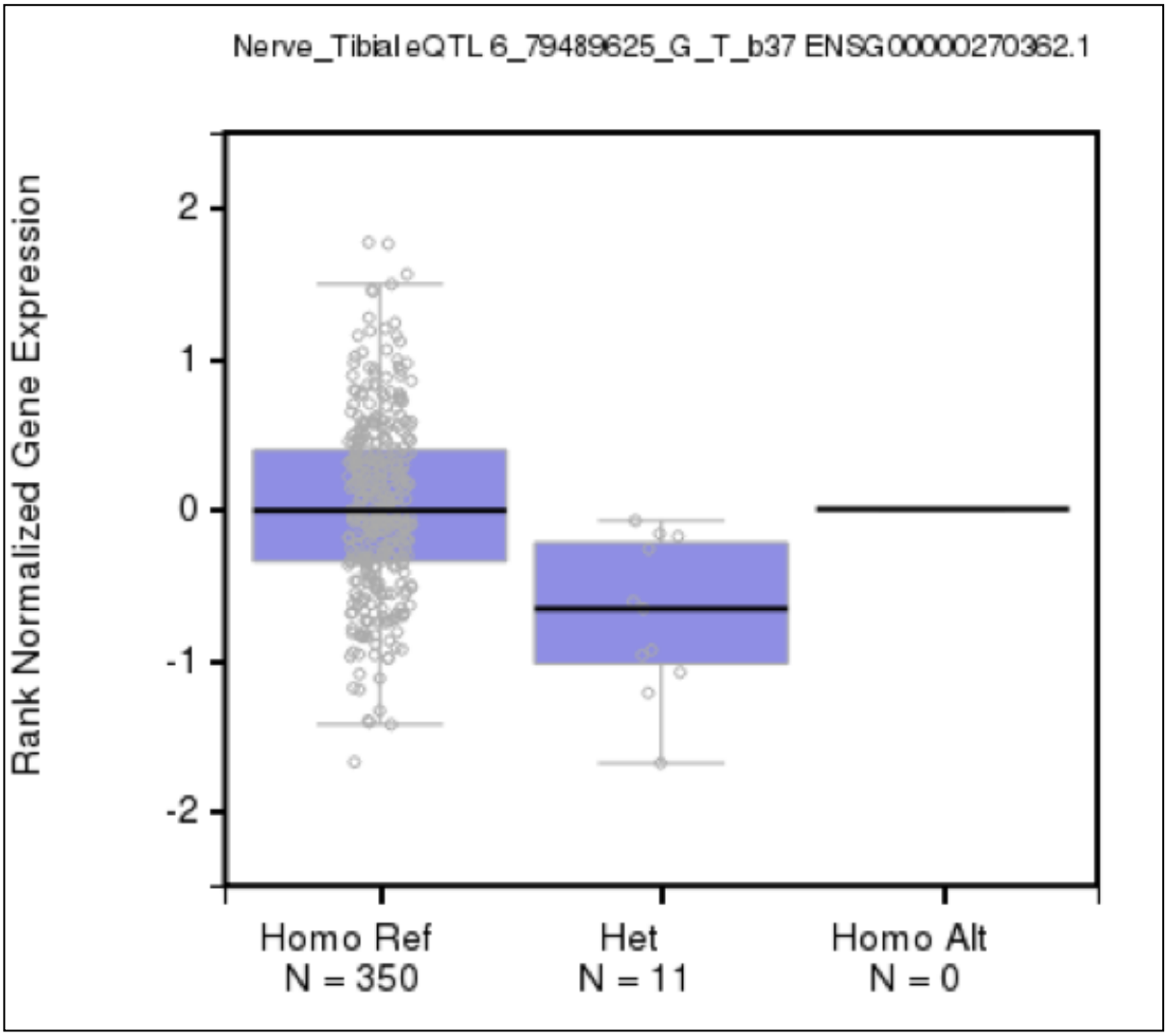
Term*	Genes list	Bonferroni-adjusted p value	FDR, %
hsa05212:Pancreatic cancer	TGFBR1, BCL2L1, PIK3R1, AKT2	0.05	0.47
GO:0046326~positive regulation of glucose import	PIK3R1, TERT, AKT2	0.22	0.74
Ubl conjugation	XRCC4, RGS3, TGFBR1, DRD4, AURKA, PIK3R1, TERT, AKT2	0.06	0.61
hsa05220:Chronic myeloid leukemia	TGFBR1, BCL2L1, PIK3R1, AKT2	0.06	0.64
GO:0005829~cytosol	XRCC4, RGS3, RB1CC1, AURKA, GSTO2, GNB3, BCL2L1, PIK3R1, RPTOR, AKT2, MRE11	0.06	0.79
GO:0007165~signal transduction	RGS12, TGFBR1, GATA3, CXCR2, GNB3, PIK3R1, AKT2	0.47	1.89
hsa05166:HTLV-I infection	TGFBR1, BCL2L1, PIK3R1, TERT, AKT2	0.24	2.63
Phosphoprotein	XRCC4, TGFBR1, CXCR2, AURKA, BCL2L1, RPTOR, MRE11, RGS12, CD80, RGS3, GATA3, RB1CC1, TERT, PIK3R1, AKT2	0.29	3.45
Transferase	TGFBR1, AURKA, GSTO2, UGT1A1, PIK3R1, TERT, AKT2	0.31	3.78
GO:0005515~protein binding	XRCC4, TGFBR1, DRD4, CXCR2, AURKA, BCL2L1, RPTOR, MRE11, CD80, RGS3, GATA3, RB1CC1, GSTO2, GNB3, TERT, PIK3R1, AKT2	0.37	4.23

*As listed in DAVID
FDR-false discovery rate.

Supplementary Figure 5.1 eQTL effect of rs144383242 (6q14.1) across multiple tissues (source: GTEx).



Supplementary Figure 5.2. Boxplot of an eQTL effect of rs144383242 (6q14.1) in nerve tissue (source: GTEx).



Supplementary Table 6.1 Previously reported polymorphisms in association with bladder cancer recurrence.

Outcome	SNP	Locus	Gene	EA	RA	EAF*	Discovery population	References
High-risk NMIBC Recurrence (BCG-treated)	rs2070744	7q36.1	NOS3	T	C	0.77	European (Sweden)	Ryk et al.[1]
High-risk NMIBC Recurrence (BCG-treated)	rs1799983	7q36.1	NOS3	G	T	0.82	European (Sweden)	Ryk et al.[1]
Low-risk NMIBC recurrence	rs744154	16p13.12	ERCC4	C	A	0.22	Chinese	Wang et al. [2]
Low-risk NMIBC recurrence	rs798766	4p16.3	TACC3/FGFR3	T	C	0.24	European (multiple)	Kiemeney et al. [3]
MIBC Recurrence	rs4957014	5p15.33	PDCD6	G	T	0.65	Chinese	Zhou et al. [4]
MIBC recurrence	rs4758680	12q24.31	IL31	A	C	0.29	Chinese	Li et al. [5]
NMIBC and UBC Recurrence	rs2505568	10p11.21 (corrected from the originally reported) 7q22.3	NAMPTP1 (corrected from originally reported NAMPT)	A	T	0.57	Chinese	Zhang et al. [6]
NMIBC Recurrence	rs2910164	5q33.3	MIR146A	C	G	0.28 (ExAC)	Chinese	Wang et al. [7]
NMIBC Recurrence	rs511918	1q25.3	RGS16	T	G	0.47	Caucasian (Northern American)	Lee et al. [8]
NMIBC Recurrence	rs16829458	1q31.2	RGS2	A	G	0.12	Caucasian (Northern American)	Lee et al. [8]
NMIBC Recurrence	rs3795617	1q31.2	RGS13	A	G	0.38	Caucasian (Northern American)	Lee et al. [8]
NMIBC Recurrence	rs11199005	10q26.11	RGS10	A	G	0.35	Caucasian (Northern American)	Lee et al. [8]
NMIBC Recurrence	rs1323291	1q31.2	RGS1	C	A	0.15	Caucasian (Northern American)	Lee et al. [8]
NMIBC Recurrence	rs5742714	12q23.2	IGF1	C	G	0.9	Caucasian (Northern American)	Andrew et al. [9]
NMIBC Recurrence	rs2238151	12q24.12	ALDH2	C	T	0.7	Caucasian (Northern American)	Andrew et al. [9]
NMIBC Recurrence	rs2169830	15q14	THBS1	G	A	0.42	Chinese	Yang et al. [10]
NMIBC recurrence (<64-year-old group)	rs1050450	3p21.31	GPX1	T	C	0.22	Caucasian (Northern American)	Zhao et al. [11]
NMIBC recurrence (BCG-treated)	rs16260	16q22.1	CDH1	A	C	0.24	Caucasian (Northern American)	Lin et al. [12]
NMIBC recurrence (BCG-treated)	rs4645978	1p36.21	CASP9	G	A	0.42	Indian	Gangwar et al. [13]
NMIBC Recurrence (BCG-treated)	rs7003908	8q11.21	PRKDC	G	T	0.33	Indian	Gangwar et al. [14]

NMIBC Recurrence (BCG-treated)	rs9904341	17q25.3	BIRC5	C	G	0.39	Indian	Jaiswal et al. [15]
NMIBC Recurrence (BCG-treated)	rs804267	8p23.1	NEIL2	C	T	0.31	Caucasian (Northern American)	Wei et al. [16]
NMIBC Recurrence (BCG-treated)	rs8191604	8p23.1	NEIL2	C	A	0.18	Caucasian (Northern American)	Wei et al. [16]
NMIBC recurrence (BCG-treated)	rs17235409	2q35	SLC11A1	A	G	0.07	Caucasian (Canadian), Chinese	Decobert et al. [17], Chiong et al. [18]
NMIBC recurrence (BCG-treated)	rs1799782	19q13.2	XRCC1	T	C	0.12	Indian	Mittal et al. [19]
NMIBC recurrence (BCG-treated)	rs2228526	10q11.23	ERCC6	C	G	0.18	Caucasian (Northern American)	Gu et al. [20]
NMIBC recurrence (BCG-treated)	rs25487	19q13.2	XRCC1	A	G	0.26	Indian	Mittal et al. [19]
NMIBC recurrence (BCG-treated)	rs1799793	19q13.3	ERCC2	A	G	0.19	Indian	Gangawar et al. [21]
NMIBC recurrence (BCG-treated)	rs2430561	12q15	IFN-G	A	T	0.28	Indian	Ahirwar et al. [22]
NMIBC Recurrence (BCG-treated)	rs2228001	3p25.1	XPC	C	A	0.32	Indian	Gangwar et al. [23]
NMIBC Recurrence (BCG-treated)	rs6463089	7p14.1	GLI3	A	G	0.09	Caucasians (Northern American) + European (Spain) for replication	Chen et al. [24]
NMIBC Recurrence (BCG-treated)	rs3801192	7p14.1	GLI3	A	G	0.07	Caucasians (Northern American) + European (Spain) for replication	Chen et al. [24]
NMIBC Recurrence (BCG-treated)	rs1233560	7q36.3	SHH	G	A	0.46	Caucasians (Northern American) + European (Spain) for replication	Chen et al. [24]
NMIBC Recurrence (BCG-treated)	rs11685068	2q14.2	GLI2	A	G	0.06	Caucasians (Northern American) + European (Spain) for replication	Chen et al. [24]
NMIBC Recurrence (BCG-treated)	rs243865	16q12.2	MMP2	C	T	0.86	Indian	Srivastava et al. [25]
NMIBC Recurrence (BCG-treated)	rs804276	8p23.1	NEIL2	G	A	0.62	Caucasian (Northern American)	Wei et al. [16]
NMIBC Recurrence (BCG-treated)	rs4639	8p23.1	NEIL2	G	A	0.47	Caucasian (Northern American)	Wei et al. [16]
NMIBC Recurrence (BCG-treated)	rs2173962	21q22.11	SOD1	G	A	0.06	Caucasian (Northern American)	Wei et al. [16]
NMIBC Recurrence (BCG-treated)	rs187238	11q23.1	IL18	C	G	0.79	Indian	Jaiswal et al. [26]
NMIBC Recurrence (BCG-treated)	rs1695	11q13.2	GSTP1	G	A	0.35	Chinese	Deng et al. [27]
NMIBC Recurrence (BCG-treated)	rs4925	10q25.1	GSTO1	A	C	0.1	Chinese	Deng et al. [27]
NMIBC Recurrence (BCG-treated)	rs3138056	14q13.2	NFKBIA	T?	C?	0.38 (T)	Northern American	Williams et al. [28]
NMIBC Recurrence (BCG-treated)	rs1544410	12q13.11	VDR	A	G	0.3	Chinese	Wang et al. [29]
NMIBC Recurrence (BCG-treated), NMIBC Recurrence	rs2279744	12q15	MDM2	G	T	0.37	Indian, Chinese	Gangwar et al. [30], Xie et al. [31]
NMIBC recurrence (BCG-treated), NMIBC recurrence (maintenance BCG-treated)	rs1800795	7p15.3	IL6	C	G	0.14	Indian, Caucasian (White)	Ahirwar et al. [32], Leibovici et al. [33]

NMIBC recurrence (BCG-treated), NMIBC Recurrence (TUR- and BCG-treated)	rs1799964	6p21.33	TNFA	C	T	0.22	Indian, European (Southern Portugal)	Ahirwar et al. [34], Lima et al. [35]
NMIBC Recurrence (Epirubicin-treated)	rs915927	19q13.2	XRCC1	G	A	0.32	Chinese	Li et al. [36]
NMIBC Recurrence (Epirubicin-treated)	rs2854501	19q13.2	XRCC1	T	C	0.18	Chinese	Li et al. [36]
NMIBC recurrence (non-BCG-treated)	rs1801282	3p25.2	PPARG	G	C	0.07	Caucasian (White)	Leibovici et al. [33]
NMIBC Recurrence (TUR- and BCG-treated)	rs1799864 (corrected from author-reported rs391835)	3p21.31	CCR2	A	G	0.41	European (Southern Portugal)	Lima et al. [35]
NMIBC Recurrence (TUR- and BCG-treated)	rs5498	19p13.2	ICAM1	G	A	0.36	European (Southern Portugal)	Lima et al. [35]
NMIBC Recurrence (TUR- and BCG-treated)	rs2275913	6p12.2	IL17A	A	G	0.29	European (Southern Portugal)	Lima et al. [35]
NMIBC Recurrence (TUR- and BCG-treated)	rs13278062	8p21.3	TNFRSF10A	G	T	0.6	European (Southern Portugal)	Lima et al. [35]
NMIBC Recurrence (TUR- and BCG-treated)	rs3746162	19p13.3	GPX4	A	G	0.16	Caucasians (European decent)	Ke et al. [37]
NMIBC Recurrence (TUR- and BCG-treated)	rs7265992	20q11.22	GSS	A	G	0.19	Caucasians (European decent)	Ke et al. [37]
NMIBC Recurrence (TUR- and BCG-treated)	rs6060124	20q11.22	GSS	A	C	0.26	Caucasians (European decent)	Ke et al. [37]
NMIBC Recurrence (TUR- and BCG-treated)	rs7260770	20q11.22	GSS	A	G	0.22	Caucasians (European decent)	Ke et al. [37]
NMIBC Recurrence (TUR- and BCG-treated)	rs4911455	20q11.22	GSS	C	A	0.28	Caucasians (European decent)	Ke et al. [37]
NMIBC Recurrence (TUR-treated + Epirubicin)	rs2854509	19q13.31	XRCC1	A	C	0.82	Chinese	Deng et al. [38]
NMIBC Recurrence (TUR-treated + Epirubicin)	rs3213255	19q13.31	XRCC1	C	T	0.32	Chinese	Deng et al. [38]
NMIBC Recurrence (TUR-treated)	rs1042522	17p13.1	TP53	C	G	0.54	Japanese	Horikawa et al. [39]
NMIBC Recurrence (TUR-treated)	rs197412	1p13.2	DDX20	T	C	0.53	Caucasian (Northern American)	Ke et al. [40]
NMIBC Recurrence (TUR-treated)	rs12186785	5p13.3	DROSHA	C	T	0.05	Caucasian (Northern American)	Ke et al. [40]
NMIBC Recurrence-(BCG-treated)	rs804256	8p23.1	NEIL2	C	T	0.26	Caucasian (Northern American)	Wei et al. [16]
NMIBC Recurrence, NMIBC Recurrence (non-BCG-treated)	rs1052133	3p25.3	OGG1	G	C	0.3	Korean, Indian	Kim et al. [41], Gangwar et al. [14]
Recurrence	rs2042329	5q12.3	CWC27	T	G	0.34	Chinese	Wang et al. [42]
UBC Recurrence	rs2292016	5p13.1	OSMR	T	G	0.08	Chinese	Deng et al. [43]

UBC Recurrence	rs2278329	5p13.1	OSMR	A	G	0.08	Chinese	Deng et al. [43]
UBC Recurrence (BCG-treated)	rs4073	4q13.3	CXCL8	A	T	0.52	Indian	Ahirwar et al. [44]

BCG-Bacillus Calmette-Guérin; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; RA-reference allele; SNP-single nucleotide polymorphism; TUR-transurethral resection; UBC-urinary bladder cancer.
*Global, based on 1000 Genomes Project.

REFERENCES:

[1] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.

[2] Wang M, Wang M, Yuan L, Wu D, Zhang Z, Yin C, Fu G, Wei Q, Zhang Z. A Novel Xpf -357a>C Polymorphism Predicts Risk and Recurrence of Bladder Cancer. Oncogene. 2010;29(13):1920-8.

[3] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.

[4] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.

[5] Li Q, Tang T, Zhang P, Liu C, Pu Y, Zhang Y, Song H, Wang Y, Song Y, Su M, Zhou B, Zhang L. Correlation of Il-31 Gene Polymorphisms with Susceptibility and Clinical Recurrence of Bladder Cancer. Familial cancer. 2018;17(4):577-85.

[6] Zhang K, Zhou B, Zhang P, Zhang Z, Chen P, Pu Y, Song Y, Zhang L. Genetic Variants in Nampt Predict Bladder Cancer Risk and Prognosis in Individuals from Southwest Chinese Han Group. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4031-40.

[7] Wang M, Chu H, Li P, Yuan L, Fu G, Ma L, Shi D, Zhong D, Tong N, Qin C, Yin C, Zhang Z. Genetic Variants in Mirnas Predict Bladder Cancer Risk and Recurrence. Cancer research. 2012;72(23):6173-82.

[8] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.

[9] Andrew AS, Gui J, Hu T, Wyszynski A, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Ekstrom RM, Li Z, Zens MS, Borsuk M, Moore JH, Karagas MR. Genetic Polymorphisms Modify Bladder Cancer Recurrence and Survival in a USA Population-Based Prognostic Study. BJU international. 2015;115(2):238-47.
[10] Yang X, Li P, Yang X, Qin C, Cao Q, Zhang Z, Wang M, Cai H, Gu J, Tao J, Gu M, Lu Q, Yin C. Tsp-1-1223 a/G Polymorphism as a Potential Predictor of the Recurrence Risk of Bladder Cancer in a Chinese Population. International Journal of Genomics. 2013;2013:9.
[11] Zhao H, Liang D, Grossman HB, Wu X. Glutathione Peroxidase 1 Gene Polymorphism and Risk of Recurrence in Patients with Superficial Bladder Cancer. Urology. 2005;66(4):769-74.
[12] Lin J, Dinney CP, Grossman HB, Jhamb M, Zhu Y, Spitz MR, Wu X. E-Cadherin Promoter Polymorphism (C-160a) and Risk of Recurrence in Patients with Superficial Bladder Cancer. Clinical genetics. 2006;70(3):240-5.
[13] Gangwar R, Mandhani A, Mittal RD. Caspase 9 and Caspase 8 Gene Polymorphisms and Susceptibility to Bladder Cancer in North Indian Population. Annals of surgical oncology. 2009;16(7):2028-34.
[14] Gangwar R, Ahirwar D, Mandhani A, Mittal RD. Do DNA Repair Genes Ogg1, Xrcc3 and Xrcc7 Have an Impact on Susceptibility to Bladder Cancer in the North Indian Population? Mutation research. 2009;680(1-2):56-63.
[15] Jaiswal PK, Goel A, Mandhani A, Mittal RD. Functional Polymorphisms in Promoter Survivin Gene and Its Association with Susceptibility to Bladder Cancer in North Indian Cohort. Molecular biology reports. 2012;39(5):5615-21.
[16] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.
[17] Decobert M, Larue H, Bergeron A, Harel F, Pfister C, Rousseau F, Lacombe L, Fradet Y. Polymorphisms of the Human Nramp1 Gene Are Associated with Response to Bacillus Calmette-Guerin Immunotherapy for Superficial Bladder Cancer. The Journal of urology. 2006;175(4):1506-11.
[18] Chiong E, Kesavan A, Mahendran R, Chan YH, Sng JH, Lim YK, Kamaraj R, Tan TM, Esuvaranathan K. Nramp1 and Hgpx1 Gene Polymorphism and Response to Bacillus Calmette-Guerin Therapy for Bladder Cancer. Eur Urol. 2011;59(3):430-7.
[19] Mittal RD, Singh R, Manchanda PK, Ahirwar D, Gangwar R, Kesarwani P, Mandhani A. Xrcc1 Codon 399 Mutant Allele: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Cancer biology & therapy. 2008;7(5):645-50.
[20] Gu J, Zhao H, Dinney CP, Zhu Y, Leibovici D, Bermejo CE, Grossman HB, Wu X. Nucleotide Excision Repair Gene Polymorphisms and Recurrence after Treatment for Superficial Bladder Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(4):1408-15.
[21] Gangawar R, Ahirwar D, Mandhani A, Mittal RD. Impact of Nucleotide Excision Repair Ercc2 and Base Excision Repair Apex1 Genes Polymorphism and Its Association with Recurrence after Adjuvant Bcg Immunotherapy in Bladder Cancer Patients of North India. Medical oncology (Northwood, London, England). 2010;27(2):159-66.

[22] Ahirwar DK, Agrahari A, Mandhani A, Mittal RD. Cytokine Gene Polymorphisms Are Associated with Risk of Urinary Bladder Cancer and Recurrence after Bcg Immunotherapy. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2009;14(4):213-8.
[23] Gangwar R, Mandhani A, Mittal RD. Xpc Gene Variants: A Risk Factor for Recurrence of Urothelial Bladder Carcinoma in Patients on Bcg Immunotherapy. Journal of cancer research and clinical oncology. 2010;136(5):779-86.
[24] Chen M, Hildebrandt MA, Clague J, Kamat AM, Picornell A, Chang J, Zhang X, Izzo J, Yang H, Lin J, Gu J, Chanock S, Kogevinas M, Rothman N, Silverman DT, Garcia-Closas M, Grossman HB, Dinney CP, Malats N, Wu X. Genetic Variations in the Sonic Hedgehog Pathway Affect Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Cancer prevention research (Philadelphia, Pa). 2010;3(10):1235-45.
[25] Srivastava P, Kapoor R, Mittal RD. Association of Single Nucleotide Polymorphisms in Promoter of Matrix Metalloproteinase-2, 8 Genes with Bladder Cancer Risk in Northern India. Urologic oncology. 2013;31(2):247-54.
[26] Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of Il-12, Il-18 Variants and Serum Il-18 with Bladder Cancer Susceptibility in North Indian Population. Gene. 2013;519(1):128-34.
[27] Deng X, Yang X, Cheng Y, Liu X, Li X, Zhao R, Qin C, Lu Q, Yin C. Gstp1 and Gsto1 Single Nucleotide Polymorphisms and the Response of Bladder Cancer Patients to Intravesical Chemotherapy. Scientific reports. 2015;5:14000.
[28] Williams SB, Kamat AM, Mmeje C, Ye Y, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variants in the Inflammation Pathway as Predictors of Recurrence and Progression in Non-Muscle Invasive Bladder Cancer Treated with Bacillus Calmette-Guerin. Oncotarget. 2017;8(51):88782-91.
[29] Wang Z, Lim YK, Lim HCC, Chan YH, Ngiam N, Raman Nee Mani L, Esuvaranathan K, Ng CF, Teoh J, Chan E, Mahendran R, Chiong E. The Role of Vitamin D Receptor Polymorphisms in Predicting the Response to Therapy for Nonmuscle Invasive Bladder Carcinoma. The Journal of urology. 2018;200(4):737-42.
[30] Gangwar R, Mittal RD. Association of Selected Variants in Genes Involved in Cell Cycle and Apoptosis with Bladder Cancer Risk in North Indian Population. DNA and cell biology. 2010;29(7):349-56.
[31] Xie L, Sun Y, Chen T, Tian D, Li Y, Zhang Y, Ding N, Shen Z, Xu H, Nian X, Sha N, Han R, Hu H, Wu C. Association between Mdm2 Snp309 T>G Polymorphism and the Risk of Bladder Cancer: New Data in a Chinese Population and an Updated Meta-Analysis. OncoTargets and therapy. 2015;8:3679-90.
[32] Ahirwar D, Kesarwani P, Manchanda PK, Mandhani A, Mittal RD. Anti- and Proinflammatory Cytokine Gene Polymorphism and Genetic Predisposition: Association with Smoking, Tumor Stage and Grade, and Bacillus Calmette-Guerin Immunotherapy in Bladder Cancer. Cancer genetics and cytogenetics. 2008;184(1):1-8.
[33] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.

[34] Ahirwar DK, Mandhani A, Dharaskar A, Kesarwani P, Mittal RD. Association of Tumour Necrosis Factor-Alpha Gene (T-1031c, C-863a, and C-857t) Polymorphisms with Bladder Cancer Susceptibility and Outcome after Bacille Calmette-Guerin Immunotherapy. BJU international. 2009;104(6):867-73.
[35] Lima L, Oliveira D, Ferreira JA, Tavares A, Cruz R, Medeiros R, Santos L. The Role of Functional Polymorphisms in Immune Response Genes as Biomarkers of Bacille Calmette-Guerin (Bcg) Immunotherapy Outcome in Bladder Cancer: Establishment of a Predictive Profile in a Southern Europe Population. BJU international. 2015;116(5):753-63.
[36] Li P, Zhang X, Deng X, Tao J, Qin C, Yang X, Cheng Y, Lu Q, Wang Z, Yin C. Pharmacogenetic Association between Xrcc1 Polymorphisms and Improved Outcomes in Bladder Cancer Patients Following Intravesical Instillation of Epirubicin. International journal of clinical and experimental medicine. 2015;8(7):11167-73.
[37] Ke HL, Lin J, Ye Y, Wu WJ, Lin HH, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Glutathione Pathway Genes Predict Cancer Recurrence in Patients Treated with Transurethral Resection and Bacillus Calmette-Guerin Instillation for Non-Muscle Invasive Bladder Cancer. Annals of surgical oncology. 2015;22(12):4104-10.
[38] Deng X, Zhang X, Cheng Y, Yang X, Zhao R, Liu X, Li X, Qin C, Lu Q, Yin C. Xrcc1 Polymorphisms Associated with Survival among Chinese Bladder Cancer Patients Receiving Epirubicin and Mitomycin C. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015;36(6):4591-6.
[39] Horikawa Y, Nadaoka J, Saito M, Kumazawa T, Inoue T, Yuasa T, Tsuchiya N, Nishiyama H, Ogawa O, Habuchi T. Clinical Implications of the Mdm2 Snp309 and P53 Arg72pro Polymorphisms in Transitional Cell Carcinoma of the Bladder. Oncology reports. 2008;20(1):49-55.
[40] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.
[41] Kim EJ, Jeong P, Quan C, Kim J, Bae SC, Yoon SJ, Kang JW, Lee SC, Jun Wee J, Kim WJ. Genotypes of Tnf-Alpha, Vegf, Hogg1, Gstm1, and Gstt1: Useful Determinants for Clinical Outcome of Bladder Cancer. Urology. 2005;65(1):70-5.
[42] Wang M, Li Z, Chu H, Lv Q, Ye D, Ding Q, Xu C, Guo J, Du M, Chen J, Song Z, Yin C, Qin C, Gu C, Zhu Y, Xia G, Liu F, Zhang Z, Yuan L, Fu G, Hu Z, Tong N, Shen J, Liu K, Sun J, Sun Y, Li J, Li X, Shen H, Xu J, Shi Y, Zhang Z. Genome-Wide Association Study of Bladder Cancer in a Chinese Cohort Reveals a New Susceptibility Locus at 5q12.3. Cancer research. 2016;76(11):3277-84.
[43] Deng S, He SY, Zhao P, Zhang P. The Role of Oncostatin M Receptor Gene Polymorphisms in Bladder Cancer. World journal of surgical oncology. 2019;17(1):30.
[44] Ahirwar DK, Mandhani A, Mittal RD. Il-8 -251 T > a Polymorphism Is Associated with Bladder Cancer Susceptibility and Outcome after Bcg Immunotherapy in a Northern Indian Cohort. Archives of medical research. 2010;41(2):97-103.

Supplementary Table 6.2. Previously reported polymorphisms in association with bladder cancer death.

Outcome	SNP	Locus	Gene	EA	RA	EAF*	Discovery population	References
High-risk NMIBC Cancer-specific death (BCG-treated)	rs1799983	7q36.1	NOS3	G	T	0.82	European (Sweden)	Ryk et al. [1]
MIBC Cancer-specific death	rs1800795	7p15.3	IL6	C	G	0.14	Caucasian (White)	Leibovici et al.[2]
MIBC Cancer-specific death	rs334358	9q22.33	TGFBR1	T	G	0.12	European (Spain)	Castillejo et al. [3]
MIBC Cancer-specific death	rs868	9q22.33	TGFBR1	G	A	0.12	European (Spain)	Castillejo et al. [3]
MIBC Cancer-specific death (Cystectomy-treated)	rs1042522	17p13.1	TP53	C	G	0.54	Japanese	Horikawa et al. [4]
MIBC Cancer-specific death (Radiotherapy-treated)	rs7180135	15q15.1	RAD51	G	A	0.27	European (United Kingdom)	Teo et al. [5]
MIBC Cancer-specific death (Radiotherapy-treated)	rs1805363	11q21	MRE11	A	G	0.03	European (Denmark)	Teo et al. [6]
MIBC Overall survival	rs9906827	17q25.3	RPTOR	A	G	0.42	Caucasian (Northern American)	Chen et al. [7]
MIBC Overall survival	rs1051013	9q32	RGS3	A/C	T	0.84	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs1395960	1q23.3	RGS5	A	G	0.24	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs762861	4p16.3	RGS12	C	G	0.36	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs17855750	16p12.1-p11.2	IL27	G	T	0.07	Chinese	Zhou et al. [9]
MIBC Overall survival	rs10515074	5q13.1	PIK3R1	G	A	0.2	Caucasian (Northern American)	Chen et al. [7]
MIBC Overall survival	rs3730050	19q13.2	AKT2	A	G	0.25	Caucasian (Northern American)	Chen et al. [7]
MIBC Overall survival	rs2344673	1q23.3	RGS5	A	G	0.05	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs10917690	1q23.3	RGS5	G	A	0.29	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs1890398	1q31.2	RGS2	C	T	0.56	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs12035879	1q23.3	RGS5	G	A	0.68	Caucasian (Northern American)	Lee et al. [8]

MIBC Overall survival	rs10753605	1q23.3	RGS5	C	T	0.35	Caucasian (Northern American)	Lee et al. [8]
MIBC Overall survival	rs156697	10q25.1	GSTO2	G	A	0.56	European (Serbia)	Djukic et al. [10]
MIBC Overall survival	rs4925	10q25.1	GSTO1	A	C	0.18	European (Serbia)	Djukic et al. [10]
MIBC Overall survival (Platinum-based chemotherapy-treated)	rs11615	19q13.32	ERCC1	T	C	0.33	Chinese	Xu et al. [11]
NMIBC Overall survival	rs2662238	5q14.2	XRCC4	A	G	0.36	Caucasian (Northern American)	Andrew et al. [12]
NMIBC Overall survival	rs4987059	11p15.5	DRD4	A	G	0.04	Caucasian (Northern American)	Andrew et al. [12]
NMIBC Overall survival	rs3756712	5p15.33	PDCD6	G	T	0.4	Chinese	Zhou et al. [13]
NMIBC Overall survival	rs2292016	5p13.1	OSMR	T	G	0.08	Chinese	Deng et al. [14]
UBC Cancer-specific death	rs2279744	12q15	MDM2	G	T	0.37	Japanese	Shinohara et al. [15]
UBC Cancer-specific death	rs4129009	4p14	TLR10	T	C	0.85	European (Spain)	Guirado et al. [16]
UBC Cancer-specific death	rs1801133	1p36.22	MTHFR	T	C	0.25	European (Sweden)	Sanyal et al. [17]
UBC Cancer-specific death	rs5443	12p13.31	GNB3	T	C	0.49	European (Germany)	Eisenhardt et al. [18]
UBC Cancer-specific death	rs9302752	16q12.1	NOD2	A	G	0.5	European (Spain)	Guirado et al. [16]
UBC Overall survival	rs13181	19q13.3	ERCC2	C	A	0.24	European (Sweden)	Sanyal et al. [19]
UBC Overall survival	rs2854461	1q42.12	EPHX1	C	A	0.64	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs2279115	18q21.33	BCL2	A	C	0.39	European (Germany)	Hess et al. [21]
UBC Overall survival	rs11543848 (merged into rs2227983)	7p11.2	EGFR	A	G	0.29	Caucasian (Northern American)	Mason et al. [22]
UBC Overall survival	rs2017000	7p11.2	EGFR	G	A	0.33	Caucasian (Northern American)	Mason et al. [22]
UBC Overall survival	rs4986998 (merged into rs1131341)	16q22.1	NQO1	T	C	0.02	European (Sweden)	Sanyal et al. [17]

UBC Overall survival	rs6024840	20q13.2	AURKA	C	T	0.45	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs1042640	2q37.1	UGT1A1	C	G	0.82	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs1126579	2q35	CXCR2	C	T	0.6	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs528778	10p14	GATA3	T	C	0.14	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs1800067	16p13.12	ERCC4	A	G	0.03	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs1994251	20q11.21	BCL2L1	C	A	0.24	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs9282638	3q13.33	CD80	A	G	0.87	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs35402311	8q11.23	RB1CC1	T	C	0.02	Caucasian (Northern American)	Andrew et al. [20]
UBC Overall survival	rs1801018	18q21.33	BCL2	A	G	0.76	European (Germany)	Hess et al. [21]
UBC Overall survival	rs2293347	7p11.2	EGFR	T	C	0.14	Caucasian (Northern American)	Mason et al. [22]
UBC Overall survival (Chemotherapy-treated)	rs915927	19q13.2	XRCC1	G	A	0.32	European (Italy)	Sacerdote et al. [23]
UBC Overall survival (Chemotherapy-treated)	rs762507	19q13.2	XRCC1	A	G	0.29	European (Italy)	Sacerdote et al. [23]
UBC Overall survival (Chemotherapy-treated)	rs2854501	19q13.2	XRCC1	T	C	0.82	European (Italy)	Sacerdote et al. [23]
UBC Overall survival (Chemotherapy-treated)	rs2854509	19q13.2	XRCC1	A	C	0.18	European (Italy)	Sacerdote et al. [23]
UBC Overall survival (Chemotherapy-treated)	rs3213255	19q13.2	XRCC1	C	T	0.32	European (Italy)	Sacerdote et al. [23]
UBC Overall survival (Chemotherapy-treated)	rs171140	19q13.3	ERCC2	C	A	0.37	European (Italy)	Sacerdote et al. [23]

BCG-Bacillus Calmette-Guérin; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; RA-reference allele; SNP-single nucleotide polymorphism; TUR-transurethral resection; UBC-urinary bladder cancer.
*Global, based on 1000 Genomes
Project.

REFERENCES:

[1] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.

[2] Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q, Wu X. Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(24):5746-56.

[3] Castillejo A, Rothman N, Murta-Nascimento C, Malats N, Garcia-Closas M, Gomez-Martinez A, Lloreta J, Tardon A, Serra C, Garcia-Closas R, Chanock S, Silverman DT, Dosemeci M, Kogevinas M, Carrato A, Soto JL, Real FX. Tgfb1 and Tgfbr1 Polymorphic Variants in Relationship to Bladder Cancer Risk and Prognosis. International journal of cancer. 2009;124(3):608-13.

[4] Horikawa Y, Nadaoka J, Saito M, Kumazawa T, Inoue T, Yuasa T, Tsuchiya N, Nishiyama H, Ogawa O, Habuchi T. Clinical Implications of the Mdm2 Snp309 and P53 Arg72pro Polymorphisms in Transitional Cell Carcinoma of the Bladder. Oncology reports. 2008;20(1):49-55.

[5] Teo MT, Landi D, Taylor CF, Elliott F, Vaslin L, Cox DG, Hall J, Landi S, Bishop DT, Kiltie AE. The Role of Microrna-Binding Site Polymorphisms in DNA Repair Genes as Risk Factors for Bladder Cancer and Breast Cancer and Their Impact on Radiotherapy Outcomes. Carcinogenesis. 2012;33(3):581-6.

[6] Teo MT, Dyrskjot L, Nsengimana J, Buchwald C, Snowden H, Morgan J, Jensen JB, Knowles MA, Taylor G, Barrett JH, Borre M, Orntoft TF, Bishop DT, Kiltie AE. Next-Generation Sequencing Identifies Germline Mre11a Variants as Markers of Radiotherapy Outcomes in Muscle-Invasive Bladder Cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2014;25(4):877-83.

[7] Chen M, Gu J, Delclos GL, Killary AM, Fan Z, Hildebrandt MA, Chamberlain RM, Grossman HB, Dinney CP, Wu X. Genetic Variations of the Pi3k-Akt-Mtor Pathway and Clinical Outcome in Muscle Invasive and Metastatic Bladder Cancer Patients. Carcinogenesis. 2010;31(8):1387-91.

[8] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.

[9] Zhou B, Zhang P, Tang T, Liao H, Zhang K, Pu Y, Chen P, Song Y, Zhang L. Polymorphisms and Plasma Levels of Il-27: Impact on Genetic Susceptibility and Clinical Outcome of Bladder Cancer. BMC cancer. 2015;15:433.

[10] Djukic TI, Savic-Radojevic AR, Pekmezovic TD, Matic MG, Pljesa-Ercegovac MS, Coric VM, Radic TM, Suvakov SR, Krivic BN, Dragicevic DP, Simic TP. Glutathione S-Transferase T1, O1 and O2 Polymorphisms Are Associated with Survival in Muscle Invasive Bladder Cancer Patients. PloS one. 2013;8(9):e74724.

[11] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).

[12] Andrew AS, Gui J, Hu T, Wyszynski A, Marsit CJ, Kelsey KT, Schned AR, Tanyos SA, Pendleton EM, Ekstrom RM, Li Z, Zens MS, Borsuk M, Moore JH, Karagas MR. Genetic Polymorphisms Modify Bladder Cancer Recurrence and Survival in a USA Population-Based Prognostic Study. BJU international. 2015;115(2):238-47.
[13] Zhou B, Zhang P, Tang T, Zhang K, Wang Y, Song Y, Liao H, Zhang L. Prognostic Value of Pdcd6 Polymorphisms and the Susceptibility to Bladder Cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7547-54.
[14] Deng S, He SY, Zhao P, Zhang P. The Role of Oncostatin M Receptor Gene Polymorphisms in Bladder Cancer. World journal of surgical oncology. 2019;17(1):30.
[15] Shinohara A, Sakano S, Hinoda Y, Nishijima J, Kawai Y, Misumi T, Nagao K, Hara T, Matsuyama H. Association of Tp53 and Mdm2 Polymorphisms with Survival in Bladder Cancer Patients Treated with Chemoradiotherapy. Cancer science. 2009;100(12):2376-82.
[16] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.
[17] Sanyal S, Ryk C, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Nqo1 and the Clinical Course of Urinary Bladder Neoplasms. Scandinavian journal of urology and nephrology. 2007;41(3):182-90.
[18] Eisenhardt A, Siffert W, Rosskopf D, Musch M, Mosters M, Roggenbuck U, Jockel KH, Rubben H, Lummen G. Association Study of the G-Protein Beta3 Subunit C825t Polymorphism with Disease Progression in Patients with Bladder Cancer. World journal of urology. 2005;23(4):279-86.
[19] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.
[20] Andrew AS, Gui J, Sanderson AC, Mason RA, Morlock EV, Schned AR, Kelsey KT, Marsit CJ, Moore JH, Karagas MR. Bladder Cancer Snp Panel Predicts Susceptibility and Survival. Human genetics. 2009;125(5-6):527-39.
[21] Hess J, Stelmach P, Eisenhardt A, Rubben H, Reis H, Schmid KW, Bachmann HS. Impact of Bcl2 Polymorphisms on Survival in Transitional Cell Carcinoma of the Bladder. Journal of cancer research and clinical oncology. 2017;143(9):1659-70.
[22] Mason RA, Morlock EV, Karagas MR, Kelsey KT, Marsit CJ, Schned AR, Andrew AS. Egfr Pathway Polymorphisms and Bladder Cancer Susceptibility and Prognosis. Carcinogenesis. 2009;30(7):1155-60.
[23] Sacerdote C, Guarrera S, Ricceri F, Pardini B, Polidoro S, Allione A, Critelli R, Russo A, Andrew AS, Ye Y, Wu X, Kiemeney LA, Bosio A, Casetta G, Cucchiarale G, Destefanis P, Gontero P, Rolle L, Zitella A, Fontana D, Vineis P, Matullo G. Polymorphisms in the Xrcc1 Gene Modify Survival of Bladder Cancer Patients Treated with Chemotherapy. International journal of cancer. 2013;133(8):2004-9.

Supplementary Table 6.3. Previously reported polymorphisms in association with age at the time of bladder cancer diagnosis.

Outcome	SNP	Locus	Gene	EA	RA	EAF*	Discovery population	References
Age, years	rs798766	4p16.3	TACC3/FGFR3	T	C	0.24	European (Multiple)	Kiemeney et al. [1]
Age (≥50 years)	rs25487	19q13.2	XRCC1	A	G	0.26	Caucasian (Northern American)	Kelsey et al. [2]
Age (>56 years)	rs710521	3q28	TP63	A	G	0.8	Caucasian (Northern American)	Stern et al. [3]
Age (>60 years)	rs874945	12q13.13	HOTAIR	A	G	0.36	Chinese	Wang et al. [4]
Age (>60 years)	rs710886	8q24.21	PCAT1	G	A	0.47	Chinese	Lin et al. [5]
Age (≤60 years)	rs217727	11p15.5	H19 (lncRNA)	A	G	0.2	Chinese	Hua et al. [6]
Age (>65 years)	rs1052133	3p25.3	OGG1	G	C	0.3	Chinese	Ma et al. [7]
Age (>65 years)	rs884225	7p11.2	EGFR	C	T	0.19	Chinese	Chu et al. [8]
Age (>65 years)	rs7003908	8q11.21	PRKDC	T	G	0.33	Chinese	Wang et al. [9]
Age (>65 years)	rs1057868	7q11.23	POR	T	C	0.29	Chinese	Xiao et al. [10]
Age (≤65 years / Healthy controls)	rs9642880	8q24.21	CASC11	T	G	0.54	Chinese	Wang et al. [11]
Age (≥65 years / Healthy controls)	rs9344	11q13.3	CCND1	A	G	0.41	Chinese	Yuan et al. [12]
Age (</≥70.2)	rs41515546	7q31.33	-	C	T	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs17149636	7q31.33	-	G	A	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs17149628	7q31.33	-	T	C	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs12666814	7q31.33	-	T	C	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs73223045	7q31.33	-	C	G	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs12673089	7q31.33	-	T	C	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs17149580	7q31.33	-	G	A	0.14	European	Lipunova et al. [13]
Age (</≥70.2)	rs17149630	7q31.33	-	T	C	0.14	European	Lipunova et al. [13]

EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; RA-reference allele; SNP-single nucleotide polymorphism

*Global, based on 1000 Genomes Project.

REFERENCES:

[1] Kiemeney LA, Sulem P, Besenbacher S, Vermeulen SH, Sigurdsson A, Thorleifsson G, Gudbjartsson DF, Stacey SN, Gudmundsson J, Zanon C, Kostic J, Masson G, Bjarnason H, Palsson ST, Skarphedinsson OB, Gudjonsson SA, Witjes JA, Grotenhuis AJ, Verhaegh GW, Bishop DT, Sak SC, Choudhury A, Elliott F, Barrett JH, Hurst CD, de Verdier PJ, Ryk C, Rudnai P, Gurzau E, Koppova K, Vineis P, Polidoro S, Guarrera S, Sacerdote C, Campagna M, Placidi D, Arici C, Zeegers MP, Kellen E, Gutierrez BS, Sanz-Velez JI, Sanchez-Zalabardo M, Valdivia G, Garcia-Prats MD, Hengstler JG, Blaszkewicz M, Dietrich H, Ophoff RA, van den Berg LH, Alexiusdottir K, Kristjansson K, Geirsson G, Nikulasson S, Petursdottir V, Kong A, Thorgeirsson T, Mungan NA, Lindblom A, van Es MA, Porru S, Buntinx F, Golka K, Mayordomo JI, Kumar R, Matullo G, Steineck G, Kiltie AE, Aben KKH, Jonsson E, Thorsteinsdottir U, Knowles MA, Rafnar T, Stefansson K. A Sequence Variant at 4p16.3 Confers Susceptibility to Urinary Bladder Cancer. Nature genetics. 2010;42(5):415-9.

[2] Kelsey KT, Park S, Nelson HH, Karagas MR. A Population-Based Case-Control Study of the Xrcc1 Arg399gln Polymorphism and Susceptibility to Bladder Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2004;13(8):1337-41.

[3] Stern MC, Van Den Berg D, Yuan JM, Conti DV, Gago-Dominguez M, Pike MC, Xiang YB, Gao YT, Cortessis VK. Sequence Variant on 3q28 and Urinary Bladder Cancer Risk: Findings from the Los Angeles-Shanghai Bladder Case-Control Study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(11):3057-61.

[4] Wang X, Wang W, Zhang Q, Gu D, Zhang K, Ge Y, Chu H, Du M, Xu B, Wang M, Lv X, Zhang Z, Yuan L, Gong W. Tagging Snps in the Hotair Gene Are Associated with Bladder Cancer Risk in a Chinese Population. Gene. 2018;664:22-6.

[5] Lin Y, Ge Y, Wang Y, Ma G, Wang X, Liu H, Wang M, Zhang Z, Chu H. The Association of Rs710886 in Lncrna Pcat1 with Bladder Cancer Risk in a Chinese Population. Gene. 2017;627:226-32.

[6] Hua Q, Lv X, Gu X, Chen Y, Chu H, Du M, Gong W, Wang M, Zhang Z. Genetic Variants in Lncrna H19 Are Associated with the Risk of Bladder Cancer in a Chinese Population. Mutagenesis. 2016;31(5):531-8.

[7] Ma L, Chu H, Wang M, Shi D, Zhong D, Li P, Tong N, Yin C, Zhang Z. Hogg1 Ser326cys Polymorphism Is Associated with Risk of Bladder Cancer in a Chinese Population: A Case-Control Study. Cancer science. 2012;103(7):1215-20.

[8] Chu H, Wang M, Jin H, Lv Q, Wu D, Tong N, Ma L, Shi D, Zhong D, Fu G, Yuan L, Qin C, Yin C, Zhang Z. Egfr 3'utr 774t>C Polymorphism Contributes to Bladder Cancer Risk. Mutagenesis. 2013;28(1):49-55.

[9] Wang SY, Peng L, Li CP, Li AP, Zhou JW, Zhang ZD, Liu QZ. Genetic Variants of the Xrcc7 Gene Involved in DNA Repair and Risk of Human Bladder Cancer. International journal of urology : official journal of the Japanese Urological Association. 2008;15(6):534-9.

[10] Xiao X, Ma G, Li S, Wang M, Liu N, Ma L, Zhang Z, Chu H, Zhang Z, Wang SL. Functional Por A503v Is Associated with the Risk of Bladder Cancer in a Chinese Population. Scientific reports. 2015;5:11751.

[11] Wang M, Wang M, Zhang W, Yuan L, Fu G, Wei Q, Zhang Z. Common Genetic Variants on 8q24 Contribute to Susceptibility to Bladder Cancer in a Chinese Population. Carcinogenesis. 2009;30(6):991-6.

[12] Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, Zhang Z. Cyclin D1 G870a Polymorphism Is Associated with Risk and Clinicopathologic Characteristics of Bladder Cancer. DNA and cell biology. 2010;29(10):611-7.
[13] Lipunova N, Wesselius A, Cheng KK, van Schooten F-J, Bryan RT, Cazier J-B, Galesloot TE, Kiemeney LALM, Zeegers MP. Genome-Wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non–Muscle-Invasive Bladder Cancer. European Urology Oncology.

Supplementary Table 6.4. Previously reported polymorphisms in association with bladder cancer progression.

Outcome	SNP	Locus	Gene	EA	RA	EAF*	Discovery population	References
NMIBC Progression	rs17350793 (has since merged into rs12628)	11p15.5	HRAS	C	T	0.30	European (Sweden)	Sanyal et al. [1]
UBC Progression	rs2297518	17q11.2	NOS2	T	C	0.17	European (Sweden)	Ryk et al. [2]
NMIBC Progression	rs10917690	1q23.3	RGS5	G	A	0.29	Caucasian (Northern American)	Lee et al. [3]
NMIBC Progression	rs4075958	5q35.3	RGS14	A	G	0.18	Caucasian (Northern American)	Lee et al. [3]
NMIBC Progression	rs10926466	1q23.1	RGS7	T	C	0.63	Caucasian (Northern American)	Lee et al. [3]
NMIBC Progression	rs12038803	1q23.1	RGS7	C/G/T? (Not reported)	A	0.66	Caucasian (Northern American)	Lee et al. [3]
High-risk NMIBC Progression (BCG-treated)	rs2070744	7q36.1	NOS3	T	C	0.77	European (Sweden)	Ryk et al. [4]
High-risk NMIBC Progression (BCG-treated)	rs1799983	7q36.1	NOS3	G	T	0.82	European (Sweden)	Ryk et al. [4]
UBC Progression (metastases present)	rs2910164	5q33.3	MIR146A	G	C	0.32 (TOPMED)	Chinese	Deng et al. [5]
NMIBC Progression	rs3088440	9p21	CDKN2A	T	C	0.17	European (Sweden)	Sakano et al. [6]
NMIBC progression (BCG-treated)	rs1800896	1q32.1	IL10	G	A	0.27	Turkish	Basturk et al. [7]
NMIBC progression (BCG-treated)	rs2243248	5q31.1	IL4	G	T	0.11	Turkish	Basturk et al. [7]
NMIBC progression (BCG-treated)	rs1800470	19q13.2	TGFB1	T	C	0.55	Turkish	Basturk et al. [7]
NMIBC progression (BCG-treated)	rs1800471	19q13.2	TGFB1	G	C	0.05	Turkish	Basturk et al. [7]
Progression (metastases present)	rs9302752	16q12.1	NOD2	G	A	0.50	European (Spain)	Guirado et al. [8]
NMIBC Progression	rs3890995	12q24.11	UNG	C	T	0.22	Caucasian (Northern American)	Wei et al. [9]
NMIBC Progression (TUR-treated)	rs720012	22q11.21	DGCR8	A	G	0.22	Caucasian (Northern American)	Ke et al. [10]
NMIBC Progression (TUR-treated)	rs2073778	22q11.21	DGCR8	T	C	0.22	Caucasian (Northern American)	Ke et al. [10]
NMIBC Progression	rs1323291	1q31.2	RGS1	C	A	0.15	Caucasian (Northern American)	Lee et al. [3]
NMIBC Progression	rs6678136	1q23.3	RGS4	A	G	0.46	Caucasian (Northern American)	Lee et al. [3]
NMIBC Progression	rs11585883	1q23.3	RGS5	C	T	0.03	Caucasian (Northern American)	Lee et al. [3]

MIBC Progression	rs11615	19q13.32	ERCC1	T	C	0.33	Chinese	Xu et al. [11]
UBC Progression	rs1801018	18q21.33	BCL2	A	G	0.76	European (Germany)	Hess et al. [12]

BCG-Bacillus Calmette-Guérin; EA-effect allele; EAF-effect allele frequency; MIBC-muscle-invasive bladder cancer; NMIBC-non-muscle-invasive bladder cancer; RA-reference allele; SNP-single nucleotide polymorphism; TUR-transurethral resection; UBC-urinary bladder cancer.
*Global, based on 1000 Genomes Project (unless otherwise specified).

REFERENCES:

[1] Sanyal S, De Verdier PJ, Steineck G, Larsson P, Onelov E, Hemminki K, Kumar R. Polymorphisms in Xpd, Xpc and the Risk of Death in Patients with Urinary Bladder Neoplasms. Acta oncologica (Stockholm, Sweden). 2007;46(1):31-41.

[2] Ryk C, Wiklund NP, Nyberg T, De Verdier PJ. Ser608leu Polymorphisms in the Nitric Oxide Synthase-2 Gene May Influence Urinary Bladder Cancer Pathogenesis. Scandinavian journal of urology and nephrology. 2011;45(5):319-25.

[3] Lee EK, Ye Y, Kamat AM, Wu X. Genetic Variations in Regulator of G-Protein Signaling (Rgs) Confer Risk of Bladder Cancer. Cancer. 2013;119(9):1643-51.

[4] Ryk C, Koskela LR, Thiel T, Wiklund NP, Steineck G, Schumacher MC, de Verdier PJ. Outcome after Bcg Treatment for Urinary Bladder Cancer May Be Influenced by Polymorphisms in the Nos2 and Nos3 Genes. Redox biology. 2015;6:272-7.

[5] Deng S, Wang W, Li X, Zhang P. Common Genetic Polymorphisms in Pre-Micrornas and Risk of Bladder Cancer. World journal of surgical oncology. 2015;13:297.

[6] Sakano S, Berggren P, Kumar R, Steineck G, Adolfsson J, Onelov E, Hemminki K, Larsson P. Clinical Course of Bladder Neoplasms and Single Nucleotide Polymorphisms in the Cdkn2a Gene. International journal of cancer. 2003;104(1):98-103.

[7] Basturk B, Yavascaoglu I, Oral B, Goral G, Oktay B. Cytokine Gene Polymorphisms Can Alter the Effect of Bacillus Calmette-Guerin (Bcg) Immunotherapy. Cytokine. 2006;35(1-2):1-5.

[8] Guirado M, Gil H, Saenz-Lopez P, Reinboth J, Garrido F, Cozar JM, Ruiz-Cabello F, Carretero R. Association between C13orf31, Nod2, Ripk2 and Tlr10 Polymorphisms and Urothelial Bladder Cancer. Human immunology. 2012;73(6):668-72.

[9] Wei H, Kamat A, Chen M, Ke HL, Chang DW, Yin J, Grossman HB, Dinney CP, Wu X. Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guerin. PloS one. 2012;7(6):e38533.

[10] Ke HL, Chen M, Ye Y, Hildebrandt MA, Wu WJ, Wei H, Huang M, Chang DW, Dinney CP, Wu X. Genetic Variations in Micro-Rna Biogenesis Genes and Clinical Outcomes in Non-Muscle-Invasive Bladder Cancer. Carcinogenesis. 2013;34(5):1006-11.

[11] Xu ZC, Cai HZ, Li X, Xu WZ, Xu T, Yu B, Zou Q, Xu L. Ercc1 C118t Polymorphism Has Predictive Value for Platinum-Based Chemotherapy in Patients with Late-Stage Bladder Cancer. Genetics and molecular research : GMR. 2016;15(2).

[12] Hess J, Stelmach P, Eisenhardt A, Rubben H, Reis H, Schmid KW, Bachmann HS. Impact of Bcl2 Polymorphisms on Survival in Transitional Cell Carcinoma of the Bladder. Journal of cancer research and clinical oncology. 2017;143(9):1659-70.

Supplementary Table 6.5. Minor allele frequencies and imputation scores (info) for tested SNPs.

Outcome	SNP	Position (BP)	Allele 1	Allele 2	MAF	Minor Allele	Info score
Age	rs1052133	9798773	C	G	0.23	G	1
	rs710521	189645933	T	C	0.27	C	1
	rs798766	1734239	T	C	0.19	T	1
	rs884225	55274084	T	C	0.10	C	1
	rs1057868	75615006	C	T	0.28	T	1
	rs41515546	125998959	T	C	0.15	C	0.99774
	rs17149636	126018952	A	G	0.15	G	0.99804
	rs17149628	126006965	C	T	0.15	T	0.997627
	rs12666814	125979540	C	T	0.14	T	0.993985
	rs73223045	125992106	G	C	0.15	C	0.99768
	rs12673089	126006133	C	T	0.15	T	0.996984
	rs17149580	125978216	A	G	0.15	G	0.991705
	rs17149630	126006996	C	T	0.15	T	0.997626
	rs7003908	48770702	C	A	0.33	C	0.824001
	rs710886	128026860	C	T	0.37	T	0.998081
	rs9642880	128718068	G	T	0.46	T	1
	rs9344	69462910	G	A	0.44	A	1
	rs217727	2016908	G	A	0.18	A	0.974512
	rs874945	54355451	C	T	0.34	T	0.993033
	rs25487	44055726	T	C	0.36	T	1
Recurrence	rs4645978	15852034	C	T	0.47	C	0.999525
	rs197412	112308953	T	C	0.40	C	1
	rs511918	182579602	G	T	0.41	G	0.992327
	rs1323291	192548543	T	G	0.09	G	0.993577
	rs3795617	192603690	C	T	0.47	T	0.991678
	rs16829458	192768324	G	A	0.07	A	1
	rs11685068	121541012	C	T	0.06	T	0.9919
	rs17235409	219259732	G	A	0.02	A	1
	rs1801282	12393125	C	G	0.12	G	1
	rs1050450	49394834	G	A	0.30	A	0.99929
	rs1052133	9798773	C	G	0.23	G	1
	rs2228001	14187449	G	T	0.40	G	0.995546
	rs1799864	46399208	G	A	0.078308	A	1
	rs4073	74606024	A	T	0.46	A	1
	rs798766	1734239	T	C	0.19	T	1
	rs2910164	159912418	C	G	0.24	C	0.999062
	rs12186785	31410608	T	C	0.09	C	1
	rs4957014	288014	T	G	0.24	T	0.997295
	rs2042329	64067752	T	G	0.41	T	1
	rs2292016	38845860	G	T	0.02	T	0.976753
	rs2278329	38921788	G	A	0.01	A	1
	rs1799964	31542308	T	C	0.21	C	1
	rs2275913	52051033	G	A	0.35	A	0.968288
	rs1800795	22766645	C	G	0.41	C	1
	rs2070744	150690079	C	T	0.38	C	0.987305
	rs1799983	150696111	T	G	0.33	T	0.983157
	rs6463089	42152856	G	A	0.10	A	0.995015
	rs3801192	42161527	C	T	0.09	T	1
	rs1233560	155593438	G	A	0.47	G	0.845252
	rs7003908	48770702	C	A	0.33	C	0.824001
	rs804267	11629241	G	A	0.32	G	0.991159
	rs8191604	11636884	T	G	0.26	G	0.988574
	rs804256	11636862	T	C	0.35	C	0.99245
	rs804276	11625008	G	A	0.42	A	0.990034
	rs4639	11644751	A	G	0.44	G	0.992205

	rs13278062	23082971	G	T	0.50	G	1
	rs2228526	50678717	T	C	0.20	C	1
	rs11199005	121296029	G	A	0.09	A	1
	rs4925	106022789	C	A	0.30	A	1
	rs2505568	36811336	T	A	0.42	T	0.98455
	rs187238	112034988	C	G	0.27	G	0.994289
	rs1695	67352689	A	G	0.35	G	1
	rs2279744	69202580	T	G	0.35	G	0.99522
	rs5742714	102789852	C	G	0.09	G	0.994077
	rs2238151	112211833	T	C	0.35	C	1
	rs4758680	122655352	T	G	0.33	T	0.996788
	rs1544410	48239835	C	T	0.40	T	1
	rs2430561	68552522	T	A	0.12	A	0.612221
	rs3138056	35868514	C	T	0.32	T	0.980789
	rs2169830	39872056	T	C	0.30	C	0.991923
	rs744154	14015081	G	C	0.28	C	0.996449
	rs243865	55511806	C	T	0.25	T	1
	rs16260	68771034	C	A	0.28	A	0.996119
	rs1042522	7579472	G	C	0.26	G	1
	rs9904341	76210367	G	C	0.31	C	0.992725
	rs2854509	44074597	T	G	0.22	T	0.990178
	rs3213255	44077507	G	A	0.42	G	0.991363
	rs915927	44057227	T	C	0.44	C	0.999689
	rs2854501	44060001	A	G	0.24	A	1
	rs1799782	44057574	G	A	0.06	A	1
	rs25487	44055726	T	C	0.36	T	1
	rs1799793	45867259	C	T	0.33	T	1
	rs5498	10395683	A	G	0.42	G	1
	rs3746162	1114119	C	T	0.22	T	0.998325
	rs7265992	33525407	G	A	0.18	A	0.976046
	rs6060124	33536897	C	A	0.31	A	0.992465
	rs7260770	33552653	G	A	0.33	A	0.996424
	rs4911455	33553062	A	C	0.33	C	0.997023
	rs2173962	33022020	T	C	0.04	C	0.98769
	rs1801133	11856378	G	A	0.33	A	1
	rs1395960	163115522	C	T	0.12	T	0.99613
	rs2854461	226011644	C	A	0.35	A	0.994428
	rs2344673	163118052	G	A	0.14	A	1
	rs10917690	163142168	A	G	0.30	G	0.921387
	rs1890398	192771127	C	T	0.35	T	0.997693
	rs12035879	163142555	G	A	0.40	A	1
	rs10753605	163145390	T	C	0.30	C	0.974848
	rs1126579	219000734	T	C	0.47	T	0.999164
	rs1042640	234681544	G	C	0.20	G	1
	rs9282638	119263770	T	C	0.15	C	0.997356
	rs4129009	38774889	T	C	0.15	C	1
Death	rs762861	3442011	G	C	0.25	C	0.979271
	rs2662238	82499307	G	A	0.45	A	0.992607
	rs10515074	67566193	A	G	0.20	G	0.998354
	rs3756712	309096	A	C	0.38	A	0.996364
	rs2292016	38845860	G	T	0.02	T	0.976753
	rs1799983	150696111	T	G	0.33	T	0.983157
	rs1800795	22766645	C	G	0.41	C	1
	rs2227983	55229255	G	A	0.26	A	1
	rs2017000	55242609	A	G	0.28	G	0.996934
	rs2293347	55268916	C	T	0.11	T	0.991363
	rs35402311	53627403	G	A	0.04	A	1
	rs334358	101910613	G	T	0.20	T	0.994179
	rs868	101911656	A	G	0.20	G	0.994126

	rs1051013	116359339	T	C	0.25	T	1
	rs156697	106039185	A	G	0.35	G	1
	rs4925	106022789	C	A	0.30	A	1
	rs528778	8112143	T	C	0.21	T	0.993889
	rs4987059	636433	G	A	0.05	A	1
	rs1805363	94226952	C	T	0.09	T	1
	rs2279744	69202580	T	G	0.35	G	0.99522
	rs5443	6954875	C	T	0.32	T	1
	rs7180135	41024094	G	A	0.43	G	0.998991
	rs9302752	50719103	T	C	0.29	T	1
	rs17855750	28515228	A	C	0.05	C	1
	rs1800067	14029033	G	A	0.08	A	1
	rs1131341	69748869	G	A	0.04	A	1
	rs1042522	7579472	G	C	0.26	G	1
	rs9906827	78665405	C	T	0.45	T	0.997786
	rs1801018	60985879	T	C	0.42	C	1
	rs2279115	60986837	G	T	0.47	G	0.998126
	rs13181	45854919	T	G	0.36	G	1
	rs915927	44057227	T	C	0.44	C	0.999689
	rs762507	44058098	T	C	0.43	T	0.999682
	rs2854501	44060001	A	G	0.24	A	1
	rs2854509	44074597	T	G	0.22	T	0.990178
	rs3213255	44077507	G	A	0.42	G	0.991363
	rs3730050	40770982	T	C	0.29	T	0.997599
	rs11615	45923653	A	G	0.39	G	1
	rs171140	45865002	C	A	0.45	C	0.995683
	rs1994251	30287328	T	G	0.22	G	0.995433
	rs6024840	54956707	A	G	0.25	G	0.980223
Progression	rs10917690	163142168	A	G	0.30	G	0.921387
	rs10926466	241521721	C	T	0.26	C	0.980052
	rs11585883	163104742	T	C	0.06	C	0.995822
	rs12038803	241522325	A	G	0.25	A	0.976809
	rs1323291	192548543	T	G	0.10	G	0.993577
	rs1800896	206946897	T	C	0.50	C	0.993185
	rs6678136	163037317	G	A	0.42	A	0.9972
	rs2243248	132008644	T	G	0.07	G	1
	rs2910164	159912418	C	G	0.24	C	0.999062
	rs4075958	176784512	G	A	0.26	A	1
	rs1799983	150696111	T	G	0.33	T	0.983157
	rs2070744	150690079	C	T	0.38	C	0.987305
	rs3088440	21968159	G	A	0.09	A	0.992401
	rs12628	534242	A	G	0.34	G	1
	rs3890995	109533529	T	C	0.18	C	0.990296
	rs9302752	50719103	T	C	0.29	T	1
	rs2297518	26096597	G	A	0.19	A	1
	rs1801018	60985879	T	C	0.42	C	1
	rs11615	45923653	A	G	0.39	G	1
	rs1800470	41858921	G	A	0.38	G	0.997955
	rs1800471	41858876	C	G	0.08	G	1
	rs2073778	20074575	C	T	0.13	T	0.995009
	rs720012	20098582	G	A	0.13	A	0.99538

MAF-Minor Allele Frequency; SNP-Single Nucleotide Polymorphism.