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Genome-wide Association Study for Tumour Stage, Grade, Size, and Age at Diagnosis of Non–muscle-invasive Bladder Cancer

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Article info

Article history:
Accepted August 23, 2018

Associate Editor:
Alberto Briganti

Keywords:
Age
Grade
Genome-wide association study
Non–muscle-invasive bladder cancer
Size
Stage

Abstract

Background: 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.
Objective: To investigate genetic associations with NMIBC tumour and patient characteristics at the time of diagnosis.
Design, setting, and participants: A sample of 653 NMIBC cases comes from the Bladder Cancer Prognosis Programme. Replication of the significant findings was conducted in the Nijmegen Bladder Cancer Study cohort ($N = 1470$).
Outcome measurements and statistical analysis: A genome-wide association study (GWAS) was carried out for outcomes of tumour size (as a 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 yr as a cut-off] variables).
Results and limitations: 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 that rs180940944 (13q13.3 locus, *NBEA*) was associated with tumour size as a continuous variable (ß = 0.9 cm, $p = 2.92E{-}09$). However, other single nucleotide polymorphisms in this region did not show evidence of association in the meta-analysis.
Conclusions: Our study suggests that 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.
Patient summary: The current study reports on a genome-wide association study on non–muscle-invasive bladder cancer tumour and patient characteristics. We suggest that *NBEA* gene might be associated with increased tumour size at the time of diagnosis. The result must be replicated to establish validity.

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https://doi.org/10.1016/j.euo.2018.08.020

1. 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 a significant burden on healthcare systems due to high recurrence and progression rates (5-yr recurrence rate: 50–70%, 5-yr 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 genome-wide association studies (GWASs) 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 that the issue is more complex. However, a later study utilised a relatively small panel of SNPs (170 000), which has lower power of discovering significant loci in comparison with genotype-imputed sets harbouring millions of variants for analysis [8]. The interstudy lack of consensus might be due to several reasons: spurious findings, lack of statistical power, and variation in outcome definition.

Other studies also suggest that significant genetic signals might be present only 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, European Organisation for Research and Treatment of Cancer [EORTC] risk category), 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).

2. Patients and methods

2.1. Participants and genotyping

BCPP is a prospective cohort that initially recruited 1544 eligible patients and is described in more detail elsewhere [11]. Clinical data on stage, grade, and size of tumours, and demographic information (age and gender) were gathered with bespoke case report forms. Tumour size of the largest tumour was established visually while performing cystoscopy. Blood samples of 888 participants with confirmed UBC were genotyped on the Illumina Infinium OmniExpress-24 BeadChip array (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.

2.2. Quality control

Quality control (QC) procedures were carried out using PLINK v1.90 [12]. The exact thresholds applied and the number of exclusions per step are outlined in Figure 1.

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 excluded SNPs deviating from the Hardy-Weinberg equilibrium, those exceeding acceptable missing rate, and rare variants.

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

2.3. 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 minor allele frequencies (MAFs) of $>1\%$, resulting in a dataset containing 11 914 228 markers available for genetic association analyses.

2.4. Statistical analysis

Statistical analyses were performed using SNP test 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 $< \geq$70 yr]). Tumour size (cm) was tested as a continuous and categorical variable ($</\geq 3$ cm [17]). Stage (Tis and T1 vs Ta) and grade (G3 vs 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 adjust for any potential residual population stratification bias. An association was held significant for $p < 5E{-}08$ and promising for $p < 5E{-}06$.

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

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

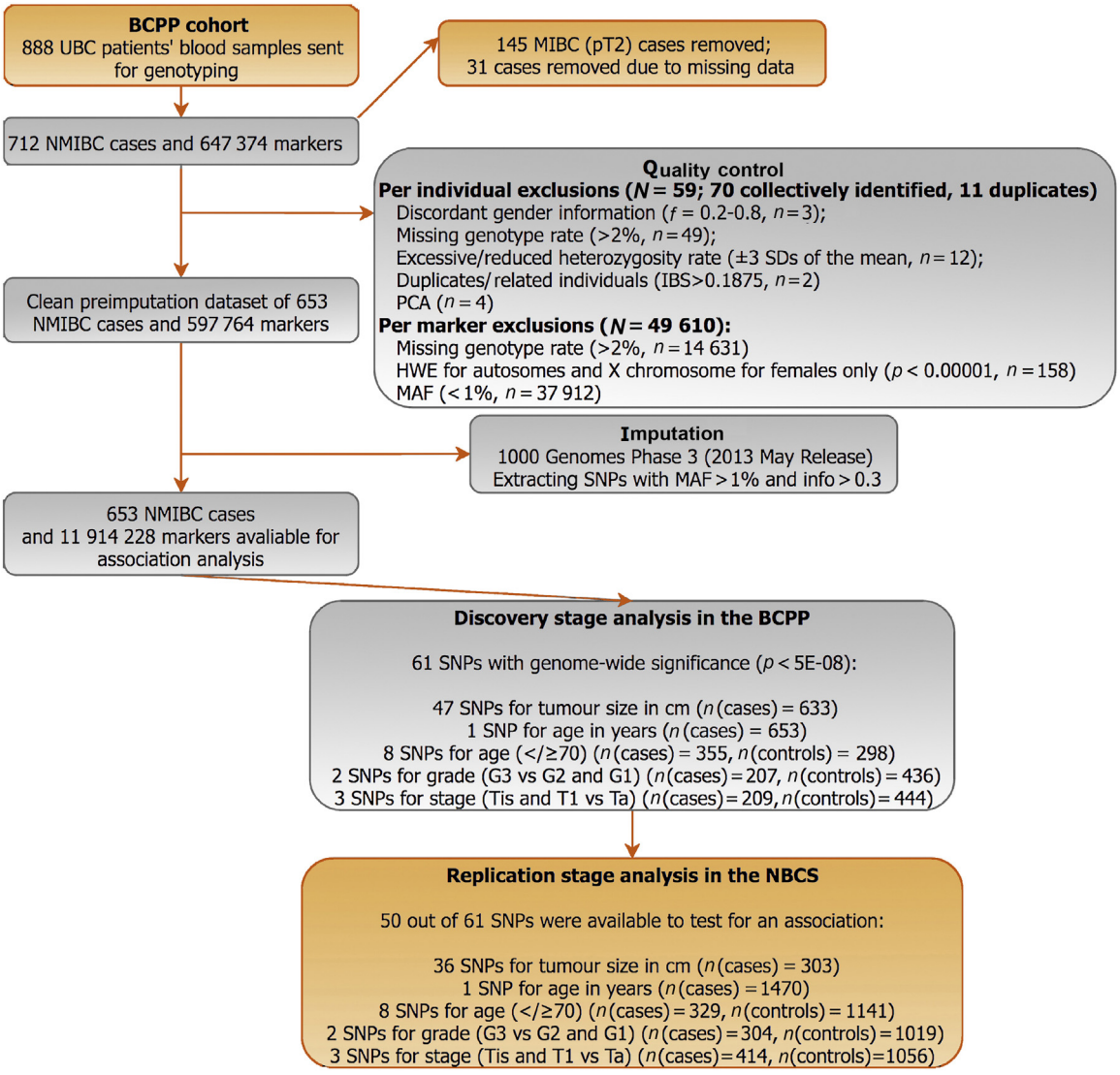

Fig. 1 – A flowchart of main steps in the GWAS analysis. BCPP = Bladder Cancer Prognosis Programme; HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; MIBC = muscle-invasive bladder cancer; NBCS = Nijmegen Bladder Cancer Study; NMIBC = non–muscle-invasive bladder cancer; PCA = principal component analysis; QC = quality control; SD = standard deviation; SNP = single nucleotide polymorphism; UBC = urinary bladder cancer.

2.5. *Functional annotation*

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

2.6. *Replication*

Genome-wide significant hits were attempted to replicate in a sample of 1470 NMIBC cases from the NBCS [22] (Fig. 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 yr; additional data were 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 are provided 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].

3. Results

Baseline clinical characteristics of the discovery and replication cohorts are shown in Table 1.

The majority of cases in BCPP were male (78.1%), with an average age of 70 yr. The mean tumour size 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 was similar between the BCPP and NBCS cohorts.

In the discovery-stage analysis, a total of 61 SNPs, corresponding to 29 different regions, showed genome-

Table 1 – Descriptive characteristics of the discovery (BCPP) and replication (NBCS) cohorts.

Variables	Discovery set (*N* = 653)	Replication set (*N* = 1470)
Age (yr)		
Mean (SD)	70.2 (10.5)	62.5 (9.7)
Median (range)	71.5 (34.3–91.5)	64 (25.0–91.0)
Age (yr)		
<70 (%)	298 (45.6)	329 (22.4)
≥70 (%)	355 (54.4)	1141 (77.6)
Sex		
Male (%)	510 (78.1)	1208 (82.2)
Female (%)	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; NA = not available; NBCS = Nijmegen Bladder Cancer Study; SD = standard deviation.

wide statistically significant associations with at least one of the outcomes. Out of those, 20 loci were mapped to genes (all intronic regions; Table 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) cm (Table 2).

One SNP in 9q22.32, rs142492877, showed a statistically significant association with decreased age at diagnosis of almost 1 yr (ß = –0.95, standard error [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% confidence interval {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 (Fig. 2) also shows that there are several polymorphisms in linkage disequilibrium (LD) with the leading SNP (Manhattan plots for all other tested outcomes are available in the Supplementary Fig. 1–6).

Regional association plot of 13q13.3 (Fig. 3) 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 Supplementary Figures 7–33.

In the replication stage, 50 out of 61 SNPs were available for test in NBCS (Table 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, a low I^2 estimate (I^2 = 0%, *p* [heterogeneity] = 0.75) indicated that there was no significant heterogeneity between the two cohorts for the replicated SNP. A conditional association analysis on rs180940944 showed that the associations in the *NBEA* gene are likely to be driven by the top SNP, as none of the variants have reached genome-wide significance when controlled for the effect of rs180940944 (Supplementary Fig. 34). Nevertheless, the analysis also suggests that there is a region in the *NBEA* gene of mildly inflated *p* values, independent of the rs180940944.

4. Discussion

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

Multiple loci were identified in the discovery stage, which 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 mostly been 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 intracellular organisation [24]. An in-depth analysis revealed that 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 intracellular 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

Table 2 – Genetic associations with NMIBC tumour and patient characteristics at baseline in the discovery (BCPP) and replication (NBCS) stages and a joint analysis.

Phenotype	rsID	BP	Locus	REF	ALT	Discovery cohort (BCPP)				Replication cohort (NBCS)				*p* (joint)	Annotation
						MAF	ß	OR (95% CI)	*p* value	MAF	ß	OR (95% CI)	*p* value		
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 (yr)	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 yr)	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 yr)	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 yr)	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 yr)	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 yr)	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 yr)	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 yr)	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 yr)	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	–

Table 2 (*Continued*)

Phenotype	rsID	BP	Locus	REF	ALT	Discovery cohort (BCPP)				Replication cohort (NBCS)				*p* (joint)	Annotation
						MAF	ß	OR (95% CI)	*p* value	MAF	ß	OR (95% CI)	*p* value		
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

ALT = alternative allele; BCPP = Bladder Cancer Prognosis Programme; BP = base-pair; CI = confidence interval; MAF = minor allele frequency (corresponds to the alternative allele); NA = not available; NBCS = Netherlands Bladder Cancer Study; NMIBC = non–muscle-invasive bladder cancer; OR = odds ratio; REF = reference allele; rsID = SNP identification number; SNP = single nucleotide polymorphism.
The most promising SNP is marked in bold.

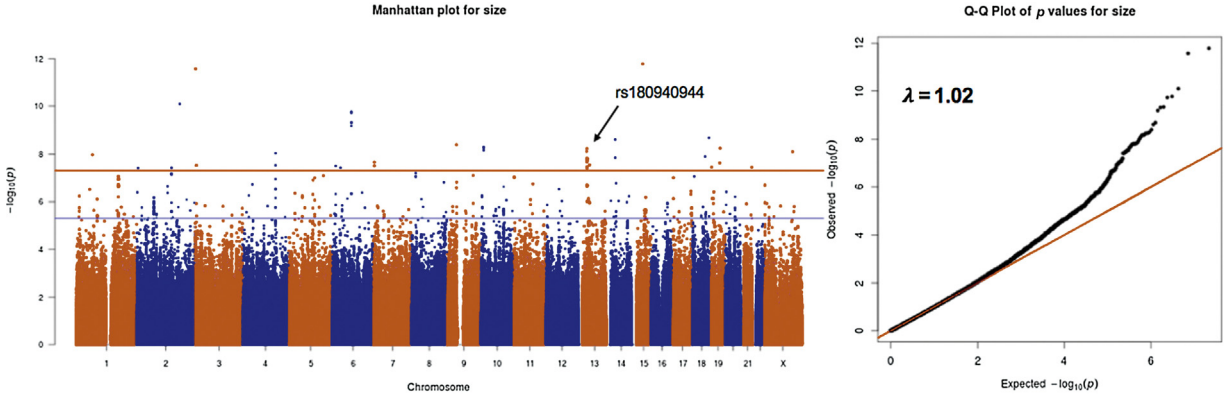

Fig. 2 – Manhattan and quantile-quantile plots for tumour size (cm) in the BCPP cohort. Blue and red horizontal lines indicate *p* values of <5E–06 and <5E–08, respectively. Highlighted variant shows the SNP reaching statistical significance in the meta-analysis of BCPP and NBCS (independent association was observed in the BCPP, and no significant effect was detected among NBCS participants only). BCPP = Bladder Cancer Prognosis Programme; NBCS = Nijmegen Bladder Cancer Study; Q-Q = quantile-quantile; SNP = single nucleotide polymorphism.

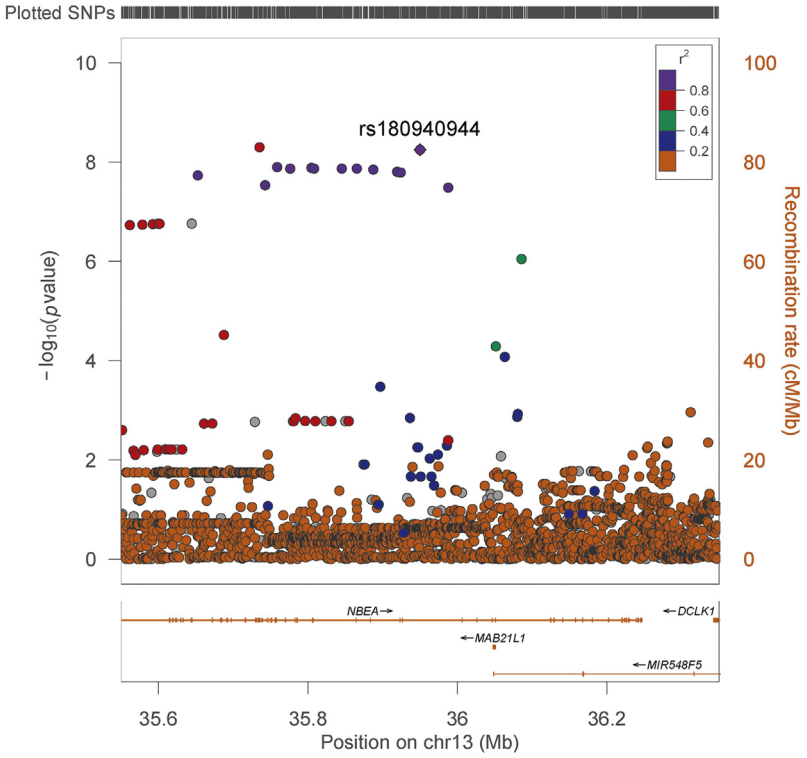

Fig. 3 – Regional association plot for 13q13.3 locus with tumour size (cm) in NMIBC patients of the BCPP cohort (annotated SNP has reached statistical significance in the meta-analysis of BCPP and NBCS cohorts). BCPP = Bladder Cancer Prognosis Programme; NBCS = Nijmegen Bladder Cancer Study; SNP = single nucleotide polymorphism.

oropharyngeal squamous cell carcinomas [27], with promising results. Collectively, these observations implicate the pleiotropic nature of *NBEA* effect across a variety of traits.

In our study, we suggest that 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 run on bigger cohorts (eg, 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).

Furthermore, tumour size measurements are subject to variability, the 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 focused only 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 the use of an independent prognostic marker of NMIBC (ie, tumour size) in our study instead of recurrence and/or progression as an outcome. A 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 (eg, size) carries a different weighting [17], collectively contributing to an endpoint (eg, recurrence).

Importantly, powerful studies on UBC risk have already shown some signals to be associated only 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 as a connecting thread between all studies. We therefore believe that separate genetic relationships are likely to be present for NMIBC determinants, rather than overall prognostic outcomes.

5. Conclusions

Our study suggests that 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.

Author contributions: Nadezda Lipunova had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Statistical analysis: Lipunova, Wesselius, Cazier, Bryan, Galesloot.
Obtaining funding: Cheng, Zeegers, Bryan.
Administrative, technical, or material support: Wesselius, Bryan, Zeegers, Galesloot, Kiemeney.
Supervision: Wesselius, Zeegers, Bryan, Cazier, van Schooten, Cheng.
Other: None.

Financial disclosures: Nadezda Lipunova certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euo.2018.08.020.

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