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Research Report

Bladder Cancer Genetic Susceptibility. A Systematic Review

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Abstract.
Background: The variant/gene candidate approach to explore bladder cancer (BC) genetic susceptibility has been applied in many studies with significant findings reported. However, results are not always conclusive due to the lack of replication by subsequent studies.
Objectives: To identify all epidemiological investigations on the genetic associations with BC risk, to quantify the likely magnitude of the associations by applying metaanalysis methodology and to assess whether there is a potential for publication/reporting bias.
Methods: To address our aims, we have catalogued all genetic association studies published in the field of BC risk since 2000. Furthermore, we metaanalysed all polymorphisms with data available from at least three independent case-control studies with subjects of Caucasian origin analyzed under the same mode of inheritance.
Results: The characterization of the genetic susceptibility of BC is composed of 28 variants, GWAS contributing most of them. Most of the significant variants associated with BC risk are located in genes belonging to chemical carcinogenesis, DNA repair, and cell cycle pathways. Causal relationship was also provided by functional analysis for *GSTM1-null, NAT2-slow, APOBEC*-rs1014971, *CCNE1*-rs8102137, *SLC14A1*-rs10775480, *PSCA*-rs2294008, *UGT1A*-rs1189203, and *TP63*-rs35592567.
Conclusions: Genetic susceptibility of BC is still poorly defined, with GWAS contributing most of the strongest evidence. The systematic review did not provide evidence of further genetic associations. The potential public health translation of the existing knowledge on genetic susceptibility on BC is still limited.

Keywords: Bladder cancer, genetic susceptibility, genetic variant, gene, pathway, metaanalysis

INTRODUCTION

The role of genetic susceptibility in bladder cancer (BC) carcinogenesis has been well recognized for a long time ago, the first studies dating from the early eighties and mainly focusing on xenobiotic metabolic genetic heterogeneity [1]. Because of the difficulties in applying molecular genotyping analysis, these first reports used phenotypic approaches to assess bladder cancer risk such as the caffeine tests to assess NAT2 metabolic variability.

Technological advances allowed the determination of genetic variants early in the 1990 s and bladder cancer was one of the first cancers benefiting from these advances; and, again, it was associated with xenobiotic metabolic genes variation [1]. The reason for focusing the attention on the genes participating in both phase 1 and phase 2 carcinogen metabolism was based upon the well-known risk factors for bladder cancer: smoking and occupational exposure to

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aromatic and heterocyclic amines. Therefore, the main hypothesis driving these preliminary studies focused on the candidate genetic variants that could modify the risk of bladder cancer conferred by the above-mentioned carcinogens. Indeed, N-acetyl transferase 2 (*NAT2*) and Glutathione-s-transferase M1 (*GSTM1*) variants were the first showing a gene*smoking interaction with bladder cancer risk [2].

The variant/gene candidate approach to explore bladder cancer genetic susceptibility has been applied in many studies and significant findings have been reported. However, results are not always conclusive due to the lack of replication by subsequent studies or to the lack of reports on a specific variant/gene, a fact that may point to a potential publication bias. The candidate pathway approach in bladder cancer genetic susceptibility has mainly focused on DNA repair and cell cycle pathways [3–9].

The first genome-wide association study (GWAS) in bladder cancer was published in 2008 [10]. Together with seven additional GWAS published in the following years, a total of 23 loci distributed in 18 regions of 13 chromosomes were identified, in addition to confirm the *GSTM1*-null genotype in 1p13.3 [reviewed in 11].

While the GWAS methodology is robust enough to provide conclusive results and quantify the risk of the identified variants, there is a need for a quantitative summary for genetic variants identified through candidate approaches. Therefore, the goals of this systematic review were to identify all epidemiological investigations on the genetic associations with bladder cancer risk, to quantify the likely magnitude of the associations by applying metaanalysis methodology and to assess whether there is a potential for publication/reporting bias.

MATERIAL AND METHODS

To address the aims of this systematic review, we have catalogued all genetic association studies published in the field of bladder cancer risk since 2000. Furthermore, for each polymorphism, we metaanalyzed all summary data available from at least three independent case-control studies of Caucasian origin using the same mode of inheritance (MOI).

Literature search strategy

We combined searches from PubMed and ISI Web of Knowledge (WoK) electronic databases. Search strategies used keywords relevant to genetic association studies and bladder cancer risk for the period Jan 1, 2000 – May 1, 2017. A detailed search strategy was developed for use in Pubmed: a combination of genetic and phenotype keywords and Medical Subject Headings (MeSH) terms: (polymorphism OR SNP OR genetic) AND ("urinary bladder neoplasm" [MeSH] AND (risk OR susceptibility). Since Web Knowledge of Science does not accept MeSH terms, the search strategy designed for Pubmed was then adapted for Web Knowledge of Science database: Topic: (("urinary bladder neoplasm" OR "bladder neoplasm" OR "bladder tumor" OR "bladder tumour" OR "urinary bladder cancer" OR "malignant tumor of urinary bladder" OR "malignant tumour of urinary bladder" OR "cancer of the bladder" OR "bladder cancer" OR "cancer of bladder") AND ("snp" OR "polymorphism" OR "genetics") AND ("risk" OR "susceptibility")) AND Year of publication: (2000–2017) AND Language: (English). Then, since the search system in Web of Knowledge of Science database does not allow the selection of papers by month of publication, the papers published after May 1, 2017 were then discarded.

Selection of eligible articles

Studies were included in this review if they: 1) were published in a peer-reviewed journal in English; 2) included adult, Caucasian human subjects; and 3) reported associations between SNPs and urothelial bladder cancer risk, our outcome of interest, using both case-control and cross-sectional designs. We included association studies testing for any genetic polymorphism at the nucleotide level, including SNPs, deletions, duplications, and copy-number variants, but excluded larger microscopic variants at the karyotype level. Reviews, metaanalyses, editorials, letters to the editor, book reviews, commentaries and proceedings to congresses were excluded. When it was not clear whether the study met these criteria, the reviewer gathered the full text. Two reviewers (ELM and MR) independently assessed the eligibility of all potentially eligible full-text studies, and any discrepancies were resolved through discussion with a third reviewer NM. We used the metagear R package [12] to assess the eligibility of the studies.

Information extraction

Co-authors ELM, MR, CA and OS independently abstracted data from all studies selected according to

the inclusion criteria. The reviewers were not blinded to the names of authors, journal or institutions. Data extracted included information on the setting of each study, details of the sampling strategy and sampled population (age, gender, ethnicity), overall and by cases and controls sample size, the definition of cases and controls, genotyping method, gene, polymorphism, MOI considered, and risk allele.

Quality assessment

Independent duplication of steps in the review process was performed, particularly in the selection of studies and extraction of data, reducing biases and minimizing errors. After the first screening of the papers, both eligible and not eligible papers were reviewed by independent reviewers. Each polymorphism was assigned to the corresponding dbSNP rs IDs using the *BiomaRt* R package, which is an interface to BioMart databases according to the GRCh37.p13 version [13, 14]. If a SNP ID (*rs* number) was merged with another, the most updated ID was used. Genes and pathways were annotated using VEP and the KEGG API, respectively.

Metaanalysis

Multiple reports of the same study were merged to obtain a single best estimator for the study prior to inclusion in the metaanalysis. For polymorphisms assessed in at least three studies using the same MOI, we conducted both random and fixed effects metaanalyses using the *metafor* R package [15]. The I^2 statistic was used as a measure of between-studies heterogeneity. In addition, we assessed the funnel plot asymmetry to test possible reporting biases or heterogeneity [16, 17]. Moreover, we used contour-enhanced funnel plots, which are funnel plots centered at 0 and with various levels of statistical significance of the points/studies indicated by the shaded regions. In particular, the unshaded (i.e., white) region in the middle corresponds to *p*-values greater than 0.10, the grey-shaded region corresponds to *p*-values between 0.10 and 0.05, the dark grey-shaded region corresponds to *p*-values between 0.05 and 0.01, and the region outside of the funnel corresponds to *p*-values below 0.01. Funnel plots drawn in this way are more useful for detecting publication bias due to the suppression of non-significant findings.

Reporting of this review complies with recommendations of the PRISMA statements [18].

RESULTS

Article selection

Figure 1 shows the flow diagram detailing the article selection steps according to PRISMA guidelines. The search strategy generated 1946 records after removing the duplicates produced when the searches from the two electronic databases (Pubmed and WoK) were pooled. From these abstracts, 386 articles were fully reviewed for eligibility. Overall, 8,865 variants were considered for association in the 154 papers considered to be eligible for metaanalysis and for which we retrieved the full-text. A list with the references of these articles is provided as Supplementary Material and the list of all those variants, together with their annotated genes, is provided in Supplementary Table 1.

After excluding the variants for which the OR for the association with bladder cancer was not reported, those without a risk allele specified, and those that appeared less than three times considering their MOI, quantitative syntheses were possible for 58 polymorphisms in or near 62 genes from 136 papers. Only the last estimate of repetitive studies conducted in the same population was included in the metaanalysis. Variants detected in GWAS studies were not considered in the metaanalysis because of their high level of evidence and we took their results for granted. Finally, only 17 out of 58 polymorphisms reported in 25 papers were considered for metaanalysis. Most of the studies were published before 2014 (Fig. 2) and they considered both men and women. Each metaanalysis included at most 16 studies or subgroups. Codominant was the most used MOI, followed by the dominant MOI. *GSTT1* was the only polymorphism included in the metaanalysis that was analyzed using a recessive MOI.

Publication bias and selective analysis

A visual inspection of the funnel plots suggested that, in most cases when asymmetry is present, it is due to the small sample size of the studies considered rather than to a substantial publication bias. Besides, tests for funnel plots asymmetry are known to have low power and, even when a test did not provide evidence of asymmetry, bias could not be excluded [17]. For example, the funnel plots for the variant rs2228001 in *XPC* showed evidence of statistically significant asymmetry under a codominant MOI, where the estimates that drove the asymmetry

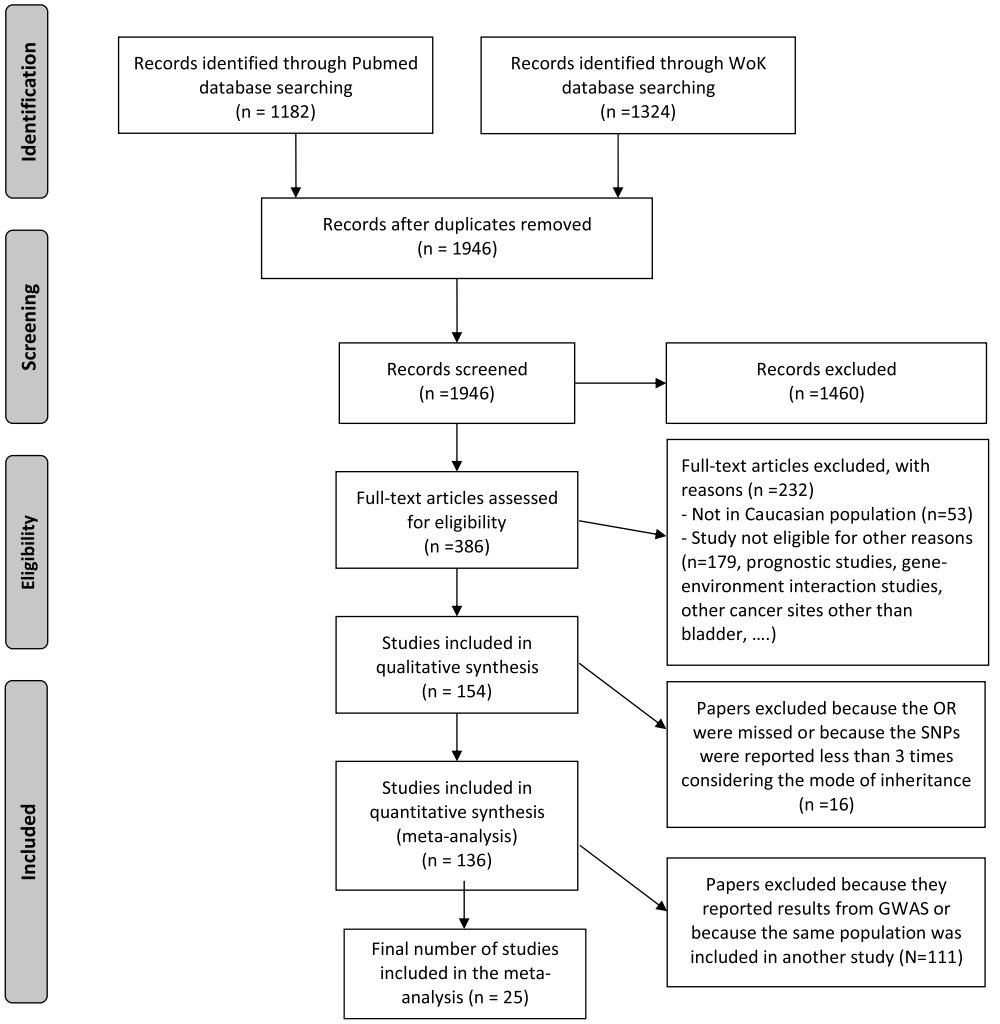

Fig. 1. PRISMA 2009 Flow Diagram detailing the article selection steps to guide the systematic review and metaanalysis on genetic variants associated with bladder cancer based on a candidate approach.

corresponded to the study of Fontana et al. [19], which showed the largest confidence interval due to the small sample size of the study (51 cases and 45 controls). Similar considerations can be extended to the funnel plot of *GSTP1*, under a codominant MOI. Overall, in most cases, only few studies were included in the metaanalysis and it was hard to detect asymmetry in the plots.

Metaanalyses

Here, we report the results from the metaanalysis for the selected variants according to their annotation in genes and KEGG pathways. Most association studies in bladder cancer genetic susceptibility focused on variation in genes involved in chemical carcinogenesis, DNA repair, and cell cycle pathways. In Table 1 we list the significant variants identified by GWAS together with those explored in the pooled analysis [20] and those included in the metaanalysis. In bold, we highlight those significantly associated with bladder cancer. Only *GSTT1* metaanalyses was based in >10 publications.

Chemical carcinogenesis

The variants included in the metaanalysis and annotated in this pathway were: *GSTT1*-null (16 studies: metaOR = 1.06, 95%CI 0.88–1.27, I2 = 46.5%), *GSTP1*-rs1695 (4 studies for the dominant and 5 the codominant MOI), *NAT1*-slow (5 studies), and *CYP1B1*-rs1056836 (6 studies analyzed the codominant MOI). None of them showed a significant

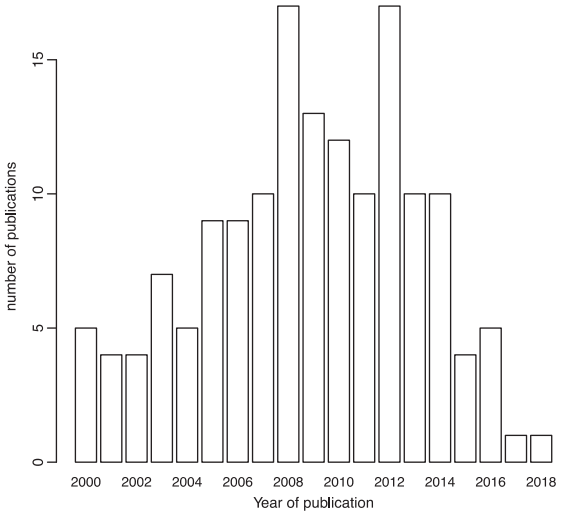

Fig. 2. Number of published papers considered in the systematic review according to their year of publication.

association with bladder cancer. Asymmetries in the forest plots may be due to the small number of papers published on this topic or to a potential publication/reporting bias (Supplementary Figure 1). On the contrary, *GSTM1-null* and *NAT2-slow* variants have been reported to be associated with bladder cancer by all approaches, including GWAS [20]. The other two variants identified through GWAS were *CLK3/CYP1A2*-rs11543198, *UGT1A8/UGT1A10*-rs1189203.

Base-excision DNA repair

APEX1-rs1130409 (3 studies) was metaanalized using a dominant MOI, although the association was not significant. *XRCC1*-rs25487 and *XRCC1*-rs1799782 were metaanalized (4 studies for both dominant and codominant MOI) and were also analyzed in the pooled study by Stern et al., although the associations were not significant in either approach [21].

Nucleotide-excision DNA repair

ERCC2-rs13181 (3 studies for the codominant and 6 for the dominant MOI), *ERCC2*-rs1799793 (3 studies for the codominant MOI), *ERCC5*-rs17655 (3 studies for the codominant MOI), *XPC*-rs2228001 (4 studies for the codominant, 3 for the dominant, and 3 for the recessive MOI), and *XPC*-polyAT (4 studies for the codominant MOI) were included in the metaanalysis. None of the metaOR were significant. Noteworthy, Stern et al. found significant associations between *ERCC2*-rs13181, *ERCC2*-rs1799793 and BC risk [21]. The same authors also analyzed *ERCC4*- rs1799801 and *XPC*-rs2228001, although none of these variants were significantly associated with bladder cancer risk [21].

Homologous recombination DNA repair

The variant *XRCC3*-rs861539 was analyzed using a codominant (4 studies) and a dominant (3 studies) MOI, although none of the estimates reached the significance threshold. This variant was also reported as not associated with BC risk in Stern et al. [21]. Interestingly, *NBN*-rs1805794 was found significantly associated with BC risk under the dominant and the additive MOI in the pooled analysis [21].

Cell cycle

The only variant annotated in this pathway that was considered for the metaanalysis was *CCND1*-rs9344 (3 studies applied a codominant MOI) and its association with BC risk was not found to be significant. The following hits in GWAS studies also annotated in the cell cycle pathway are: *CCNE1*-rs8102137, *MYC/POU5F1B/PVT1*-rs10094872, *MYC/BC042052/CASC11*-rs9642880. Interestingly, the latter variant was the only one that provided significant results when we metaanalyzed the non-GWAS available data with a random-effect model (Fig. 3) [22–24]. Three estimates were reported by Golka et al. [22] from three independent series and two reported negative but not significant OR, with a quite low estimate for heterogeneity (I = 30%). Negative and significant estimate and similar in magnitude was obtained with a fixed effect model (metaOR = 0.77, 95%CI 0.69, 0.86). The funnel plot showed no evidence for asymmetry. The study at the bottom left of the plot corresponds to Yates et al. [24], which is the one with the lowest sample size.

One carbon pool by folate

MTHFR-rs1801131 (5 studies applied the codominant and 3 the dominant MOI) and *MTHFR*-rs1801133 (5 studies applied the codominant MOI) on this pathway were included in the metaanalysis, although none of them showed a significant association with BC risk.

Peroxisome

Only *SOD2*-rs4880 with 3 studies applying a codominant MOI could be metaanalysed and the result showed no significant association with BC risk.

Table 1
Annotated pathways and genes for the variants selected in this metaanalysis (MA), and those identified by the bladder cancer GWAS and pooled analysis (PA). In bold, the variants that provided statistical significant meta-risks

Gene	VARIANT	Other pathways
	***MAIN PATHWAY*: Chemical carcinogenesis**	
GSTM1	**Null/Present** (GWAS)	Glutathione metabolism; Metabolism of xenobiotics by cytochrome P450; Drug metabolism – cytochrome P450; Platinum drug resistance; Pathways in cancer; Hepatocellular carcinoma; Fluid shear stress and atherosclerosis
GSTT1	Null/Present (MA)	Glutathione metabolism; Metabolism of xenobiotics by cytochrome P450; Drug metabolism – cytochrome P450; Platinum drug resistance; Pathways in cancer; Hepatocellular carcinoma; Fluid shear stress and atherosclerosis
GSTP1	I105V (MA)	Glutathione metabolism; Metabolism of xenobiotics by cytochrome P450; Drug metabolism – cytochrome P450; Platinum drug resistance; Pathways in cancer; Prostate cancer; Hepatocellular carcinoma; Fluid shear stress and atherosclerosis
NAT2	**rs1495741** (GWAS)	Caffeine metabolism; Drug metabolism – other enzymes
NAT1	*4/*10 (MA)	Caffeine metabolism; Drug metabolism – other enzymes
CYP1B1	rs1056836 (MA)	
CLK3/CYP1A2	**rs11543198** (GWAS)	Steroid hormone biosynthesis; Caffeine metabolism; Tryptophan metabolism; Linoleic acid metabolism; Retinol metabolism; Metabolism of xenobiotics by cytochrome P450; Drug metabolism – cytochrome P450; Metabolic pathways
UGT1A8/UGT1A10	**rs11892031** (GWAS)	Pentose and glucuronate interconversions; Ascorbate and aldarate metabolism; Steroid hormone biosynthesis; Retinol metabolism; Porphyrin and chlorophyll metabolism; Metabolism of xenobiotics by cytochrome P450; Drug metabolism – cytochrome P450; Drug metabolism – other enzymes; Metabolic pathways
	***MAIN PATHWAY*: One carbon pool by folate**	
MTHFR	rs1801131 (MA) rs1801133 (MA)	Carbon metabolism; Antifolate resistance; Metabolic pathways
	***PATHWAY*: Base excision repair**	
APEX1 *XRCC1*	rs1130409 (MA + PA) rs25487 (MA + PA) rs1799782 (PA)	
	***MAIN PATHWAY*: Nucleotide excision repair**	
ERCC2	**rs13181** (MA + **PA**) **rs1799793** (MA + **PA**)	Basal transcription factors
ERCC4	rs1799801 (PA)	Fanconi anemia pathway
ERCC5	rs17655 (MA)	
XPC	**rs2228000**(**PA**) rs2228001 (MA + PA) poly AT (MA)	
	***MAIN PATHWAY*: Homologous recombination**	
XRCC3 *NBN*	rs861539 (MA + PA) **rs1805794** (**PA**)	Cellular senescence
	***MAIN PATHWAY*: Cell cycle**	
CCND1	rs9344 (MA)	Endocrine resistance; p53 signalling pathway; PI3KAkt signalling pathway; AMPK signalling pathway; Cellular senescence; Wnt signalling pathway; Hedgehog signalling pathway; Apelin signalling pathway; Hippo signalling pathway; FoxO signalling
CCNE1	**rs8102137** (GWAS)	Cell cycle; Oocyte meiosis; p53 signaling pathway; PI3K-Akt signaling pathway; Cellular senescence; Hepatitis B; Measles; Human papillomavirus infection; Pathways in cancer; Viral carcinogenesis; MicroRNAs in cancer; Prostate cancer; Small cell lung cancer; Gastric cancer; MODULE: Cell cycle – G1/S transition

(*Continued*)

Table 1
(*Continued*)

Gene	VARIANT	Other pathways
MYC/POU5F1B/PVT1	**rs10094872** (GWAS)	MYC: MAPK signalling pathway; ErbB signalling pathway; Cell cycle; PI3K-Akt signalling pathway; Cellular senescence; Wnt signalling pathway; TGF-beta signalling pathway; Hippo signalling pathway; Signalling pathways regulating pluripotency of stem cells; Jak-STAT signalling pathway; Thyroid hormone signalling pathway; Hepatitis B; HTLV-I infection; Kaposi's sarcoma-associated herpesvirus infection; Epstein-Barr virus infection; Pathways in cancer; Transcriptional misregulation in cancer; Proteoglycans in cancer; MicroRNAs in cancer; Colorectal cancer; Endometrial cancer; Thyroid cancer; Bladder cancer; Chronic myeloid leukaemia; Acute myeloid leukaemia; Small cell lung cancer; Breast cancer; Hepatocellular carcinoma; Gastric cancer; Central carbon metabolism in cancer *POU5F1B*: Signalling pathways regulating pluripotency of stem cells
MYC/BC042052/CASC11	**rs9642880** (**MA+GWAS**)	MAPK signalling pathway; ErbB signalling pathway; Cell cycle; PI3K-Akt signalling pathway; Cellular senescence; Wnt signalling pathway; TGF-beta signalling pathway; Hippo signalling pathway; Signalling pathways regulating pluripotency of stem cells; Jak-STAT signalling pathway; Thyroid hormone signalling pathway; Hepatitis B; HTLV-I infection; Kaposi's sarcoma-associated herpesvirus infection; Epstein-Barr virus infection; Pathways in cancer; Transcriptional misregulation in cancer; Proteoglycans in cancer; MicroRNAs in cancer; Colorectal cancer; Endometrial cancer; Thyroid cancer; Bladder cancer; Chronic myeloid leukaemia; Acute myeloid leukaemia; Small cell lung cancer; Breast cancer; Hepatocellular carcinoma; Gastric cancer; Central carbon metabolism in cancer
***MAIN PATHWAY*: Peroxisome**		
SOD2	rs4880 (MA)	FoxO signalling pathway; Longevity regulating pathway; Huntington's disease
***MAIN PATHWAY*: Ubiquinone and other terpenoid-quinone biosynthesis**		
NQO1	rs1800566 (MA)	Ubiquinone and other terpenoidquinone biosynthesis; Hepatocellular carcinoma; Fluid shear stress and atherosclerosis
OTHER PATHWAYS		
ACTRT3/MYNN/TERC/LRRC34	**rs10936599** (GWAS)	Pathways in cancer; Hepatocellular carcinoma; Gastric cancer
TERT/CLPTM1L	**rs401681** (GWAS)	Human papillomavirus infection; HTLV-I infection; Pathways in cancer; Hepatocellular carcinoma; Gastric cancer
TP63	**rs710521** (GWAS)	MicroRNAs in cancer
JAG1	**rs62185668** (GWAS)	Endocrine resistance; Notch signalling pathway; Apelin signalling pathway; Th1 and Th2 cell differentiation; TNF signalling pathway; Human papillomavirus infection; Pathways in cancer; Breast cancer
TMEM129/TACC3/FGFR3	**rs798766** (GWAS)	EGFR tyrosine kinase inhibitor resistance; MAPK signalling pathway; Ras signalling pathway; Rap1 signalling pathway; Endocytosis; PI3K-Akt signalling pathway; Signalling pathways regulating pluripotency of stem cells; Regulation of actin cytoskeleton; Pathways in cancer; MicroRNAs in cancer; Bladder cancer; Central carbon metabolism in cancer RNA transport (TACC3)
NO PATHWAY ANNOTATED		
CWC27	**rs2042329** (GWAS)	
NR	**rs7747724** (GWAS) **rs5003154** (GWAS) **rs4907479** (GWAS)	
CDKAL1	**rs4510656** (GWAS)	
JRK/PSCA	**rs2294008** (GWAS)	

(*Continued*)

Table 1
(*Continued*)

Gene	VARIANT	Other pathways
LSP	**rs907611** (GWAS)	
SLC14A1	**rs17674580** (GWAS) **rs7238033** (GWAS)	
SLC14A2	**rs10775480** (GWAS)	
C20orf187/ LOC339593	**rs6104690** (GWAS)	
CBX6/APOBEC3A	**rs1014971** (GWAS)	

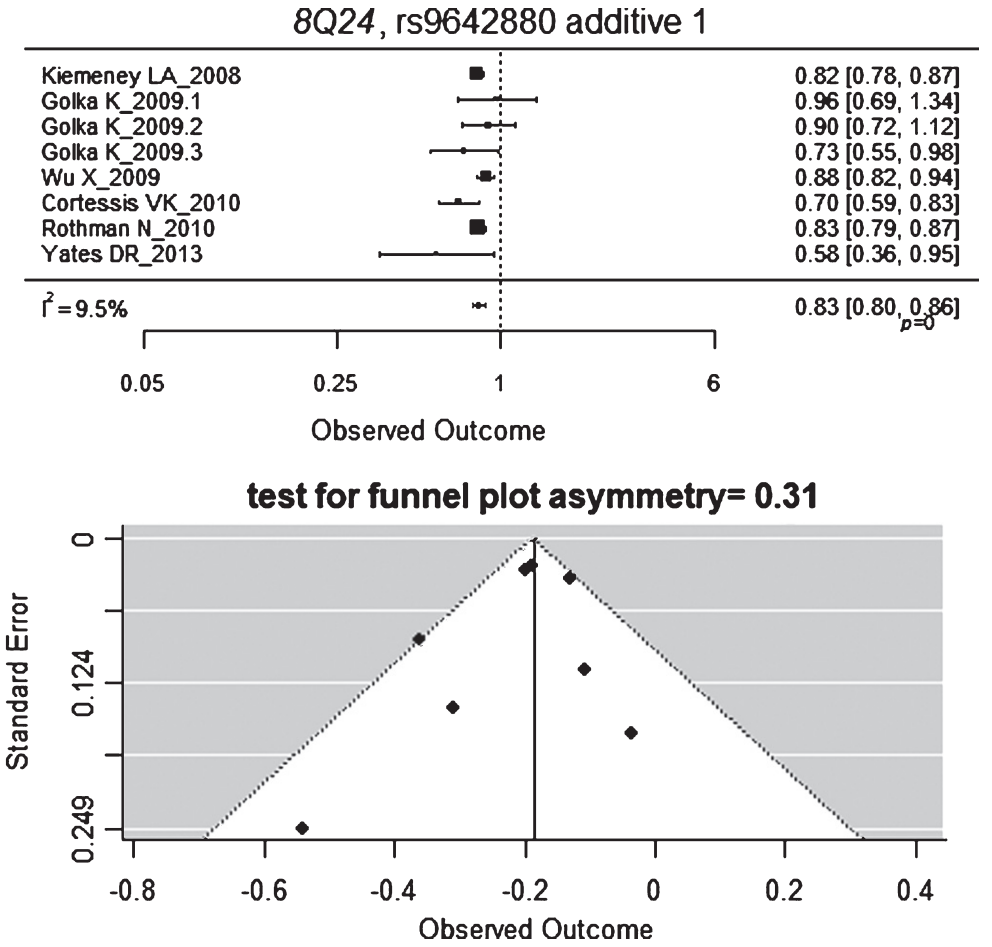

Fig. 3. Forest and funnel plots for *MYC/BC042052/CASC11*-rs9642880.

Ubiquinone and other terpenoid-quinone biosynthesis

Four studies applying the codominant MOI for *NQO1*-rs1800566 were considered in the metaanalysis showing a non-significant association with BC risk.

The GWAS reported variants *ACTRT3/MYNN/TERC/LRRC34*-rs10936599, *TERT/CLPTM1L*- rs401681, *TP63*-rs710521, *JAG1*-rs62185668, *TMEM129/TACC3/FGFR3*-rs798766 associated with BC risk were annotated in other pathways as "Pathways in cancer", whereas *CWC27*-rs2042329, *NR*-rs7747724, *NR*-rs5003154, *NR*-rs4907479, *CDKAL1*-rs4510656, *JRK/PSCA*- rs2294008, *LSP*- rs907611, *SLC14A1*-rs17674580, *SLC14A1*-rs7238033, *SLC14A2*-rs10775480, *C20orf187/ LOC339593*-rs6104690, and *CBX6/APOBEC3A*-rs1014971 were not annotated in any KEGG pathway.

DISCUSSION

A total number of 386 studies were considered in this systematic review. After applying inclusion criteria, 154 studies assessing 8,865 unique SNPs remained (Supplementary Table 1). Candidate variant-based studies were published between 2000 to 2017. We included in this systematic review the pooled study by Stern et al. [21]. However, we excluded previous metaanalyses performed on variants in *GSTM1* [2, 25, 26], *NAT2* [2, 25–27], *GSTP1* [25, 28], *hOGG1* [29], *NQO1* [25, 30–32], *TP53* [33], *XRCC1* [34–37], *XRCC3* [25, 38], *XPC* [25], *XPD* [25], *PSCA* [32], *SDF-1* [39], *TRAIL-R1* [40], *E-cadherin* [41], and *TGFBR1* [42].

The systematic review did not provide enough evidences for additional candidate variants associated with bladder cancer risk further from those already reported in the GWAS (N = 24) and the pooled analysis (N = 4) [21] (Table 1). This limited characterization of the genetic susceptibility of BC, in comparison to that of other cancer such as breast and prostate [https://www.ebi.ac.uk/gwas] is intriguing, being BC a paradigm of complex diseases. The fact that this cancer is largely environmentally-driven, may partly explain the low number of variants identified at present. Either a gene*environment interaction or an extreme-phenotype approaches are probably needed to identify those missing or hidden heritability of BC, as suggested by Lopez-Maturana et al. [43]. In this already published study, we provided evidence on the importance of applying multi-marker/SNP analyses towards a better characterization of the BC genetic risk.

It is interesting to observe that an important proportion of significant variants with strong evidence of involvement in BC carcinogenesis belong to genes involved in chemical carcinogenesis (*GSTM1, NAT2, CLK3/CYP1A2,* and *UGT1A8/UGT1A10*), DNA repair pathways (*ERCC2, XPC,* and *NBN*), and cell cycle pathway (*CCNE1, MYC/POU5F1B/PVT1,* and *MYC/BC042052/CASC1*). GWAS also identified variants in genes involved in many other pathways as well as in yet not-annotated pathways. A recent pathway analysis based on GWAS data further identified variants in 9 novel genes (*RAPGEF1*, *SKP1*, *HERPUD1*, *CACNB2*, *CACNA1C*, *CACNA1S*, *COL4A2*, *SRC*, and *CACNA1C*) [44]. Functional analyses have also provided evidences of causality for *GSTM1-null, NAT2-slow, APOBEC*-rs1014971, *CCNE1*-rs8102137, *SLC14A1*-rs10775480, *PSCA*-rs2294008, *UGT1A*-rs1189203, and *TP63*-rs35592567 [45–52]. These signals should act as clues to further explore the gene and pathway variation in association with BC risk. Actually, a rapid consultation of the variants included in Supplementary Table 1 using GWAS data would provide conclusive results for most of them.

Pleiotropy at all levels is noteworthy with most variants, genes, and pathways being shared with genetic susceptibility of other cancers and non-malignant diseases. This fact would support a holistic genetic susceptibility approach across diseases (malignant and non-malignant) using a competing-risk analysis to identify the common and the specific actors promoting and preventing their development.

Except for GWAS results, the knowledge of genetic susceptibility scenario of BC has not changed in the last 10 years. Extending the publication period to 1990–1999 did not provide further genetic variants suitable to contribute to this metaanalysis in addition to those in *GSTM1*, *GSTT1*, *NAT2*, and *NAT1* that were already included. Despite the thousands of candidate genetic variants being analyzed, only 6 have provided consistent results of an association with BC risk in Caucasian population-based studies, including *GSTM1*-null and *NAT2*-slow acetylation. The lack of replication of most of the results from candidate genetic variant based-studies should preclude the conduction of such type of studies and, instead, move towards the GWAS approach.

With the exception of GWAS, the quality of the evidence provided by this systematic review on the null-associated variants is limited. The validity of our metaanalysis relies upon the validity of the studies included within it and most studies are small and heterogeneous. In addition, since effect magnitudes in genetic association studies are generally small, small biases may be relevant in concluding about their role on genetic susceptibility of BC. We are also aware of potential biases related to the studies considered, which could be related to the case definition (histologically confirmed urothelial bladder carcinoma), population stratification, and methods in the collection and processing of DNA as well as the determination of genotypes. In addition, potential biases related to the collection of studies as a whole may be possible though we explored two sources of publications, as well as reporting biases (including publication biases and selective reporting), and bias in the selection of genetic variants to study in each paper. Particularly in GWAS and also in some candidate gene approaches, only the significant associations are reported in the papers. Asymmetries were difficult to be identified in the funnel plots paired to each forest

plots in Supplementary Figure 1 because of the small number of papers included in each metaanalysis rather than indicating lack of bias. Also, the investigation of a polymorphism-disease association maybe based on already published results, and interesting findings are the most likely targets for replication [17, 53].

The above-mentioned evidences regard to low penetrance variants frequent in the population. Nevertheless, it is worth noting that a higher risk of BC has also been reported in germline *MSH2* mutation carriers and first degree relatives of Lynch syndrome families [54, 55], in Costello syndrome (*HRAS* germline mutations) affected individuals [56, 57], and in retinoblastoma (*RB1* germline mutations) affected survivors [58, 59], the latter ones showing the higher risk for BC. While germline mutations of these genes are rare in the population, somatic mutations of them are common in BC tumors.

In conclusion, the characterization of the genetic susceptibility of BC is still limited, with 28 variants showing strong evidence of association, mainly through GWAS. The systematic review did not provide evidences of further genetic associations. Most of the significant variants associated with BC risk are located in genes belonging to chemical carcinogenesis, DNA repair, and cell cycle pathways. Causal relationship has been also provided by functional analysis for *GSTM1-null, NAT2-slow, APOBEC*-rs101 4971, *CCNE1*-rs8102137, *SLC14A1*-rs10775480, *PSCA*-rs2294008, *UGT1A*-rs1189203, and *TP63*-rs 35592567.

There is a vast list of potentially associated variants, genes and pathways suggested by candidate-based approaches that can act as clues to further explore the genetic variation in association with BC risk using already available GWAS data. While the public health application of the existing knowledge on genetic susceptibility on BC based on individual SNPs is limited, the short-term perspectives to analyze them through system approaches will probably allow to identify genetic susceptibility signatures with a higher translational potential.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

SUPPLEMENTARY MATERIAL

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