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Clinical significance of fibroblast growth factor receptor-3 mutations in bladder cancer: a systematic review and meta-analysis

X. Liu[1]*, W. Zhang[2]*, D. Geng[3]*, J. He[2], Y. Zhao[2] and L. Yu[1]

[1]State Key Laboratory of Genetic Engineering, Institute of Genetics, Fudan University, Shanghai, China
[2]Institutes for Advanced Interdisciplinary Research, East China Normal University, Shanghai, China
[3]Department of Orthopaedic Surgery, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

*These authors contributed equally to this study.
Corresponding author: L. Yu
E-mail: yulong201@yahoo.cn

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ABSTRACT. Mutations in the fibroblast growth factor receptor-3 (*FGFR3*) gene are frequently found in bladder cancer, but their prognostic value remains controversial. To globally summarize the association between *FGFR3* mutations and the grade and stage of bladder cancer, and to analyze the predictive role of *FGFR3* mutations with respect to survival, eligible studies were identified and assessed for quality through multiple search strategies. Risk ratio (RR) data were collected from studies comparing the number of *FGFR3* mutants among low-grade and early-stage bladder cancer patients to the number among high-grade and late-stage patients. Hazard ratio (HR) data were collected from studies comparing survival in patients with mutant *FGFR3* genes to those with wild-type genes. Studies were pooled, and the RRs of grade and stage and the HRs of survival were

calculated. Thirty studies were included in the present meta-analysis. *FGFR3* mutations were found to be closely associated with low-grade and early-stage bladder cancer, showing pooled RRs = 2.948 [95% confidence interval (CI) = 2.357-3.688] and 2.845 (95%CI = 2.145-3.773), respectively. Notably, patients with *FGFR3* mutations tended to show better disease-, progress-, and recurrence-free survival (HR = 0.561, 95%CI = 0.405-0.779), and better disease-specific survival (HR = 0.363, 95%CI = 0.266-0.496). This study demonstrated that *FGFR3* mutations are closely related to low grade, early stage, and better survival among bladder cancer patients.

Key words: FGFR3; Bladder cancer; Prognosis; Grade; Staging

INTRODUCTION

Bladder cancer (BC) is the seventh most common cancer worldwide, accounting for approximately 336,000 new cases each year. It is the seventh most common cause of death from cancer in men and the eighth most common cause in women. In most western countries, bladder cancer predominantly involves urothelial cell carcinoma (UC or UCC); however, in other countries, most bladder cancers are squamous cell carcinomas (Kaufman et al., 2009; Cheng et al., 2011). Conventional clinical and pathological indexes are widely used to grade and stage tumors and to eventually predict clinical outcome. However, their predictive value is limited because of low accuracy in BC patients. In the last decade, with the development of molecular mechanisms of tumorigenesis, a variety of biomarkers involved in key pathways in carcinogenesis have been shown to be clinically relevant. They may be useful as diagnostic and prognostic molecular markers. Among these candidates, the fibroblast growth factor receptor 3 (FGFR3) is one of the most attractive. It is detectable and measurable in patient specimens and can be considered representative of various tumor properties.

FGFR3 belongs to a family of tyrosine kinase receptors, and is encoded by four different genes, *FGFR1-4*. These receptors are glycoproteins composed of two to three extracellular immunoglobulin-like domains, a transmembrane domain, and a split tyrosine-kinase domain. FGFs, which are the ligands for FGFRs, bind to their extracellular domains to trigger downstream signaling, which regulates cell proliferation, differentiation, migration, and apoptosis. *FGFR3* gene mutations in the germline are well-known causes of skeletal syndromes. Somatic *FGFR3* mutations have been found in malignant neoplasms. *FGFR3* appears to be the most frequently mutated oncogene in bladder cancer. *FGFR3* mutations have also been detected in >70% of non-muscle-invasive bladder tumors, but they have only been detected in 10-20% of tumors that invade the bladder muscle. Although a number of studies have reported that *FGFR3* mutations are significantly associated with low tumor grade and early cancer stages, other studies have shown that they are not (Bodoor et al., 2010; Miyake et al., 2010). Therefore, the prognostic value of FGFR3 remains controversial (Cheng et al., 2011; Mukhtar and Perry, 2011). Al-Ahmadie et al. (2011) and Bodoor et al. (2010) suggested that FGFR3 might not be a significant prognostic indicator of survival. However, Hernández et al. (2006) showed that *FGFR3* mutations were associated with a lower rate of death from bladder cancer (P = 0.002). To date, however, no individual study has emerged with sufficient power to determine whether or not *FGFR3* mutations have any significant independent prognostic value.

The objective of the present study was to conduct a systematic review and meta-analysis of associations between stage or grade and *FGFR3* mutations in bladder cancer. The impact of *FGFR3* mutations with respect to clinical outcome, as represented by progress-free survival (PFS) and disease-specific survival (DSS), was also analyzed.

MATERIAL AND METHODS

We performed a meta-analysis in accordance with the guidelines issued by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group (Stroup et al., 2000).

Search strategy

We carefully searched articles in PubMed published from 1966 to January 30, 2012 to identify relevant studies. Two distinct sets of key words were used: "FGFR3 and bladder cancer" and "FGFR3 and bladder carcinoma''. The following criteria were used for preliminary screening: 1) the research team identified *FGFR3* mutations in patients with bladder or urothelial cancer; 2) the research team compared the frequency of *FGFR3* mutations in low-grade or early-stage BC patients to the frequency in high-grade or late-stage patients; 3) the research team evaluated the potential association between *FGFR3* mutations and the survival outcome of BC. Eligible studies were required to meet the first criterion and either of the latter two criteria. Articles were excluded based on the following criteria: 1) review articles or letters; 2) non-English articles; 3) laboratory studies; 4) articles lacking key information, such as the sample size of the classified groups.

The titles, abstracts, full texts, and reference lists of all of the identified reports were examined independently by two reviewers. Each reviewer was responsible for collecting data for FGFR3 (X. Liu, W. Zhang, and D. Geng). Each reviewer's extracted data were double-checked by both other reviewers. Disagreements were resolved by consensus among the three readers or consultation with a fourth reviewer (Y. Zhao). The authors of the studies were contacted by e-mail to request additional information or data for meta-analytic calculations. When duplicate studies were retrieved, studies involving more patients or that were conducted most recently were chosen over those with fewer patients or those conducted earlier. A flow diagram of the study selection process is presented in Figure 1.

Quality assessment

According to a critical review checklist issued by the Dutch Cochrane Centre and proposed by MOOSE, we systematically assessed the quality of all studies included in the meta-analysis (Stroup et al., 2000). The key points of the current checklist included: 1) clear definition of study population and their disease; 2) clear definition of mutations and the method of detection; 3) clear definition of each subgroup according to grade or stage; 4) clear definition of outcome in survival studies; and 5) follow-up of sufficient duration. The classification of tumor grade was performed using the World Health Organization (WHO) grading system. LMPN, G1, and G2 were considered low-grade tumor; G3 and all other higher grades were considered high-grade tumor. Pathological stages were divided using the tumor-node-metastasis (TNM) classification. LMPN, CIS, pTa, and pT1 were considered early-stage, and T2, T3, and T4 were considered late-stage BC.

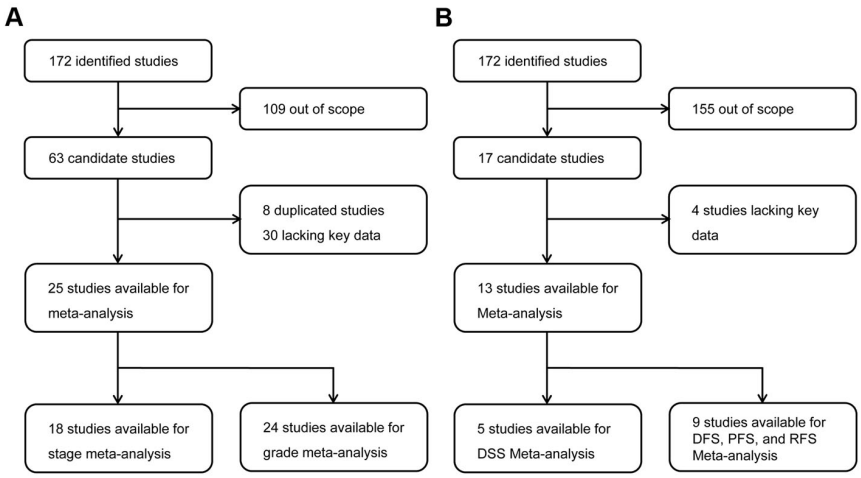

Figure 1. Study selection process. **A.** Grade and stage studies. **B.** Survival studies. DSS = disease-specific survival; DFS = disease-free survival; PFS = progress-free survival; RFS = recurrence-free survival.

Data extraction and conversion

The following elements were included among the extracted data elements in this review: 1) publication details: first author's last name, year of publication, and country of origin of the studied population; 2) characteristics of the studied population: sample size, age, gender ratio, stage of disease, and grade of disease; 3) sample size of each group; 4) risk ratio (RR) of *FGFR3* mutation in low-grade or early-stage disease; 5) hazard ratio (HR) of mutant *FGFR3* for survival. RRs were calculated using standard methods, so that the frequency of *FGFR3* mutations in low-grade and early-stage individuals was divided by its frequency in high-grade and late-stage BC individuals (Borenstein et al., 2009). HRs were collected directly from the articles or were calculated using Kaplan-Meier survival curves and previously described methods (Parmar et al., 1998).

Statistical analysis

A test of heterogeneity of combined HRs was conducted using the Cochran Q test and Higgins I-squared statistic. P values less than 0.05 were considered to be significant. A random-effect model (DerSimonian and Laird method) was used if heterogeneity was observed ($P < 0.05$). Otherwise, the fixed-effect model was used. Publication bias was evaluated using the funnel plot with the Egger bias indicator test. All analyses were conducted using the Stata: Data Analysis and Statistical Software V10.1 (http://www.stata.com).

RESULTS

One hundred and seventy-two records were identified from a primary literature

search in PubMed. After manually screening the titles, abstracts, and key data, 109 studies were excluded because they were review articles, letters, not written in English, laboratory studies, or were irrelevant to the current analysis. Of the 63 reports selected for detailed evaluation, 33 were excluded as duplications or because they lacked key data. The final meta-analysis was carried out on the remaining 30 studies (Billerey et al., 2001; Kimura et al., 2001; Bakkar et al., 2003; Rieger-Christ et al., 2003; van Rhijn et al., 2003, 2004; Hernández et al., 2005; Jebar et al., 2005; van der Aa et al., 2005; Wallerand et al., 2005; Hernández et al., 2006; Lindgren et al., 2006; Tomlinson et al., 2007; van Oers et al., 2007; Burger et al., 2008; Junker et al., 2008; Eltze et al., 2009; Ouerhani et al., 2009; van Oers et al., 2009; Zieger et al., 2009; Bakkar et al., 2010; Bodoor et al., 2010; Kompier et al., 2010; Miyake et al., 2010; van Rhijn et al., 2010; Al-Ahmadie et al., 2011; Dodurga et al., 2011; Serizawa et al., 2011; Sjödahl et al., 2011; van Rhijn et al., 2012). Twenty-five of the studies (Table 1A) investigated the association between *FGFR3* mutations and grade or stage, while the other 13 studies (Table 1B) investigated the prognostic value of *FGFR3* mutations for BC.

The main features of the eligible studies are summarized in Table 1A and B. We collected data from 30 studies in total, which included data obtained from 5025 participants from Denmark, France, Germany, Japan, Jordan, the Netherlands, Spain, Sweden, Tunisia, Turkey, the United Kingdom, and the United States. As shown in Table 2, of all the studies that evaluated either grade or stage, 24 studies (N = 3999) evaluated grade and 18 studies (N = 2491) evaluated stage. Of the survival studies, 9 (N = 2015) analyzed disease-free survival (DFS), recurrence-free survival (RFS), or PFS. Five studies (N = 1579) examined DSS. All studies recruited patients with bladder cancer or urothelial carcinoma, and three studies included only patients with non-muscle-invasive BC. The methods used for identifying mutants included SNaPshot, polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), sequencing, denaturing high-performance liquid chromatography (DHPLC), and PCR-restricted fragment length polymorphism (RFLP). Mutations were identified mainly on exons 7, 10, and 15. The point mutations S249C (C746G) and Y375C (A1124G) were especially common.

Among studies that evaluated either grade or stage, there appeared to be some heterogeneity between *FGFR3* mutations and wild-type ($P < 0.05$). For this reason, a random model was used to calculate a pooled RR and its 95%CI.

We found the frequency of *FGFR3* mutations to be significantly higher among low-grade and early-stage BC groups, which showed pooled RR = 2.948 (95%CI = 2.357-3.688) for grade (Figure 2A) and RR = 2.845 (95%CI = 2.145-3.773) for stage (Figure 2B). Considering that BC patients with low-grade or early-stage conditions tend to benefit from better survival rates, we also analyzed the association between *FGFR3* mutations and survival. Among studies that evaluated DFS, PFS, or RFS, the pooled HR = 0.561 (95%CI = 0.405-0.779) (Figure 2C). For studies that evaluated DSS, the combined HR = 0.363 (95%CI = 0.266-0.496) (Figure 2D). These results indicated that *FGFR3* mutations might indicate favorable outcomes for BC.

Finally, the publication bias of the included studies was evaluated using funnel plots and the Egger test. As shown in Figure 3 and Table 2, two of the four meta-analyses showed no publication bias ($P > 0.05$), but the others did ($P < 0.05$).

Table 1. Meta-analysis of bladder cancer.

A. Study	Population	Number of subjects	Age (years)	Male (%)	Disease	Mutant	Assay
Al-Ahmadie et al., 2011	U.S.	245	68.6	75.6	UC	Y375C, S249C, etc.	MS, sequencing
Bakkar et al., 2003	France	81	64	-	UCC	exons (7, 10, and 15)	DHPLC, sequencing
Bakkar et al., 2010	France	170	64	100.0	BC	S249C, Y375C, A248C, etc.	SNaPshot
Billerey et al., 2001	France, Netherlands	132	-	-	BC	exons (7, 10, 15, and 19)	PCR
Bodoor et al., 2010	Jordan	121	63	87.6	BC	exons (7, 10, and 15)	PCR
Burger et al., 2008	Germany, Netherlands	221	68	77.0	NMI-UCC	S249C, Y375C, R248C	SNaPshot
Dodurga et al., 2011	Turkey	56	65.5	87.5	BC	A248C, S249C, G372C, T375C	PCR-RFLP, sequencing
Hernández et al., 2006	Spain	764	68	87.0	NMI-UC	exons (7 and 10)	PCR, sequencing
Jebar et al., 2005	UK	98	-	-	UCC	exons (7, 10, and 15)	SSCP, sequencing
Junker et al., 2008	Germany	92	-	-	BC	Y375C, G372C, R248C, etc.	SNaPshot
Kimura et al., 2001	Japan	81	65.6	86.4	BC	exons (7, 10, and 15)	RFLP, SSCP, sequencing
Kompier et al., 2010	Netherlands	257	65.7	75.0	BC	exons (7, 10, and 15)	SNaPshot
Lindgren et al., 2006	Sweden	75	-	-	BC	exons (7, 10, 13, and 15)	PCR, sequencing
Miyake et al., 2010	Japan	45	63	77.8	NMI-BC	exons (7, 10, and 15)	PCR, sequencing
Ouerhani et al., 2009	Tunisia	90	66.86	87.7	BC	exons (7, 10, and 15)	PCR, SNaPshot
Rieger-Christ et al., 2003	USA	192	67.16	78.1	BC	exons (7, 10, and 15)	SSCP, sequencing
Serizawa et al., 2011	Denmark	105	70.2	86.7	BC	S249C, Y375C, R248C, etc.	PCR
Sjödahl et al., 2011	Sweden	145	-	20.0	UC	exons (7, 10, and 15)	PCR
Tomlinson et al., 2007	UK	158	71.7	64.6	BC	exons (7, 10, and 15)	PCR, sequencing
van Oers et al., 2007	Germany	208	70	75.0	BC	S249C, Y375C, R248C, etc.	SNaPshot
van Oers et al., 2009	UK, France	117	70	68.0	BC	S249C, Y375C, R248C, etc.	SNaPshot
van Rhijn et al., 2003	Netherlands	286	65.7	76.0	UCC	exons (7, 10, and 15)	PCR-SSCP, sequencing
van Rhijn et al., 2012	Netherlands	132	68.7	82.0	BC	exons (7, 10, and 15)	SNaPshot, IHC
Wallerand et al., 2005	France	110	67	-	BC	exons (7, 10, and 15)	DHPLC, PCR, sequencing
Zieger et al., 2009	Denmark	218	-	-	BC	exons (7 and 10)	Sequencing, SNP-microarray, qPCR

Characteristics of grade and stage studies included in the meta-analysis. UC = carcinoma; UCC = urothelial cell carcinoma; BC = bladder cancer; NMI = non-muscle-invasive; MS = mass spectrometry; DHPLC = denatured high-performance liquid chromatography; RFLP = restriction fragment length polymorphism; SSCP = single-strand conformation polymorphism; qPCR = quantitative real-time polymerase chain reaction; SNP = single-nucleotide polymorphism; - = not available.

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Table 1. Continued.

B.

Study/Year	Population	Number of subjects	Age (years)	Male (%)	Disease	Mutant	Assay	Follow-up (m)	Survival	HR
Al-Ahmadie et al., 2011	U.S.A	245	68.6	75.6	UC	Y375C, S249C, etc.	MS, sequencing	15.6	PFS	SC
Bodoor et al., 2010	Jordan	121	63	87.6	BC	exons (7, 10, and 15)	PCR	30	DFS	SC
Burger et al., 2008	Germany, Netherlands	221	68	77.0	NMI-UCC	S249C, Y375C, R248C	SNaPshot	35	PFS	SC
Eltze et al., 2009	Germany	154	70	71.4	UC	S249C, Y375C,				
R248C, etc.	SNaPshot	67.5	RFS	TR						
Hernández et al., 2005	Spain	119	67	86.6	BC	exons (7, 10, and 15)	PCR, sequencing	54	DFS	SC
Hernández et al., 2006	Spain	764	68	87.0	BC	exons (7, 10, and 15)	PCR, sequencing	62.6	PFS, DSS	SC
van der Aa et al., 2005	Netherlands	63	68	-	UCC	-	PCR-SSCP	55	PFS	TR
van Oers et al., 2007	Germany	208	70	75.0	BC	S249C, Y375C, R248C, etc.	SNaPshot	75	DSS	TR
van Oers et al., 2009	UK, France	117	70	68.0	BC	S249C, Y375C, R248C, etc.	SNaPshot	216	DSS	SC
van Rhijn et al., 2003	Netherlands	286	65.7	76.0	UCC	exons (7, 10, and 15)	PCR-SSCP, sequencing	66	PFS	SC
van Rhijn et al., 2004	Netherlands	260	67.2	75.0	UCC	exons (7, 10, and 15)	PCR-SSCP	180	DSS	SC
van Rhijn et al., 2010	Netherlands	230	65.1	76.0	BC	exons (7, 10, and 15)	SSCP, sequencing	103	DSS	SC
van Rhijn et al., 2012	Netherlands	132	68.7	82.0	BC	exons (7, 10, and 15)	SNaPshot	78	PFS	SC

Characteristics of survival studies included in the meta-analysis. UC = carcinoma; UCC = urothelial cell carcinomas; BC = bladder cancer; MS = mass spectrometry; SSCP = single-strand conformation polymorphism; qPCR = quantitative real-time polymerase chain reaction; SNP = single-nucleotide polymorphism; DFS = disease-free survival; PFS = progression-free survival; RFS = recurrence-free survival; DSS = disease-specific survival; TR = text reported; SC = survival curve; (-) = not available.

Table 2. Comparison of the predictive value of low-grade *FGFR3* mutations with early-stage *FGFR3* mutations in bladder cancer patients, and of DFS, PFS, and RFS in mutated groups.

	Grade	Stage	DFS/PFS/RFS	DSS
RR or HR (95%CI)	2.948 (2.357, 3.688)	2.845 (2.145, 3.773)	0.561 (0.405, 0.779)	0.363 (0.266, 0.496)
Heterogeneity (P value)	0.000	0.000	0.000	0.227
Model	Random	Random	Random	Fixed
Bias (P value)	0.300	0.000	0.032	0.286
Number of subjects	3999	2491	2015	1579
Number of studies	24	18	9	5

DFS = disease-free survival; PFS = progress-free survival; RFS = recurrence-free survival; DSS = disease-specific survival.

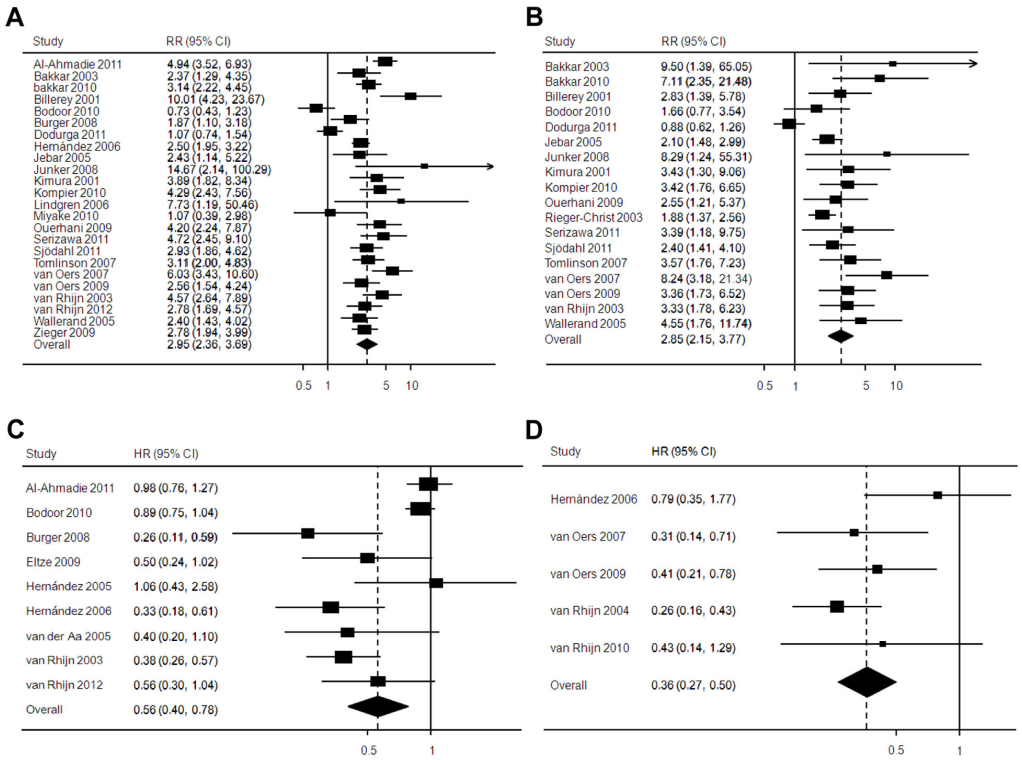

Figure 2. Forest plots and meta-analysis of studies evaluating risk ratios (RR) of mutated specimens relative to wild-type specimens. **A.** Grade. **B.** Stage. **C.** DFS/PFS/RFS. **D.** DSS. For abbreviations, see legend to Figure 1.

DISCUSSION

This systemic review and meta-analysis revealed that a higher frequency of *FGFR3* mutations was associated with lower histological grade and lower clinical stage in BC patients, with RR values of 2.948 and 2.845, respectively. Furthermore, *FGFR3* mutations predicted better survival, with respect to both PFS and DSS.

These results confirmed the clinical value of *FGFR3* mutations in bladder cancer patients. Nonetheless, our conclusions must be interpreted with caution. The current meta-

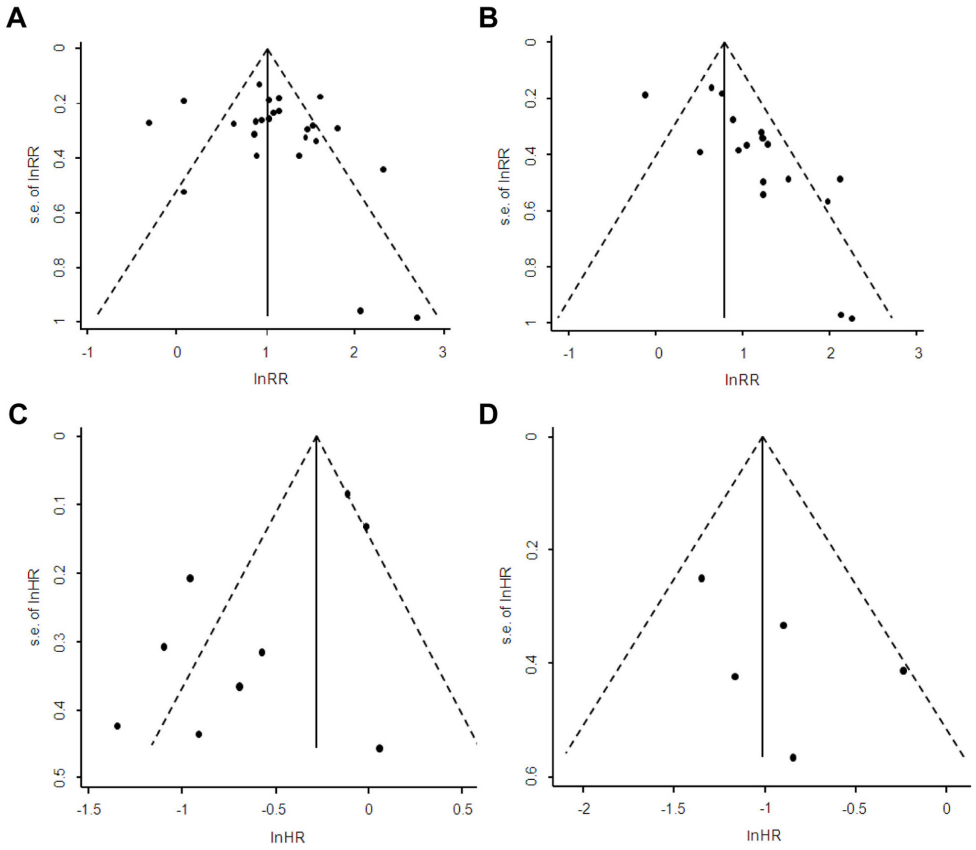

Figure 3. Funnel plots for studies included in the four meta-analyses. Plots are arranged as follows: **A.** Grade; **B.** Stage; **C.** DFS/PFS/RFS; **D.** DSS. For abbreviations, see legend to Figure 1.

analysis has several limitations. First, marked heterogeneity was observed in three of the four distinct groups of subjects. The heterogeneity of the population was most likely due to differences in the baseline characteristics of patients (race, age, and tumor stage), method of detecting mutations, duration of follow-up, and other parameters. We attempted to minimize the effects of these differences by applying a random-effect model. Publication bias was detected in the stage and DFS/PFS/RFS meta-analyses, and this cannot be adequately overcome with any statistical techniques currently available.

Previous experimental studies established a clear link between the presence of FGFR3-activating mutations and tumorigenesis. Mouse fibroblast NIH-3T3 cells transfected with an S249C *FGFR3* mutant construct showed characteristics reminiscent of tumorigenesis, such as rapid proliferation, colony formation, and tumor xenograft formation in mice (Bernard-Pierrot et al., 2006). In the UC cell line MGH-U3, which contains a Y375C-activating mutation, cell growth and proliferation were suppressed by an FGFR inhibitor and by an *FGFR3* knockdown (Bernard-Pierrot et al., 2006). S249C, Y375C, and K652E mutations in *FGFR3* were found to phosphorylate Plcgamma1 and to induce morphological transformation, cell

proliferation, and anchorage-independent growth (Di Martino et al., 2009). Mutational activation with constitutive receptor dimerization and overexpression of wild-type *FGFR3* are two distinct mechanisms that may account for *FGFR3*-dependent tumorigenesis in UC. *FGFR3* mutations are observed more frequently in lower-grade and earlier-stage tumors, and in cases that ultimately show favorable clinical outcomes, whereas the overexpression of wild-type receptors is associated with higher-grade and later-stage tumors and with worse outcomes (Iyer and Milowsky, 2013). Further studies should be conducted to evaluate the involvement of FGFR3-mediated downstream signaling for cell growth and proliferation and ultimate therapeutic tractability of these two mechanisms.

Currently, the molecular analysis of BC is one of the most popular fields in both clinical studies and scientific research. These molecular markers may allow more complete characterization of individual urothelial neoplasms than is currently possible using histological evaluation alone. To date, a variety of molecular markers, such as FGFR3, EGFR, pRB, p53, Ki-67, VEGF, and CK20, have been found to be associated with tumor grade and staging, recurrence, progression, and survival (Bryan et al., 2010; Cheng et al., 2011). These markers may participate in the regulation of the cell cycle, cell proliferation, signal transduction, apoptosis, extracellular matrix modulating, and angiogenesis (Cheng et al., 2011). In this meta-analysis, *FGFR3* mutations were found to be more frequent in BC patients with low-grade or early-stage conditions. More importantly, the mutant *FGFR3* was found to predict better outcomes. Overexpression of EGFR has been shown to be associated with late-stage and high-grade tumor. Overexpression of pRB has been shown to be associated with poor clinical outcomes in BC. p53 mutations are associated with high grade, late stage, and poor clinical outcome. Overexpression of Ki-67, VEGF, and CK20 are related to tumor grade, stage, progression, and recurrence. In addition to the markers listed above, p21, Her-2, Bax/bcl-2, and CD40 also merit further research (Youssef et al., 2009). For accurate grading, staging, and prediction of the outcome of BC, more clinical studies must be conducted using multiple assays and combinations of several urinary biomarkers.

In summary, *FGFR3* mutations were found to be significantly associated with low grade and early stage in BC patients, and with better survival.

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Conflicts of interest

The authors declare no conflict of interest.

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