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Clinical validation of Urine DNA-based PENK methylation test for detecting bladder cancer in hematuria patients

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Background

- The most common sign of bladder cancer (BC) is hematuria with the risk of BC that is approx. 16.5%.
- Cystoscopy, which is a gold standard examination for BC, is invasive and painful with potential side effects and with sensitivities of 85% to 94%.
- Voided urine cytology is commonly used method but it has poor sensitivity and overall accuracy.
- The raise for the specific molecular marker for early detection of BC from urine has been developed for several years but have limitation and low sensitivity and specificity.
- Therefore, in order to reduce the burden on patients with hematuria, it is necessary to have an accurate and non-invasive detection method to identify BC in patients with hematuria.
- We have previously identified and verified **aberrant methylation of the *PENK* gene (me*PENK*) as a specific novel biomarker for early detection of BC with sensitivity and specificity >90% in bladder tissues and urine.**

Objective

- To demonstrate the clinical validity and utility of urine DNA-based *PENK* methylation assay as a useful adjunct to determine the further necessity of cystoscopy by distinguishing BC patients from hematuria patients.

Materials & Methods

- **Study design:** Two retrospective cohort studies and one prospective cohort study.
- **Subject:** Patients with hematuria before cystoscopy or surgical treatment and patients with benign urologic disorders or with the other urologic cancer (OC).
- **DNA extraction from urine:** DNA were extracted from the cell sediments of voided urine using bead-based method and treated with bisulfite for the conversion of unmethylated cytosine.
- ***PENK* methylation assay:** The bisulfite (BS)-treated DNA were subjected for PCR reactions; Linear Target Enrichment (LTE) and methylation specific real time PCR (qMSP). (See Appendix 1)

Table1. Subjects' characteristics

Study type	Retrospective-Training		Retrospective-Validation		
Total subjects	179		567		
Variables	Control	BC	Control	BC	OC*
No.	143	36	328	177	62
Sex - no. (%)					
Male	68 (47.6)	24 (66.6)	112 (34.1)	143 (80.8)	52 (83.8)
Female	75 (52.4)	12 (33.3)	216 (65.9)	34 (19.2)	10 (16.1)
Age - mean±SD	61.6±14.4	71.5±10.6	61.2±13.4	70.7±10.4	68.2±9.7
Stage - no. (%)					
Ta LG		11 (30.5)		43 (24.3)	
Ta HG		8 (22.2)		30 (16.9)	
T1		14 (38.8)		83 (46.9)	
T2-T4		3 (8.1)		21 (11.8)	

*OC: Other urological cancers (kidney cancer, prostate cancer, ureter cancer)

Figure 1. Significant association between *PENK* methylation and development of bladder cancer

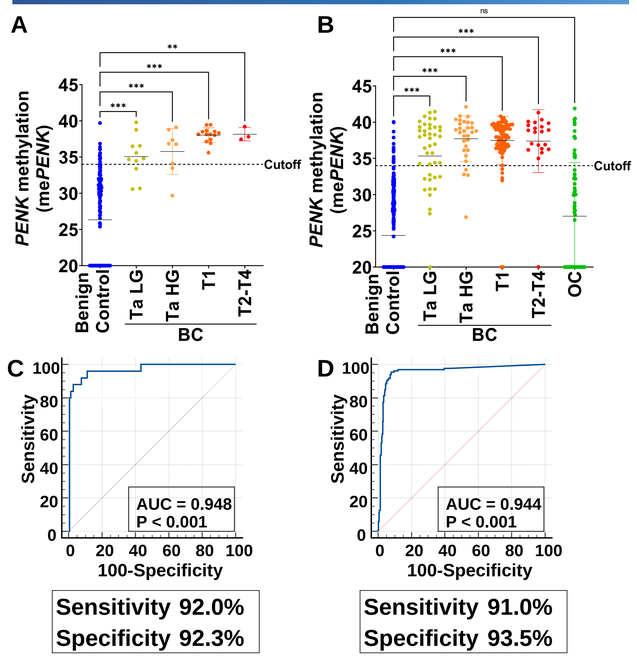

Fig1. (A-B) Methylation level of PENK from urine DNA in different cohort studies were analyzed using me*PENK*-LTE/qMSP (2-step). (C-D) ROC curve analysis for discrimination of the subjects with Ta HG and advanced stages from hematuria. (A,C) Training set (n=179) and (B,D) Validation set (n=567).

Table2. Subjects' characteristics

Study type	Retrospective-Training		Prospective-Validation	
Total subjects	106*		183	
Variables	Control	BC	Control	BC
No.	42	64	104	79
Sex - no. (%)				
Male	15 (35.7)	52 (81.3)	45 (43.3)	65 (82.3)
Female	27 (64.3)	12 (18.8)	59 (56.7)	14 (17.7)
Age - mean±SD	62.3±12.8	71.8±9.8	66.7±10.1	72.3±9.9
Stage - no. (%)				
Tis		0 (0)		2 (2.5)
Ta LG		11 (17.2)		18 (22.8)
Ta HG		12 (18.8)		18 (22.8)
T1		36 (56.3)		35 (44.3)
T2-T4		5 (7.9)		6 (7.6)

*The samples were randomly selected from the retrospective study (n=567).

Figure 2. Consistent high sensitivity and specificity of me*PENK* assay in prospective study

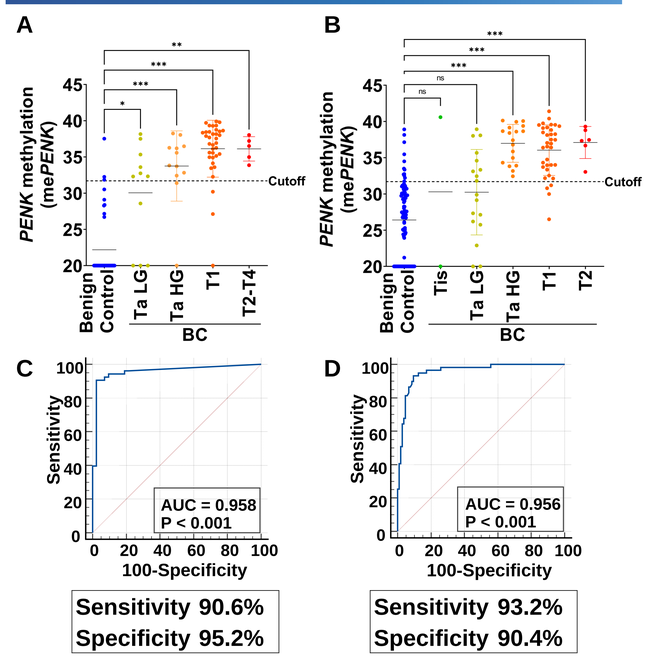

Fig2. (A-B) Methylation level of PENK from urine DNA in different cohort studies were analyzed using me*PENK*-LTE-qMSP (1-step). (C-D) ROC curve analysis for discrimination of the subjects with Ta HG and advanced stages from hematuria. (A,C) Retrospective training set (n=106) and (B,D) Prospective validation set (n=183).

Summary & Conclusion

- ***PENK* methylation become abundant in urine DNA in BC specifically and is well associated with the stages of BC.**
- **Both sensitivity and specificity of *PENK* methylation assay are over 90% for detection of Ta HG and advanced stages.**
- **In conclusion, the noninvasive urine DNA-based *PENK* methylation test can be used for early diagnosis of BC either diagnostic test or adjunct to cystoscopy.**
- **This assay is expected to distinguish the patients who need cystoscopy from patients with hematuria and reduce unnecessary procedures and burden.**

Future research direction

- **Urine-based *PENK* methylation test has a high potential for early detection of BC. A large-scale of prospective study will be warranted to evaluate the clinical performance of *PENK* methylation test for detecting BC from the patients with hematuria.**

Appendix 1. CystoChek assay flow chart