

Evaluation of STR typings (TH01, TPOX and CSF1PO loci) using urine obtained from bladder cancer patients

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Abstract. We examined urine and blood DNA obtained from bladder cancer patients with commercially available STR kits such as those for TH01, TPOX and CSF1PO loci. These loci were amplified by PCR, and polymorphic alleles were visualized as bands on gels. The results were compared with each paired DNA. Microsatellite alternation was observed in 5 out of 52 (9.6%) urine DNAs from the patients with bladder cancer. These loss of heterozygosity (LOH) and microsatellite instability (MSI) were observed such as the combination of an intense and a faint band. Complete LOH causing incorrect typing results was not observed. We were able to avoid incorrect typing results when using urine DNA under careful observation. © 2003 Elsevier B.V. All rights reserved.

Keywords: Microsatellite; Urine DNA; Loss of heterozygosity; Instability; Bladder cancer

1. Introduction

In forensic casework, microsatellite analysis of urine provides important information for DNA profiling [1–4]. Several investigators using polymorphic microsatellite markers have reported microsatellite alternations such as loss of heterozygosity (LOH) and microsatellite instability (MSI) in urine sediments from patients with bladder cancer and urologic diseases [5–7]. Microsatellite alteration is characterized by expansion or deletion of a repeat unit which has been related to malignancy and inflammatory condition. It should be considered to affect the microsatellite typing results.

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This study was performed to evaluate commercially available kits for microsatellite typing of urine DNA.

2. Materials and methods

All samples from Japanese patients with urologic disease were obtained with informed consent and were collected at the Depart. of Urology, Juntendo Univ. School of Medicine.

Blood DNA was isolated from whole blood using the QIAamp DNA Mini Kit (QIAGEN, Germany). Urine DNA was isolated from urine as follows. Two to five milliliters of urine was centrifuged for 5 min at 5000 rpm and the pelleted cells were used for extraction by the phenol–chloroform method.

PCR amplification was completed with reagents and protocols provided by Promega GenePrint STR kits (Promega, Tokyo) TH01, TPOX and CSF1PO for loci located on chromosomes 11, 2 and 5, respectively. The amplified products were separated by polyacrylamide gel electrophoresis and visualized using Promega's DNA silver staining system (Promega, Tokyo).

3. Results and discussion

Microsatellite alternations such as LOH and MSI were observed in 5 out of 52 (9.6%) urine DNAs from the patients. In two out of five urine DNAs, the alternation was simultaneously observed at the TH01 and TPOX loci (Table 1). The remaining three urine DNAs showed two instances at the TH01 locus and one instance at the CSF1PO locus. As shown in Fig. 1, microsatellite alternation was observed, such as the combination of an intense and a faint band. Complete LOH causing incorrect typing results was not observed.

The frequency of microsatellite alternation in our study was lower than that found by other investigators [5–7]. This might have been due to differences in choice of microsatellite loci, or number of microsatellite markers studied. Our results may be attributed to a panel of three microsatellite markers. Furthermore, as the DNA derived from urine is contained in leukocytes and exfoliated cells including renal tubular, transitional urothelial, squamous and cancer cells, the mixture ratio of cancer or inflammatory cells in the urine may also have an influence. This may be attributable to the preparation of DNA samples.

Table 1
Microsatellite alternation in urine DNA

Sample no.	Microsatellite markers		
	TH01	TPOX	CSF1PO
19	MSI	MSI	–
44	LOH	–	–
52	–	–	LOH
56	LOH	LOH	–
59	MSI	–	–

–: no genetic alternation detected between blood and urine DNA; LOH: loss of heterozygosity, MSI: microsatellite instability.

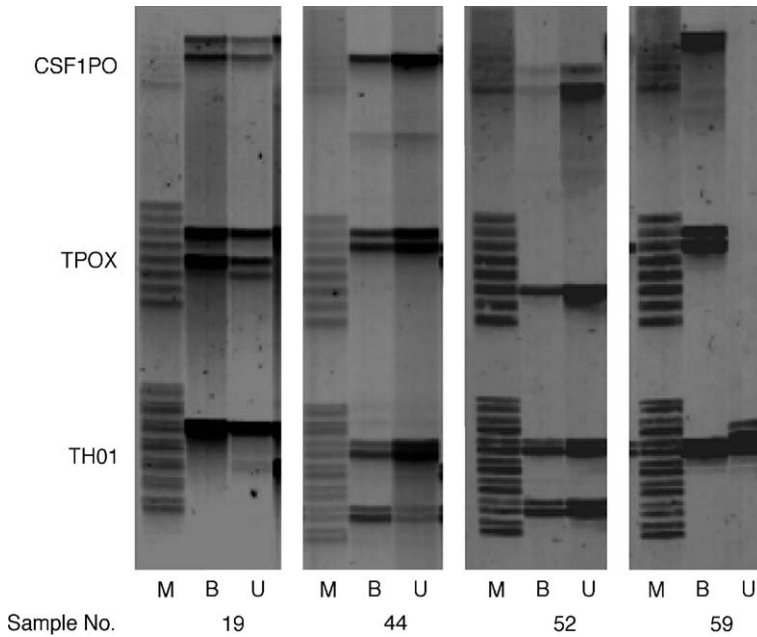


Fig. 1. Electrophoretic patterns of microsatellite analysis of paired blood DNA (B) and urine DNA (U). Sample No. 19; MSI of urine DNA in TH01 and TPOX loci, No. 44; LOH of urine DNA in TH01 locus, No. 52; LOH of urine DNA in CSF1PO locus; No. 59; MSI of urine DNA in TH01 locus.

Microsatellite analysis of urine represents a novel and potentially powerful clinical tool for the detection of recurrent bladder cancer. For this purpose, choice of microsatellite loci, number of microsatellite markers studied and preparation of urine DNA were considered to be important factors to detect a high frequency of microsatellite alternation.

Microsatellite alternation in urine DNA is presumed to affect the DNA typing results. In this study, however, we were able to avoid incorrect typing results under careful observation.

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