RESEARCH COMMUNICATION

Gene Amplification using DNA from Human Spot Urine Samples

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Abstract

The purpose of this study was to test the amplification of DNA from human urinary sediment for molecular epidemiological studies. Twenty-six urine samples were obtained from healthy volunteers. Polymerase chain reactions (PCR) for methylenetetrahydrofolate reductase (MTHFR), β-globin, and N-acetyltransferase 2 (NAT2) was conducted using genomic DNA isolated from the urine. The MTHFR and β-globin genes were amplified successfully from all the urine DNA samples while the NAT2 gene was amplified in 88.5% of cases. The median yield of DNA was 0.28 μg from the 10 ml urine samples, sufficient amounts of DNA being contained in urinary sediments for amplification of all three genes. This result indicates that urine can be used as a DNA source for PCR-based molecular epidemiological studies.

Key Words: Urine - DNA - gene amplification - PCR

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Introduction

Most of molecular epidemiological studies are usually done with DNA isolated from blood leukocytes, but blood samples have not always been collected in large cohort studies. Another difficulty to get blood DNA is that people living in the certain areas like most Asian countries are reluctant to provide their blood samples. Urine has been known to contain erythrocytes, leukocytes, and squamous cell, even in normal subjects, and these cells are examined in the clinical laboratory as a part of routine testing. Therefore, the possibility of using urine specimens for molecular epidemiological analysis in population-based studies need to be evaluated (Botezatu et al., 2000; van Noord, 2003; Prinz et al., 1993; van der Hel et al., 2002; van Duijnhoven et al., 2002; Yokota et al. 1998). To evaluate the feasibility of molecular studies using DNA extracted from urine, we examined the quantity of DNA extracted from urine of twenty-six subjects. We also tested its ability to provide an adequate template for amplification of three genes, methylenetetrahydrofolate reductase (MTHFR), β-globin, and N-acetyltransferase 2 (NAT2).

Materials and Methods

Study Population

Twenty-six urine samples were obtained from healthy volunteers (14 males and 12 females) whose age ranges from 8 to 41 years old. Spot urine samples were collected by urine bottle and immediately frozen and kept at -70°C overnight. Information on demographic characteristics such as smoking and alcohol consumption was collected using a structured questionnaire and written informed consent was provided by all study subjects.

DNA Isolation

Frozen urine (40 ml) was thawed at 4˚C and mixed vigorously, and then 10 ml urine was taken for DNA isolation. Cells and nuclei were centrifuged at 2,500 g for 10 min and then washed with phosphate-buffered saline (PBS). Urine sediment was collected into a polypropylene tube (1.5 ml) and DNA was isolated using Wizard genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's protocol (Miller et al., 1988; Promega, 1999). The quantity of DNA was measured by spectrophotometric analysis at 260 nm, using a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan). The quality of DNA was evaluated by estimating the ratio of absorbency at 260 nm and 280 nm.

PCR Analysis

Polymerase chain reactions (PCR) for MTHFR, β-globin, and NAT2 genes were performed using genomic DNA isolated from the urine to evaluate DNA recovery. Twenty

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Table 1. PCR Conditions and Primer Sequences

Gene	Fragment length	Program	Sequence
MTHFR	198	94˚C for 4 min; 35 cycles of 94˚C for 40s, 58˚C for 40s, 72˚C for 2 min; 72˚C for 10 min	Forward 5'-aggacggtgcggtgagagtg-3' Reverse 5'-tgaaggagaaggtgtctgcggga-3'
β-globin	268	94˚C for 4 min; 35 cycles of 94˚C for 1 min, 55˚C for 45 s, 72˚C for 1 min; 72˚C for 5 min	Forward 5'-ccacctcatccacgttcacc-3' Reverse 5'-gaagagccaaggacaggtac-3'
NAT2	547	95˚C for 5 min; 35 cycles of 95˚C for 1 min, 58˚C for 1 min, 72˚C for 2 min; 72˚C for 10 min	Forward 5'-gctgggtctggaagctcctc-3' Reverse 5'-ttgggtgatacatacacaaggg-3'

Table 2. Amplification of MTHFR, β-globin, and NAT2 Genes

Gene	Samples amplified by PCR (%)
MTHFR	26 (100)
β-globin	26 (100)
NAT2	23 (88.5)

μl of PCR reaction mixture used for amplification of MTHFR, β-globin, or NAT2 genes contained 10 mM of Tris-HCl (pH 9.0), 40 mM of KCl, 1.5 mM of MgCl2, 0.25 mM of each dNTP, 1 unit of Taq polymerase (Bioneer, Seoul, Korea), 20 pmole of the forward and reverse primers, and 50 ng of isolated genomic DNA as a template. PCR of reaction mixtures was carried out using a thermal cycler, PTC-200 (MJ Research, Watertown, MA, USA). Primer sequences and PCR conditions are given in Table 1 (van der Hel et al., 2002). PCR amplified fragments were detected by electrophoresis on 2% agarose gels. Positive amplification of the MTHFR, β-globin, and NAT2 genes was determined based on the presence of 198-bp, 286-bp, and 547-bp products.

Statistical analysis

Yield of DNA isolated from male urine was compared to those from female urine using the t-test. A probability level of 0.05 was used as the criterion of the statistical significance. SAS, version 8.1 (SAS institute Inc., Cary, NC, USA), was used for the statistical analysis.

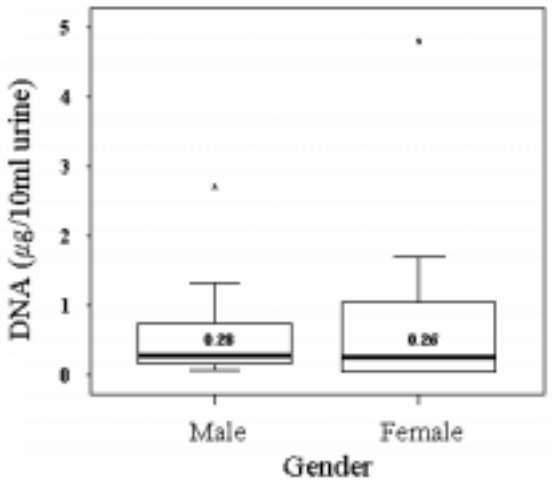

Figure 1. Quantity of DNA Isolated from Urine Samples

Results

From the 10 ml urine samples, total DNA yield ranged from trace levels to 4.80 μg. The median yield of DNA isolated from the urine was 0.28 μg for male and 0.26 μg for female (Figure 1). Although the urine from males had more DNA than those from females, the difference was not significant (p = 0.49). The number of samples from which DNA was extracted less than 0.5 μg, between 0.5 and 1.5 μg, and over 1.5 μg was 16 (61.5%), 6 (23.1%), and 4 (15.4%), respectively. When the quality of isolated DNA was evaluated by estimating the ratio of absorbency at 260 nm and 280 nm, median value of the quality showed 1.6. When PCR was performed, MTHFR and β-globin genes were amplified successfully from all the genomic DNA samples while NAT2 gene was amplified in 23 of 26 (88.5%) (Figure 2 and Table 2).

Discussion

In this study, urine specimen was tested as a suitable DNA source for molecular epidemiological studies. Our result that MTHFR and β-globin genes were amplified successfully from all the genomic DNA samples suggests that urine samples can be used as a DNA source for determining genotypes. The urinary DNA originates from cells dying in various tissues including kidney and bladder or the urinary

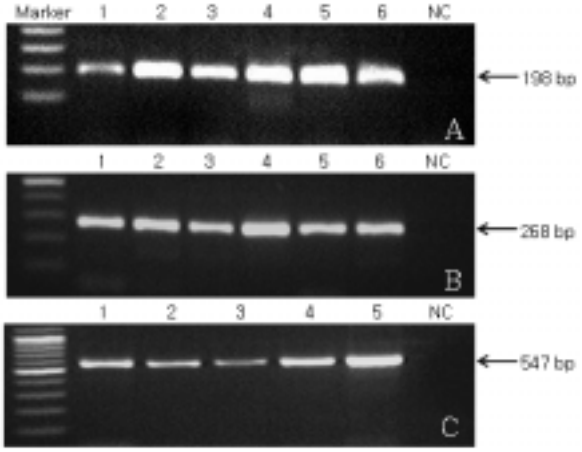

Figure 2. PCR Products of MTHFR, β-globin, and NAT2 Genes. Commercialize markers (100-bp) and negative control (NC) are shown on the left and right, respectively. Amplification of the MTHFR, β-globin, and NAT2 genes was determined based on presence of 198-bp (A), 286-bp (B), and 547-bp (C) products

tract of an organism (Bret et al., 1990). Taking into account that about 10^{11} cells die daily in human adult, total amount of released DNA could be about 0.6 g (Botezatu et al., 2000). Moreover, there are some advantages of urinary DNA-based technology compared with using other DNA sources such as serum or plasma when DNA repository is not available: (i) urine can be easily obtained from study subject for analysis because urine-based tests are absolutely noninvasive (Botezatu et al., 2000; van Noord, 2003); (ii) urine is not contaminated with various pathogens including human immunodeficiency virus (HIV) to give low chance of infection to researchers (Botezatu et al., 2000); (iii) using urine is technically easier than using serum in DNA isolation because the protein concentration in urine is less than 1,000-fold lower than those in serum (Botezatu et al., 2000; Yokota et al., 1998); (iv) urine has high PCR productivity because they have low level of PCR inhibitors compared to blood (Botezatu et al., 2000; Yokota et al., 1998). These facts support our findings that urine can be a good source for molecular epidemiological studies.

Prinz et al. (1993) reported that total amount of human DNA that could be extracted from 20 ml of fresh urine was 20 ng to 40 ng for males and 400 ng to 800 ng for females, and the DNA yields dropped to 1 ng to 2 ng for male and 10 ng to 20 ng for female after urines were stored at 4°C for 6 months. However, DNA extracted from urine samples stored at -20°C for 6 months showed yields equivalent to fresh samples and no problems with amplification. (Prinz et al., 1993). In our study, we extracted similar amount of DNA between male and female using 10 ml of urine samples stored at -70°C overnight. Further studies employing different temperatures and durations of storage are needed to evaluate the sexual difference in DNA recovery.

The percentage of amplification showed a decreasing trend with the length of amplicon in our study. This result indicates that isolated DNA is degraded into various lengths of fragments since urine DNA originates from dying cells (Bret et al., 1990) and amplification is conducted with this fragmented genomic DNA as a template for PCR. Even though age, sex, alcohol consumption, smoking, kidney and urethra disorder, and menstruation cycle in women have been known to affect DNA isolation, these factors did not affect the amount of isolated DNA in our study ($P > 0.05$).

There are some limitations for using the urine DNA in molecular epidemiological studies because DNA concentration varies depending on samples. In addition, urine can be contaminated easily with DNA of microorganisms such as bacteria, yeast, and fungi during extraction. Nevertheless, we suggest that urine specimen is a suitable DNA source for epidemiological studies "when blood samples cannot be obtained or have not been stored."

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