International Congress Series 1239 (2003) 685–688

# Microsatellite instability in mononuclear cells from non-tumorigenic human tonsils and its use in forensic evaluation

R. Kobayashi [a,b], Y. Itoh [b,*]

[a] *Department of Microbiology, Tokyo Medical University, Tokyo, Japan*
[b] *Department of Forensic Medicine, Juntendo University School of Medicine, Hongo, Tokyo 113-8421, Japan*

**Abstract**

Microsatellite instability (MSI) is characterized by small deletions or expansions within short tandem repeats in tumor DNA as compared with matching normal DNA. The additional bands (also called stutter bands or shadow bands) are observed in genetic mapping studies by PCR amplification of the loci with mono-, di-, tri- and sometimes tetra-nucleotide repeats. In addition, it is very difficult to distinguish the mutation events that have occurred during DNA replication or repair in vivo from those that occurred during PCR. To determine the origin of the stutter bands, TH01 typing was performed by semi-nested PCR using single somatic cells isolated from non-tumorigenic tonsils. One out of 178 cells (0.56%) examined had a product 4 bp shorter than the major band. These results indicate that the typing data from PCR amplification may reflect somatic mosaicism in the individual. However, we conclude that the existence of the mutated cells in this proportion may have no effect on forensic examination.


*Keywords:* Microsatellite; TH01; Stutter band; PCR; DNA

## 1. Introduction

Microsatellites are highly polymorphic and scattered more or less throughout the whole genome. The detection of polymorphic microsatellite DNA sequences has become extremely important for personal identification in forensic science. However, microsatellite instability (MSI) has also been reported to be a landmark for some tumors of the colon and rectum. The goal of this study was to investigate whether MSI existed or not

* Corresponding author.
*E-mail address:* yitoh@med.juntendo.ac.jp (Y. Itoh).

0531-5131/03 © 2003 Elsevier Science B.V. All rights reserved.
PII: S0531-5131(02)00388-6

in normal individuals and to evaluate whether the MSI had a bad effect on the results of forensic studies.

## 2. Materials and methods

### 2.1. Cell sources

Mononuclear cells were isolated from tonsils extracted from patients with chronic tonsillitis using Ficoll Hypaque density-gradient separation.

### 2.2. Primers

TH01$[AATG]_n$ PCR primers were as follows (5′→ 3′ ): TH01F, att caa agg gta tct ggg ctc tgg; TH01R, gtg ggc tga aaa gct ccc gat tat [1]. TH01Ff, cca ttg gcc tgt tcc tcc ctt att, was also synthesized for second-round PCR. The tonsillar cells were diluted with PBS to $5\times10^2$ cells/ml (one cell/2 μl) and then PCR amplification using 2 μl of this, that is, single cells, performed as described by Zhang et al. [2].

### 2.3. PCR amplification

Two microliters of the diluted cell suspension was placed into the wells of a 96-well microtiter plate containing 5 μl of an alkaline lysis solution (200 mM KOH/50 mM dithiothreitol). After 10 min incubation at 65 °C, 5 μl of the neutralization solution (900 mM Tris–HCl, pH 8.3/300 mM KCl/200 mM HCl) was added. To the lysed and neutralized sample were added 0.5 μl of TH01F (1 pmol/μl), 0.5 μl of TH01R (10 pmol/μl), 2.5 μl of $10\times K^+$ free PCR buffer [15 mM $MgCl_2$/gelatin (1 mg/ml)/100 mM Tris–HCl, pH 8.3], 1.25 μl of 1% W-1 (Gibco RBL, MD), 0.5 μl of mixture of the four

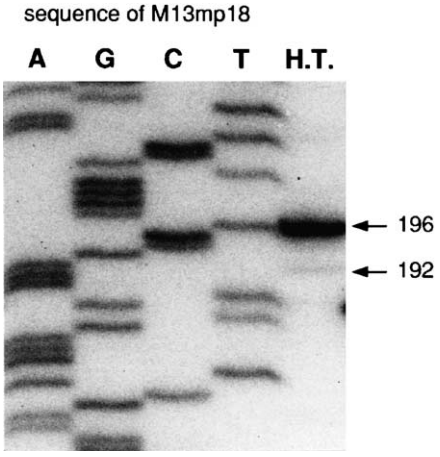


Fig. 1. Stutter band in TH01 typing using tonsillar DNA (TH01=9-9).

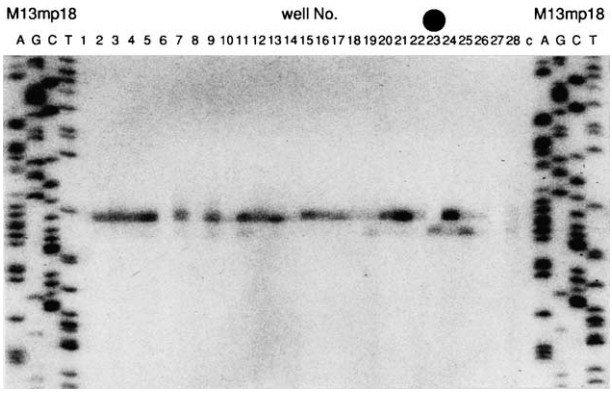


Fig. 2. Microsatellite alteration at TH01 type 9-9 individual. It is marked by a closed cycle (lane 23).

dNTPs (each at 10 mM; Amersham Pharmacia Biotech, Uppsala), and 0.125 μl of Taq DNA polymerase (5 units/μl; Gibco BRL), and the volume was made up to 25 μl with water. Thirty primer-extension cycles were carried out in a thermal cycler (MJ Research, MA). First- and second-round amplifications consisted of a 45-s denaturation step at 94 °C, a 30-s annealing step at 60 °C, and a 30-s extension at 72 °C. Before second-round amplification, TH01Ff was labeled with $^{32}P$ and the PCR products were resolved on a 6% polyacrylamide gel with 8.3 M urea at 1400 V for 4 h and the gel was exposed to X-ray film for 16–96 h. The M13mp18 sequence ladder was used as a size standard.

## 3. Results

TH01 typing was performed using tonsillar DNA. Stutter bands were found using tonsillar DNA (Fig. 1). This band is about 16 times less intense and 4 bp shorter than the major band. To study the origin of the stutter bands, TH01 typing was carried out using single somatic cells by semi-nested PCR. The TH01 type 9-9 cells were serially 10-fold diluted from $5\times10^5$ to $5\times10^2$ cells/ml and 2 μl of these cell suspensions was used to determine the TH01 typing. One out of 178 cells (0.56%) examined by semi-nested PCR had a band 4 bp shorter than the major band (Fig. 2). The cells that had TH01 type 7-11 were also examined, but no bands differing from the major bands could be observed (data not shown).

## 4. Conclusions

Studies presented here indicate that the typing data from single cells may represent not only a slippage event of DNA polymerase during PCR amplification but may also reflect somatic mosaicism in the individual. However, the existence of the mutated cells in this proportion may have no effect on forensic examination.

## References

[1] A. Edwards, A. Civitello, H.A. Hammond, C.T. Caskey, DNA typing and genetic mapping with trimeric and tetrameric tandem repeats, Am. J. Hum. Genet. 49 (1991) 746–756.
[2] L. Zhang, X. Cui, K. Schmitt, R. Hubert, W. Navidi, N. Arnheim, Whole genome amplification from a single cell: implications for genetic analysis, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 5847–5851.