

## DETECTION OF DAPSONE RESISTANT MYCOBACTERIUM LEPRAE BY DNA SEQUENCE ANALYSIS FROM A TURKISH RELAPSED LEPROSY PATIENT

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### SUMMARY

*Mycobacterium leprae* obtained from the relapsed case after 43-year dapsone monotherapy was submitted to sequence analysis of the genes associated with resistance to antileprosy drugs for drug susceptibility testing. Single nucleotide substitution was revealed at the codon 55 of the *folP* gene, which had been reported to confer resistance to dapsone. No mutations in the *rpoB* and *gyrA* genes indicated this isolate was susceptible to rifampicin and fluoroquinolones respectively. Drug susceptibility was conducted easily and rapidly by the sequence analysis compared with that by mouse footpad method.

**Key words:** Leprosy, *Mycobacterium leprae*, Drug resistance, Dapsone

### INTRODUCTION

Many cases of relapse have been observed among the patients treated by dapsone monotherapy (11) or multidrug therapy (5). High prevalence of drug resistance was also indicated in the relapsed cases from the patients previously treated by single drug therapy (3, 7). Clarification of the susceptibility to antileprosy drugs enhances the effective treatment. Drug susceptibility test has been conducted by mouse footpad method for many years but hampered by the cumbersome nature of the methods (10). Mutations of 1 genes associated with drug resistance to antileprosy drugs were revealed and confirmed (1,4,6,12). These advances enabled us to examine the susceptibility to antileprosy drugs by sequence analysis especially in the countries where the examination with mouse footpad test is not feasible.

We report here the drug resistance profile of *M. leprae* obtained from the relapsed case with a prolonged dapsone monotherapy by the sequence analysis for the genes relevant

to dapsone, rifampicin and quinolones resistance.

### MATERIALS and METHODS

**Patient:** A slit skin sample was collected from a 60-year old Turkish man, who was first diagnosed with leprosy at the age of 15, by the same method for BI test. The patient had been treated with DDS monotherapy for 43 years until the year 2000 and he faced no clinical health problem during the time. After stopping the administration, he had erythematous maculae in the face, extremities and trunk. The BI was +5 in skin lesion when sample was collected in 2002, however, no data of BI test during dapsone monotherapy was available. Slit skin smear sample on the blade was soaked in 70% ethanol until further analysis.

**Preparation of template and sequence analysis:** Bacilli on the surface of the blade were removed and suspended in 70% ethanol followed by washing with PBS. Genomic DNA

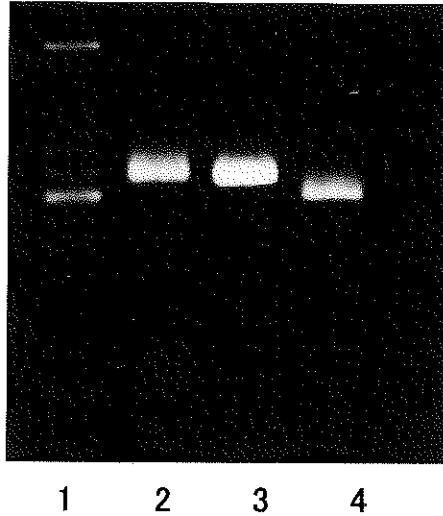
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Figure 1. PCR products for sequencing of genes. Lane 1, 50bp ladder DNA marker. Lane 2, *folP1* (390bp). Lane 3, *rpoB* (381bp). Lane 4, *gyrA* (350bp).



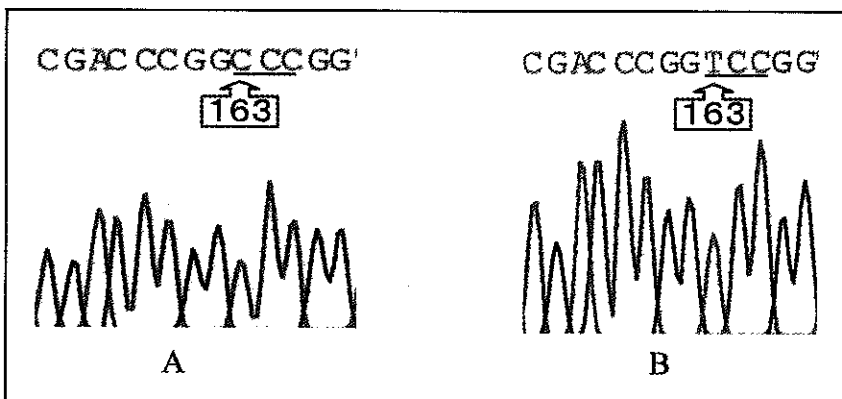
was prepared as described previously (2). DNA fragments were amplified by G mixture of FailSafe PCR System (EPICENTRE, Madison, WI, USA) that corresponded to the regions of the *rpoB* (forward primer: 5'-CAGGACGTCGACGCGATCAC-3', reverse primer: 5'-CAGCGGTCAAGTATTCGATC-3'), *folP1* (forward primer: 5'-GCTTCTCGTGCCGAAGCGCTC-3', reverse primer: 5'-GCCATCGCGG-GATCTGCTCGCCCA-3') and *gyrA* (5'-for-

ward primer: CAGGTGACGGTCTATACAG-3', reverse primer: 5'-TACCCGGC-GAACCGAAATTG-3') genes associated with resistance to rifampicin, dapsone and fluoroquinolones, respectively. The PCR products were sequenced as the same manner as previous methods (7,9), however, DNA samples were recovered by MinElute Gel Extraction Kit (QIAGEN, GmbH, Germany) instead of EASYTRAP (Takara, Siga, Japan) after electrophoresis of PCR products.

## RESULTS

DNA fragments of the target regions of the *folP1*, *rpoB* and *gyrA* genes were amplified and electrophoresed on an agarose gel. The PCR product of the expected length was observed by ethidium bromide staining and UV irradiation (Figure 1). Sequence analysis revealed a single mutation in the *folP1* gene at codon 55 (CCC→TCC) resulting in the substitution of a proline for a serine (Figure 2). The results suggested that the clinical isolate was resistant to dapsone. No mutations of amino acids in *rpoB* at 513, 516, 526, 531 and 533, and amino acids in *gyrA* at 89 and 91 were detected. The results indicated that this isolate was sensitive to rifampicin and fluoroquinolones respectively.

Figure 2. The mutation in *folP* at codon 55 (CCC→TCC). A: Wild type, *M. leprae* Thai- 53 B: *M. leprae* isolate from the relapsed case.



## DISCUSSION

Detection of drug resistance to antileprosy drugs is frequently observed in relapsed cases (3,7), therefore, the relapsed case examined in this study could be predicted to be resistant to some drugs.

Several mutations in the *folP1* gene were

Picture 1-2. Active skin lesions on the knees and gluteal region



reported at codons 53 and 55, namely ACC→GCC, AC→ATC at codon 53 and CCC→CTC, CCC→CGC at codon 55 in high level of dapsone resistant *M. leprae*, which was confirmed by mouse footpad method (6,7,8,12). The mutation detected in this isolate was CCC→TCC at codon 55, substituting proline to serine. This mutation

was recently revealed to associate with the intermediate level of dapsone resistance in clinical samples from a Japanese patient (9). This is the first case of dapsone resistant leprosy case detected by molecular biological analysis in Turkey so far.

Detection of mutations by sequence analysis

is a simple and rapid method compared with that by mouse footpad test to investigate the susceptibility to antileprosy drugs. We recommend surveying the prevalence of drug resistant *M. leprae* from leprosy patients especially from the relapsed and/or intractable cases for effective treatment and the control of leprosy in Turkey.

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