

## Laboratory and Epidemiology Communications

### Molecular Epidemiology of Intra-Familial Tuberculosis Transmission

Makoto Takahara\*, Yoshiharu Yajima, Sumio Miyazaki, Michiyo Aiyoshi, Tomoko Fujino<sup>1</sup>, Yayoi Otsuka<sup>1</sup>, Jun-ichiro Sekiguchi<sup>1</sup>, Katsutoshi Saruta<sup>1</sup>, Tadatoshi Kuratsuji<sup>1</sup> and Teruo Kirikae<sup>1</sup>

*National Nishi-Kofu Hospital, Yamanashi 400-0075 and  
International Medical Center of Japan, Tokyo 162-8655*

Communicated by Hiroshi Yoshikura

(Accepted July 22, 2003)

The risk of tuberculosis transmission has been estimated to be relatively high among household contacts of index tuberculosis patients (1). We report here two cases of tuberculosis transmission from index patients to family members, based on restriction fragment length polymorphism (RFLP)-analysis of the pathogens.

Case 1: A 43-year-old male (patient A1) visited his home doctor complaining of fever and a left chest pain. He had smoked 15 cigarettes per day for 27 years. At the age of 28, he had experienced right side pneumothorax. He worked in a factory. He was single and lived with his father and sister. A chest radiograph revealed a cavitory lesion in the left apical region and left pleural effusion. A CT scan confirmed the cavitory lesion. He was referred to a city hospital in Yamanashi Prefecture. His sputum was negative for acid-fast bacilli (AFB). His bronchial lavage was positive for both AFB and *Mycobacterium tuberculosis* cultures. His gastric juice was positive for *M. tuberculosis* in polymerase chain reaction (PCR) (COBAS AMPLICOR™ MTB, Roche Diagnostics, Branchburg, N.J., USA). He was referred to a national hospital for treatment of pulmonary tuberculosis. The household contact investigation then performed revealed that his father (patient A2), but not his sister, had contracted tuberculosis.

His father had no symptoms. He was a 73-year-old retired man, who had smoked 30 cigarettes per day for 50 years. He had hypertension. His sputum was positive for AFB and *M. tuberculosis* culture. A chest radiograph showed multiple small cavitory lesions in the bilateral upper lung fields. His chest CT scan revealed centrilobular infiltrations in the right upper lung field.

Case 2: A 54-year-old male (patient B1) visited his home doctor in October 2001 with complaints of fatigue, fever, shortness of breath, and a 20 kg loss of body weight over the previous 3 months. He had smoked 20 cigarettes per day for 37 years and was a carpenter. His father had died of tuberculosis after being ill for 10 years. His sputum was positive for AFB and *M. tuberculosis*-positive in culture and PCR. His chest radiograph revealed multiple cavitory lesions in the bilateral upper and middle lung fields. He was referred to the national hospital for treatment of pulmonary tuberculosis in October 2001. His son (patient B2), a 25-year-old office worker who lived with him until 2000, was referred to the same hospital in February 2002 when a routine radiograph check revealed an abnormal shadow. He had smoked 20 cigarettes per day for 6 years. His sputum was positive for AFB and *M. tuberculosis*-positive in culture and PCR. A chest radiograph revealed a cavitory lesion in the left upper field and infiltration shadows in the left middle and right upper lung fields. His chest CT scan showed a cavitory lesion in the left apical region. He was admitted for treatment of pulmonary tuberculosis.

\*Corresponding author: Mailing address: National Nishi-Kofu Hospital, Yamamiya 3368, Kofu-shi, Yamanashi 400-0075, Japan. Fax: +81-55-251-5882, E-mail: takahara@wkoufu.hosp.go.jp

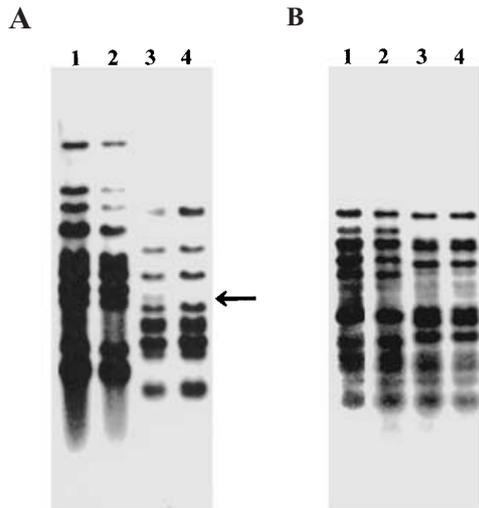


Fig. 1. *IS6110* and  $(CGG)_5$  restriction fragment length polymorphism (RFLP) typing. *Pvu*II-digested genomic DNA was analyzed by Southern blot hybridization with a peroxidase-labeled DNA probe for *IS6110* (A) and  $(CGG)_5$  (B). Lane 1: *M. tuberculosis* isolate from patient A1 (isolate A1); lane 2: isolate A2; lane 3: isolate B1; lane 4: isolate B2.

To determine whether the strains of *M. tuberculosis* infecting the respective patients and their contacts were of the same origin, chromosomal DNAs from these isolates were analyzed by RFLP using a *IS6110* probe (2) (Fig. 1A) and a trinucleotide repeat sequence  $(CGG)_5$  probe (Y. Otsuka et al., in preparation for a paper) (Fig. 1B). The *IS6110* patterns

were identical for isolates from patients A1 and A2 (lanes 1 and 2). The *IS6110* patterns of isolates from patients B1 and B2 were identical except in one band (indicated by an arrow in Fig. 1A), and differed from the pattern observed in isolates A1 and A2. The  $(CGG)_5$  pattern was identical between isolates A1 and A2, and between isolates B1 and B2, respectively. All these isolates were sensitive to isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. No mutations were found in the drug resistance-related genes, including *katG* and *inhA* responsive for isoniazid-resistance, *rpoB* for rifampin-resistance, *pncA* for pyrazinamide, *embB* for ethambutol-resistance, *rrsL* and *rrs* for streptomycin, and *gyrA* for fluoroquinolones. The data indicated that *M. tuberculosis* isolates A1 and A2, and isolates B1 and B2, respectively, were of the same origin. The data clearly supported transmission of *M. tuberculosis* within a family.

The authors thank Dr. F. Ikeda, Nirasaki City Hospital, for critical comments on Case 1. Patients A2 and B2 requested the epidemiological analysis described herein.

#### REFERENCES

1. Wang, P. D. and Lin, R. S. (2000): Tuberculosis transmission in the family. *J. Infect.*, 41, 249-251.
2. Otal, I., Martin, C., Vincent-Levy-Frebault, V., Thierry, D. and Gicquel, B. (1991): Restriction fragment length polymorphism analysis using *IS6110* as an epidemiological marker in tuberculosis. *J. Clin. Microbiol.*, 29, 1252-1254.