Memorial Lecture by the Imamura Award Winner

# DISEASE PROGRESSION OF *MYCOBACTERIUM AVIUM* PULMONARY INFECTION AND THE MYCOBACTERIAL VARIABLE NUMBER TANDEM REPEAT (VNTR) TYPING

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**Abstract**: Nontuberculous mycobacteriosis may progress to fatal chronic respiratory infections. Some cases remain stable over a relatively long period of time. With no well established progression predictors yet available, we conducted a retrospective analysis of the association between mycobacterial variable numbers of tandem repeat (VNTR) and clinical progression in 37 patients who were seen at the Department of Respiratory Medicine, Tohoku University Hospital between 2005 and 2006 and from whose respiratory tract specimens *M.avium* was isolated and cultured. The disease type in the 15 patients who began an antimicrobial therapy within 1 year after a bacteriological diagnosis was defined as progressive, and that in the 9 patients who began an antimicrobial therapy 2 years or longer after diagnosis was defined as stable. A cluster analysis of the mycobacterial VNTR genotypes showed concentrations of the progressive-type isolates and the stable-type isolates in different clusters. Furthermore, the study demonstrated that multiple logistic regression analysis can be used to construct a model for estimating, with statistical significance, progression of nontuberculous mycobacteriosis based on the mycobacterial VNTR genotype. These results indicated that whether a nontuberculous mycobacteriosis is progressive can be estimated by the VNTR genotyping of the nontuberculous mycobacterium.

**Key words**: Nontuberculous mycobacteriosis, Treatment standard, Minisatellite repeat, Computational biology, Cluster analysis

## Introduction

Nontuberculous mycobacterioses (NTM) are granulomatous infections caused by *Mycobacteria* other than the tuberculosis complex and *Mycobacterium leprae*. With no established chemotherapies having reliable curative potential, NTM may progress to fatal chronic respiratory infections.[1)~3)] The disease progression of NTM, however, is variable. Some cases progress relatively rapidly, and yet some cases remain stable for decades.[1)~3)] The reasons for the variable disease progression are unknown, and no NTM progression predictors have been established. Treatment guidelines for NTM, therefore, suggest that making the diagnosis does not, *per se*, necessitate the initiation of therapy, and that it is difficult to distinguish between patients who require immediate therapy and those in whom such a decision can be withheld.[3)~5)]

Given the above context, this study aims to estimate the probability of disease progression of NTM and to provide a guideline on treatment suitability by a retrospective analysis of the association between mycobacterial genotypes and the clinical progression in NTM patients with *Mycobacterium avium*, the most common pathogen associated with NTM.[6)]

## Patient Population and Methods

The patient population comprised all 37 patients from whose specimens *M.avium* was cultured by the Department of Respiratory Medicine, Tohoku University Hospital between January 2005 and December 2006. Of these cases, 26 were pulmonary NTM diagnosed on clinical progression and chest imaging, and the other 11 were believed to be transient infections that were non-pulmonary NTM (Table). Of the 26 patients with pulmonary NTM, one died of complication of lung cancer and could not be followed up; another one had hypersensitivity pneumonitis; 15 began an antimicrobial therapy within 10 months of *M.avium* detection, and whose disease type was defined as progressive; and 9 began an antimicrobial therapy 2 years or longer after *M.avium* detection, and whose disease type was defined as stable.

The genotype of *M.avium* was determined by the variable numbers of tandem repeats (VNTR) genotyping method (Fig. 1) based on the number of repeat units in the tandemly repetitive sequence in 16 loci selected from the minisatellite regions

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**Table** Demographic and clinical characteristics of subjects

| | *M. avium* lung disease (N=26) | No *M. avium* lung disease (N=11) | *M. avium* lung disease (N=24) | |
|---|---|---|---|---|
| | | | Progressive disease (N=15) | Stable disease (N=9) |
| Mean age (male/female) | 58 (7/19) | 63 (7/4) | 58 (5/10) | 54 (0/9) |
| Fibrocavitary lung disease | 3 | | 3 | 0 |
| Nodular bronchiectatic lung disease | 22 | | 12 | 9 |
| Hypersensitivity-like lung disease | 1 | | | |

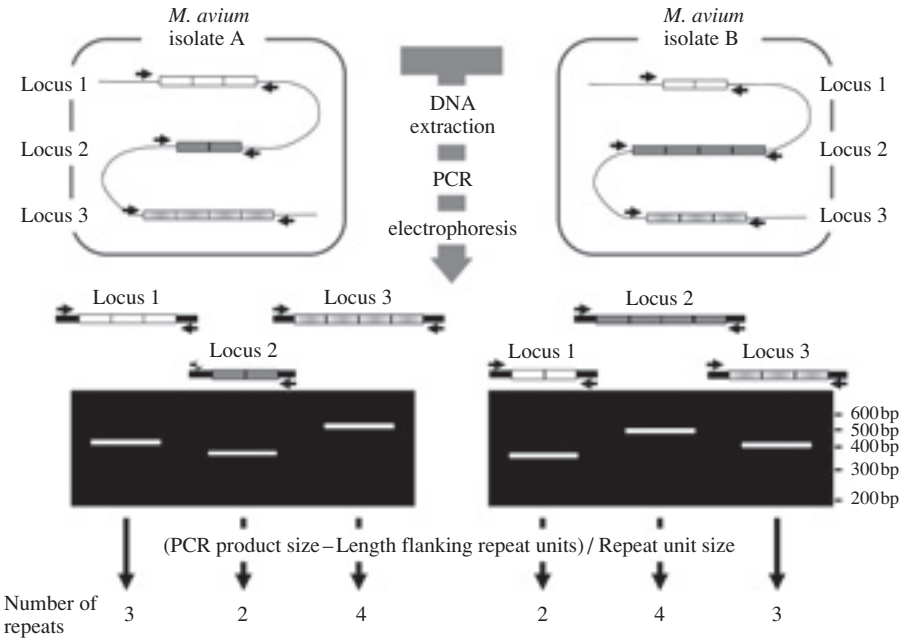


**Fig. 1** The polymerase chain reaction (PCR) -based method to profile variable numbers of tandem repeats (VNTR). Using the DNA extracted from *M. avium* isolates, mycobacterial interspersed repetitive unit (MIRU) loci were amplified by PCR. The PCR products were run on an agarose gel to determine the size of each amplicon. From the size of the PCR product, the number of repeat units was calculated.

interspersed in the genome.[7)~13)] The dissimilarity among mycobacterial genotypes was calculated by the Manhattan distance and analyzed using a neighbor-joining method to create a phylogenetic diagram.[14) 15)] In addition, a multiple logistic regression analysis was used to construct a model for estimating the probability for exacerbation of pulmonary NTM based on the *M.avium* genotype.

## Results

The genotypes of the *M.avium* isolates from all 37 patients are shown in Fig. 2. Two isolates each that differed from one another in genotype were obtained from 3 subjects (2, 13, 22). A phylogenetic-diagram analysis on all 40 *M.avium* isolates showed 3 clusters (Clusters A, B, and C) as shown in Fig. 3. Cluster A has a significant ($p=0.02$) concentration of the isolates from patients with the stable-type pulmonary NTM, whereas Cluster C has a significant ($p=0.01$) concentration of the isolates from patients with the progressive-type pulmonary NTM.

A model for estimating the probability for progression of pulmonary NTM based on the *M.avium* genotype was created with a multiple logistic regression analysis. The most significant predictive model was constructed with Manhattan distances of isolates from Ma-31 (age-adjusted odd ratio, 1.95; 95% confidence interval, 1.16−3.30; $p=0.01$). This model was verified in 13 patients with the stable-type pulmonary NTM and 19 patients with the progressive-type pulmonary NTM, including those diagnosed with pulmonary NTM at the Department of Respiratory Medicine, Tohoku University Hospital in 2007. The results showed a mean probability for progression of 0.77 for the progressive-type and 0.30 for the stable-type, with the ratio being significantly higher in the progressive-type ($p=0.003$, Mann-Whitney non-parametric test), a sensitivity of 79% (percentage of the progressive-type

| *M. avium* Isolate | MIRU Locus | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MATR -1 | MATR -2 | MATR -3 | MATR -4 | MATR -5 | MATR -6 | MATR -7 | MATR -8 | MATR -9 | MATR -10 | MATR -11 | MATR -12 | MATR -13 | MATR -14 | MATR -15 | MATR -16 |
| Ma-1 | 2 | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-2a | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 1 | 4 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-2b | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-3 | 1 | 1 | 5 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 1 | 3 | 0 | 2 | 2 | 3 |
| Ma-4 | 2 | 2 | 2 | 0 | 3 | 3 | 3 | 2 | 2 | 0 | 4 | 3 | 2 | 4 | 3 | 2 |
| Ma-5 | 2 | 2 | 1 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 3 |
| Ma-6 | 2 | 0 | 1 | 1 | 2 | 1 | 6 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-7 | 2 | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-8 | 2 | 1 | 5 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-9 | 2 | 1 | 1 | 1 | 2 | 1 | 6 | 2 | 2 | 2 | 2 | 3 | 0 | 2 | 2 | 3 |
| Ma-10 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-11 | 1 | 0 | 4 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 3 | 2 | 3 |
| Ma-12 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-13a | 1 | 1 | 5 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 1 | 3 | 0 | 2 | 2 | 3 |
| Ma-13b | 1 | 0 | 5 | 1 | 2 | 1 | 6 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-14 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-15 | 2 | 0 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 0 | 2 | 2 | 3 |
| Ma-16 | 2 | 2 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 0 | 2 | 3 | 0 | 3 | 2 | 1 |
| Ma-17 | 2 | 2 | 5 | 2 | 2 | 1 | 4 | 1 | 2 | 2 | 4 | 3 | 1 | 2 | 4 | 2 |
| Ma-18 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-19 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-20 | 2 | 2 | 2 | 0 | 3 | 1 | 3 | 4 | 2 | 1 | 3 | 3 | 2 | 5 | 4 | 3 |
| Ma-21 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-22a | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 2 | 2 | 2 |
| Ma-22b | 2 | 2 | 2 | 3 | 3 | 1 | 2 | 1 | 3 | 3 | 1 | 3 | 0 | 2 | 2 | 2 |
| Ma-23 | 2 | 0 | 5 | 1 | 2 | 1 | 6 | 2 | 2 | 3 | 2 | 3 | 0 | 3 | 2 | 3 |
| Ma-24 | 1 | 0 | 5 | 1 | 2 | 1 | 6 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-25 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 2 | 3 | 3 | 1 | 3 | 0 | 3 | 4 | 1 |
| Ma-26 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-27 | 2 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-28 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-29 | 1 | 0 | 5 | 1 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 3 | 0 | 2 | 2 | 3 |
| Ma-30 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-31 | 2 | 0 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-32 | 2 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 |
| Ma-33 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-34 | 1 | 1 | 5 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 1 | 3 | 0 | 2 | 2 | 3 |
| Ma-35 | 1 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Ma-36 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 3 | 0 | 3 | 2 | 2 |
| Ma-37 | 2 | 0 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 3 | 2 | 3 | 0 | 2 | 2 | 3 |

**Fig. 2** VNTR profiles of 40 clinical *M. avium* isolates. The numbers of tandem repeat units at 16 MIRU loci are shown for each *M. avium* isolate. Two different *M. avium* isolates were cultured from respiratory samples of subjects 2, 13 and 22. They are referred to as Ma-2a/b, Ma-13a/b and Ma-22a/b, respectively.

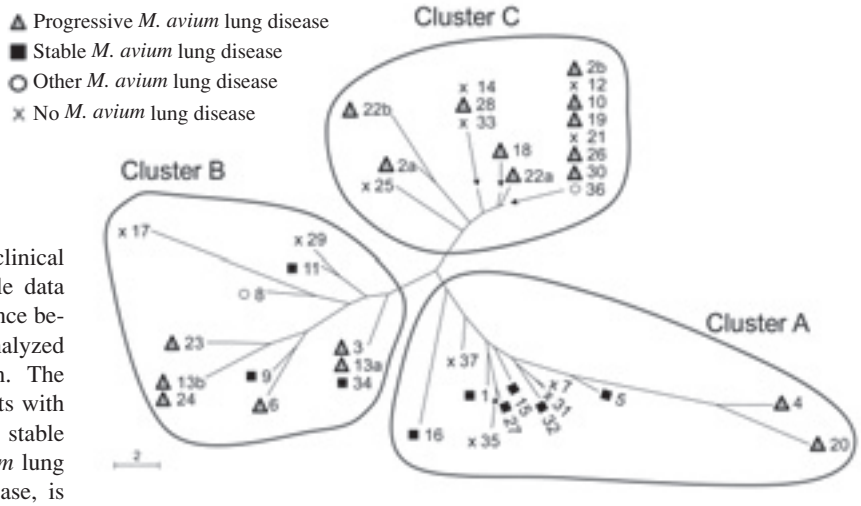


**Fig. 3** Cluster analysis of *M.avium* clinical isolates. Based on the VNTR profile data shown in Fig. 2, the Manhattan distance between each pair was calculated and analyzed using a neighbor-joining algorithm. The distribution of *M.avium* from subjects with progressive *M.avium* lung disease, stable *M.avium* lung disease, other *M.avium* lung disease, and no *M.avium* lung disease, is shown as a radial dendrogram. The scale bar indicates genetic distance.

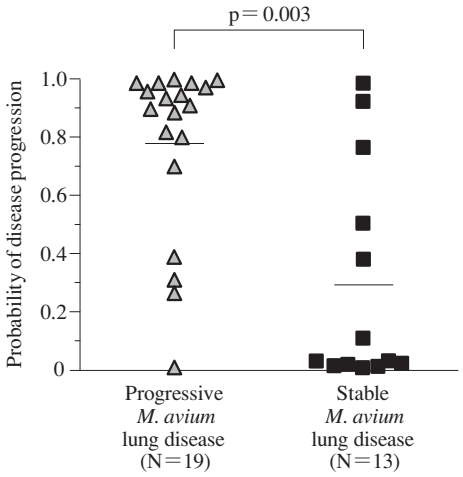


**Fig. 4** Estimates of *M. avium* lung disease progression. The probabilities of disease progression were calculated from the logistic regression analysis according to the Manhattan distances of *M. avium* isolates from Ma-31. Results are shown for subjects with progressive or stable *M. avium* lung disease. Horizontal lines indicate mean values for the results.

[probability for progression＞0.5]), and a specificity of 77% (percentage of the stable-type [probability for progression ＜0.5]) (Fig. 4).

## Summary

The results of this study showed an association between disease progression in pulmonary NTM caused by *M.avium* and mycobacterial genotypes, and an investigation of mycobacterial genotypes indicated that the probability of progression of pulmonary NTM can be estimated.

Several research papers have suggested an association between disease progression in NTM and *M.avium* characteristics. For example, a study reported that *M.avium* isolates of the serotype 1/4/8 are highly virulent, another reported that the smooth-transparent colonial morphotype is associated with a greater capacity for replication and inducing cytokine production, and yet another reported that NTMs caused by the serotype 4 strain have a poor prognosis.[16)~18)] Further study is warranted on the correlation between these reported serotypes/characteristics of *M.avium* and the mycobacterial VNTR genotypes used in this study. In addition, given that this study is a retrospective analysis on a small number of patients, a prospective analysis on a greater number of patients is being planned to investigate whether the disease progression parameters used in this study are clinically justified.

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