MAJOR ARTICLE

Mycobacterial Genotypes Are Associated With Clinical Manifestation and Progression of Lung Disease Caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*

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Background. *Mycobacterium abscessus* and *Mycobacterium massiliense*, which cause lung disease, are variable in their clinical manifestation and progression. We hypothesized that mycobacterial genotypes represent their pathogenic phenotypes, which would result in particular genotypes being associated with disease progression.

Methods. Variable number tandem repeat (VNTR) loci were selected to establish a genotype assay that was capable of differentiating patients with heterogeneous prognoses in the development cohort (48 isolates). The analysis was reevaluated in the validation cohort (63 isolates).

Results. A total of 53 *M. abscessus* and 58 *M. massiliense* isolates were assembled into 3 clusters based on their VNTR genotyping. The patients in cluster A were more likely to have stable disease of the nodular bronchiectatic form; 100% of *M. abscessus* patients and 96% of *M. massiliense* patients were followed without antibiotic treatment for >24 months after diagnosis. In contrast, the patients in cluster B were more likely to have progressive disease of the nodular bronchiectatic form; 96% of *M. abscessus* patients and 81% of *M. massiliense* patients started antibiotic treatment within 24 months after diagnosis. All patients in cluster C had fibrocavitary disease and started antibiotic treatment immediately after diagnosis. The genetic distance of each clinical isolate from the reference strain was associated with the highest likelihood of disease progression and a disease phenotype of the fibrocavitary form ($P < .001$).

Conclusions. Mycobacterial genotyping of *M. abscessus* and *M. massiliense* may provide valuable information for predicting disease phenotype and progression.

Keywords. nontuberculous mycobacterium; *Mycobacterium abscessus*; *Mycobacterium massiliense*; genotype; disease progression.

Pulmonary diseases that are caused by nontuberculous mycobacteria (NTM) are being diagnosed with increasing frequency worldwide [1]. Typically, NTM are divided into slow-growing and rapid-growing species. *Mycobacterium abscessus* is the most common etiology of lung disease that is caused by rapidly growing mycobacteria [1]. *M. abscessus* is resistant to many antibiotics in vitro, which results in unsatisfactory treatment results [2–5]. Recently, *M. abscessus* was divided into *M. abscessus* sensu stricto, *Mycobacterium massiliense*, and *Mycobacterium bolletii* [6–8]. *M. massiliense* is now recognized as a separate species from *M. abscessus*, and treatment response rates to clarithromycin-based antibiotic therapy are much higher in patients

Received 13 October 2012; accepted 7 March 2013; electronically published 19 March 2013.

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Clinical Infectious Diseases 2013;57(1):32–9

DOI: 10.1093/cid/cit172

with *M. massiliense* than in those with *M. abscessus* lung disease [9–11].

The traditionally recognized presentation of NTM lung disease is apical fibrocavitary lung disease [1]. This type of disease usually develops in older males with underlying lung disease, such as previous tuberculosis. Alternatively, NTM lung disease can present with nodular infiltrates that frequently involve the right middle lobe or lingular segment of the left upper lobe. This form of disease is termed "nodular bronchiectatic disease," and it occurs predominantly in postmenopausal, nonsmoking females and tends to have a much slower progression than cavitary disease [12, 13].

A diagnosis of NTM lung disease does not necessitate the initiation of antibiotic therapy, which is a decision that is based on the potential risks and benefits of therapy for individual patients [1]. There is a wide range of clinical manifestations in patients with NTM lung disease; patients may remain stable for years or the illness may progress rapidly [2–5]. However, little is known regarding the mycobacterial factors that determine the clinical course of *M. abscessus* and *M. massiliense* lung disease.

Previous studies revealed an extensive genetic divergence within NTM species [14, 15]. This finding suggests that particular subgroups of mycobacterial strains may be associated with the pathogenesis of NTM infection [16]. Variable number tandem repeat (VNTR) analyses have been used for the molecular genotyping of mycobacterial species and species discrimination [17–19]. A recent study revealed that specific VNTR genotypes were associated with disease progression in *Mycobacterium avium* lung disease [20]. However, the clinical implication of mycobacterial genotyping has never been studied in other NTM lung disease. The recent availability of the complete genomic sequence of *M. abscessus* has made the identification of informative VNTR loci for species-level identification possible [19, 21]. In this study, we performed VNTR genotyping for the clinical isolates of *M. abscessus* and *M. massiliense* and determined whether there was any association between the mycobacterial genotype and disease phenotype and progression.

METHODS

Study Subjects

Patients with *M. abscessus* and *M. massiliense* lung disease and the stored isolates were identified from January 2001 to December 2008 using the database of the NTM Registry of Samsung Medical Center (a 1950-bed referral hospital in Seoul, Korea) [9].

During this 8-year period, 189 patients were diagnosed with *M. abscessus* or *M. massiliense* lung disease, after excluding 13 patients with mixed infection with *M. avium-intracellulare* and rapidly growing mycobacteria. All clinical isolates of *M. abscessus* and *M. massiliense* were recovered from patients who met the diagnostic criteria of the American Thoracic Society for NTM lung disease [1]. Patients without stored isolates (n = 22), patients with previous treatment for NTM disease (n = 28), patients with a concurrent malignancy (n = 17), and patients who were followed up for less than 24 months (n = 11) were excluded. Therefore, 111 patients were included in this study. The etiologic organisms included *M. abscessus* in 53 patients and *M. massiliense* in 58 patients. No patients were positive for the human immunodeficiency virus. *M. abscessus* and *M. massiliense* were identified as previously described (see Supplementary Material) [9, 19, 22, 23]. Permission was obtained from Samsung Medical Center's Institutional Review Board to review and publish information from the patient records.

The study population was divided into 2 cohorts according to the study period. Initially, VNTR profiles were determined in the development cohort, which consisted of 48 isolates of *M. abscessus* (n = 24) and *M. massiliense* (n = 24) that were obtained from patients who were diagnosed from January 2001 to December 2004. After the VNTR genotyping and assay optimization were evaluated in this development cohort, genotyping analyses were validated in the isolates with *M. abscessus* (n = 29) and *M. massiliense* (n = 34), which were obtained from patients who were diagnosed between January 2005 and December 2008.

Patient Management Data Analysis

We classified chest radiography and high-resolution computed tomography findings obtained at the time of diagnosis as showing either fibrocavitary disease or nodular bronchiectatic disease (see Supplementary Material) [2, 9, 10].

Disease progression was determined by using the time interval between date of diagnosis and initiation of antibiotic therapy. We determined whether the condition was progressive (treatment required within 24 months of diagnosis) or stable (treatment delayed until after 24 months); these criteria were slightly modified from the previous study (see Supplementary Material) [20].

VNTR Genotyping and Phylogenetic Analysis

Tandem-repeat–containing loci with a high allelic diversity in the genome sequence of *M. abscessus* ATCC19977^T (GenBank accession no. CU458896.1) were selected using the tandem repeat finder program (http://tandem.bu.edu/trf/trf/html); the number of repeat units of each isolate was determined by polymerase chain reaction as previously described (see Supplementary Material) [19]. Information regarding the VNTR loci that were selected for each species is provided in Supplementary Table 1 and Figure 1. VNTR genotyping assays were performed without knowledge of the clinical information of patients.

Next, we analyzed the disease phenotype, disease progression, and phylogenetic relationships between the clinical isolates. The

phylogenetic distribution was generated according to the genotypic diversity of the clinical isolates using a neighbor-joining algorithm (see Supplementary Material).

Statistical Analysis

All data are presented as the medians and interquartile ranges for the continuous variables and as numbers (percentages) for the categorical variables. The data were compared using Student *t* test for the continuous variables and a χ^2 or Fisher exact test for the categorical variables. The accuracy rates were used to evaluate clustering performance [24], and the 95% confidence intervals for accuracy rates were computed using the method developed by Clopper and Pearson for exact small-sample inference [25]. The probability of disease phenotype and progression from the stable disease was calculated by the logistic regression analysis as previously described [20]. All statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC); a 2-sided $P < .05$ was considered significant.

RESULTS

Patient Characteristics

The baseline characteristics of the patients are summarized in Table 1. No significant differences were found between the development cohort and the validation cohort regarding any of the baseline characteristics in patients with *M. abscessus* and *M. massiliense* lung disease. In addition, distributions of the radiographic type of disease were similar: the majority of patients with *M. abscessus* (46/53, 87%) and *M. massiliense* (44/58, 76%) lung disease had the nodular bronchiectatic form of the disease.

All patients with the fibrocavitary form of *M. abscessus* (7/53, 13%) and *M. massiliense* (14/58, 24%) received antibiotic therapy after diagnosis of disease. In patients with the nodular bronchiectatic form of *M. abscessus* lung disease, 52% (24/46) of patients were treated within 24 months of diagnosis because of the progression of the disease, and treatment was withheld for more than 24 months in 48% (22/46) of patients because of the stable course of the disease. In patients with the nodular bronchiectatic form of *M. massiliense* lung disease, 39% (17/44) of patients were treated within 24 months of diagnosis, and 61% (27/44) of patients were followed without antibiotic treatment for more than 24 months. The overall proportion of patients who received antibiotic therapy at 24 months was similar (31/53 [58%] in cases of *M. abscessus* vs 30/58 [52%] in cases of *M. massiliense*; $P = .732$).

Selection of the VNTR Loci, Genotyping, and Clustering Analysis in the Development Cohort

A total of 30 VNTR loci were typed in each *M. abscessus* isolate. These loci were also used for the investigation of the VNTR profiles of *M. massiliense*. The initial genotyping analyses included the reference strain *M. abscessus* ATCC19977^T and *M. massiliense* CIP108297^T. Therefore, including each reference

Table 1. Demographic and Clinical Characteristics of Patients With *Mycobacterium abscessus* and *Mycobacterium massiliense* Lung Disease in the Development and Validation Cohorts

	Mycobacterium abscessus (n = 53)			*Mycobacterium massiliense* (n = 58)		
Characteristic	Development Cohort (n = 24)	Validation Cohort (n = 29)	*P* Value	Development Cohort (n = 24)	Validation Cohort (n = 34)	*P* Value
Sex, male	6 (25)	5 (17)	.518	1 (4)	7 (21)	.123
Age, y	57 (42–63)	60 (52–65)	.357	52 (42–60)	57 (45–65)	.425
Body mass index, kg/m^2	20.6 (18.6–21.8)	20.8 (18.5–22.1)	.908	20.3 (19.2–22.4)	20.2 (19.2–22.4)	.782
Nonsmoker	23 (96)	27 (93)	1	23 (96)	30 (88)	.392
Associated diseases						
Previous tuberculosis	13 (54)	17 (59)	.745	12 (50)	20 (59)	.506
Bronchiectasis	20 (83)	26 (90)	.688	20 (83)	26 (77)	.744
Chronic heart disease	4 (17)	2 (7)	.392	1 (4)	4 (12)	.392
Diabetes mellitus	2 (8)	0 (0)	.2	1 (4)	2 (6)	1
Laboratory findings						
Positive AFB smear	19 (79)	22 (76)	.775	14 (58)	20 (59)	.97
CRP, mg/dL	0.22 (0.08–0.62)	0.23 (0.04–0.92)	.804	0.30 (0.08–2.02)	0.08 (0.04–0.89)	.17
Type of disease						
Fibrocavitary form	4 (17)	3 (10)	.688	7 (29)	7 (21)	.452
Nodular bronchiectatic form	20 (83)	26 (90)		17 (71)	27 (79)	

Abbreviations: AFB, acid-fast bacilli; CRP, C-reactive protein.

The data are expressed as the median (interquartile range) or the number (%).

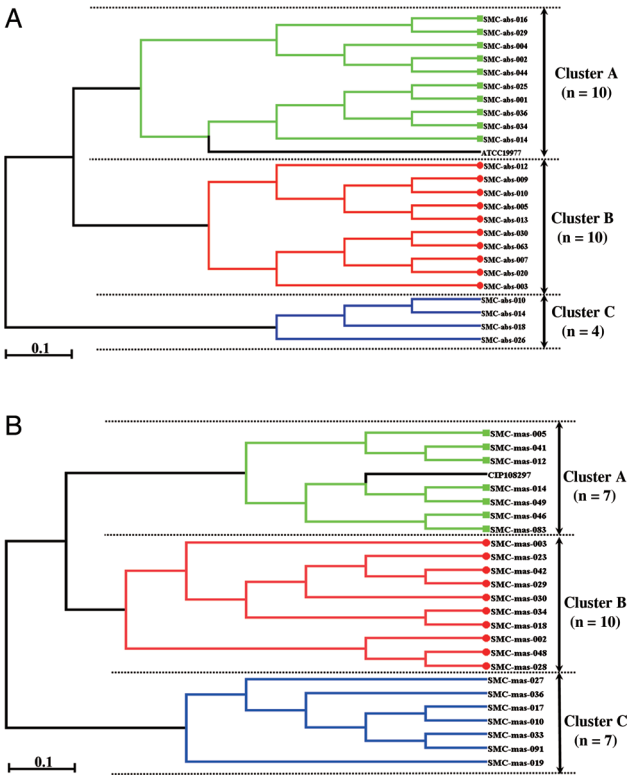

Figure 1. Phylogenetic clustering of the (*A*) *Mycobacterium abscessus* (n = 25) and (*B*) *Mycobacterium massiliense* (n = 25) clinical isolates that belonged to the development cohort, including each reference strain. Among 30 VNTR loci, 10 VNTR loci for *M. abscessus* and 9 VNTR loci for *M. massiliense* that were capable of distinguishing the radiographic type of disease and disease progression were selected in this development cohort. The red lines indicate the progressive nodular bronchiectatic form, the green lines indicate the stable disease, and the blue branches indicate the fibrocavitary form of disease. The 3 major branches of the *M. abscessus* and *M. massiliense* isolates were designated as clusters *A*, *B*, and *C*. The scale bar at the bottom indicates the Manhattan distance.

strain, the VNTR profiles were determined for 25 *M. abscessus* isolates and 25 *M. massiliense* isolates.

Thirty VNTR loci were amplified from each isolate obtained from patients in the development cohort, and allele sizes were determined by electrophoresis (Supplementary Figure 2). As a complementary analysis of the 30 candidate VNTR loci, 9 VNTR loci (Mab-1, 4, 7, 9, 14, 18, 23, 24, and 28) for *M. abscessus* and 10 VNTR loci (Mab-1, 4, 7, 11, 14, 18, 21, 24, 28, and 29) for *M. massiliense* were selected for VNTR genotyping. The selected loci for each species yielded a high discriminatory index (h) with great allelic variations in clinical isolates of each species ($h > 0.84$; Supplementary Tables 2 and 3).

In the phylogenetic tree, 25 *M. abscessus* isolates and 25 *M. massiliense* isolates were grouped into 3 major clusters that were designated as A, B, and C (Figure 1*A* and 1*B*). The reference strains *M. abscessus* ATCC19977^T and *M. massiliense* CIP108297^T were unaffectedly located in cluster A. As shown in Table 2, patients with the stable or progressive nodular bronchiectatic form of the disease were grouped in cluster A or B, respectively. All patients with the fibrocavitary form of *M. abscessus* and *M. massiliense* lung disease were grouped in cluster C.

Genotyping and Clustering Analysis in a Validation Cohort

The established genotyping assay, which was based on the VNTR profiles, was used to evaluate 29 *M. abscessus* isolates and 34 *M. massiliense* isolates that were recovered from patients in the validation cohort. All isolates of each species were grouped into clusters A, B, and C (Figure 2*A* and 2*B*). Similar to the initial development cohort, all patients with the fibrocavitary form, except 1 patient who had *M. massiliense* lung disease, were grouped into cluster C. Patients with the stable or progressive nodular bronchiectatic form were more likely to be grouped into cluster A or B, respectively (Table 3).

Table 2. Radiographic Type and Disease Progression in 48 Patients With *Mycobacterium abscessus* and *Mycobacterium massiliense* Lung Disease According to the VNTR Clusters (Initial Development Cohort)

Species	VNTR Cluster	Nodular Bronchiectatic Form		Fibrocavitary Form	Accuracy of Discrimination (95% CI)
		Stable	Progressive		
Mycobacterium abscessus (n = 24)	Cluster A (n = 10)	10			100% (59.1%–100%)
	Cluster B (n = 10)		10		
	Cluster C (n = 4)			4	
Mycobacterium massiliense (n = 24)	Cluster A (n = 7)	7			100% (59.1%–100%)
	Cluster B (n = 10)		10		
	Cluster C (n = 7)			7	

Abbreviation: CI, confidence interval.

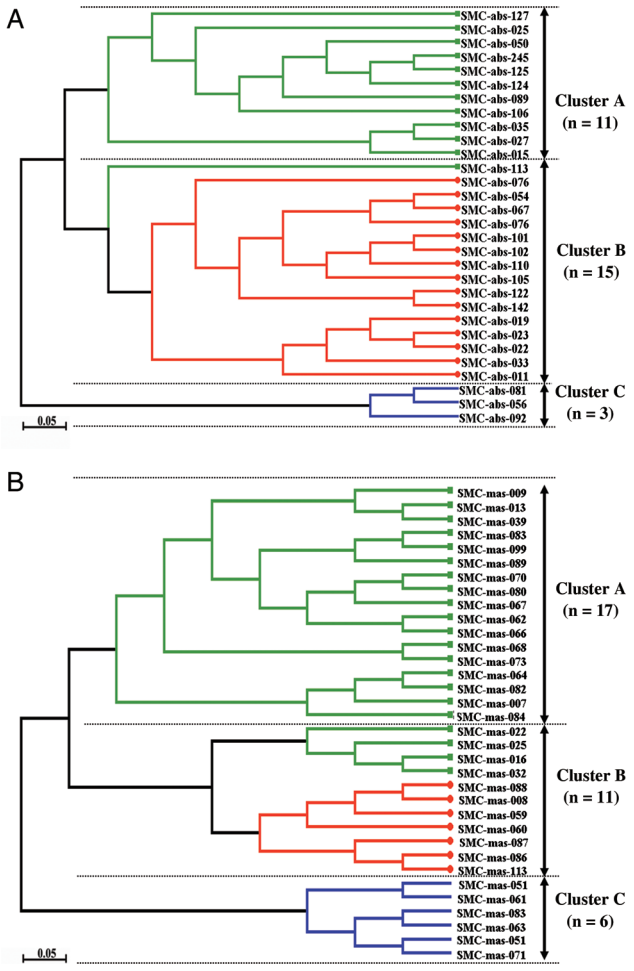

Figure 2. Validation of the association between the mycobacterial genotype, which was based on VNTR profiling data, and the radiographic type of disease and disease progression in a validation cohort. The phylogenetic clustering of the (*A*) *Mycobacterium abscessus* (n = 29) and (*B*) *Mycobacterium massiliense* (n = 34) clinical isolates in the validation cohort are displayed as 3 major branches: clusters *A*, *B*, and *C* as demonstrated in Figure 1. Patients with the fibrocavitary form were more likely grouped into cluster C, and patients with the stable or progressive nodular bronchiectatic form were more likely to be grouped into cluster *A* or *B*, respectively ($P < .001$). The scale bar at the bottom indicates the Manhattan distance.

Clinical Relevance of the Genotypic Diversity in an Entire Cohort

To determine whether genetic distances of the clinical isolates in each cluster could predict disease phenotype and progression, we performed logistic regression analysis using *M. abscessus* ATCC19977^T and *M. massiliense* CIP108297^T, which belonged to the cluster of the stable disease of each species, as clinically unbiased standards to estimate the Manhattan distances of the individual isolates of each species. Among the 53 *M. abscessus* clinical isolates, the genetic distance between each clinical isolate and the reference isolate was associated with the highest likelihood of disease progression (patients with the progressive nodular bronchiectatic form vs patients with the stable nodular bronchiectatic form, $P < .001$; patients with the progressive nodular bronchiectatic form vs patients with the fibrocavitary form, $P < .001$; Figure 3*A*).

The same analysis was performed for the *M. massiliense* isolates. Among the 58 *M. massiliense* clinical isolates, the genetic distance between each clinical isolate and the reference strain was associated with the highest likelihood of disease progression (patients with the progressive nodular bronchiectatic form vs patients with the stable nodular bronchiectatic form, $P < .001$; patients with the progressive nodular bronchiectatic form vs patients with the fibrocavitary form, $P < .001$; Figure 3*B*).

DISCUSSION

In this study we examined more than 100 patients with *M. abscessus* or *M. massiliense* lung disease and found that *M. abscessus* and *M. massiliense* isolates with a distinctive VNTR profile resulted in destructive and progressive disease. The *M. abscessus* and *M. massiliense* isolates from the fibrocavitary and nodular bronchiectatic forms were clustered differently. Among the clinical isolates from patients with the nodular bronchiectatic form, the isolates from patients with progressive disease and the isolates from patients with stable disease were clustered differently. Therefore, VNTR-based genotyping of *M. abscessus* and *M. massiliense* isolates could be a useful strategy for predicting disease progression.

A diagnosis of NTM lung disease does not always require antibiotic therapy [1]. Treatment may require long-term antibiotic and often complicated treatment, especially for *M. abscessus* lung disease [2–5]. Patients may meet the diagnostic criteria but not have progressive or severe disease; therefore, these patients are closely monitored with regular sputum collection [1]. In previous studies, long-term antibiotic therapy was administered to approximately 50% of patients after a diagnosis of *M. abscessus* or *M. massiliense* lung disease [2, 9]. Physicians may opt for a "wait and see" approach before prescribing antibiotics, even after disease recognition, because of the reported low treatment efficacy and high drug toxicity [7, 26].

Mycobacterial virulence and host predisposition influence the progression of NTM lung disease [1]. The strain-specific virulence of *M. avium* complex was implicated in disease progression in a previous study of *M. avium* serovar 4, which was isolated from patients [27]. A clinical strain that was derived from patients with progressive *M. avium* complex lung disease exhibited strong virulence [16]. In addition, several *M. massiliense* strains have been invasive and pathogenic in postsurgical outbreaks [28, 29]. Moreover, a recently published study of 37 patients with *M. avium* lung disease in Japan revealed that

Table 3. Radiographic Type and Disease Progression in 63 Patients With *Mycobacterium abscessus* and *Mycobacterium massiliense* Lung Disease According to the VNTR Clusters (Validation Cohort)

Species	VNTR Cluster	Nodular Bronchiectatic Form		Fibrocavitary Form	Accuracy of Discrimination (95% CI)
		Stable	Progressive		
Mycobacterium abscessus (n = 29)	Cluster A (n = 11)	11			96.6% (59.0–100%)
	Cluster B (n = 15)	1	14		
	Cluster C (n = 3)			3	
Mycobacterium massiliense (n = 34)	Cluster A (n = 17)	16		1	85.3% (50.1–100%)
	Cluster B (n = 11)	4	7		
	Cluster C (n = 6)			6	

Abbreviation: CI, confidence interval.

specific genotypes of *M. avium* were associated with disease progression [20]. However, these preliminary findings have not been validated in a large cohort or with other etiologies of NTM lung disease.

Some studies have been conducted to understand the host factors that are involved in determining the clinical course of NTM lung disease [30–33]. However, no host predictors for disease progression of *M. abscessus* and *M. massiliense* lung disease have been clearly established to date. Therefore, we hypothesized that the progression of *M. abscessus* and *M. massiliense* lung disease depends on the mycobacterial genotype of the clinical isolates.

This study was based on the identification of VNTR loci in the preliminary *M. abscessus* genome and the polymorphisms of the most interesting VNTR loci candidates using the clinical isolates and reference strains of *M. abscessus* and *M. massiliense*. To differentiate the patients with stable and progressive disease, 9 and 10 VNTR loci were selected for the *M. abscessus* and *M. massiliense* isolates, respectively. The VNTR analysis exhibited a Hunter-Gaston's Discriminatory Index value >0.84 (see Supplementary Material), which suggests that this method is highly informative and possesses great discriminatory power for the identification of genetically similar strains. The association between the mycobacterial genotype and disease progression

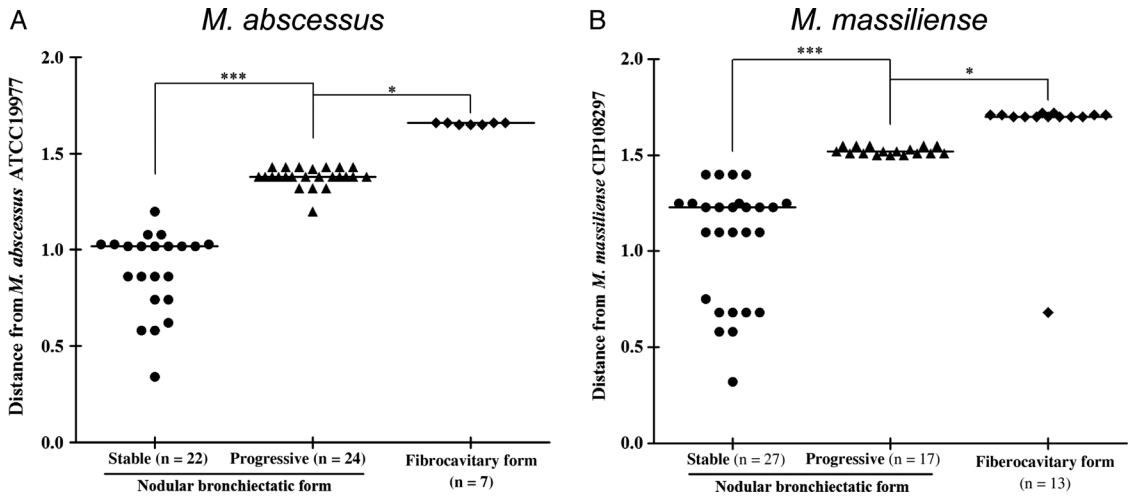

Figure 3. Relationships between VNTR genotype, disease phenotype, and disease progression in *Mycobacterium abscessus* and *Mycobacterium massiliense* lung disease. The genetic distance was calculated as the Manhattan distance of each (*A*) *M. abscessus* and (*B*) *M. massiliense* clinical isolate from the reference strains ATCC19977^T and CIP108297^T, respectively. To determine whether the individual characteristics of disease could be discriminated based on the VNTR genotype, a comparison was made between the patients with stable and progressive disease of the nodular bronchiectatic form and the fibrocavitary form. The horizontal bar through each scatter plot indicates the mean value of genetic distance for the group. *$P < .05$ and ***$P < .001$ were considered significant.

was confirmed in the validation cohort. These results provide compelling evidence that the VNTR profiles of *M. abscessus* and *M. massiliense* isolates could serve as a potential predictor of disease progression.

Patients with NTM lung disease fall into 2 general categories: fibrocavitary and nodular bronchiectatic disease. [1]. Traditionally, fibrocavitary lung disease occurs in patients with preexisting lung lesions, including chronic obstructive pulmonary disease and previous tuberculosis. This type of disease leads to massive lung destruction over a period of several years. Host factors are important in the development of fibrocavitary and nodular bronchiectatic disease. Interestingly, we found that the patients in our study with the fibrocavitary form differed significantly from the patients with the nodular bronchiectatic form of *M. abscessus* and *M. massiliense* lung disease based on the VNTR profile classification. All *M. abscessus* isolates (7/7, 100%) and the majority of *M. massiliense* isolates (13/14, 93%) from the fibrocavitary form were grouped into cluster C. We previously reported that *M. abscessus* isolates from fibrocavitary disease are more virulent than isolates from nodular bronchiectatic disease [34]. The results from this study were consistent with our previous observations. In addition, our results suggest that particular mycobacterial genotypes are more closely associated with disease progression and phenotype. Thus, mycobacterial genotypes could be important factors that are involved in the pathogenesis of *M. abscessus* and *M. massiliense* lung disease.

Of note, the majority of *M. abscessus* and *M. massiliense* isolates recovered from the fibrocavitary form showed a rough morphotype, whereas a significant correlation between bacterial surface morphotype and disease progression was not found among the isolates recovered from the nodular bronchiectatic form in our study. It is well documented that some *M. abscessus* strains are more invasive and induce a more vigorous host immune responses, which is dependent on their surface glycopeptidolipid production [35, 36]. In addition, some VNTR regions are associated with certain virulence factors, including cell wall biosynthesis, cell wall biogenesis, and lipid metabolism (Supplementary Table 4). Thus, further studies to investigate polymorphisms of the VNTR loci or adjunct regions should be performed to determine any association with pathogenesis.

This study has several limitations. First, it was conducted at a single center; therefore, it was not representative of conditions throughout Korea due to referral bias. In addition, there have been reports of mycobacterial diversity across geographical distributions [37]; thus, the usefulness of the VNTR genotyping assay in other countries should be verified by determining the association between VNTR loci and clinical outcomes in these countries. Second, this study did not include all consecutive patients who were diagnosed with *M. abscessus* and *M. massiliense* lung disease during the study period. Patients whose isolates were not kept in storage and patients who were followed up for <24 months were excluded from this study. Exclusion of these patients could represent a potential bias. Third, the decision to initiate long-term combination antibiotic therapy did not depend on firmly established objective criteria. However, we believe that the probability of bias was distributed equally among patients with specific VNTR genotypes because information regarding the mycobacterial genotypes was not available to the attending physician during the study period. Finally, this study was preliminary because we did not identify the mechanism of virulence of the clinical isolates with specific genotypes.

In conclusion, this study provides evidence that *M. abscessus* and *M. massiliense* genotypes influence clinical disease phenotype and disease progression. The development and progression of *M. abscessus* and *M. massiliense* lung disease may depend on the balance between mycobacterial virulence and host defense. Future studies of the genetic differences between the *M. abscessus* and *M. massiliense* isolates described in this study may reveal new insights regarding the pathogenicity of these difficult-to-treat mycobacteria.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Financial support. This work was supported by the Mid-career Researcher Program through a National Research Foundation grant funded by the Korean Ministry of Education, Science, and Technology [2011-0015546] and a grant from the Korea Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea [A100027].

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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