ANNALSATS Articles in Press. Published on 19-November-2013 as 10.1513/AnnalsATS.201309-297OC

Association between polyclonal and mixed mycobacterial *Mycobacterium avium* complex infection and environmental exposure

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T. K. collected and analyzed data and revised the manuscript.

K. M. collected data.

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Financial support: This study was supported by Grants-in-Aid for Scientific Research by the Japanese Society for the Promotion of Science (24591479)

Running head: Factors associated with polyclonal MAC infection

Classification of the article: 11.5: Non-tuberculous Mycobacterial Disease

Key words: variable number of tandem repeats; environmental exposure; soil; asthma

Word count for the body of the manuscript: 3962 words

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

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Abstract

Rationale: Polyclonal and mixed mycobacterial *Mycobacterium avium* complex (MAC) infection is observed in pulmonary MAC disease. Human living environments contain multiple species or genotypes of non-tuberculous mycobacterial strains and are considered sources of infection.

Objectives: To investigate the association of environmental exposure with polyclonal and mixed mycobacterial infection in pulmonary MAC disease after adjustments for potential confounding diseases and conditions, and radiographic findings.

Methods: We collected two separate sputum samples from 102 patients and single sputum samples from 18 patients in whom the second MAC strain was not isolated in our prospective cohort of pulmonary MAC disease. MAC isolates from sputum samples and patients' residential soils were used for variable number of tandem repeats (VNTR) analyses. Polyclonal and mixed mycobacterial MAC infections were defined as having different VNTR genotypes and other mycobacterial species, respectively. Monoclonal MAC infection was defined as all isolates showing a single VNTR genotype. Associations of the type of infection with clinical and radiographic findings, and environmental exposure were measured.

Measurements and Main Results: Polyclonal and mixed mycobacterial MAC and monoclonal infections were observed in 42 and 78 patients, respectively. By stepwise regression analysis, patients with polyclonal and mixed mycobacterial MAC infections were associated with history of asthma [odds ratio (OR) 11.56, 95% confidence interval (CI) 1.41–255.77, P=0.021], high soil exposure (≥2 hours per week, OR 4.31, 95% CI 1.72–11.45, P<0.01), shower use in a bathroom (OR 4.57, 95% CI 1.28–23.23, P=0.018) and swimming in a pool (OR 9.69, 95% CI 1.21–206.92, P<0.01).

Conclusions: Environmental exposure was associated with polyclonal and mixed mycobacterial MAC infection in pulmonary MAC disease.

Word count for the abstract: 266 words

ANNALSATS Articles in Press. Published on 19-November-2013 as 10.1513/AnnalsATS.201309-297OC

Introduction

In recent years, more attention has been focused on pulmonary *Mycobacterium avium* complex (MAC) disease because of its increasing prevalence (1-3). Non-tuberculous mycobacteria including MAC are widely distributed in water and soils in human living environments (4-12) which are considered sources of infection (13).

Several studies have demonstrated that polyclonal MAC or mixed mycobacterial infections have been observed in acquired immunodeficiency syndrome (AIDS) patients with disseminated MAC disease (14-17). Albeit et al. initially reported that two AIDS patients with bacteremic *M. avium* infections were concurrently infected with two distinct genotypes of strains (14). In the same cohort, von Reyn et al. reported hospital hot water as the source of *M. avium* infection (5). Wallace et al. reported that polyclonal MAC infection was also observed in human immunodeficiency virus (HIV)-negative patients with pulmonary MAC disease (18) and that new infections were observed after completing macrolide therapy (19).

We previously reported that high soil exposure was an independent risk factor for pulmonary MAC disease (20) and that about one-half of patients' residential soil contained one or more variable number of tandem repeats (VNTR) genotypes of MAC strains, with residential soils likely sources of MAC infection (12). Therefore, environmental exposure may lead to polyclonal MAC infection in patients with pulmonary MAC disease, but the association is unknown. We investigated the incidence of polyclonal MAC or mixed mycobacterial MAC infection in patients with pulmonary MAC disease and its association with environmental exposure after adjustments of confounding diseases and conditions, and radiographic findings. Some of the results of these studies have been previously reported in abstract form (21).

Methods

Study population

Between January 2007 and April 2013, we enrolled 218 pulmonary MAC patients (169 pulmonary *M. avium* patients, 48 *M. intracellulare* patients, and 1 unspecified MAC patient) who met the American Thoracic Society guidelines for diagnosis of MAC infection at Kyoto University Hospital (22) in our prospective consecutive cohort. We excluded 10 patients who did not want to participate in the study, 64 patients for whom clinical *M. avium* or *M. intracellulare* strains were unavailable, 10 patients who were transferred to another hospital, 9 patients who discontinued hospital visits, and 5 patients who died during the study period. Finally, 120 pulmonary MAC patients (94 *M. avium* patients and 26 *M. intracellulare* patients) were analyzed in this study.(Figure 1). We included 71 of 100 patients with pulmonary MAC disease in our previous study and 1 non-infected patient who developed pulmonary MAC disease after the previous study (12) into this study. The remaining 29

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patients were excluded, because they had no follow-up or did not provide sputum samples. No patient had evidence of HIV infection or active malignant disease. All patients signed a written consent form. The institutional review of Kyoto University board approved this prospective study.

Data collection

All patients completed a standardized questionnaire about underlying diseases and conditions, smoking status, alcohol usage, and experience of environmental exposure, such as soil exposure from farming and gardening, or any activities involving soil exposure and types of soil (farm, yard, and potting soil) to which patients had been exposed, and water exposure from taking baths, showering, washing dishes, and swimming in a pool. (20) We defined a drinking habit as a patient who drank alcohol at least once each week. As we previously reported that patients with pulmonary MAC diseased experienced significantly more soil exposure (2 or more hours per week) than controls (12, 20), high soil exposure was defined as 2 or more hours per week of these activities. Japanese individuals, especially the elderly, usually fill a bathtub with hot water and scoop the hot water without using a shower when washing their bodies. We asked about the usual use of a shower when taking a bath, swimming habits, and where the patients went swimming (pool, river, lake, or sea). Gastroesophageal reflux disease symptoms were assessed by self-reported questionnaires (23, 24). Severe pneumonia was defined as hospitalization for pneumonia. We collected the last laboratory data before or while collecting sputum samples. High-resolution computed tomography (HRCT) images (contiguous, 2 mm-thick lung images) of the 119 patients were obtained. Blind readings of these HRCT images were performed by one board-certified thoracic radiologist who had no prior knowledge of the patient profiles or the results of laboratory data.

Clinical and soil MAC strains subjected to VNTR analyses

We collected two separate sputum samples at separate hospital visits from 102 patients (*M. avium* in 81 patients and *M. intracellulare* in 21 patients). Single sputum samples were analyzed in 18 patients (*M. avium* in 13 patients and *M. intracellulare* in 5 patients) because the second MAC strain was not isolated during the follow-up periods. Soil samples from 72 patients in our previous study (12) were used for this study. Patients collected approximately 5 g of soil from their residences to which they had been exposed and mailed the soil samples to Kyoto University Hospital. The choice of soil samples and depth of soil were left to the individual patients. Soil samples were processed as previously reported (25). After cultivation using the BACTEC MGIT 960 system [Becton Dickinson and Company (BD), NJ, USA], positive cultures were subjected to either PCR analysis for the identification of *M. avium* or *M. intracellulare* using the COBAS TaqMan MAI test (Roche Diagnostics, Basel, Switzerland), or DNA-DNA hybridization for identification of other non-tuberculous mycobacteria species of clinical isolates using DDH Mycobacteria Kyokuto (Kyokuto Pharmaceutical Industrial

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Co., Tokyo, Japan). Positive cultures were routinely subcultured on Kudo PD agar (Japan BCG Laboratory, Tokyo, Japan) in our microbiological laboratory. Joined multiple colonies on the agar were picked for VNTR analysis and subcultured on Middlebrook 7H11 agar (BD) to separate single colonies. When single DNA bands were seen at every VNTR allele from the joined, multiple colonies, two distinct single colonies were selected to confirm that each of the two VNTR profiles showed the same single genotype. When multiple, separate DNA bands were seen at any VNTR allele from the multiple MAC colonies, multiple, distinct, single colonies were selected until a determination of each of the multiple VNTR genotypes could be made.

VNTR analysis procedure

Primer sets for 15 *M. avium* VNTR loci and 16 *M. intracellulare* VNTR loci were used in the VNTR analysis as described previously (26, 27, Tables E1 and E2). The PCR amplification condition for *M. avium* was as follows: 1 cycle of 95°C for 10 min, followed by 38 cycles of 98°C for 10 s, 68°C for 30 s, and 72°C for 1 min, and then 1 cycle of 72°C for 7 min. For *M. intracellulare*, the PCR condition was 1 cycle of 95°C for 10 min, followed by 38 cycles of 98°C for 10 s, 64°C for 30 s, and 72°C for 1 min, and then 1 cycle of 72°C for 7 min. PCR products of the same VNTR allele of all strains from the same patient ran on the chip of the QIAxcel capillary electrophoresis system (QIAGEN, Valencia, CA, USA) side by side to compare their DNA sizes. Sample analyses were performed in combination with a QIAxcel

DNA High Resolution kit, with a built-in OM500 method and the following parameters: sample injection voltage at 5 kV for 10 s and a separation voltage at 5 kV for 500 s, and with BioCalculator Software. An alignment marker (15 bp/3 kb) and a 100 bp DNA size marker (Takara Bio, Shiga, Japan) were run simultaneously with the samples for size estimation of the allelic ladders (28). Finally, the calculated values were rounded to the closest whole number.

Definition of monoclonal and polyclonal MAC infections

Polyclonal MAC infection was defined when the VNTR analysis for the same *M. avium* strains or *M. intracellulare* strains, respectively, revealed a change at 1 or more out of the 15 *M. avium* VNTR loci or 16 *M. intracellulare* VNTR loci; mixed mycobacterial MAC infection was defined when other mycobacterial species were isolated. Monoclonal MAC infection was defined when all the colonies isolated from sputum samples represented a single VNTR pattern.

Statistical analysis

JMP version 9.0.0 (SAS Institute, NC, USA) was used for all statistical analyses. Group comparisons were made using the Chi-square test, Fisher's exact test, and Wilcoxon test. More than two group comparisons were made by analysis of variance. Variables were included in stepwise regression analysis if the probability values were less than 0.05 by univariate analysis. Odds ratios (ORs) and their respective 95% confidence intervals (CIs)

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were computed as estimates of relative risk. A P value less than 0.05 was considered statistically significant.

Results

Genetic diversity of MAC infection

Seventy-eight of 120 patients (65.0%) had a monoclonal MAC infection: 64 patients (82.1%) were infected with *M. avium,* and 14 patients (17.9%) were infected with *M. intracellulare*. Among 60 patients provided two different samples, 59 patients had two or more positive MAC cultures in a single year around the time of the second sputum sample collection, and one patient had a single positive *M. intracellulare* culture after sputum conversion. Eighteen patients provided single sputum samples and showed single genotypes in each of the samples. They were classified into monoclonal infection (Tables E3 and E4, Figures E1 and E2).

Twenty-seven of 120 patients (22.5%) had a polyclonal MAC infection: 20 patients (74.1%) were infected with *M. avium,* and 7 patients (25.9%) were infected with *M. intracellulare*. All patients provided two sputum samples containing MAC strains and had two or more positive MAC cultures in a single year around the time of the second sputum sampling event. Sixteen patients with an *M. avium* infection and 5 patients with an *M. intracellulare* infection had different VNTR genotypes of MAC strains from each of the two separate sputum samples (subsequent infections in independent events). One patient with an *M. avium* infection and one patient with an *M. intracellulare* infection had two different VNTR genotypes of strains from one sputum sample (simultaneous polyclonal infections). Three patients with an *M. avium* infection and one patient with an *M. intracellulare* infection all had two different VNTR genotypes of strains from one sputum sample and another VNTR genotype of a strain from another sputum sample (in total, three different VNTR genotypes from two sputum samples) (Tables E3 and E4, Figures E1 and E2).

Fifteen patients had mixed mycobacterial MAC infections. *M. avium* strains changed to *M. intracellulare* in six patients, to *M. abscessus* in two patients, *M. fortuitum* in one patient, and to an unidentified *Mycobacterium* spp. in one patient. *M. intracellulare* strains changed to *M. avium* in two patients, to *M. abscessus* in two patients, and to an unidentified *Mycobacterium* spp. in one patient. Except for the last patient, all patients had two or more positive cultures of the same mycobacterial species in a single year around the time of the second sputum sampling event.

In total, 42 patients (35.0%) were included in the polyclonal and mixed mycobacterial MAC infection group. Both patients with monoclonal MAC infections and polyclonal and mixed mycobacterial MAC infections had similar sampling intervals of their two sputum cultures (22.2±13.8 months *vs.* 24.2±17.7, respectively, P=0.89, Table 1).

We found 107 *M. avium* VNTR genotypes of *M. avium* strains, consisting of one

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genotype from each of 64 monoclonal infections, two genotypes from each of 17 subsequent polyclonal infections, and three genotypes from each of 3 subsequent and simultaneous polyclonal infections. Eighty-four genotypes were singletons, and 23 genotypes sharing identical genotypes were classified into nine different genotypes (types 2, 18, 21, 24, 26, 44, 61, 71, and 73, Table E3, Figure E1). Eventually, we found 93 different *M. avium* VNTR genotypes among the 107 genotypes (86.9% diversity). Twenty-nine *M. intracellulare* VNTR genotypes consisted of one genotype from each of 14 monoclonal infections, two genotypes from each of six subsequent polyclonal infections, and three genotypes from one subsequent and simultaneous infection. Twenty-five genotypes were singletons and four genotypes were classified into two different genotypes (types 2 and 10, Table E4, Figure E2). Eventually, 27 different genotypes were found among the 29 genotypes (93.1% diversity). *M. avium* and *M. intracellulare* strains from 15 patients with mixed mycobacterial MAC infections were not analyzed for VNTR genotypes.

Characteristics of pulmonary MAC patients with monoclonal MAC and polyclonal/mixed mycobacterial MAC infections

Patients with polyclonal and mixed mycobacterial MAC infections were more often male and had a higher prevalence of asthma than those with monoclonal MAC infections in univariate analysis (42.9% *vs*. 15.4%, respectively, OR 4.13, 95% CI 1.73–9.81, P<0.01; 16.7% *vs*. 1.3%, respectively, OR 15.40, 95% CI 1.82–129.98, P=0.013, Table 2). No significant differences were found in body mass index, duration of MAC disease from diagnosis to enrollment of the study, other underling diseases and conditions, use of immunosuppressants, smoking status, alcohol drinking habits, or laboratory findings. Seven patients currently used inhaled corticosteroids: fluticasone in one patient, budesonide in one patient, and a combination of fluticasone and salmeterol in five patients. The median daily dose of inhaled corticosteroids was 500 μg/day equivalents of fluticasone (200–500 μg/day). Seven patients with asthma and six patients using inhaled corticosteroids had subsequent polyclonal infections.

In both patients with monoclonal MAC and polyclonal and mixed mycobacterial MAC infections, nodules and bronchiectasis were the most common findings, and either the right middle lobe or left lingular area was predominantly involved (97.4% and 90.5%, respectively). Cavitation (cavitary form and cavitary and nodular bronchiectasis form) was more common among patients with monoclonal MAC infections than those with polyclonal and mixed mycobacterial MAC infections in univariate analysis (44.2% vs. 21.4%, P=0.014). There was no significant difference in the distribution of lung involvement or abnormalities of the thoracic skeleton between patients with monoclonal MAC and polyclonal/mixed mycobacterial MAC infections (Table 3).

Among 43 patients with cavitation, 34 patients had monoclonal MAC infections: 4 patients (11.8%) had a history of smoking, 14 patients (41.2%) had a drinking habit, 3 (8.8%) patient had a previous history of tuberculosis, and 1 patient (2.9%) had chronic obstructive

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pulmonary disease (COPD). Of the remaining nine patients who had polyclonal and mixed mycobacterial MAC infections, no patient had a history of smoking, two (22.2%) patients had a drinking habit, and two patients (22.2%) had a previous history of tuberculosis.

Among 102 patients who provided two sputum samples, 64 (62.7%) patients received treatment. Eight patients were treated prior to collecting the two samples. The remaining 56 patients provided the first sample before or after starting treatment; 41 and 15 patients provided the second samples during treatment and after discontinuing treatment, respectively. Among the former 41 patients, 14 patients (34.1%) converted sputum and 23 patients did not convert sputum. Among the 23 patients who provided the second samples after treatment, 9 patients (39.1%) converted and relapsed, and 14 patients did not convert sputum. Among the 38 patients without treatment, 26 (68.4%) patients maintained positive cultures, 12 patients converted sputum, and 5 patients relapsed. Eventually, 7 of 38 patients (18.4%) converted sputum. Among the 18 patients who provided a single sample, 11 patients received treatment and 7 patients did not receive treatment. All 18 patients converted sputum without relapse.

Association between types of MAC infection and environmental exposure

Patients with polyclonal and mixed mycobacterial MAC infections experienced significantly higher soil exposure, used a bathroom shower more frequently, and swam more frequently in a pool than patients with monoclonal MAC infections (61.9% *vs*. 30.8%, respectively, $P<0.01$;

90.5% *vs*. 70.5%, respectively, P=0.013; 16.7% *vs*. 1.3%, respectively, P<0.01, respectively, Table 4). Duration of soil exposure and experience of soil exposure before the diagnosis of pulmonary MAC disease and during the follow-up periods were not different between patients with polyclonal and mixed mycobacterial MAC infections and those with monoclonal MAC infections. Eight patients swam in a pool two to eight times per month. Six of the eight patients (75.0%) experienced swimming before the diagnosis of pulmonary MAC.

Association of types of soil or recovery of MAC strain with characteristic of patients or types of MAC infection

Fifty, 45, and 20 patients experienced high soil exposure (≥ 2 hours/week), low soil exposure (< 2 hours/week) and no exposure, respectively. Among the 95 patients with any soil exposure, 6, 36, and 53 patients, respectively, were involved with farm, potting, and yard soils. When the characteristics and environmental exposure of the patients were stratified with the types of soil, patients with high soil exposure were involved with more farm and potting soils (66.7% in farm soil, 66.7% in potting soil, and 41.5% in yard soil, P= 0.05).

M. avium and *M. intracellulare* strains were recovered from 33 (45.8%) of 72 soil samples (3 farm soil samples, 25 potting soil samples, and 37 yard soil samples); 22 samples (30.6%) contained monoclonal strains, and 11 samples (15.3%) contained polyclonal strains. Types of MAC infection, either monoclonal MAC or polyclonal and mixed mycobacterial MAC infection, were not associated with types of soils (farm, potting, or yard soils), soil

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without MAC strains, soil with monoclonal MAC strains, or soil with polyclonal MAC strains (Table 5). Clinical MAC and corresponding soil isolates with identical VNTR genotypes were identified from three patients with polyclonal infections and two patients with monoclonal infections. All five patients experienced high soil exposure (MA18-1a/bP, MA30a/bP, MA64a/bP, and MA77a/bP in Table E3 and MI21a/bP in Table E4).

Factors associated with polyclonal/mixed mycobacterial MAC infection

In the multivariate stepwise regression analysis, history of asthma (OR 11.56, 95% CI 1.41–255.77, P=0.021), high soil exposure (OR 4.31, 95% CI 1.72-11.45, P<0.01), shower use in a bathroom (OR 4.57, 95% CI 1.28–23.23, P=0.018), and swimming in a pool (OR 9.69, 95% CI 1.21–206.92, P=0.032) were significantly associated with polyclonal and mixed mycobacterial MAC infection (Table 6). We also found similar frequencies in each variable in *M. avium* and *M. intracellulare* infection. All significant variables were associated with polyclonal and mixed mycobacterial MAC infection due to *M. avium* infection, whereas no variables reached statistical significance in cases of *M. intracellulare* infection due to the small number of patients (Tables E5, E6, and E7).

Discussion

This is the first study to demonstrate that polyclonal and mixed mycobacterial infection in pulmonary MAC disease is significantly associated with environmental exposure after adjusting for confounding clinical and radiographic findings.

We demonstrated that high soil exposure was most significantly associated with polyclonal and mixed mycobacterial MAC infection after adjusting for confounding clinical and radiographic findings (Tables 4 and 6). All five patients with identical VNTR genotypes of pairs of MAC clinical and soil strains experienced high soil exposure. These patients experienced a long duration of soil exposure both before and after development of pulmonary MAC disease and were considered to have repetitive soil exposure from their residences. In a recent study, Wallace et al. showed that the *M. intracellulare* strain was absent from household water and biofilm samples and that pulmonary *M. intracellulare* patients are thought to acquire their pathogens from environmental sources other than household water (29). Because the *M. intracellulare* strain was isolated from soil (11, 12), soil could be a possible source of *M. intracellulare* infection.

Furthermore, we found both shower use in a bathroom and swimming in a pool were associated with polyclonal and mixed mycobacterial MAC infection. MAC is an environmental organism in water and has been found in a showerhead in a bathroom and a spa pool (7-9). A previous study reported that *M. avium* was recovered from showerheads and shower water in bathrooms that were genetically related to clinical isolates (8). Spraying plants with a spray bottle was also reported to be associated with pulmonary MAC disease

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(30). As we focused on susceptible hosts in this study, our findings cannot automatically be extended to the general population. However, repetitive exposure to soil or water is likely to cause new MAC or mixed mycobacterial infections in susceptible hosts.

Wallace et al. reported that monoclonal MAC infection was predominantly observed in cavitary disease (18). Although few patients had typical cavitary disease (male smokers with COPD or a previous history of tuberculosis) in our cohort, patients with monoclonal infections were more common than those with polyclonal and mixed mycobacterial MAC infections (Table 1, 2, and 3). Moreover, monoclonal infection was associated with female sex and cavitation (cavitary form and cavitary and nodular bronchiectasis form) (Tables 2, 3, and 5). Cavitary disease was often concomitant with nodules or bronchiectasis in our cohort as well as another cohort of pulmonary MAC disease in Japan (31). Because there have been a decreasing number of patients with tuberculosis and its associated morbidity, typical cavitary disease caused by previous events of tuberculosis has been greatly reduced. In the United States in the 1980s, patients with pulmonary MAC disease were predominantly elderly males with chronic lung disease and cavitary disease, whereas in the 2000s, the patient demographic changed to elderly women (32, 33). Nodular infiltrates and cylindrical bronchiectasis can progress to fibrocavitary disease that is indistinguishable from pre-existing bronchiectatic lung disease (34).

Various host traits, including asthma (35, 36), COPD (30, 36), gastroesophageal reflux disease (37), severe pneumonia (30), abnormalities of the thoracic skeleton such as thin scoliosis and pectus excavatum (38), and use of immunosuppressing agents (30, 35) or inhaled corticosteroids (35, 36), have been reported as risk factors for non-tuberculous mycobacterial pulmonary diseases. Although we found that a history of asthma was significantly associated with polyclonal and mixed mycobacterial MAC infection, the number of asthma patients was very low in each group. (Tables 2 and 6)

In addition to pulsed-field gel electrophoresis and restriction fragment length polymorphisms (5-9, 11, 13-16), VNTR analysis has become one of the most widely used genotyping methods and has the following advantages: easy to perform, time saving, cost efficient, and acceptable discriminatory power (26-28, 39, 40). The VNTR analysis showed good discriminatory power (86.9% diversity of *M. avium* genotypes and 93.1% diversity of *M. intracellulare* genotypes) in this study.

There were some limitations in our study. First, if we collected more than two sputum samples or colonies during the study period, more patients might have been diagnosed with polyclonal infections. However, different clones were rarely found between two separate cultures when those cultures contained the identical clone (15). Although we collected joined, multiple colonies from each sample, we determined only one or two genotypes. Second, as we collected the second samples about 2 years apart from the first samples without any consideration of clinical significance, we could not distinguish infection from colonization.

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However, as the patients primarily had two or more positive mycobacterial cultures in a single year around the time of the second sputum sample collection, we consider that almost all of the second strains were actual infections. Third, our sample size was insufficient to show host traits other than asthma that was associated with polyclonal and mixed mycobacterial MAC infection. Finally, because we collected soil samples from 60.0% of the participants but no water samples, our microbiological findings may support a limited picture of the route of transmission. Soil samples were self-collected by patients, and choice of soil samples and depth of soil were left to the individual patients. It was quite possible that relevant groups made different choices of soils. Actually, patients with high soil exposure were involved with farm and potting soils, probably due to planting or gardening. However, we reported that the types of soil did not affect recovery of MAC strains (12). Furthermore, we showed that types of MAC infection were not associated with types of soil, presence of MAC strains, and clonality (Table 5). As MAC is ubiquitous environmental bacteria, we considered that patient characteristics or behavioral activities would predict polyclonal and mixed mycobacterial MAC infection more than the results of the source of infection.

In conclusion, we demonstrate a strong association between environmental exposure and polyclonal and mixed mycobacterial MAC infection in patients with pulmonary MAC disease after adjusting for confounding clinical and radiographic findings, which would provide beneficial information of patients' care for preventing repetitive MAC infection in

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susceptible patients.

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Table 1. Genetic diversity of pulmonary *Mycobacterium avium* complex infections

	Patients with monoclonal MAC infections (n=78)	Patients with polyclonal MAC infections (n=27)	Patients with mixed mycobacterial MAC infections (n=15)
M. avium	64 (82.1)	20 (74.1)	[†] 10 (66.7)
M. intracellulare	14 (17.9)	7 (25.9)	[‡] 5 (33.3)
Sputum sampling intervals, month	22.2±13.8	24.2±17.7	

Definition of abbreviations: MAC = *Mycobacterium avium* complex.

Data show number (%) of patients or mean ± standard deviation.

[†] *M. avium* change to *M. intracellulare* 6; *M. abscessus* 2; *M. fortuitum* 1; *Mycobacterium* spp. 1.

[‡] *M. intracellulare* change to *M. avium* 2; *M. abscessus* 2; *Mycobacterium* spp. 1.

Table 2. Characteristics of patients with monoclonal infections and polyclonal and mixed mycobacterial *Mycobacterium avium* complex infections in pulmonary MAC disease

Variable	Patients with monoclonal MAC infections (n=78)	Patients with polyclonal and mixed mycobacterial MAC infections (n=42)	P value
Age, years	63.0 (55.8–70.0)	65.0 (60.0–71.3)	0.21
Gender, male	12 (15.4)	18 (42.9)	<0.01
Body mass index, kg/m^2	18.7 (17.4–20.5)	19.1 (17.1–21.6)	0.36
Duration of MAC disease, years	5.5 (3.0–9.0)	6.0 (4.0–8.0)	0.73
Underlying disease			
Lung disease	19 (24.4)	13 (31.0)	0.44
COPD	4 (5.1)	1 (2.4)	0.66
Asthma	1 (1.3)	7 (16.7)	<0.01
Previous tuberculosis	7 (9.0)	4 (9.2)	>0.99
Severe pneumonia	20 (25.6)	5 (11.9)	0.077
Previous malignant disease	14 (18.0)	6 (14.3)	0.61
Diabetes mellitus	3 (3.9)	2 (4.8)	>0.99
Liver disease	4 (5.1)	1 (2.4)	0.66
Renal disease	2 (2.6)	0 (0.0)	0.54
Autoimmune disease	10 (12.8)	4 (11.7)	0.77
Rheumatoid arthritis	5 (6.4)	4 (9.5)	0.72
Gastroesophageal reflux disease symptoms	22 (28.2)	10 (23.8)	0.60
Immunosuppressing agents	4 (5.3)	4 (9.5)	0.45
Inhaled corticosteroids	2 (2.6)	5 (11.9)	0.050
Smoking status (never)	66 (84.6)	30 (71.4)	0.085
Alcohol drinking habit	33 (42.3)	15 (35.7)	0.48
Laboratory findings			
White blood cell, $\times 10^3/\mu L$	5.60 (4.48–6.76)	5.40 (4.58–6.53)	0.74
Hemoglobin, g/dL	12.7 (12.0–13.9)	13.3 (12.3–14.7)	0.077
Hematocrit, %	38.8 (36.7–41.4)	40.3 (37.5–44.3)	0.070
Platelet, $\times 10^6/\mu L$	2.14 (1.73–2.47)	1.98 (1.55–2.51)	0.37
C-reactive protein, mg/dL	0.20 (0.0–0.98)	0.10 (0.0–0.40)	0.20
Erythrocyte sedimentation rate, mm/hour	17.5 (10.0–38.8)	13.0 (5.0–35.0)	0.22
Total protein, g/dL	7.2 (6.8–7.5)	6.9 (6.7–7.5)	0.33

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Albumin, g/dL	4.1 (3.8–4.3)	4.1 (3.9–4.4)	0.40
Treatment for MAC disease			
Not receiving treatment	[†]29 (37.2)	16 (38.1)	0.92
Receiving treatment	[‡]49 (62.8)	26 (61.9)	

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; MAC = *Mycobacterium avium* complex.

Data show either number (%) of patients or median (interquartile ranges). An alcohol drinking habit was defined as at least one drink per week. [†]Seven patients who provided a single sample were included. [‡]Eleven patients who provided a single sample were included.

Table 3. High-resolution computed tomography features of patients with monoclonal *Mycobacterium avium* complex infections and polyclonal and mixed mycobacterial infections

Variable	Patients with monoclonal MAC infections (n=77)	Patients with polyclonal and mixed mycobacterial MAC infections (n=42)	P value
Radiographic pattern			
Nodular and bronchiectasis form	36 (46.8)	26 (61.9)	0.11
Cavitary form	16 (20.8)	5 (11.9)	0.32
Cavitary + nodular and bronchiectasis form	18 (23.4)	4 (9.5)	0.084
Unclassified form	7 (9.0)	7 (16.7)	0.22
HRCT findings			
Nodule	70 (90.9)	39 (92.9)	0.71
Bronchiectasis	69 (89.6)	35 (83.3)	0.32
Cavity	34 (44.2)	9 (21.4)	0.014
Consolidation	55 (71.4)	30 (71.4)	>0.99
Location			
Right upper lobe	48 (62.3)	26 (61.9)	0.96
Right middle lobe	68 (88.3)	36 (85.7)	0.68
Right lower lobe	51 (66.2)	25 (59.5)	0.54
Left upper lobe	35 (45.5)	19 (45.2)	0.98
Lingular	63 (81.8)	34 (81.0)	0.91
Left lower lobe	43 (55.8)	19 (45.2)	0.27
Thoracic abnormality			
Scoliosis	20 (25.6)	12 (28.6)	0.73
Pectus excavatum	9 (11.5)	6 (14.3)	0.66

Definition of abbreviations: HRCT = High-resolution computed tomography, MAC = *Mycobacterium avium* complex.

Data show number (%) of patients. One patient did not undergo HRCT.

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Table 4. Environmental exposures of patients with monoclonal *Mycobacterium avium* complex infections and polyclonal and mixed mycobacterial infections

Variable	Patients with monoclonal MAC infections (n=78)	Patients with polyclonal and mixed mycobacterial MAC infections (n=42)	P value
Soil exposure			
High (≥2 hours per week)	24 (30.8)	26 (61.9)	<0.01
*Duration of soil exposure	16.8 (50-50.0)	19.2 (2.0-54.0)	0.87
[¶]Soil exposure before diagnosis	21 (87.5)	22 (84.6)	0.63
[§]Soil exposure during the followed-up periods	21 (87.5)	23 (88.5)	0.85
Water exposure			
Bathing (≥2 per day)	1 (1.3)	0 (0.0)	>0.99
Shower use in a bathroom	55 (70.5)	38 (90.5)	0.013
Dish washing (≥2 per day)	60 (76.9)	27 (64.3)	0.20
Swimming in a pool	1 (1.3)	7 (16.7)	<0.01

Definition of abbreviations: MAC = *Mycobacterium avium* complex.

Data show number (%) of patients. Soil exposure includes farming and gardening, or any activities involving soil exposure.

Table 5. Recovery of *Mycobacterium avium* complex strains from soil samples at the residences of patients with pulmonary MAC disease

Type of soil samples	Patients with monoclonal MAC infections (n=46)	Patients with polyclonal and mixed mycobacterial MAC infections (n=26)	P value
Farm	2 (4.3)	3 (11.5)	0.24
Potting	16 (34.8)	12 (46.1)	
Yard	28 (60.9)	11 (42.3)	
No MAC strain	22 (47.8)	17 (65.4)	
Monoclonal MAC strains	17 (37.0)	5 (19.2)	0.27
Polyclonal MAC strains	7 (15.2)	4 (15.4)	
MAC strains of identical VNTR genotypes with one clinical isolates	3 (6.5)	2 (7.7)	>0.99

Definition of abbreviations: MAC = *Mycobacterium avium-intracellulare* complex; VNTR = variable number of tandem repeats.

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Table 6. Factors associated with polyclonal and mixed mycobacterial infections of pulmonary *Mycobacterium avium* complex disease

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
Male gender	4.13 (1.73–9.81)	<0.01	2.94 (0.98–9.12)	0.054
History of asthma	15.40 (1.82–129.98)	0.013	11.56 (1.41–255.77)	0.021
No cavitation	2.90 (1.22–6.88)	0.014	2.50 (0.91–7.48)	0.075
High soil exposure	3.66 (1.19–11.70)	<0.01	4.31 (1.72–11.45)	<0.01
Shower use in a bathroom	3.73 (1.19–11.70)	0.018	4.57 (1.28–23.23)	0.018
Swimming in a pool	15.40 (1.82–129.98)	<0.01	9.69 (1.21–206.92)	0.032

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

Cavitation includes cavitary form and cavitary and nodular bronchiectasis form.

All values were included in multivariate analysis if the probability values were less than 0.05 by univariate analysis.

Figure legends

Figure 1. Flow chart of the study.

MAC = *Mycobacterium avium* complex

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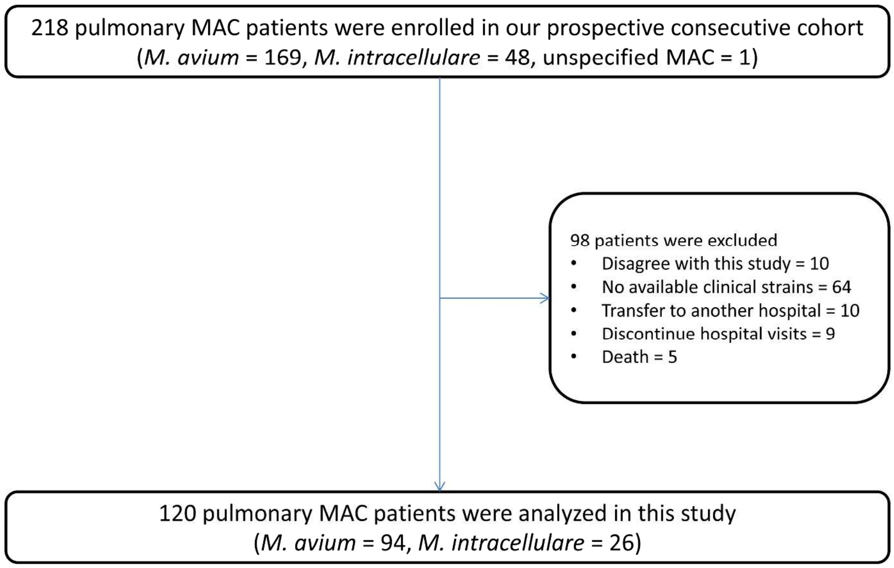

Figure 1. Flow chart of the study.
MAC = *Mycobacterium avium* complex

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Association between polyclonal and mixed mycobacterial *Mycobacterium avium* complex infection and environmental exposure

ONLINE DATA SUPPLEMENT

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Figure E1. *M. avium* phylogenetic tree

The phylogenetic tree was constructed by variable number of tandem repeats. Phylip software ver. 3.69 was used for analysis. Black, red, and blue fonts indicate monoclonal, polyclonal, and both monoclonal and polyclonal infection, respectively.

Figure E2. *M. intracellulare* phylogenetic tree

The phylogenetic tree was constructed by variable number of tandem repeats. Phylip software ver. 3.69 was used for analysis. Black and red fonts indicate monoclonal and polyclonal infection, respectively.

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Table E1. Primer sets for *M. avium* VNTR analysis

VNTR locus	PCR primer sequences	PCR product size (bp)	Repeat unit size (bp)
MATR-1	5':GAACGTTGGGCCGAATGCGA 5':GTGTCGGACCCCTCCCGTAA	334	53
MATR-2	5':TTGAGCAGCTCGTAAAGCGT 5':CGCGCTCAAGGAGATGGTTC	300	53
MATR-3	5':CCAATCACAACGGCACCATC 5':TCCTCGACAATCAGCACACT	460	53
MATR-4	5':GACAATGGCATGCCGATCCT 5':CGCTACGGCCTTCTCCATCT	274	53
MATR-5	5':CTTGCAGCAGGACGATCAGG 5':GTGGTCGAAGTCGCTGTTGG	307	58
MATR-6	5':TCGCAGGAAACCAACCTCAA 5':GCGTGATCGACTCGAAGACC	384	58
MATR-7	5':CCGAGGAAGAGACGAAACCC 5':TCGTCACCCACAACATGCAG	391	57
MATR-8	5':CAGGTCCAGGGCATGTTTCC 5':TCCCGATAATCCGTTGCATGAC	334	57
MATR-9	5':CTGTTGGAGCGCAGCCGTTT 5':ACCCAGTCGTCGACGGTGTT	435	55
MATR-11	5':TGGCTGCTGTTCAATTGGATG 5':TCGTCGGTCAATTGCACCTT	559	55
MATR-12	5':TGATGGCGACCACCGACAAGG 5':TGGATGCGGCCGACCAACA	542	57
MATR-13	5':CCTCGAAGGTGGCGGACTTG 5':ACCAGGATGGTGCCCAAACC	347	56
MATR-14	5':TGGTCGCCGCACACCTACT 5':GCCCTTACTGGGCAGGTCCTTC	447	58
MATR-15	5':GGAAGGCAGCAAGGGTCAAC 5':TCAGGTCCAGCGACAGCTTC	422	57
MATR-16	5':GTGGTCAGCACCCGGAGAGT 5':ACCACCGACTGCTCGACCTT	418	59

Definition of abbreviations: VNTR = variable number of tandem repeat; MATR = *Mycobacterium avium* tandem repeat. *M. avium* strain 104 was used as reference.

Table E2. Primer sets for *M. intracellulare* VNTR analysis

VNTR locus	PCR primer sequences	PCR product size (bp)	Repeat unit size (bp)
MITR-1	5’:TCGCCGAGGACTTCGTCT	273	57
	5’:GTCACCACGAGGAAGATCG		
MITR-2	5’:AGGGTGGTGAACGCGTAG	299	57
	5’:CTCTGGCAGCCCGATACC		
MITR-3	5’:AGAGGTGCTGCCGATTACAC	222	58
	5’:TCCTTGTCCGGTTCCTTTTG		
MITR-4	5’:AGGCTTCAATTCGGGTGAC	271	55
	5’:TTCCGACCACCTACATCGAG		
MITR-5	5’:GACGACGACGGTGTTGGT	223	54
	5’:GATCGTCTCGCTGGTGGAC		
MITR-6	5’:GGTCGATCCGGTCAGCTC	228	56
	5’:CACCTTGATGGGCGATGT		
MITR-7	5’:TTTCATGGTTCGCCCTCTAC	274	53
	5’:GTTCGTCGGAGGTCATGGT		
MITR-8	5’:TCAAACTCATTTGCGCTGAG	270	57
	5’:CGACATCTGGTTCTTCGACTC		
MITR-9	5’:GGTCACTGGCTTCTCTCCTG	256	56
	5’:CACAGCTACGCCGCAGAC		
MITR-10	5’:GGCTGGTTCTTCTGGTGAC	353	57
	5’:CGCGTCAAGGAACGTCAT		
MITR-11	5’:TGTTGCGCTGAGGTCATATC	189	57
	5’:ACAGGTTGTCGGTCATTGGT		
MITR-12	5’:AGACCAACCCAGAAAAGTGC	245	53
	5’:GTCGTGATACGCCGAATTG		
MITR-13	5’:GTTCAGCGAGCCGGTATCT	292	50
	5’:AGCTCTCGCAGCTTGGTTC		
MITR-14	5’:ATGCCGGTTAGTCTCTCACG	258	56
	5’:GCTCGTCGATCCAGAAAGAG		
MITR-15	5’:GCAAACGCAGTGGTACTCAG	219	58
	5’:GATGATGCCGAGCACCTG		
MITR-16	5’:GGACGCTTTGTATCCGAGTT	265	55
	5’:ACCGTGTCGGTGACTTGAAC		

Definition of abbreviations: VNTR = variable number of tandem repeat; MITR = *Mycobacterium intracellulare* tandem repeat. *M. intracellulare* ATCC 13950 was used as reference.

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1 Table E3. VNTR profiles of *Mycobacterium avium* strains isolated from patients with *M. avium* infection

Patient ID	Isolate	MATR genotype	MATR locus														
			1	2	3	4	5	6	7	8	9	11	12	13	14	15	16
MACstudy1	MA1-1aP	Type1	3	1	2	2	3	2	1	1	2	2	3	0	2	2	3
MACstudy1	MA1-1bP	Type1	3	1	2	2	3	2	1	1	2	2	3	0	2	2	3
MACstudy2	MA2-1aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy2	MA2-1bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy2	MA2-2aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy2	MA2-2bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy3	MA3-1aP	Type3	2	1	1	1	2	2	6	2	3	2	3	0	2	2	3
MACstudy3	MA3-1bP	Type3	2	1	1	1	2	2	6	2	3	2	3	0	2	2	3
MACstudy6	MA6-1aP	Type4	2	0	1	1	2	1	6	2	3	2	3	0	2	2	3
MACstudy6	MA6-1bP	Type4	2	0	1	1	2	1	6	2	3	2	3	0	2	2	3
MACstudy6	MA6-2aP	Type4	2	0	1	1	2	1	6	2	3	2	3	0	2	2	3
MACstudy6	MA6-2bP	Type4	2	0	1	1	2	1	6	2	3	2	3	0	2	2	3
MACstudy8	MA8-1aP	Type5	2	1	5	1	2	1	3	2	1	2	3	0	2	2	3
MACstudy8	MA8-1bP	Type5	2	1	5	1	2	1	3	2	1	2	3	0	2	2	3
MACstudy8	MA8-2aP	Type5	2	1	5	1	2	1	3	2	1	2	3	0	2	2	3
MACstudy8	MA8-2bP	Type5	2	1	5	1	2	1	3	2	1	2	3	0	2	2	3
MACstudy9	MA9-1aP	Type6	2	0	5	1	2	1	6	2	1	2	3	2	2	2	3
MACstudy9	MA9-1bP	Type6	2	0	5	1	2	1	6	2	1	2	3	2	2	2	3
MACstudy9	MA9-2aP	Type6	2	0	5	1	2	1	6	2	1	2	3	2	2	2	3

MACstudy9	MA9-2bP	Type6	2	0	5	1	2	1	6	2	1	2	3	2	2	2	3
MACstudy10	MA10-1aP	Type7	2	0	5	1	2	1	1	2	2	2	3	2	2	2	3
MACstudy10	MA10-1bP	Type7	2	0	5	1	2	1	1	2	2	2	3	2	2	2	3
MACstudy10	MA10-2aP	Type7	2	0	5	1	2	1	1	2	2	2	3	2	2	2	3
MACstudy10	MA10-2bP	Type7	2	0	5	1	2	1	1	2	2	2	3	2	2	2	3
MACstudy11	MA11-1aP	Type8	2	3	1	1	2	1	1	1	0	1	3	1	2	2	2
MACstudy11	MA11-1bP	Type8	2	3	1	1	2	1	1	1	0	1	3	1	2	2	2
MACstudy11	MA11-2aP	Type9	4	2	4	0	3	2	3	1	0	1	3	1	2	2	2
MACstudy11	MA11-2bP	Type9	4	2	4	0	3	2	3	1	0	1	3	1	2	2	2
MACstudy12	MA12-1aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy12	MA12-1bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy12	MA12-2aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy12	MA12-2bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy13	MA13-1aP	Type11	3	1	1	1	2	2	6	2	2	2	3	0	3	2	3
MACstudy13	MA13-1bP	Type11	3	1	1	1	2	2	6	2	2	2	3	0	3	2	3
MACstudy13	MA13-2aP	Type11	3	1	1	1	2	2	6	2	2	2	3	0	3	2	3
MACstudy13	MA13-2bP	Type11	3	1	1	1	2	2	6	2	2	2	3	0	3	2	3
MACstudy14	MA14-1aP	Type12	3	2	3	2	3	1	1	2	2	1	3	2	1	2	3
MACstudy14	MA14-1bP	Type12	3	2	3	2	3	1	1	2	2	1	3	2	1	2	3
MACstudy14	MA14-2aP	Type13	0	0	2	1	1	1	4	2	1	1	2	0	2	2	3
MACstudy14	MA14-2bP	Type13	0	0	2	1	1	1	4	2	1	1	2	0	2	2	3
MACstudy16	MA16-1aP	Type14	1	0	5	1	2	2	2	2	2	2	3	1	2	2	3
MACstudy16	MA16-1bP	Type14	1	0	5	1	2	2	2	2	2	2	3	1	2	2	3

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MACstudy16	MA16-2aP	Type14	1	0	5	1	2	2	2	2	2	2	3	1	2	2	3
MACstudy16	MA16-2bP	Type14	1	0	5	1	2	2	2	2	2	2	3	1	2	2	3
MACstudy17	MA17-1aP	Type15	2	1	4	1	2	2	2	2	2	2	2	2	3	2	3
MACstudy17	MA17-1bP	Type16	2	1	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy17	MA17-2aP	Type17	4	3	2	1	2	2	2	1	2	2	3	2	2	2	3
MACstudy17	MA17-2bP	Type17	4	3	2	1	2	2	2	1	2	2	3	2	2	2	3
MACstudy18	MA18-1aP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy18	MA18-1bP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy18	MA18-2aP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy18	MA18-2bP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy20	MA20-1aP	Type19	2	0	1	1	2	2	2	2	3	2	3	2	2	2	3
MACstudy20	MA20-1bP	Type19	2	0	1	1	2	2	2	2	3	2	3	2	2	2	3
MACstudy20	MA20-2aP	Type19	2	0	1	1	2	2	2	2	3	2	3	2	2	2	3
MACstudy20	MA20-2bP	Type19	2	0	1	1	2	2	2	2	3	2	3	2	2	2	3
MACstudy23	MA23-1aP	Type20	1	1	1	1	2	2	1	2	2	1	3	2	2	2	3
MACstudy23	MA23-1bP	Type20	1	1	1	1	2	2	1	2	2	1	3	2	2	2	3
MACstudy23	MA23-2aP	Type20	1	1	1	1	2	2	1	2	2	1	3	2	2	2	3
MACstudy23	MA23-2bP	Type20	1	1	1	1	2	2	1	2	2	1	3	2	2	2	3
MACstudy28	MA28-1aP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy28	MA28-1bP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy28	MA28-2aP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy28	MA28-2bP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy29	MA29-1aP	Type22	1	0	4	1	1	1	6	2	2	2	3	0	3	2	3

MACstudy29	MA29-1bP	Type23	1	0	4	1	2	0	4	1	2	2	3	0	3	2	3
MACstudy29	MA29-2aP	Type24	1	0	4	1	2	0	4	1	2	1	3	0	2	2	2
MACstudy29	MA29-2bP	Type24	1	0	4	1	2	0	4	1	2	1	3	0	2	2	2
MACstudy30	MA30-1aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy30	MA30-1bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy30	MA30-2aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy30	MA30-2bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy31	MA31-1aP	Type26	2	0	5	2	2	1	6	2	2	2	3	0	2	2	1
MACstudy31	MA31-1bP	Type26	2	0	5	2	2	1	6	2	2	2	3	0	2	2	1
MACstudy31	MA31-2aP	Type27	2	0	5	2	2	1	6	2	2	2	3	0	2	2	3
MACstudy31	MA31-2bP	Type27	2	0	5	2	2	1	6	2	2	2	3	0	2	2	3
MACstudy32	MA32-1aP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy32	MA32-1bP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy32	MA32-2aP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy32	MA32-2bP	Type18	1	1	1	1	2	1	1	1	3	1	3	0	3	2	2
MACstudy33	MA33-1aP	Type28	1	0	1	0	1	0	0	0	2	2	3	1	3	2	2
MACstudy33	MA33-1bP	Type28	1	0	1	0	1	0	0	0	2	2	3	1	3	2	2
MACstudy33	MA33-2aP	Type29	2	2	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy33	MA33-2bP	Type29	2	2	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy34	MA34-1aP	Type30	1	1	1	1	2	2	1	1	3	1	3	0	3	2	3
MACstudy34	MA34-1bP	Type30	1	1	1	1	2	2	1	1	3	1	3	0	3	2	3
MACstudy34	MA34-2aP	Type30	1	1	1	1	2	2	1	1	3	1	3	0	3	2	3
MACstudy34	MA34-2bP	Type30	1	1	1	1	2	2	1	1	3	1	3	0	3	2	3

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MACstudy35	MA35-1aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy35	MA35-1bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy35	MA35-2aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy35	MA35-2bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy36	MA36-1aP	Type31	2	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy36	MA36-1bP	Type31	2	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy36	MA36-2aP	Type31	2	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy36	MA36-2bP	Type31	2	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy38	MA38-1aP	Type32	1	1	1	2	2	1	1	1	3	1	3	0	3	2	2
MACstudy38	MA38-1bP	Type32	1	1	1	2	2	1	1	1	3	1	3	0	3	2	2
MACstudy38	MA38-2aP	Type32	1	1	1	2	2	1	1	1	3	1	3	0	3	2	2
MACstudy38	MA38-2bP	Type32	1	1	1	2	2	1	1	1	3	1	3	0	3	2	2
MACstudy39	MA39-1aP	Type33	2	0	4	1	2	1	4	2	2	2	3	0	2	2	3
MACstudy39	MA39-1bP	Type33	2	0	4	1	2	1	4	2	2	2	3	0	2	2	3
MACstudy40	MA40-1aP	Type34	1	1	1	1	2	2	5	2	3	1	3	0	3	2	3
MACstudy40	MA40-1bP	Type34	1	1	1	1	2	2	5	2	3	1	3	0	3	2	3
MACstudy40	MA40-2aP	Type34	1	1	1	1	2	2	5	2	3	1	3	0	3	2	3
MACstudy40	MA40-2bP	Type34	1	1	1	1	2	2	5	2	3	1	3	0	3	2	3
MACstudy41	MA41-1aP	Type35	1	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy41	MA41-1bP	Type35	1	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy41	MA41-2aP	Type35	1	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy41	MA41-2bP	Type35	1	0	1	1	2	1	2	2	3	2	3	0	3	2	3
MACstudy43	MA43-1aP	Type35	2	1	1	1	2	1	5	5	1	1	3	0	1	2	3

MACstudy43	MA43-1bP	Type36	2	1	1	1	2	1	5	5	1	1	3	0	1	2	3
MACstudy43	MA43-2aP	Type36	2	1	1	1	2	1	5	5	1	1	3	0	1	2	3
MACstudy43	MA43-2bP	Type36	2	1	1	1	2	1	5	5	1	1	3	0	1	2	3
MACstudy47	MA47-1aP	Type37	1	1	1	1	2	1	1	1	4	1	3	0	2	2	2
MACstudy47	MA47-1bP	Type37	1	1	1	1	2	1	1	1	4	1	3	0	2	2	2
MACstudy48	MA48-1aP	Type27	2	0	5	2	2	1	6	2	2	2	3	0	2	2	1
MACstudy48	MA48-1bP	Type27	2	0	5	2	2	1	6	2	2	2	3	0	2	2	1
MACstudy48	MA48-2aP	Type38	1	2	1	1	2	1	1	1	4	1	3	0	2	2	2
MACstudy48	MA48-2bP	Type38	1	2	1	1	2	1	1	1	4	1	3	0	2	2	2
MACstudy50	MA50-1aP	Type39	2	0	1	2	2	2	1	2	2	2	3	0	2	2	3
MACstudy50	MA50-1bP	Type39	2	0	1	2	2	2	1	2	2	2	3	0	2	2	3
MACstudy50	MA50-2aP	Type40	0	0	3	0	2	0	5	2	2	1	2	2	2	2	3
MACstudy50	MA50-2bP	Type40	0	0	3	0	2	0	5	2	2	1	2	2	2	2	3
MACstudy51	MA51-1aP	Type41	3	1	0	4	2	1	0	2	2	0	3	1	2	2	2
MACstudy51	MA51-1bP	Type41	3	1	0	4	2	1	0	2	2	0	3	1	2	2	2
MACstudy51	MA51-2aP	Type42	2	2	2	2	3	2	2	1	4	1	3	0	3	2	2
MACstudy51	MA51-2bP	Type42	2	2	2	2	3	2	2	1	4	1	3	0	3	2	2
MACstudy52	MA52-1aP	Type43	1	0	5	1	2	1	6	2	3	2	3	2	2	2	3
MACstudy52	MA52-1bP	Type43	1	0	5	1	2	1	6	2	3	2	3	2	2	2	3
MACstudy56	MA56-1aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy56	MA56-1bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy56	MA56-2aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy56	MA56-2bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2

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MACstudy57	MA57-1aP	Type44	3	0	1	1	2	2	6	2	2	2	3	0	2	2	3
MACstudy57	MA57-1bP	Type44	3	0	1	1	2	2	6	2	2	2	3	0	2	2	3
MACstudy57	MA57-2aP	Type44	3	0	1	1	2	2	6	2	2	2	3	0	2	2	3
MACstudy57	MA57-2bP	Type44	3	0	1	1	2	2	6	2	2	2	3	0	2	2	3
MACstudy58	MA58-1aP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy58	MA58-1bP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy58	MA58-2aP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy58	MA58-2bP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy60	MA60-1aP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy60	MA60-1bP	Type45	2	0	1	1	2	2	2	2	3	1	3	2	2	2	3
MACstudy61	MA61-1aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy61	MA61-1bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy61	MA61-2aP	Type46	1	2	1	1	2	1	1	1	2	1	3	0	2	2	2
MACstudy61	MA61-2bP	Type46	1	2	1	1	2	1	1	1	2	1	3	0	2	2	2
MACstudy62	MA62-1aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy62	MA62-1bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy62	MA62-2aP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy62	MA62-2bP	Type2	1	1	1	2	2	1	1	1	2	1	3	0	2	2	2
MACstudy63	MA63-1aP	Type47	2	0	1	1	2	2	6	2	2	2	3	2	2	2	3
MACstudy63	MA63-1bP	Type47	2	0	1	1	2	2	6	2	2	2	3	2	2	2	3
MACstudy63	MA63-2aP	Type47	2	0	1	1	2	2	6	2	2	2	3	2	2	2	3
MACstudy63	MA63-2bP	Type47	2	0	1	1	2	2	6	2	2	2	3	2	2	2	3
MACstudy64	MA64-1aP	Type48	2	0	1	1	2	2	1	2	3	2	3	2	2	2	3

MACstudy64	MA64-1bP	Type48	2	0	1	1	2	2	1	2	3	2	3	2	2	2	3
MACstudy64	MA64-2aP	Type49	1	2	1	1	2	1	1	2	2	1	3	0	2	2	2
MACstudy64	MA64-2bP	Type49	1	2	1	1	2	1	1	2	2	1	3	0	2	2	2
MACstudy65	MA65-1aP	Type50	2	0	1	1	2	2	1	2	2	2	3	2	2	2	3
MACstudy65	MA65-1bP	Type50	2	0	1	1	2	2	1	2	2	2	3	2	2	2	3
MACstudy65	MA65-2aP	Type50	2	0	1	1	2	2	1	2	2	2	3	2	2	2	3
MACstudy65	MA65-2bP	Type50	2	0	1	1	2	2	1	2	2	2	3	2	2	2	3
MACstudy66	MA66-1aP	Type51	2	1	5	1	2	2	2	2	2	2	3	2	3	2	3
MACstudy66	MA66-1bP	Type51	2	1	5	1	2	2	2	2	2	2	3	2	3	2	3
MACstudy66	MA66-2aP	Type52	2	2	3	2	3	2	2	2	4	3	3	0	3	2	2
MACstudy66	MA66-2bP	Type52	2	2	3	2	3	2	2	2	4	3	3	0	3	2	2
MACstudy69	MA69-1aP	Type53	3	1	2	2	2	1	2	2	4	3	4	0	2	2	3
MACstudy69	MA69-1bP	Type53	3	1	2	2	2	1	2	2	4	3	4	0	2	2	3
MACstudy69	MA69-2aP	Type53	3	1	2	2	2	1	2	2	4	3	4	0	2	2	3
MACstudy69	MA69-2bP	Type53	3	1	2	2	2	1	2	2	4	3	4	0	2	2	3
MACstudy70	MA70-1aP	Type54	1	1	3	2	3	3	2	1	2	2	3	2	2	2	2
MACstudy70	MA70-1bP	Type54	1	1	3	2	3	3	2	1	2	2	3	2	2	2	2
MACstudy70	MA70-2aP	Type55	3	3	5	2	3	2	6	2	4	2	3	2	3	2	2
MACstudy70	MA70-2bP	Type55	3	3	5	2	3	2	6	2	4	2	3	2	3	2	2
MACstudy72	MA72-1aP	Type56	1	1	1	2	2	1	1	2	3	1	3	0	3	2	3
MACstudy72	MA72-1bP	Type56	1	1	1	2	2	1	1	2	3	1	3	0	3	2	3
MACstudy72	MA72-2aP	Type56	1	1	1	2	2	1	1	2	3	1	3	0	3	2	3
MACstudy72	MA72-2bP	Type56	1	1	1	2	2	1	1	2	3	1	3	0	3	2	3

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MACstudy73	MA73-1aP	Type57	2	0	1	1	2	2	1	2	2	1	3	2	2	2	3
MACstudy73	MA73-1bP	Type57	2	0	1	1	2	2	1	2	2	1	3	2	2	2	3
MAVstudy73	MA73-2aP	Type58	2	3	0	1	2	0	6	4	3	3	3	0	2	2	3
MAVstudy73	MA73-2bP	Type59	3	2	2	1	2	2	1	2	3	1	3	4	2	2	3
MACstudy74	MA74-1aP	Type60	2	0	5	1	2	1	4	2	2	2	3	0	2	2	3
MACstudy74	MA74-1bP	Type60	2	0	5	1	2	1	4	2	2	2	3	0	2	2	3
MACstudy75	MA75-1aP	Type61	2	0	5	0	2	1	4	2	2	2	3	2	3	2	2
MACstudy75	MA75-1bP	Type61	2	0	5	0	2	1	4	2	2	2	3	2	3	2	2
MACstudy75	MA75-2aP	Type61	2	0	5	0	2	1	4	2	2	2	3	2	3	2	2
MACstudy75	MA75-2bP	Type61	2	0	5	0	2	1	4	2	2	2	3	2	3	2	2
MACstudy76	MA76-1aP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy76	MA76-1bP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy76	MA76-2aP	Type63	2	0	2	2	2	2	1	1	2	1	3	0	2	2	2
MACstudy76	MA76-2bP	Type63	2	0	2	2	2	2	1	1	2	1	3	0	2	2	2
MACstudy77	MA77-1aP	Type64	2	0	2	2	2	2	2	2	2	2	3	2	3	2	3
MACstudy77	MA77-1bP	Type64	2	0	2	2	2	2	2	2	2	2	3	2	3	2	3
MACstudy77	MA77-2aP	Type64	2	0	2	2	2	2	2	2	2	2	3	2	3	2	3
MACstudy77	MA77-2bP	Type64	2	0	2	2	2	2	2	2	2	2	3	2	3	2	3
MACstudy79	MA79-1aP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy79	MA79-1bP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy79	MA79-2aP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy79	MA79-2bP	Type21	1	2	1	2	2	1	1	1	2	1	3	0	3	2	2
MACstudy80	MA80-1aP	Type65	2	0	1	2	2	1	2	2	2	2	3	1	2	2	3

MACstudy80	MA80-1bP	Type65	2	0	1	2	2	1	2	2	2	2	3	1	2	2	3
MACstudy80	MA80-2aP	Type66	1	2	5	1	2	1	1	4	1	1	3	1	3	2	2
MACstudy80	MA80-2bP	Type67	2	2	4	2	3	1	1	4	1	2	3	1	3	2	2
MACstudy84	MA84-1aP	Type68	1	0	1	1	2	2	1	2	1	2	3	2	2	2	3
MACstudy84	MA84-1bP	Type68	1	0	1	1	2	2	1	2	1	2	3	2	2	2	3
MACstudy84	MA84-2aP	Type68	1	0	1	1	2	2	1	2	1	2	3	2	2	2	3
MACstudy84	MA84-2bP	Type68	1	0	1	1	2	2	1	2	1	2	3	2	2	2	3
MACstudy87	MA87-1aP	Type69	4	2	3	2	2	3	2	3	4	3	4	3	4	3	4
MACstudy87	MA87-1bP	Type69	4	2	3	2	2	3	2	3	4	3	4	3	4	3	4
MACstudy87	MA87-2aP	Type70	2	1	3	1	2	1	5	2	2	2	4	0	3	2	3
MACstudy87	MA87-2bP	Type70	2	1	3	1	2	1	5	2	2	2	4	0	3	2	3
MACstudy88	MA88-1aP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy88	MA88-1bP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy88	MA88-2aP	Type71	1	1	1	1	1	0	1	2	0	2	3	0	3	2	2
MACstudy88	MA88-2bP	Type71	1	1	1	1	1	0	1	2	0	2	3	0	3	2	2
MACstudy89	MA89-1aP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy89	MA89-1bP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy89	MA89-2aP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy89	MA89-2bP	Type62	1	1	1	2	2	1	1	1	4	1	3	0	3	2	2
MACstudy90	MA90-1aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy90	MA90-1bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy90	MA90-2aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy90	MA90-2bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3

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MACstudy91	MA91-1aP	Type72	2	0	1	1	2	2	2	2	2	2	3	0	3	2	2
MACstudy91	MA91-1bP	Type72	2	0	1	1	2	2	2	2	2	2	3	0	3	2	2
MACstudy91	MA91-2aP	Type72	2	0	1	1	2	2	2	2	2	2	3	0	3	2	2
MACstudy91	MA91-2bP	Type72	2	0	1	1	2	2	2	2	2	2	3	0	3	2	2
MACstudy92	MA92-1aP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy92	MA92-1bP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy92	MA92-2aP	Type74	2	1	1	1	2	2	2	2	3	2	3	2	3	2	3
MACstudy92	MA92-2bP	Type74	2	1	1	1	2	2	2	2	3	2	3	2	3	2	3
MACstudy93	MA93-1aP	Type75	2	1	2	1	2	1	2	1	3	1	3	0	3	2	2
MACstudy93	MA93-1bP	Type75	2	1	2	1	2	1	2	1	3	1	3	0	3	2	2
MACstudy93	MA93-2aP	Type75	2	1	2	1	2	1	2	1	3	1	3	0	3	2	2
MACstudy93	MA93-2bP	Type75	2	1	2	1	2	1	2	1	3	1	3	0	3	2	2
MACstudy94	MA94-1aP	Type76	1	1	1	2	2	2	1	2	1	1	3	0	3	2	3
MACstudy94	MA94-1bP	Type76	1	1	1	2	2	2	1	2	1	1	3	0	3	2	3
MACstudy94	MA94-2aP	Type76	1	1	1	2	2	2	1	2	1	1	3	0	3	2	3
MACstudy94	MA94-2bP	Type76	1	1	1	2	2	2	1	2	1	1	3	0	3	2	3
MACstudy96	MA96-1aP	Type77	2	1	1	1	2	1	1	1	3	1	3	0	2	2	2
MACstudy96	MA96-1bP	Type77	2	1	1	1	2	1	1	1	3	1	3	0	2	2	2
MACstudy96	MA96-2aP	Type77	2	1	1	1	2	1	1	1	3	1	3	0	2	2	2
MACstudy96	MA96-2bP	Type77	2	1	1	1	2	1	1	1	3	1	3	0	2	2	2
MACstudy98	MA98-1aP	Type78	2	0	3	1	2	2	1	2	3	2	3	0	2	2	3
MACstudy98	MA98-1bP	Type78	2	0	3	1	2	2	1	2	3	2	3	0	2	2	3
MACstudy99	MA99-1aP	Type79	2	1	1	1	3	2	1	4	6	2	3	1	3	2	2

MACstudy99	MA99-1bP	Type80	2	2	2	1	3	1	1	1	3	2	3	1	3	2	2
MACstudy99	MA99-2aP	Type80	2	2	2	1	3	1	1	1	3	2	3	1	3	2	2
MACstudy99	MA99-2bP	Type80	2	2	2	1	3	1	1	1	3	2	3	1	3	2	2
MACstudy103	MA103-1aP	Type81	2	1	5	1	2	1	1	2	3	2	3	0	3	2	3
MACstudy103	MA103-1bP	Type81	2	1	5	1	2	1	1	2	3	2	3	0	3	2	3
MACstudy103	MA103-2aP	Type81	2	1	5	1	2	1	1	2	3	2	3	0	3	2	3
MACstudy103	MA103-2bP	Type81	2	1	5	1	2	1	1	2	3	2	3	0	3	2	3
MACstudy105	MA105-1aP	Type82	1	1	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy105	MA105-1bP	Type82	1	1	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy105	MA105-2aP	Type82	1	1	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy105	MA105-2bP	Type82	1	1	1	1	2	1	1	2	3	1	3	0	3	2	2
MACstudy106	MA106-1aP	Type83	1	1	1	1	2	2	1	2	3	2	3	2	2	2	3
MACstudy106	MA106-1bP	Type83	1	1	1	1	2	2	1	2	3	2	3	2	2	2	3
MACstudy106	MA106-2aP	Type83	1	1	1	1	2	2	1	2	3	2	3	2	2	2	3
MACstudy106	MA106-2bP	Type83	1	1	1	1	2	2	1	2	3	2	3	2	2	2	3
MACstudy108	MA108-1aP	Type84	1	1	1	0	2	1	2	2	3	1	2	0	2	2	2
MACstudy108	MA108-1bP	Type84	1	1	1	0	2	1	2	2	3	1	2	0	2	2	2
MACstudy109	MA109-1aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy109	MA109-1bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy109	MA109-2aP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy109	MA109-2bP	Type10	2	0	1	1	2	2	2	2	2	2	3	2	2	2	3
MACstudy112	MA112-1aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy112	MA112-1bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2

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MACstudy113	MA113-1aP	Type85	2	1	5	1	2	1	2	1	2	2	3	2	3	2	3
MACstudy113	MA113-1bP	Type85	2	1	5	1	2	1	2	1	2	2	3	2	3	2	3
MACstudy114	MA114-1aP	Type86	0	0	5	1	2	1	1	2	3	2	3	2	3	2	1
MACstudy114	MA114-1bP	Type86	0	0	5	1	2	1	1	2	3	2	3	2	3	2	1
MACstudy115	MA115-1aP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy115	MA115-1bP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy115	MA115-2aP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy115	MA115-2bP	Type73	2	0	5	1	2	1	6	2	2	2	3	0	2	2	3
MACstudy117	MA117-1aP	Type87	2	2	2	2	3	3	2	1	3	1	3	0	3	2	3
MACstudy117	MA117-1bP	Type87	2	2	2	2	3	3	2	1	3	1	3	0	3	2	3
MACstudy117	MA117-2aP	Type87	2	2	2	2	3	3	2	1	3	1	3	0	3	2	3
MACstudy117	MA117-2bP	Type87	2	2	2	2	3	3	2	1	3	1	3	0	3	2	3
MACstudy118	MA118-1aP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy118	MA118-1bP	Type25	1	1	1	1	2	1	1	1	2	1	3	0	3	2	2
MACstudy123	MA123-1aP	Type88	2	0	1	1	2	2	2	2	2	1	3	1	2	2	3
MACstudy123	MA123-1bP	Type88	2	0	1	1	2	2	2	2	2	1	3	1	2	2	3
MACstudy123	MA123-2aP	Type88	2	0	1	1	2	2	2	2	2	1	3	1	2	2	3
MACstudy123	MA123-2bP	Type88	2	0	1	1	2	2	2	2	2	1	3	1	2	2	3
MACstudy127	MA127-1aP	Type89	3	2	2	2	3	3	3	2	3	1	3	2	2	2	3
MACstudy127	MA127-1bP	Type89	3	2	2	2	3	3	3	2	3	1	3	2	2	2	3
MACstudy127	MA127-2aP	Type89	3	2	2	2	3	3	3	2	3	1	3	2	2	2	3
MACstudy127	MA127-2bP	Type89	3	2	2	2	3	3	3	2	3	1	3	2	2	2	3
MACstudy128	MA128-1aP	Type90	2	1	5	1	2	1	1	2	1	1	3	2	2	2	3

MACstudy128	MA128-1bP	Type90	2	1	5	1	2	1	1	2	1	1	3	2	2	2	3
MACstudy128	MA128-2aP	Type90	2	1	5	1	2	1	1	2	1	1	3	2	2	2	3
MACstudy128	MA128-2bP	Type90	2	1	5	1	2	1	1	2	1	1	3	2	2	2	3
MACstudy129	MA129-1aP	Type91	2	2	1	1	2	2	2	2	1	1	3	2	2	2	3
MACstudy129	MA129-1bP	Type91	2	2	1	1	2	2	2	2	1	1	3	2	2	2	3
MACstudy129	MA129-2aP	Type91	2	2	1	1	2	2	2	2	1	1	3	2	2	2	3
MACstudy129	MA129-2bP	Type91	2	2	1	1	2	2	2	2	1	1	3	2	2	2	3
MACstudy133	MA133-1aP	Type92	1	0	2	1	2	2	2	2	2	2	3	0	2	2	3
MACstudy133	MA133-1bP	Type92	1	0	2	1	2	2	2	2	2	2	3	0	2	2	3
MACstudy133	MA133-2aP	Type92	1	0	2	1	2	2	2	2	2	2	3	0	2	2	3
MACstudy133	MA133-2bP	Type92	1	0	2	1	2	2	2	2	2	2	3	0	2	2	3
MACstudy134	MA134-1aP	Type93	0	0	1	0	2	1	1	1	3	1	3	0	3	2	2
MACstudy134	MA134-1bP	Type93	0	0	1	0	2	1	1	1	3	1	3	0	3	2	2
MACstudy134	MA134-2aP	Type93	0	0	1	0	2	1	1	1	3	1	3	0	3	2	2
MACstudy134	MA134-2bP	Type93	0	0	1	0	2	1	1	1	3	1	3	0	3	2	2

Definition of abbreviations: VNTR = variable number of tandem repeats; MATR = *Mycobacterium avium* tandem repeats.

Isolates from the first and the second sputum samples are shown as “1a/b” and “2a/b,” respectively.

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Table E4. VNTR profiles of *Mycobacterium intracellulare* strains isolated from patients with *M. intracellulare* infection

Patient ID	Isolate	MITR genotype	MITR locus															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MACstudy5	MI5-1aP	Type1	2	3	2	4	3	3	3	0	2	5	4	3	2	3	2	2
MACstudy5	MI5-1bP	Type1	2	3	2	4	3	3	3	0	2	5	4	3	2	3	2	2
MACstudy5	MI5-2aP	Type2	2	3	1	3	2	2	3	2	3	4	2	2	3	2	2	2
MACstudy5	MI5-2bP	Type2	2	3	1	3	2	2	3	2	3	4	2	2	3	2	2	2
MACstudy21	MI21-1aP	Type2	2	3	1	3	2	2	3	2	3	4	2	2	3	2	2	2
MACstudy21	MI21-1bP	Type2	2	3	1	3	2	2	3	2	3	4	2	2	3	2	2	2
MACstudy21	MI21-2aP	Type3	1	2	1	3	2	2	3	0	2	4	4	2	3	2	3	2
MACstudy21	MI21-2bP	Type4	1	1	1	2	2	2	3	2	2	4	3	2	3	2	3	2
MACstudy22	MI22-1aP	Type5	2	3	1	4	0	2	2	2	3	4	4	3	3	3	3	2
MACstudy22	MI22-1bP	Type5	2	3	1	4	0	2	2	2	3	4	4	3	3	3	3	2
MACstudy25	MI25-1aP	Type6	1	1	1	1	3	3	3	0	3	4	2	2	3	2	2	2
MACstudy25	MI25-1bP	Type6	1	1	1	1	3	3	3	0	3	4	2	2	3	2	2	2
MACstudy25	MI25-2aP	Type6	1	1	1	1	3	3	3	0	3	4	2	2	3	2	2	2
MACstudy25	MI25-2bP	Type6	1	1	1	1	3	3	3	0	3	4	2	2	3	2	2	2
MACstudy37	MI37-1aP	Type7	1	2	0	2	1	2	2	0	2	3	2	2	3	2	2	2
MACstudy37	MI37-1bP	Type7	1	2	0	2	1	2	2	0	2	3	2	2	3	2	2	2
MACstudy42	MI42-1aP	Type8	2	3	0	3	2	2	2	2	2	1	2	2	3	2	2	2
MACstudy42	MI42-1bP	Type8	2	3	0	3	2	2	2	2	2	1	2	2	3	2	2	2
MACstudy42	MI42-2aP	Type8	2	3	0	3	2	2	2	2	2	1	2	2	3	2	2	2

MACstudy42	MI42-2bP	Type8	2	3	0	3	2	2	2	2	2	1	2	2	3	2	2	2
MACstudy45	MI45-1aP	Type9	3	4	1	3	2	3	2	2	2	1	4	2	3	2	2	2
MACstudy45	MI45-1bP	Type9	3	4	1	3	2	3	2	2	2	1	4	2	3	2	2	2
MACstudy45	MI45-2aP	Type10	2	1	1	1	2	2	2	0	1	1	2	2	3	2	2	2
MACstudy45	MI45-2bP	Type10	2	1	1	1	2	2	2	0	1	1	2	2	3	2	2	2
MACstudy46	MI46-1aP	Type11	2	2	2	4	2	3	3	0	2	1	3	2	2	2	3	2
MACstudy46	MI46-1bP	Type11	2	2	2	4	2	3	3	0	2	1	3	2	2	2	3	2
MACstudy46	MI46-2aP	Type11	2	2	2	4	2	3	3	0	2	1	3	2	2	2	3	2
MACstudy46	MI46-2bP	Type11	2	2	2	4	2	3	3	0	2	1	3	2	2	2	3	2
MACstudy49	MI49-1aP	Type10	2	1	1	1	2	2	2	0	1	1	2	2	3	2	2	2
MACstudy49	MI49-1bP	Type10	2	1	1	1	2	2	2	0	1	1	2	2	3	2	2	2
MACstudy49	MI49-2aP	Type12	2	2	1	4	2	3	3	0	2	4	4	3	2	3	3	2
MACstudy49	MI49-2bP	Type12	2	2	1	4	2	3	3	0	2	4	4	3	2	3	3	2
MACstudy54	MI54-1aP	Type13	2	2	1	1	0	2	2	2	2	4	2	2	3	2	1	2
MACstudy54	MI54-1bP	Type13	2	2	1	1	0	2	2	2	2	4	2	2	3	2	1	2
MACstudy68	MI68-1aP	Type14	2	3	1	3	2	4	3	0	2	2	2	3	3	2	2	2
MACstudy68	MI68-1bP	Type14	2	3	1	3	2	4	3	0	2	2	2	3	3	2	2	2
MACstudy68	MI68-2aP	Type14	2	3	1	3	2	4	3	0	2	2	2	3	3	2	2	2
MACstudy68	MI68-2bP	Type14	2	3	1	3	2	4	3	0	2	2	2	3	3	2	2	2
MACstudy71	MI71-1aP	Type15	2	1	1	1	2	3	1	0	2	1	4	2	2	2	1	2
MACstudy71	MI71-1bP	Type15	2	1	1	1	2	3	1	0	2	1	4	2	2	2	1	2
MACstudy71	MI71-2aP	Type15	2	1	1	1	2	3	1	0	2	1	4	2	2	2	1	2
MACstudy71	MI71-2bP	Type15	2	1	1	1	2	3	1	0	2	1	4	2	2	2	1	2

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MACstudy78	MI78-1aP	Type16	1	1	0	2	0	5	0	1	2	1	2	2	3	2	2	2
MACstudy78	MI78-1bP	Type16	1	1	0	2	0	5	0	1	2	1	2	2	3	2	2	2
MACstudy78	MI78-2aP	Type17	2	1	0	3	2	3	1	2	2	1	2	2	3	1	2	2
MACstudy78	MI78-2bP	Type17	2	1	0	3	2	3	1	2	2	1	2	2	3	1	2	2
MACstudy82	MI82-1aP	Type18	2	4	2	3	2	3	2	2	1	4	2	2	3	2	2	2
MACstudy82	MI82-1bP	Type18	2	4	2	3	2	3	2	2	1	4	2	2	3	2	2	2
MACstudy82	MI82-2aP	Type18	2	4	2	3	2	3	2	2	1	4	2	2	3	2	2	2
MACstudy82	MI82-2bP	Type18	2	4	2	3	2	3	2	2	1	4	2	2	3	2	2	2
MACstudy83	MI83-1aP	Type19	2	1	1	1	0	3	3	0	2	1	4	3	3	2	2	2
MACstudy83	MI83-1bP	Type19	2	1	1	1	0	3	3	0	2	1	4	3	3	2	2	2
MACstudy83	MI83-2aP	Type20	1	3	1	1	2	3	3	2	2	3	2	2	3	2	2	2
MACstudy83	MI83-2bP	Type20	1	3	1	1	2	3	3	2	2	3	2	2	3	2	2	2
MACstudy95	MI95-1aP	Type21	2	4	2	3	2	3	3	2	3	4	2	2	3	1	2	2
MACstudy95	MI95-1bP	Type21	2	4	2	3	2	3	3	2	3	4	2	2	3	1	2	2
MACstudy100	MI100-1aP	Type22	2	1	0	2	2	2	2	2	2	1	4	2	3	2	2	2
MACstudy100	MI100-1bP	Type23	2	3	0	2	2	2	2	1	2	1	4	2	3	2	2	2
MACstudy100	MI100-2aP	Type22	2	1	0	2	2	2	2	2	2	1	4	2	3	2	2	2
MACstudy100	MI100-2bP	Type22	2	1	0	2	2	2	2	2	2	1	4	2	3	2	2	2
MACstudy116	MI116-1aP	Type24	3	1	1	4	3	3	2	1	2	1	4	2	3	2	1	2
MACstudy116	MI116-1bP	Type24	3	1	1	4	3	3	2	1	2	1	4	2	3	2	1	2
MACstudy122	MI122-1aP	Type25	2	2	2	4	2	3	3	2	3	4	2	2	3	2	2	2
MACstudy122	MI122-1bP	Type25	2	2	2	4	2	3	3	2	3	4	2	2	3	2	2	2
MACstudy122	MI122-2aP	Type25	2	2	2	4	2	3	3	2	3	4	2	2	3	2	2	2

MACstudy122	MI122-2bP	Type25	2	2	2	4	2	3	3	2	3	4	2	2	3	2	2	2
MACstudy130	MI130-1aP	Type26	3	4	0	1	0	2	3	3	2	1	2	2	2	3	1	2
MACstudy130	MI130-1bP	Type26	3	4	0	1	0	2	3	3	2	1	2	2	2	3	1	2
MACstudy130	MI130-2aP	Type26	3	4	0	1	0	2	3	3	2	1	2	2	2	3	1	2
MACstudy130	MI130-2bP	Type26	3	4	0	1	0	2	3	3	2	1	2	2	2	3	1	2
MACstudy132	MI132-1aP	Type27	2	1	1	3	2	2	2	0	1	1	4	2	3	2	1	2
MACstudy132	MI132-1bP	Type27	2	1	1	3	2	2	2	0	1	1	4	2	3	2	1	2
MACstudy132	MI132-2aP	Type27	2	1	1	3	2	2	2	0	1	1	4	2	3	2	1	2
MACstudy132	MI132-2bP	Type27	2	1	1	3	2	2	2	0	1	1	4	2	3	2	1	2

Definition of abbreviations: VNTR = variable number of tandem repeats; MITR = *Mycobacterium intracellulare* tandem repeats.

Isolates from the first and the second sputum sample are shown as “1a/b” and “2a/b,” respectively.

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Table E5. Characteristics, high-resolution computed tomography features, and environmental exposures of patients with monoclonal infections and polyclonal and mixed mycobacterial *Mycobacterium avium* (MAV) infections in pulmonary MAV disease

Variable	Patients with monoclonal MAV Infection (n=64)	Patients with polyclonal and mixed mycobacterial MAV infection (n=30)	P value
Age, years	62.0 (55.3-69.0)	64.5 (59.8-70.3)	0.28
Gender, female	55 (85.9)	17 (56.7)	<0.01
Body mass index, kg/m^2	19.4 (17.8-20.9)	19.2 (18.1-21.6)	0.73
Duration of MAV disease, year	6.0 (3.0-9.8)	6.0 (4.0-9.0)	0.96
Underlying disease			
Lung disease	15 (23.4)	10 (33.3)	0.31
COPD	3 (4.7)	1 (3.3)	>0.99
Bronchial asthma	1 (1.6)	6 (20.0)	<0.01
Previous tuberculosis	6 (9.4)	3 (10.0)	>0.99
Severe pneumonia	15 (23.4)	3 (10.0)	0.16
Previous malignant disease	10 (15.6)	3 (10.0)	0.54
Diabetes mellitus	3 (4.7)	1 (3.3)	>0.99
Liver disease	4 (6.3)	1 (3.3)	>0.99
Renal disease	2 (3.1)	0 (0.0)	>0.99
Autoimmune disease	6 (9.4)	3 (10.0)	>0.99
Rheumatoid arthritis	4 (6.3)	3 (10.0)	0.68
Gastroesophageal reflux disease symptoms	18 (28.1)	8 (26.7)	>0.99
Immunosuppressing agents	1 (1.6)	3 (10.0)	0.094
Inhaled corticosteroids	1 (1.6)	4 (13.3)	0.035
Smoking status (never)	53 (82.8)	20 (66.7)	0.080
Alcohol drinking habit	26 (40.6)	10 (33.3)	0.50
Laboratory findings			
White blood cell , $\times 10^3/\mu L$	5.60 (4.42-6.48)	5.10 (4.58-6.45)	0.72
Haemoglobin, g/dL	12.8 (12.0-13.9)	13.2 (12.0-14.7)	0.23
Haematocrit, %	38.8 (36.8-41.5)	40.2 (37.2-44.3)	0.24
Platelet, $\times 10^6/\mu L$	2.11 (1.67-2.46)	1.98 (1.51-2.38)	0.38

C-reactive protein, mg/dL	0.10 (0.0-0.65)	0.10 (0.0-0.30)	0.76
Erythrocyte sedimentation rate, mm/hour	14.0 (10.0-37.8)	14.0 (4.8-27.8)	0.23
Total protein, g/dL	7.2 (6.8-7.5)	6.8 (6.6-7.4)	0.028
Albumin, g/dL	4.1 (4.0-4.4)	4.1 (3.9-4.3)	0.89
Radiographic pattern			
Nodular and bronchiectasis form	34 (53.1)	21 (70.0)	0.14
Cavitary form	13 (20.3)	2 (6.7)	0.13
Cavitary + nodular and bronchiectasis form	11 (17.2)	2 (6.7)	0.21
Unclassified form	5 (7.8)	5 (16.7)	0.19
HRCT findings			
Nodule	57 (90.5)	28 (93.3)	>0.99
Bronchiectasis	57 (90.5)	25 (83.3)	0.32
Cavity	24 (38.1)	4 (13.3)	0.016
Consolidation	44 (69.8)	22 (73.3)	0.73
Location			
Right upper lobe	38 (60.3)	18 (60.0)	>0.99
Right middle lobe	55 (87.3)	25 (83.3)	0.61
Right lower lobe	38 (60.3)	16 (53.3)	0.52
Left upper lobe	26 (41.3)	13 (43.3)	0.85
Lingular	50 (79.4)	23 (76.7)	0.77
Left lower lobe	34 (54.0)	12 (40.0)	0.21
Thoracic abnormality			
Scoliosis	13 (20.3)	8 (26.7)	0.49
Pectus excavatum	6 (9.4)	6 (20.0)	0.15
Environmental exposure			
Soil exposure			
High, ≥2h per week	20 (31.3)	18 (60.0)	<0.01
Water exposure			
Bathing, ≥2 per day	1 (1.6)	0 (0.0)	>0.99
Shower use in a bathroom	46 (71.9)	29 (96.7)	<0.01
Dish washing, ≥2 per day	49 (76.6)	19 (63.3)	0.18
Swimming in a pool	1 (1.6)	6 (20.0)	<0.01

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HRCT = high-resolution computed tomography; MAV = *Mycobacterium avium*.

Data show either number (%) of patients or median (interquartile ranges).

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Table E6. Characteristics, high-resolution computed tomography features, and environmental exposures of patients with monoclonal infections and polyclonal and mixed mycobacterial *Mycobacterium intracellulare* (MIN) infections in pulmonary MIN disease

Variable	Patients with monoclonal MIN infection (n=14)	Patients with polyclonal and mixed mycobacterial MIN infection (n=12)	P value
Age, years	66.5 (58.0-73.3)	67.5 (58.8-76.8)	0.84
Gender, female	11 (78.6)	7 (58.3)	0.40
Body mass index, kg/m^2	17.3 (15.2-18.6)	19.0 (16.6-21.8)	0.19
Duration of MIN disease, year	2.5 (1.0-8.0)	6.5 (4.0-7.8)	0.27
Underlying disease			
Lung disease	4 (28.6)	3 (25.0)	>0.99
COPD	1 (7.1)	0 (0.0)	>0.99
Bronchial asthma	0 (0.0)	1 (8.3)	0.46
Previous tuberculosis	1 (7.1)	1 (8.3)	>0.99
Severe pneumonia	5 (35.7)	2 (16.7)	0.39
Previous malignant disease	4 (28.6)	3 (25.0)	>0.99
Diabetes mellitus	0 (0.0)	1 (8.3)	0.46
Liver disease	0 (0.0)	0 (0.0)	-
Renal disease	0 (0.0)	0 (0.0)	-
Autoimmune disease	4 (28.6)	1 (8.3)	0.33
Rheumatoid arthritis	1 (7.1)	1 (8.3)	>0.99
Gastroesophageal reflux disease symptoms	4 (28.6)	2 (16.7)	0.65
Immunosuppressing agents	3 (21.4)	1 (8.3)	0.60
Inhaled corticosteroids	1 (7.1)	1 (8.3)	>0.99
Smoking status (never)	13 (92.9)	10 (83.3)	0.58
Alcohol drinking habit	7 (50.0)	5 (41.7)	0.67
Laboratory findings			
White blood cell , $\times 10^3/\mu L$	6.45 (4.63-8.13)	6.00 (4.75-6.88)	0.54
Haemoglobin, g/dL	11.7 (11.7-13.2)	13.4 (12.6-14.7)	0.094
Haematocrit, %	39.1 (35.7-40.6)	40.9 (39.1-44.6)	0.14
Platelet, $\times 10^6/\mu L$	2.16 (1.98-2.48)	2.01 (1.59-2.83)	0.63

C-reactive protein, mg/dL	0.65 (0.28-2.4)	0.10 (0.0-1.2)	0.034
Erythrocyte sedimentation rate, mm/hour	28.5 (12.5-42.0)	13.0 (6.0-43.0)	0.41
Total protein, g/dL	7.1 (6.6-7.3)	7.5 (6.8-7.9)	0.13
Albumin, g/dL	3.9 (3.5-4.2)	4.2 (3.6-4.6)	0.16
Radiographic pattern			
Nodular and bronchiectasis form	2 (14.3)	5 (41.7)	0.19
Cavitary form	3 (21.4)	3 (25.0)	>0.99
Cavitary + nodular and bronchiectasis form	7 (50.0)	2 (16.7)	0.11
Unclassified form	2 (14.3)	2 (16.7)	>0.99
HRCT findings			
Nodule	13 (92.9)	11 (91.7)	>0.99
Bronchiectasis	12 (85.7)	10 (83.3)	>0.99
Cavity	10 (71.4)	5 (41.7)	0.23
Consolidation	11 (78.6)	8 (66.7)	0.67
Location			
Right upper lobe	10 (71.4)	8 (66.7)	>0.99
Right middle lobe	13 (92.9)	11 (91.7)	>0.99
Right lower lobe	13 (92.9)	9 (75.0)	0.31
Left upper lobe	9 (64.3)	6 (50.0)	0.46
Lingular	13 (92.9)	11 (91.7)	>0.99
Left lower lobe	9 (64.3)	7 (58.3)	0.76
Thoracic abnormality			
Scoliosis	7 (50.0)	4 (33.3)	0.45
Pectus excavatum	3 (21.4)	0 (0.0)	0.22
Environmental exposure			
Soil exposure			
High, ≥2h per week	4 (28.6)	8 (66.7)	0.11
Water exposure			
Bathing, ≥2 per day	0 (0.0)	0 (0.0)	-
Shower use in a bathroom	9 (64.3)	9 (75.0)	0.68
Dish washing, ≥2 per day	11 (78.6)	8 (66.7)	0.67
Swimming in a pool	0 (0.0)	1 (8.3)	0.46

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; HRCT = high-resolution computed tomography; MIN = *Mycobacterium intracellulare*.

Data show either number (%) of patients or median (interquartile ranges).

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Table E7. Factors associated with polyclonal and mixed mycobacterial infections of pulmonary *Mycobacterium avium* disease.

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Male gender	4.67 (1.73–13.2)	<0.01	4.57 (1.17–20.10)	0.029
History of asthma	15.75 (2.51–305.61)	<0.01	18.19 (1.70–499.71)	0.015
No cavitation	4.00 (1.35–14.80)	0.011	4.06 (1.02–21.74)	0.046
High soil exposure	3.30 (1.36–8.32)	<0.01	4.02 (1.28–13.73)	0.017
Shower use in a bathroom	11.35 (2.16–209.51)	<0.01	35.72 (2.23-5006.2)	<0.01
Swimming in a pool	15.75 (2.51–305.61)	<0.01	9.20 (0.99–225.27)	0.051

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

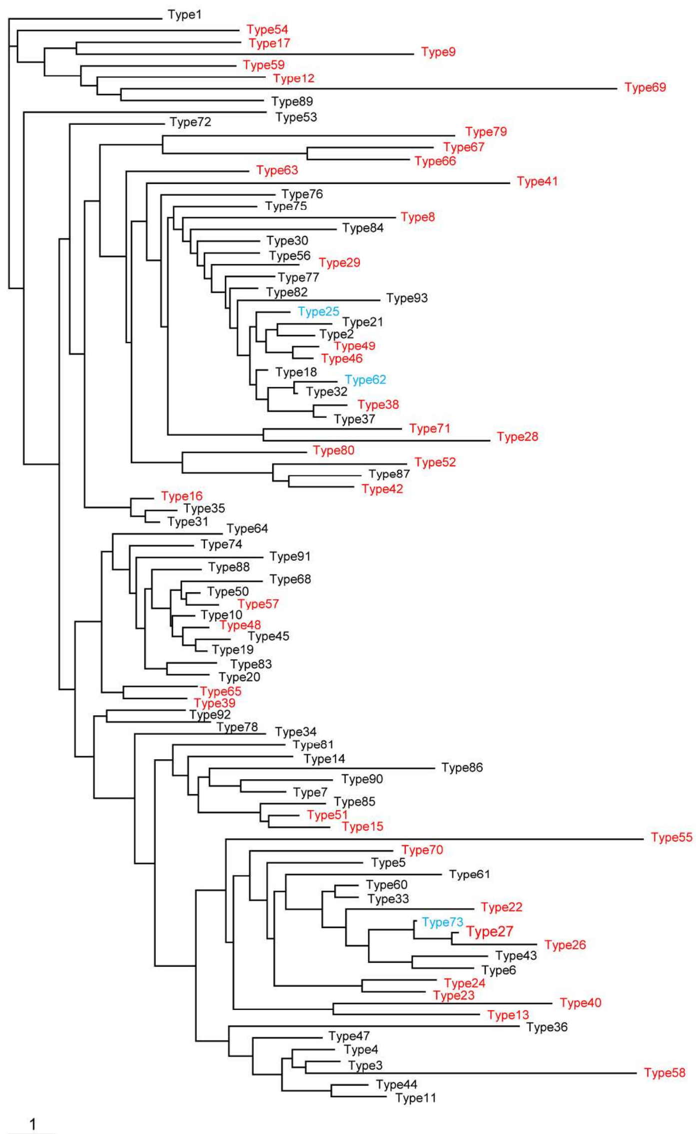

Figure E1. *M. avium* phylogenetic tree
The phylogenetic tree was constructed by variable number of tandem repeats. Phylip software ver. 3.69 was used for analysis. Black, red, and blue fonts indicate monoclonal, polyclonal, and both monoclonal and polyclonal infection, respectively.
157x235mm (300 x 300 DPI)

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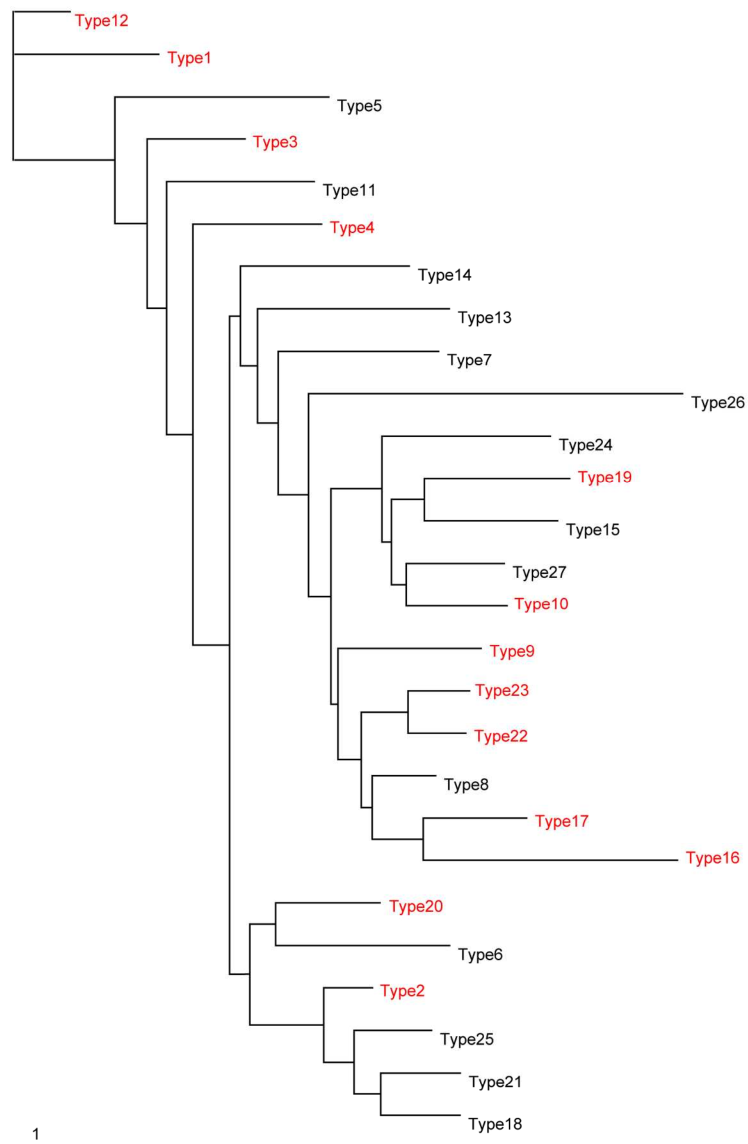

Figure E2. *M. intracellulare* phylogenetic tree
The phylogenetic tree was constructed by variable number of tandem repeats. Phylip software ver. 3.69 was used for analysis. Black and red fonts indicate monoclonal and polyclonal infection, respectively.
149x208mm (300 x 300 DPI)