2008

Association between 16S-23S internal transcribed spacer sequence groups of Mycobacterium avium complex and pulmonary disease

Jason E. Stout
*Duke University Medical Center*

Gregory W. Hopkins
*Duke University Medical Center*

Jay R. McDonald
*Washington University School of Medicine in St. Louis*

Anita Quinn
*Genomic Services Division*

Carol D. Hamilton
*Duke University Medical Center*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

**Recommended Citation**

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.
Association between 16S-23S Internal Transcribed Spacer Sequence Groups of *Mycobacterium avium* Complex and Pulmonary Disease

Jason E. Stout, Gregory W. Hopkins, Jay R. McDonald, Anita Quinn, Carol D. Hamilton, L. Barth Reller and Richard Frothingham


Updated information and services can be found at: [http://jcm.asm.org/content/46/8/2790](http://jcm.asm.org/content/46/8/2790)

**SUPPLEMENTAL MATERIAL**

Supplemental material

**REFERENCES**

This article cites 26 articles, 18 of which can be accessed free at: [http://jcm.asm.org/content/46/8/2790#ref-list-1](http://jcm.asm.org/content/46/8/2790#ref-list-1)

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](http://journals.asm.org/site/misc/reprints.xhtml)

Information about commercial reprint orders: [http://journals.asm.org/site/misc/reprints.xhtml](http://journals.asm.org/site/misc/reprints.xhtml)

To subscribe to another ASM Journal go to: [http://journals.asm.org/site/subscriptions/](http://journals.asm.org/site/subscriptions/)
Association between 16S-23S Internal Transcribed Spacer Sequence Groups of Mycobacterium avium Complex and Pulmonary Disease

Jason E. Stout, 1* Gregory W. Hopkins, 2 Jay R. McDonald, 3‡ Anita Quinn, 4§ Carol D. Hamilton, 5 L. Barth Reller, 6 and Richard Frothingham 7

Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 2Human Vaccine Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 3Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 4Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 5Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 6Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, North Carolina; 7Department of Pathology, Duke University Medical Center, Durham, North Carolina; and Human Vaccine Institute, Department of Medicine, Duke University Medical Center, Durham, North Carolina, and Infectious Diseases Section, Veterans Affairs Medical Center, Durham, North Carolina

Organisms within the Mycobacterium avium complex (MAC) may have differential virulence. We compared 33 subjects with MAC pulmonary disease to 75 subjects with a single positive culture without disease. M. avium isolates were significantly more likely to be associated with MAC pulmonary disease (odds ratio = 5.14, 95% confidence interval = 1.25 to 22.73) than M. intracellulare.

Exposure to aerosols containing organisms in the Mycobacterium avium complex (MAC) likely occurs often (11, 12), but resultant pulmonary disease is uncommon (13, 16). The MAC group of organisms consists of two relatively common named species, M. avium and M. intracellulare, and a third group of organisms that until recently were not denoted by species names. Whether some species of MAC are more likely to cause pulmonary disease than others is unknown.

(This article was previously published as an abstract [abstr. A820] and presented as an oral presentation at the May 2007 American Thoracic Society Meeting, San Francisco, CA.)

We performed a case-control study comparing persons with MAC pulmonary disease to persons with a single positive respiratory culture but no evidence of disease. Persons who had a respiratory specimen that grew MAC between 1 January 1993 and 30 June 1999 and available medical records were considered for inclusion (n = 600). Case subjects were considered for inclusion if they met the 1997 American Thoracic Society criteria for MAC pulmonary disease (1). The following criteria were used to exclude both potential cases and controls: (i) human immunodeficiency virus infection, (ii) cystic fibrosis, (iii) age under 18 years, (iv) recipient of organ or bone marrow transplant, (v) pulmonary alveolar proteinosis, and (iv) active tuberculosis. The control group was randomly selected from patients with a positive respiratory MAC culture who did not meet American Thoracic Society criteria and had a low likelihood of having MAC pulmonary disease, including no radiographic evidence of MAC pulmonary disease. All controls had (i) a single positive culture for a MAC organism from a respiratory site (as described above), (ii) negative acid-fast smears on the same specimen and all other respiratory specimens collected, and (iii) at least two mycobacterial cultures obtained from the respiratory tract. Patients were excluded from the control group if they had (i) a diagnosis of MAC pulmonary disease by an infectious disease specialist or a pulmonologist, (ii) received a specific therapy directed at MAC pulmonary disease (as defined above under inclusion criteria for cases), or (iii) granulomatous inflammation in any biopsy specimens taken from the respiratory tract.

Clinical isolates were identified by the Duke Clinical Microbiology Laboratory as MAC based on growth characteristics, lack of pigmentation, biochemical testing, and confirmation with the MAC AccuProbe (Gen-Probe, San Diego, CA). Duplicated samples comprising 10% of the isolates were analyzed as a quality control.

The internal transcribed spacer region between the 16S and 23S rRNA genes was amplified from bacterial lysates using 30 cycles of PCR with the primers CCL (5'-TTGTA

* Corresponding author. Mailing address: Box 3306, Duke University Medical Center, Durham, NC 27710. Phone: (919) 684-3279. Fax: (919) 681-7494. E-mail: stout002@mc.duke.edu.
† Supplemental material for this article may be found at http://jcm.asm.org.
‡ Present address: Department of Medicine, Washington University School of Medicine, St. Louis, MO.
§ Present address: Genomic Services Division, Almac Diagnostics, Durham, NC.
Published ahead of print on 11 June 2008.
CAGCCGCGTC-3') and 23S (5'-TCTCGATGCCAA GGCACTACACCC-3') (5). Purified PCR products were sequenced directly using the internal primer 5BS (5'-GACG AAGTCTAACAAGGTAGC-3') at the Duke University Medical Center's DNA Sequencing Facility. Sequences were aligned by using CLUSTAL W (version 1.83; ftp://ftp.ebi.ac.uk /pub/software/dos/clustalw/clustalw1.83.XP.zip), and isolates were assigned to known sequevars based on exact matching. A phylogenetic tree was constructed based on maximum likelihood using PHYLIP 3.65, (http://evolution.gs.washington.edu/phylip.html). The data were resampled with 100 bootstrap replications. Groupings found on more than 70% of the bootstrap replications are marked on the phylogenetic tree. The relationship between sequevar group and case status was assessed by using odds ratios and exact 95% confidence intervals with SAS version 9.1 (SAS Systems, Cary, NC).

Forty case subjects were selected, but isolates were only available for thirty-three. Eighty control subjects were selected, but four did not have isolates and one had a dual/ambiguous sequence, leaving seventy-five subjects with analyzable sequence data (Table 1). Sequencing of the 16S-23S internal transcribed spacer revealed 22 distinct sequevars (see the supplemental material), whose phylogenetic relationships are demonstrated in the Fig. 1. Six isolates belonging to this sequevar group.

We found a significant association between M. avium sequevar group MAC isolates and MAC pulmonary disease, with a >5-fold increase in the odds of true MAC pulmonary disease in the M. avium sequevar group compared to the M. intracellulare sequevar group. However, consistent with other studies (3, 4, 8, 18, 25), the majority of MAC isolates (63.9%) among study subjects with or without MAC pulmonary disease belonged to the M. intracellulare sequevar group. M. intracellulare organisms are preferentially aerosolized (compared to M. avium) (17), resulting in relatively greater potential for respiratory exposure to M. intracellulare. Our data suggest the hypothesis that when respiratory exposure occurs, M. avium may be more likely to cause pulmonary disease than M. intracellulare; in other words, M. avium might be more virulent after respiratory exposure, all other factors being equal. M. avium possesses specific virulence factors not found in M. intracellulare.
lare (3, 19), is better able to invade and replicate inside macrophages than *M. intracellular* (14), and has been associated with more invasive forms of MAC disease (3, 10, 21, 27).

Our data differ from the findings of a recent study by Han et al., who found that *M. avium* (identified by 16S rRNA sequencing) isolated from clinical specimens was actually less likely to be associated with pulmonary disease than was *M. intracellular* (9). In that study, *M. avium* isolation was strongly associated with hematologic malignancy but did not often cause clinical disease (16.1% of isolates), while 63.1% of patients with *M. intracellular* had clinical disease. Since that study was conducted at a cancer referral hospital, the vast majority of patients had malignancies, so the results are unlikely to reflect what occurs in the general population. Furthermore, the study was cross-sectional, so misclassification of disease status may have occurred. Geographic strain differences resulting in differential exposure and resultant disease may also explain the difference between the Han et al. study and the present study.

Our data have several inherent limitations. Only one mycobacterial isolate per case subject was examined. MAC pulmonary disease is often polyclonal (25), and reinfection with new strains occurs frequently (24). Examining only one isolate may have resulted in misclassification of the infecting MAC sequvar in some case subjects. However, this misclassification would be most likely nondifferential and therefore would reduce the association between any particular sequvar and case status. Some subjects who truly had MAC pulmonary disease may have been inappropriately assigned to the control group. Again, this misclassification would tend to reduce any observed associations between case status and sequvar group. The demographics and comorbidities of the case and control groups were quite different, and observed differences in sequvar distributions may have been a result of confounding. Our study lacked statistical power to thoroughly explore this question, but in an exploratory analysis, no demographic/comorbidity was significantly associated with sequvar group (data not shown). Patients with fibrocytovac MAC pulmonary disease were not well represented in our study, and the sequvars associated with disease among patients with fibrocytovac MAC may well differ from those associated with disease among patients with nodular/bronchietac MAC.

The 2007 American Thoracic Society/Infectious Diseases Society of America guidelines for treatment of nontuberculous mycobacterial infections recommend that all clinically significant nontuberculous mycobacteria should be identified to the species level (7). Our data emphasize the importance of species identification in understanding the role of nontuberculous mycobacteria in lung disease. DNA sequencing of mycobacterial isolates is a powerful and increasingly popular method for speciation. The use of the 16S-23S internal transcribed spacer is a well-validated method to divide mycobacteria into species and subspecies groups (5, 6, 15, 26); other commonly used loci are the 16S ribosomal (3), pvoB (2), and hsp65 (20) genes. Use of these techniques has enabled provisional assignment of new species names to at least two previously unpublished organisms within MAC (22, 23). Further studies using these techniques to study well-defined patient groups will be necessary to better understand the role of MAC in human disease.

J.E.S. acknowledges support from NIH K23 AI051409. R.F. acknowledges support from the Department of Veterans Affairs, the Duke Center for Translational Research, National Institutes of Health grant P30 AI51445, and NIH training grant AI07392. We thank Scott Langdon for advice on DNA sequencing.

**REFERENCES**


