MAJOR ARTICLE

Whole-Genome Sequencing and Epidemiological Analysis Do Not Provide Evidence for Cross-transmission of *Mycobacterium abscessus* in a Cohort of Pediatric Cystic Fibrosis Patients

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Background. *Mycobacterium abscessus* has emerged as a major pathogen in cystic fibrosis (CF) patients and has been associated with poor clinical outcomes, particularly following lung transplant. We investigated the acquisition of this bacterium in a cohort of pediatric CF patients.

Methods. Demographic and patient location data were used to uncover epidemiological links between patients with genetically related strains of *M. abscessus* that had been previously typed by variable-number tandem repeat profiling. Whole-genome sequencing was applied to 27 *M. abscessus* isolates from the 20 patients in this cohort to provide definitive data on the genetic relatedness of strains.

Results. Whole-genome sequencing data demonstrated that *M. abscessus* isolates from 16 patients were unrelated, differing by at least 34 single-nucleotide polymorphisms (SNPs) from any other isolate, suggesting that independent acquisition events have occurred. Only 2 clusters of very closely related (<25 SNPs) isolates from different patients were seen. The first cluster contained 8 isolates, differing by a maximum of 17 SNPs, from a sibling pair who had intense exposure to each other both inside and outside the hospital. The second cluster contained 3 isolates, differing by a maximum of 24 SNPs, from 2 individuals with no apparent epidemiological links.

Conclusions. We have not demonstrated cross-transmission of *M. abscessus* within our hospital, except between 1 sibling pair. Alternative routes of acquisition of *M. abscessus* infection, in particular the environment, require further investigation.

Keywords. *Mycobacterium abscessus*; cystic fibrosis; cross-transmission; whole-genome sequencing; VNTR.

Mycobacterium abscessus has emerged as a major pathogen in cystic fibrosis (CF) patients and has been associated with poor clinical outcomes, particularly following lung transplant [1–3]. *Mycobacterium abscessus* is resistant to most classes of antibiotics [4, 5]. Macrolide resistance is either due to mutations in the *rrl* gene or the presence of an inducible ribosomal RNA methylase gene, *erm*(41) [6–8].

Mycobacterium abscessus is a single species that encompasses 3 subspecies (*M. abscessus* subsp *abscessus*, *M. abscessus* subsp *massiliense*, and *M. abscessus* subsp *bolletii*) [8–11]. These 3 subspecies have been

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associated with varying clinical outcomes [8, 12–14]. Accurate identification can generally be achieved by sequencing multiple gene targets [15]. Variable-number tandem repeat (VNTR) profiling can further differentiate isolates [9, 16] and, when applied to our cohort, showed that the majority of chronically infected patients were infected with 1 of 2 dominant strains. [9].

Whole-genome sequencing can provide more definitive data on the relatedness of isolates. Several publications have reported whole-genome sequences from a single *M. abscessus* complex isolate [17–20] or several isolates from a single patient [21]. Whole-genome sequences of *M. abscessus* subsp *massiliense* isolates from a cohort of adults with CF provided the first evidence that patient-to-patient spread can occur [10].

We describe the dynamics of acquisition of *M. abscessus* in a cohort of pediatric CF patients, using epidemiological and clinical data to uncover evidence of cross-transmission events and whole-genome sequencing to establish the resolution of the previously published VNTR typing scheme. Ultimately, this will lead to a better understanding of the impact of particular strains on clinical outcomes, especially following lung transplant.

MATERIALS AND METHODS

Patients and Microbiological Data Collection

Great Ormond Street Hospital is a large regional referral center for pediatric CF patients, including those patients for whom the hospital is their main CF clinic and patients from other centers undergoing assessment and then being listed for lung transplant (Table 1). All patients seen as outpatients or admitted to wards undergo regular respiratory microbiological diagnostic investigations, including specific stain and culture for mycobacteria on both sputum and bronchoalveolar lavage samples (which are performed at least on an annual basis).

Demographic and patient location data were extracted from the patient administration system and microbiological data from the laboratory information management system using SQL (structured query language) databases and Excel spreadsheets. Electronic patient records were used to capture all inpatient and outpatient episodes including location (general CF clinics, lung function testing, inpatient ward, and bed-days admitted). Additional sources of information included CF and transplant databases. Clinical case-note review was used to verify location, admission data, and clinical/radiological evidence of nontuberculous mycobacteria infection using American Thoracic Society consensus guidelines [5]. All investigations were performed in accordance with the hospital's research governance policies and procedures. We sought and obtained specific informed consent in 2 instances where patients had moved from our center to an adult CF center.

For patients who had isolated *M. abscessus* for the first time after initial contact with the hospital, all outpatient and inpatient admission episodes and cumulative bed-days were captured up to the date of their initial culture with *M. abscessus*. Exposure of these *M. abscessus*–naive patients was defined as being at the same time and same location as another patient known to be infected with *M. abscessus*. For comparison purposes, basic demographic data were collected on all patients who had isolated *Pseudomonas aeruginosa* for the first time after initial contact with our hospital. Surveillance data on *P. aeruginosa* have been prospectively gathered and stored on a database since 1994. The project was registered as a service evaluation.

Data Analysis

Graphs and (where appropriate) statistical analysis were generated using GraphPad Prism software version 6.03. Advice on statistical analysis of epidemiological exposure data was provided by the Department of Paediatric Epidemiology and Biostatistics at the Institute of Child Health. Where statistical analysis was performed, nonparametric tests were utilized (Mann–Whitney *U* test for unpaired data and Wilcoxon signed-rank tests for paired data).

Whole-Genome Sequencing

Twenty-seven *M. abscessus* isolates from 20 pediatric CF patients attending either CF or transplant clinics at our hospital between January 2004 and December 2011 were analyzed by whole-genome sequencing. All isolates had previously been identified to subspecies level by sequencing of *hsp65* and *rpoB* gene targets [15] and typed by VNTR profiling [9]. DNA was extracted from isolates as previously described [9], and the concentration was determined using a Qubit high-sensitivity (HS) assay kit (Life Technologies). Two hundred fifty nanograms of DNA was sheared on the Covaris S2 (duty cycle, 5%; intensity, 4; cycles per burst, 200; time, 90 seconds) before undergoing library preparation with the NEBNext DNA Library Prep Master Mix Set for Illumina (New England Biolabs) combined with 12 cycles of enrichment polymerase chain reaction with multiplex adapters. During library preparation, libraries were size-selected to 500 bp using Ampure XP beads (Beckman). Final libraries were quantified using the Qubit HS DNA assay, and absence of adapter-dimer was confirmed using the Bioanalsyer HS DNA Chip (Agilent). Libraries were equimolar pooled, and 12 pM was loaded onto an Illumina MiSeq to undergo 250 bp paired-end sequencing with v2 chemistry. The short reads from these studies are deposited in the short-read archive of the European Nucleotide Archive in the project PRJEB6776.

Sequence Data Analysis

Whole-genome phylogenetic analysis was performed using the sequence data from this study and from *M. abscessus* isolates from adult patients described in the study by Bryant et al [10], as deposited in the European Nucleotide Archive under study accession number ERP001039.

Table 1. Twenty Patients With Cystic Fibrosis From Whom *Mycobacterium abscessus* Was Isolated

Patient	Subspecies	VNTR Cluster	Sex	CF Genotype	Clinical Diagnosis of NTM Infection	Proportion of Sputum/ BAL AAFB Positive	No. of Positive *M. abscessus* Cultures	Specific *M. abscessus* Antimicrobial Treatment Given	Age at First *M. abscessus* Isolate, y	Years Between First *M. abscessus* Isolate and Contact With Study Center	Infection Status at First Contact to GOSH	Main CF Attending Center/Main CF Center	UK Residence HPU
2	ABS	VNTR I	F	p.Phe508del/p.Phe508del	Likely	6/32	6	Y	13.59	0	Already Infected	South of England	Surrey and Sussex
8	ABS	VNTR I	M	p.Phe508del/p.Phe508del	Likely	7/20	10	Y	10.29	10.04	Naive	Study center	South Midlands and Hertfordshire
9	ABS	VNTR I	F	p.Phe508del/p.Trp1089X	Likely	22/35	20	Y	10.93	0	Already Infected	London (other)/ study center	Northwest London
10	ABS	VNTR I	M	p.Phe508del/p.Phe508del	Likely	5/17	6	Y	13.36	4.97	Naive	Study center	South Midlands and Hertfordshire
21	ABS	VNTR I	M	p.Phe508del/p.Phe508del	Likely	4/5	5	Y	15.15	11.97	Naive	Study center	South Midlands and Hertfordshire
3	ABS	VNTR II	F	p.Phe508del/p.Phe508del	Likely	6/25	11	Y	11.33	0	Already Infected	North of England	Cumbria and Lancashire
18	ABS	VNTR II	M	p.Phe508del/p.Gly551Asp	Likely	53/62	40	Y	5.90	3.79	Naive	Study center	Northwest London
19	ABS	VNTR II	F	p.Phe508del/p.Phe508del	Likely	0/4	1	Y	17.25	16.18	Naive	Study center	Northwest London
22	ABS	VNTR II	F	Delta F508/I507/Delta F508/I507	Likely	5/25	10	Y	15.05	14.24	Naive	Study center	Northwest London
11	ABS	Unique	F	c.2988+1G>A/p.Tyr913X	Likely	0/40	3	Y	13.76	13.04	Naive	Study center	Northeast and North- central London
15	ABS	Unique	F	p.Phe508del/c.1585–1G>A	Unlikely	4/6	5	N	10.99	10.87	Naive	Study center	Anglia
17	ABS	Unique	M	p.Phe508del/p.Phe508del	Unlikely	1/13	1	No	14.07	13.81	Naive	Study center	Southeast London
24	ABS	Unique	F	UK	Likely	1/15	1	Y	13.27	0	Already Infected	London (other)	South Midlands and Hertfordshire
27	ABS	Unique	F	p.Phe508del/p.Phe508del	Likely	2/21	2	Y	14.86	5.37	Naive	Study center	South Midlands and Hertfordshire
30	ABS	Unique	F	p.Phe508del/p.Phe508del	Likely	3/10	4	Y	14.99	14.64	Naive	Study center	Essex
7	MAS	VNTR III	F	p.Phe508del/p.Phe508del	Likely	3/3	3	Y	14.49	0	Already Infected	Midlands	West Midlands West
23	MAS	VNTR III	F	p.Phe508del/p.Phe508del	Likely	25/40	30	Y	6.97	5.64	Naive	Study center	South Midlands and Hertfordshire
14	MAS	Unique	F	p.Gly551Asp/c.2052delA	Likely	0/8	1	Y	14.29	0	Already Infected	Wales	South Wales
28	MAS	Unique	F	p.Phe508del/p.Phe508del	Likely	1	1	Y	14.09	0	Already Infected	Midlands	West Midlands East
1	BOL	Unique	F	p.Phe508del/p.Phe508del	Likely	1/14	1	Y	10.08	0	Already Infected	London (other)	Northeast and North-central London

Clinical and radiological diagnosis of likely NTM infection with clinician decision to treat with long-term antibiotics considered active against *M. abscessus* was recorded in patient records.

Abbreviations: AAFB, acid-alcohol fast bacilli; ABS, *Mycobacterium abscessus* subsp *abscessus*; BAL, bronchoalveolar lavage; BOL, *Mycobacterium abscessus* subsp *bolletii*; CF, cystic fibrosis; GOSH, Great Ormond Street Hospital; HPU, Health Protection Unit; MAS, *Mycobacterium abscessus* subsp *massiliense*; NTM, nontuberculous mycobacteria; VNTR, variable-number tandem repeat.

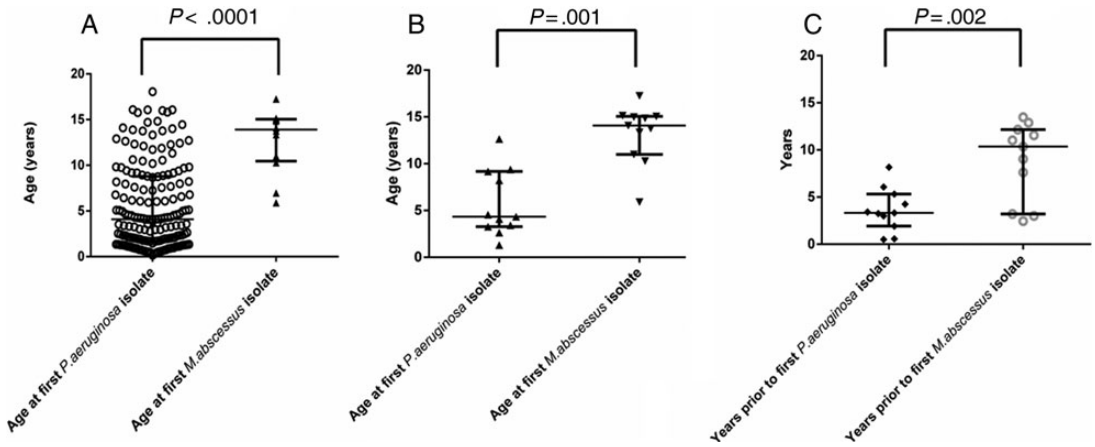

Figure 1. Comparison of age of acquisition of *Pseudomonas aeruginosa* and *Mycobacterium abscessus* in cohort of pediatric cystic fibrosis patients. *A*, Age at first acquisition of *P. aeruginosa* in *M. abscessus*–negative patients (n = 122) vs age of acquisition of *M. abscessus* ($P < .0001$, Mann–Whitney *U* test). *B*, Age of acquisition of *P. aeruginosa* vs acquisition of *M. abscessus* in all 12 patients who acquired *M. abscessus* after first contact with hospital ($P = .001$, Wilcoxon signed-rank test). *C*, Time in years after first contact with hospital and first acquisition of *P. aeruginosa* vs acquisition of *M. abscessus* (n = 12; $P = .002$, Wilcoxon signed-rank test).

The short reads were mapped to the reference ATCC 19977 (accession number CU458896) using BWA-MEM 0.7.5.a [22] and the default parameters. The sequence alignment map output from BWA was sorted and indexed to produce a binary alignment map (BAM) using Samtools 0.1.18 [23]. The Genome Analysis Toolkit 2 [24] was used to create a variant call format (VCF) file for each sequenced isolate using the BAM files as input and specifying diploid mode when calling variants. Variants within the VCF files were parsed to retain high-quality single-nucleotide polymorphisms (SNPs) based on the following conditions: DP (depth) ≥5, AD ratio (ratio between variant base and alternative bases) ≥0.8, MQ (mapping quality score) ≥30, ratio of number of reads with MQ0 (mapping quality of 0) to total number of reads ≤0.05, and distance to nearest SNP >10. All positions that fulfilled these criteria in >0.9 of the samples were joined to produce a multiple fasta format file where the sequence for each strain consists of the concatenated variants. This file was used as an input to generate a maximum likelihood tree using RAxML [25] with the following parameters: –m (substitutionModel) GTRCAT, –b (bootstrapRandomNumberSeed) 12345, -# (NumberOfRuns) 100, –c (NumberOfCategories) 25.

Multilocus sequence types (MLSTs) were identified by mapping the reads against all *M. abscessus* allele variants held in the Institut Pasteur MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) using a modification of the short-read sequence typing [26].

Antibiotic resistance analysis was performed by searching for SNPs previously discovered to be responsible for resistance mutations within the coding sequence of *rrl* and both the coding sequence and 126-bp upstream regulatory region of *erm*(41) [6]. Trimmed reads were mapped to the *rrl* and *erm*(41) gene sequences from the genome sequence of *M. abscessus* strain ATCC 19977 using bowtie2 version 2.1 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml). The resulting BAM file was converted to pileup format using samtools and the output parsed to determine the nucleotide base at the known drug-resistance locations. To generate a phylogenetic tree based on the *rrl* and *erm*(41) genes, the pileup format was converted to form a consensus sequence for each sample, aligned using muscle [27], and a maximum likelihood tree was created using FastTree [28].

RESULTS

Clinical and Microbiological Characteristics of *M. abscessus*–Infected CF Patients

In patients from whom *M. abscessus* was isolated for the first time, a number of observations were apparent. When compared with a larger cohort of *M. abscessus*–negative CF patients (n = 122) who acquired *P. aeruginosa* for the first time after contact with the hospital, children who acquired *M. abscessus* were significantly older ($P = .0001$, Mann–Whitney *U* test; Figure 1). The median age for acquiring *P. aeruginosa* was 4.35 years compared with a median age of first acquisition of *M. abscessus* of 14.07 years ($P = .005$, Wilcoxon signed-rank test; Table 1 and Figure 1). The median age difference in years between first *P. aeruginosa* isolation and first *M. abscessus* isolation was 7.56

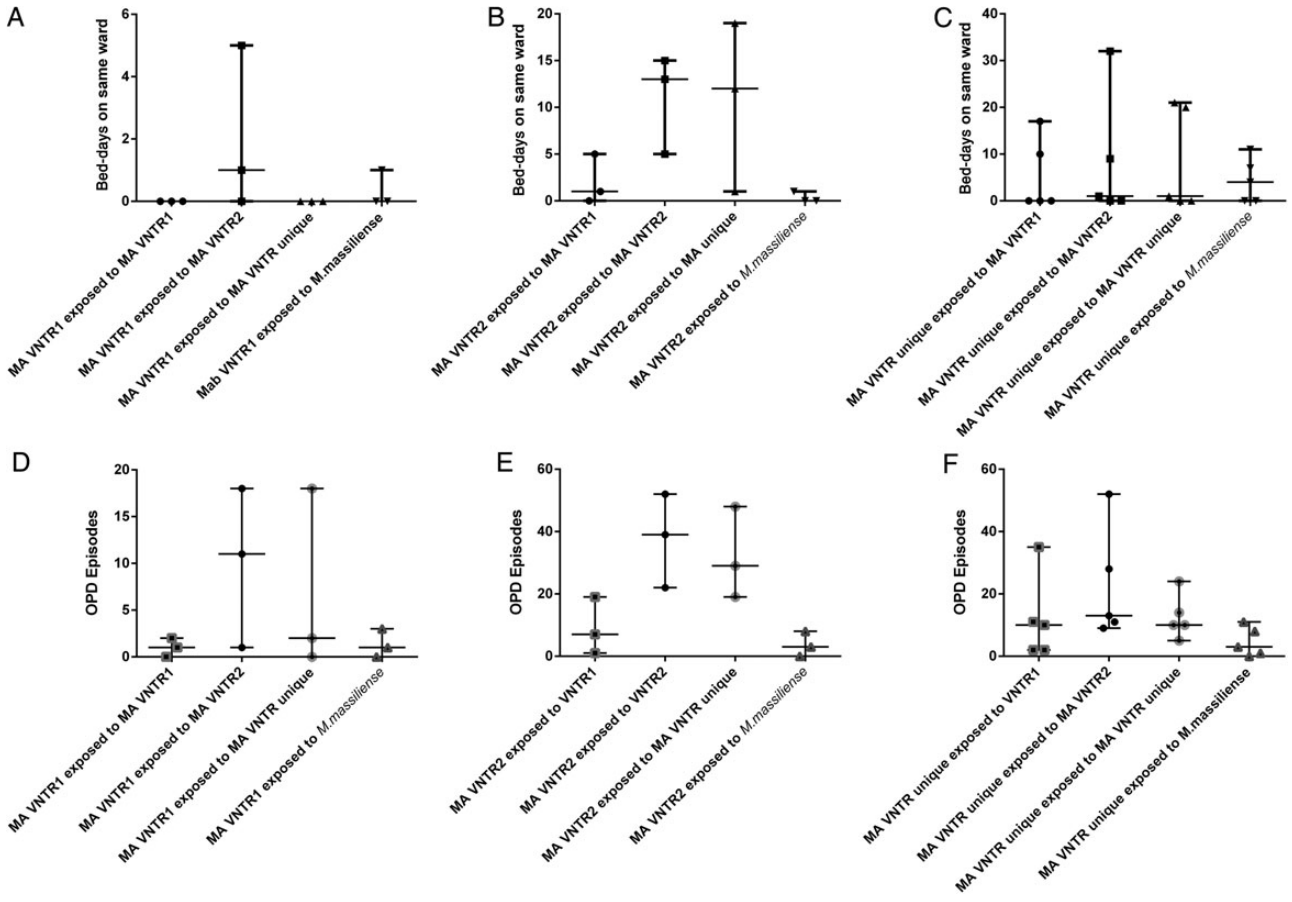

Figure 2. Exposure of patients who first acquired *Mycobacterium abscessus* after contact with our center to other *M. abscessus*-infected patients. Exposure of patients who acquired *M. abscessus* subsp *abscessus* (MA) variable-number tandem repeat (VNTR) I (n = 3; *A* and *D*), MA VNTR II (n = 3; *C* and *E*), and MA VNTR unique strains (n = 5; *C* and *F*) to other patients already infected with MA VNTR I, VNTR II, VNTR unique, and *M. abscessus* subsp *massiliense* strains. *A–C*, Total number of bed-days on the same ward at the same time that each patient was with other *M. abscessus*-infected patients. *C–E*, Total number of outpatient episodes where patients were with other *M. abscessus*-infected patients. Abbreviation: OPD, outpatients department.

years (interquartile range, 5.18–11.00 years). Taken together, in this single but large regional referral center, infection with *P. aeruginosa* always precedes *M. abscessus* infection, which usually occurs in the early to middle teenage years.

Mycobacterium abscessus strains clustered by VNTR profile (VNTR I, VNTR II, or VNTR III) or had unique VNTR profiles. Among the 12 patients who acquired *M. abscessus* for the first time during contact with our hospital, 11 acquired *M. abscessus* subsp *abscessus* strains (3 VNTR I, 3 VNTR II, and 5 with unique VNTR profiles) [9], and 1 individual acquired *M. abscessus* subsp *massiliense* (Table 1). After consulting with expert statistical colleagues, we concluded that there were insufficient numbers in each VNTR-defined outcome group to make statistically robust comparisons based on multiple comparisons of exposure to ward environments or among patients. Despite the relatively low numbers of defined acquisition events, we were able to make a number of observations.

Patient-to-Patient Contact of *M. abscessus*–Infected CF Patients

We analyzed the strength of exposure of patients who acquired *M. abscessus* after exposure to other patients with *M. abscessus* of any subspecies and VNTR profile. First, taking the patients (n = 3) who acquired VNTR I strains, there was minimal exposure to patients already infected with a VNTR I strain, either as an outpatient or as an inpatient. In contrast, patients who acquired VNTR I strains were exposed on a number of occasions both as inpatients and outpatients to other patients already infected with VNTR II strains and strains with unique VNTR profiles (Figure 2*A* and 2*D*). Where patients acquired VNTR II strains (n = 3), there was more intense exposure to patients with VNTR II strains (in particular, between patients 18 and 19) in both outpatient and inpatient settings than to patients with VNTR I strains; however, exposure to patients with strains that had a unique VNTR profile was similarly intense (Figure 2*B* and 2*E*). Patients who acquired strains with a unique VNTR

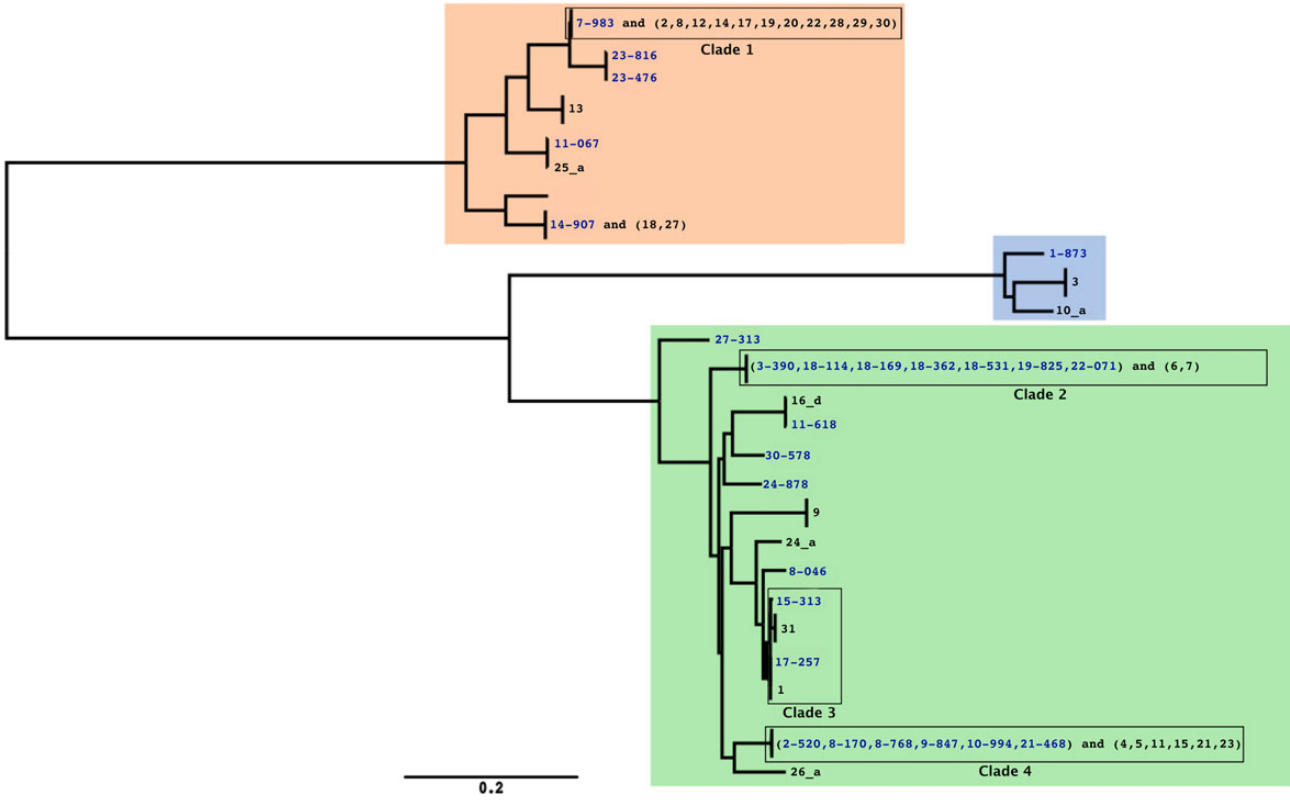

Figure 3. A maximum likelihood tree based on single-nucleotide polymorphisms in shared regions from the whole genome of *Mycobacterium abscessus* isolates from this study (dark blue text) and from the study described by Bryant et al [10] (black text). Three major lineages representing the *M. abscessus* subsp *massiliense*, *bolletti*, and *abscessus* are shaded in orange, blue, and green, respectively. The scale bar represents the number of substitutions per site across 133 683 variable sites.

profile (n = 5) were also exposed on a number of occasions, in both outpatient and inpatient environments, to patients infected with strains with different VNTR profiles, including VNTR I and VNTR II strains (Figure 2*C* and 2*F*).

Whole-Genome Sequencing of *M. abscessus* Isolates

Whole-genome sequences were obtained from 27 *M. abscessus* isolates in this study. These and sequences from the study by Bryant et al [10] were processed to produce a multiple fasta file, which was used to generate a tree. The fasta file recorded a total of 133 683 variant positions. The maximum likelihood tree showed 3 distinct clades corresponding to the 3 subspecies. Twenty isolates from this study were *M. abscessus* subsp *abscessus*, 6 were *M. abscessus* subsp *massiliense*, and 1 was *M. abscessus* subsp *bolletii*. Sixteen isolates were assigned to 1 of 4 clades. A further 9 isolates that were not closely grouped with >1 isolate were not assigned to a clade (Figure 3). Two isolates (27–313 and 28–319) did not produce sufficient read-depth to be included in the maximum likelihood tree.

Within clade 1, there were 152 SNPs; the minimum distance between any an isolate from this study and any other was 38 SNPs. Within clade 2, there were 157 SNPs. Isolates 18–114, 18–531, 18–362, 7_a, 7_b, 7_c, 7_d, 19–825, and 18–169 differed by 17 SNPs, and the smaller subset of 18–169, 19–825, 7_a, 7_b, 7_c, and 7_d differed by just 5 SNPs. Within clade 3, there were 1328 SNPs; the minimum distance between an isolate from this study and any other was 43 SNPs. Within clade 4, there were 308 SNPs. Isolates 8–768 and 8–170 were 2 SNPs different. Isolates 10–994, 21–468, and 15_a differed by 24 SNPs. Apart from these, the minimum distance between any isolate from this study and any other was 34 SNPs (Figure 4).

MLST and Resistance Mutations

MLSTs were deduced from the whole-genome sequences of all isolates in this study. All VNTR 1 strains were sequence type ST-26 and fell into sequence clade 4. All VNTR II strains were ST-1 and fell into sequence clade 2. The VNTR III strains had 2 different MLST types (ST-48 and ST-23) and fell into sequence clade 1 or no clade. All other strains had a unique VNTR profile and MLST type and did not fall into any sequence clade (Table 2).

Mutations in the *rrl* and *erm*(41) genes, known to cause antimicrobial resistance, were also examined. None of the VNTR I

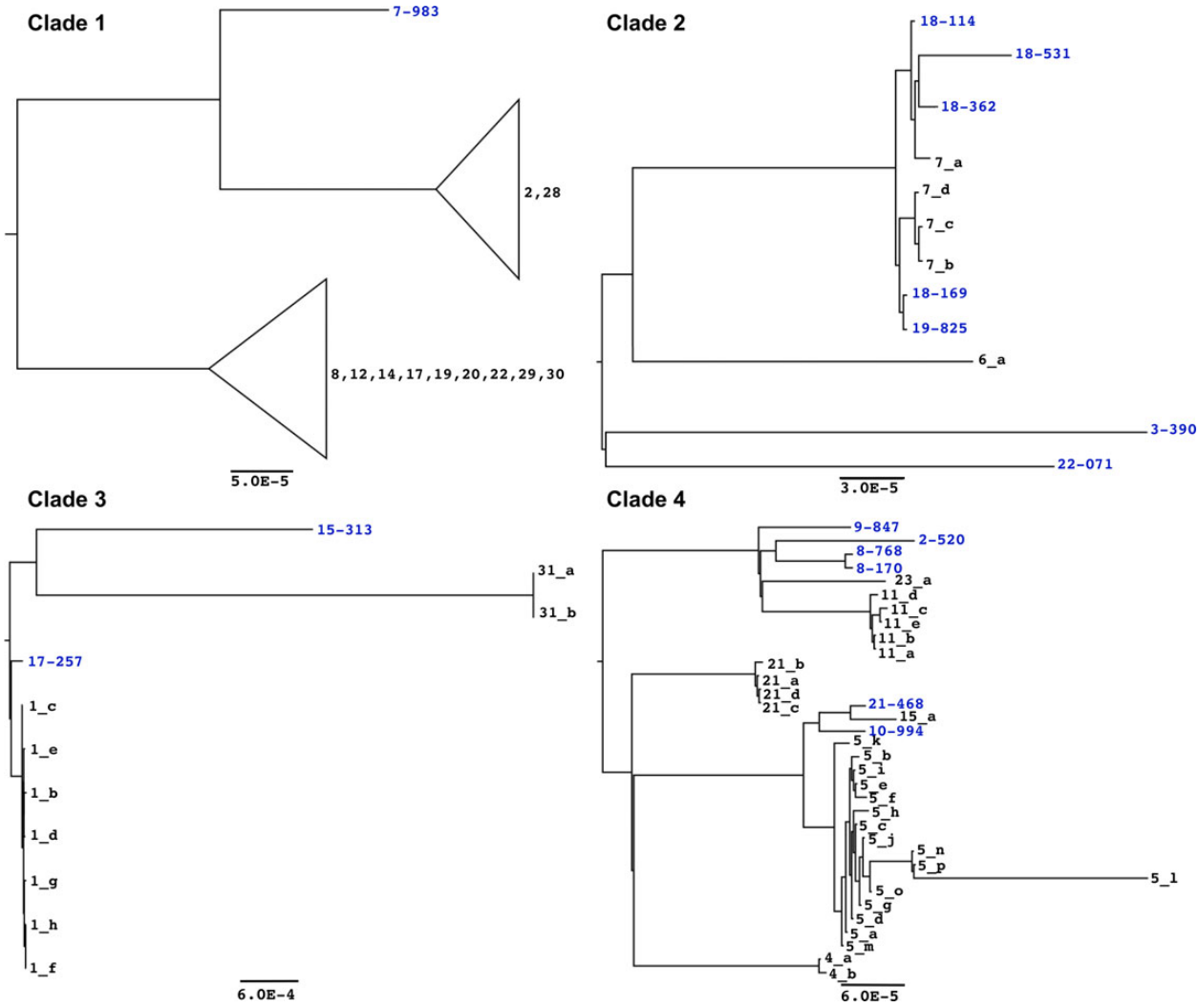

Figure 4. Subtrees from the maximum likelihood tree in Figure 3 representing detailed views of 4 clades where >2 isolates from this study were clustered. Where there are groups of isolates only from the study by Bryant et al [10], these are collapsed and displayed as triangles because they do not represent new data. Isolates from this study are shown in dark blue, and those from the study described by Bryant et al in black. The scale bar represents the number of substitutions per site.

or VNTR II strains had *erm*(41) mutations and therefore had a predicted phenotype of inducible macrolide resistance. All *M. abscessus* subsp *massiliense* strains had a truncated *erm*(41) sequence, no resistance-associated *rrl* mutations, and were predicted to be macrolide susceptible. Four *M. abscessus* strains with unique VNTR profiles had no resistance-associated *rrl* mutations and had the *erm*(41)mutation (T28C) that predicts macrolide susceptibility (Table 2). A phylogenetic tree generated from the *rrl* and *erm*(41)sequences clustered isolates identically to the trees generated from whole-genome sequences. Two isolates that did not produce sufficient read-depth to be included in the whole-genome analysis were included in this tree (Supplementary Figure).

DISCUSSION

The purpose of this retrospective cohort study of *M. abscessus* infection in pediatric CF patients was to explore the evidence for cross-infection between patients using a combination of epidemiology, VNTR profiling, and whole-genome sequencing.

Epidemiological data were used to investigate the intensity of exposure between patients with strains that were identical by VNTR profiling and strains with different VNTR profiles [9]. We hypothesized that if cross-transmission had occurred, we were likely to see more intense exposure prior to first detection of a VNTR I or VNTR II strain with a strain from the same cluster compared with exposure to strains from another cluster or

Table 2. Genotypic Data for All 27 *Mycobacterium abscessus* Isolates in This Study

Isolate	Date Isolated	Subspecies	VNTR Profile	MLST Type	Sequence Clade	*erm*(41) Mutation	*rrl* Mutation	Predicted Macrolide Resistance Phenotype
15–313	Jun 2006	ABS	Unique	ST-122	3	T28C	None	Susceptible
24–878	Mar 2009	ABS	Unique	Novel allele	None	none	None	Inducible resistance
17–257	Apr 2009	ABS	Unique	Novel ST	3	T28C	None	Susceptible
27–313	Dec 2012	ABS	Unique	ST-33	Not available[a]	none	None	Inducible resistance
30–578	Jun 2012	ABS	Unique	Novel ST	None	none	None	Inducible resistance
11–067	Oct 2008	MAS	Unique	ST-117	None	Trunc158–430	None	Susceptible
11–618	May 2012	ABS	Unique	Novel allele	None	None	None	Inducible resistance
2–520	Jan 2009	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
8–046	Jun 2007	ABS	Unique	Novel ST	None	T28C	None	Susceptible
8–170	Oct 2009	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
8–768	May 2012	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
9–847	Nov 2009	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
21–468	Oct 2009	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
10–994	Feb 2011	ABS	VNTR I	ST-26	4	None	None	Inducible resistance
18–169	Jun 2007	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
18–114	Mar 2008	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
18–362	Mar 2010	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
18–531	Jan 2012	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
3–390	Jul 2009	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
22–071	Feb 2009	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
19–825	Jun 2007	ABS	VNTR II	ST-1	2	None	None	Inducible resistance
14–907	Apr 2005	MAS	Unique	ST-69	None	Trunc158–430	None	Susceptible
7–983	Apr 2009	MAS	VNTR III	ST-23	1	Trunc158–430	None	Susceptible
23–476	Feb 2009	MAS	VNTR III	ST-48	None	Trunc158–430	None	Susceptible
23–816	Nov 2011	MAS	VNTR III	ST-48	None	Trunc158–430	None	Susceptible
28–319	Jun 2011	MAS	Unique	ST-37	Not available[a]	Trunc158–430	None	Susceptible
1–873	Dec 2004	BOL	Unique	Novel ST	None	None	None	Inducible resistance

Sequence clade, MLST types, *erm*(41) mutations, and *rrl* mutations were all deduced from the genome sequencing data.

Abbreviations: ABS, *Mycobacterium abscessus* subsp *abscessus*; BOL, *Mycobacterium abscessus* subsp *bolletii*; MAS, *Mycobacterium abscessus* subsp *massiliense*; MLST, multilocus sequence type; ST, sequence type; VNTR, variable-number tandem repeat.

[a] Insufficient sequence reads to determine sequence clade.

unique strains. There was little evidence of transmission of *M. abscessus* as a result of contact with the hospital. Only 2 patients (18 and 19), a sibling pair who both acquired VNTR II strains, had frequent exposure to each other in outpatient environments and at home.

Whole-genome sequencing data have provided a more accurate basis for assessing the degree of genetic similarity between isolates and have confirmed that VNTR profiling is an accurate method for identifying genetically related strains. Isolates with identical VNTR profiles were also of the same MLST type and belonged to the same whole-genome sequence clade, with the exception of 3 *M. abscessus* subsp *massiliense* isolates (Figure 3 and Table 2). All isolates with either the VNTR I or VNTR II profile (ST-26 and ST-1) were predicted to have a phenotype of inducible macrolide resistance. These are the dominant VNTR types in our patient cohort, and we have previously suggested a link with chronic infection [9]. Moreover, ST-1 and ST-26 are global lineages that appear to be successful clones [29]. Indeed, infections with strains that have inducible resistance to macrolides would be significantly harder to treat and therefore more likely to establish chronic infections [30]. However, it is possible that other parameters are important, such as expression of virulence factors, biofilm formation, adaptation to the CF microenvironment, and host–pathogen interactions [31], all of which warrant further study. The finding that all the patients who acquired *M. abscessus* were already infected with *P. aeruginosa* is noteworthy, although the reasons for this are unclear. It is possible that more aggressive antimicrobial therapy associated with chronic *P. aeruginosa* infection could play a role; however, the precise mechanism is as yet unknown and warrants further study.

Whole-genome sequence data provided greater resolution than either VNTR profiling or MLST, differentiating isolates within each of the 4 clades. Bryant et al [10] described different modes of similarity where those isolates belonging to a "related" cluster are <25 SNPs different. Using this cutoff, there are only 2 clusters with isolates from >1 patient in our data. The first cluster contains 4 isolates from patient 18 and 1 isolate from patient 19 (sibling). There were also 4 sequences (7 a–d) from Bryant et al [10], and we have confirmed that these were also isolates from patient 19, who was transferred to the adult service. These isolates were identical or virtually identical, differing by a maximum of 17 SNPs in patient 18 over a 5-year period. Patients 18 and 19 had a great deal of exposure to each other, particularly at home. Cross-transmission of *M. abscessus* is likely to have occurred, although, interestingly, the older sibling acquired *M. abscessus* 11 years after the younger sibling. The second cluster contains isolates from patients 10 and 21 (this study) and 15 (Bryant et al [10]), and we have confirmed that patient 10 and 15 are the same individual. Patients 10 and 21 acquired *M. abscessus* at a similar time, after their first contact with our center. However, the epidemiological data in this study did not show any contact between these 2 individuals within the hospital environment. Furthermore, they did not attend the same local hospital, outreach clinics, or school and, to the best of our knowledge, did not know each other socially. This suggested that cross-transmission between these 2 patients was unlikely and that these individuals had acquired highly genetically related strains by another route.

Isolates from the remaining 16 patients in this study differed by at least 34 SNPs from each other, which further suggested that cross-transmission was uncommon in this pediatric CF cohort. This conclusion has been reached previously by genotyping of *M. abscessus* isolates from CF patients at a single center [32]. However, this is in contrast to a recent study that demonstrated person-to-person spread of *M. abscessus* subsp *massiliense* between adult CF patients [10]. It is not yet clear why this difference is seen. It is possible that *M. abscessus* subsp *massiliense* is more transmissible, or that adults have more prolonged or intense exposures or shed a higher load of bacteria into the environment, making transmission more likely. Differences in infection control practices between centers could also explain why we have seen little evidence of person-to-person spread. Another potential route of acquisition of *M. abscessus* is a common environmental source. A recent publication demonstrated that strains isolated from potable water were genetically similar to patient isolates [33, 34]. We suggest that a better understanding of the role of the environment in the acquisition of *M. abscessus* infection is critical and has not been adequately investigated.

In conclusion, we could not demonstrate cross-transmission of *M. abscessus* within our hospital, except between 1 sibling pair that had intense exposure both in the hospital and, perhaps more importantly, the home environment. Two patients were infected with highly genetically similar strains but appeared to be epidemiologically unrelated. The role of the environment in the acquisition of *M. abscessus* infection requires further investigation.

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

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

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