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Indigenous Transmission of *Mycobacterium africanum* in Canada: A Case Series and Cluster Analysis

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Mycobacterium africanum is an important cause of human tuberculosis and is found almost exclusively in West Africa. We identified a cluster of patients in Montreal, Canada, with *M africanum* disease that share identical genotypic signatures by mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing and a putative epidemiological link, thus providing evidence of possible local transmission of *M africanum* in Montreal over a 10-year period.

Keywords. cluster; genotype; *Mycobacterium africanum*; transmission.

Mycobacterium africanum (*MAF*) is a member of the *Mycobacterium tuberculosis* (*MTB*) complex, and along with *MTB* it is a primary cause of human tuberculosis (TB). *Mycobacterium africanum* is endemic to equatorial Africa, with specimens isolated from Senegal, Nigeria, The Gambia, Côte d'Ivoire, Benin, Cameroon, Burkina Faso, Sierra Leone, and Uganda [1]. *Mycobacterium africanum* consists of 2 phylogenetically distinct lineages within the *MTB* complex (MTBC), known as MTBC lineage 5 and MTBC lineage 6 [1, 2]. Isolation of *MAF* from patients with TB in non-African countries has been mostly restricted to migrants from endemic areas in Africa [3, 4].

In the present article, we describe a case of disseminated *MAF* disease in a young Canadian patient living in Montreal. This case led to investigation and recognition of a *MAF* disease cluster in Montreal, evoking the possibility of local transmission.

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METHODS

Mycobacterium africanum isolates were identified from clinical specimens submitted from 3 different tertiary-care hospital laboratories to the Mycobacteria unit of the regional laboratory, the Laboratoire de Santé Publique du Québec. Sixteen isolates of *MAF*, identified from 2006 to 2015 (0–4 strains/year), were included in this study.

Isolates were plated on Lowenstein-Jensen slants at 37°C. Deoxyribonucleic acid was extracted from representative colonies using the BioRobot M48 (QIAGEN, Hilden, Germany) [5]. Identification to species level was done using a polymerase chain reaction (PCR)-based genomic deletion analysis of regions of difference [6].

All *MAF* clinical isolates were tested for drug susceptibility using the BACTEC 960 instrument. Critical drug concentrations used were 0.1 and 0.4 μg/mL isoniazid, 1.0 μg/mL rifampin, 100 μg/mL pyrazinamide, and 5.0 μg/mL ethambutol [7]. The isolates described in this study were from different laboratories, the patients presented over 10 years, and the characterization took place over this 10-year period, which makes laboratory cross-contamination very unlikely.

Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Typing

Genotyping was performed at the National Microbiology Laboratory, Winnipeg, Canada. Twenty-four mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) loci were amplified by PCR as previously described [7]. The number of repeats at each locus was assigned according to the electrophoretic mobility of the corresponding PCR product. Alleles were assigned numerical values according to the number of repeats present in each locus. Phylogenetic analysis was performed using the Bionumercis software V.7 and compared with the international MIRU-VNTR plus database (http://www.miru-vntrplus.org/).

RESULTS

Case

In December 2014, a 30-year-old patient presented with a slowly evolving, 2-centimeter, tender, subcutaneous lesion on the left thumb. Past medical history was significant for Crohn's disease treated with anti-tumor necrosis factor (TNF)-α injections and mercaptopurine. Hand x-ray and ultrasound showed no osteoarticular or synovial involvement. Sport-related trauma was identified as a possible causal event and the patient was treated symptomatically.

In February 2015, the patient consulted again for fever, weight loss, and night sweats. Chest radiography revealed bilateral nodular opacities in the upper third of the both lungs and hilar

adenopathy. Bronchoalveolar lavage fluid culture was negative for bacteria, mycobacteria, and fungi. Noncaseating necrotic granulomas were seen in histopathology of the lung. A diagnosis of sarcoidosis was evoked and the patient was started on prednisone.

However, the patient did not improve and he was admitted to our hospital in March 2015 for persisting fever. Patient was born and raised in Canada and history was negative for known exposure to TB or travel to Africa. Enlarged axillary lymph nodes and splenomegaly were noted. Blood work was unremarkable except for an elevated C-reactive protein at 50 mg/L. Excision biopsy of the axillary ganglion showed noncaseating granulomas. Auramine staining was negative. A presumptive diagnosis of anti-TNF-α-induced sarcoidosis was made.

Despite higher doses of steroids, the patient continued to be febrile with night sweats. A biopsy of the hand lesion was then performed. Purulent material stained positive for acid-fast bacilli and culture was positive for a slow-growing mycobacterium, which was identified as *MAF* sensitive to all 4 primary anti-TB drugs. Subsequent sputum and urine cultures were also positive for *MAF*.

Transmission Analysis

From 2006 to 2015, 16 isolates of *MAF* were identified in Quebec. The genotypic profile of these isolates is shown in Figure 1. Twelve isolates belong to MTBC lineage 6 and 4 isolates belong to lineage 5. Within lineage 6, 3 other cases (A, B, and D) shared the same MIRU-VNTR profile with our case (case C). Clinical presentation of these 4 patients is summarized in Table 1.

Investigations by local public health authorities revealed that the first case from that cluster (case A) was an immigrant from Senegal who had been studying at a commerce school in Montreal in winter 2006. At that time, contact investigation had followed a "concentric circle approach".

Ten students enrolled in 2 of the same classes as the index case were categorized as close nonhousehold contacts. Of these, 5 (50%) had positive tuberculin skin test (TST). Three of those were born in Québec, Canada, a low-prevalence setting. Screening was then extended to a bigger circle of "casual contacts", and letters were sent to those sharing at least 1 class with the contact. Of the TST results received, 45% of contacts were positive.

Case B was diagnosed in 2011. He was a student in one of the classes of the index case in 2006 and had been identified as a contact at the time but had refused latent TB testing. Patient C (our patient) studied in the same school and at the same time as the index case but had not been tested for latent TB. Epidemiological link to the fourth patient (case D) with an identical genotypic signature was less clear. He was an immigrant from Bangladesh, had never traveled to Africa, and at the time of diagnosis was working in a restaurant in Montreal within a 2-km radius of the domicile of the index case.

On the basis of the genotype cluster definition of ≥2 cases in 1 geographical area with identical 24-locus MIRU-VNTR patterns and epidemiological link [8], we identified a local cluster of *MAF* cases, consisting in 1 foreign-born patient (case A) and 2 Canadian-born (cases B and C) patients. Because *MAF* is not endemic in Bangladesh, case D probably acquired the disease locally, although an epidemiological link with our cluster was not firmly established.

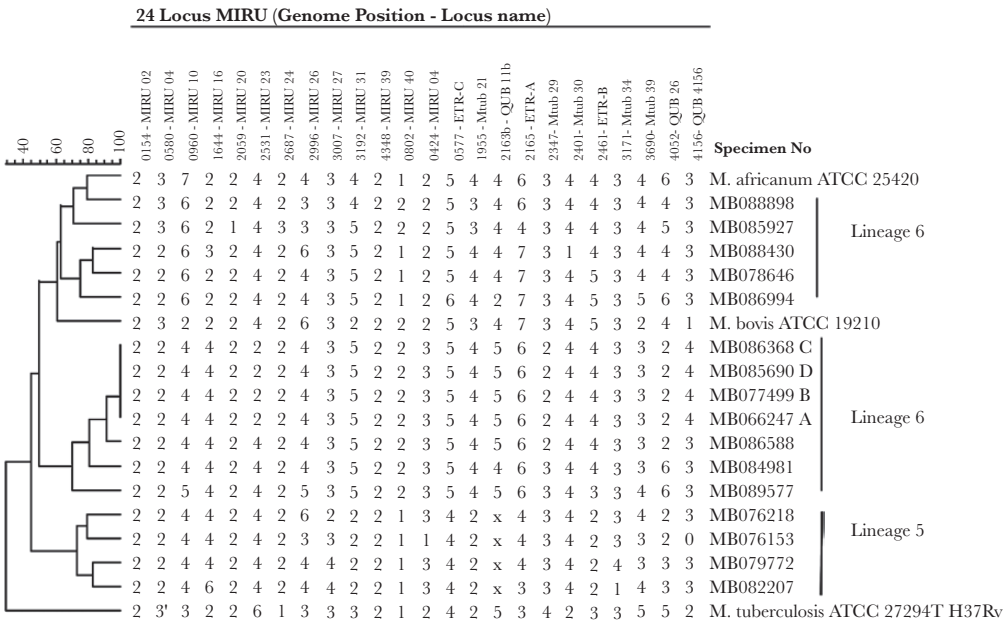

Figure 1. Genotypic profile of *Mycobacterium africanum* isolates in Quebec from 2006 to 2015.

Table 1. Clinical Characteristics of *Mycobacterium africanum* Patients in Québec Province, Canada (2006–2015)

Patient	Date of Diagnosis	Demographic Characteristics	Signs and Symptoms	Radiological Characteristics	Treatment
A (index case)	May 12, 2006	24-year-old male; immigrant from Senegal; in Canada since 2004; HIV negative	Cough, weight loss, night sweats, fatigue starting in April 2006	Right upper lobe cavitation, bilateral upper lobe infiltrates.	INH/RMP/PZA × 2 months EMB × 1 month followed by INH/RMP × 7 months
B	October 21, 2011	27-year-old Canadian-born female; no travel history to TB endemic areas; HIV negative	Cough, sputum, weight loss, night sweats starting in October 2010	Cavitary lesions in the left upper and lower lobes Right lung consolidation	INH/RMP/PZA/EMB × 2 months followed by INH/RMP × 7 months
C	April 2, 2015	30-year-old Canadian-born male; no travel history to TB endemic areas; HIV negative	Soft tissue involvement of first MCP, fever, weight loss starting in December 2014	Generalized adenopathy, miliary TB pattern	RMP/PZA/EMB[a] × 12 months
D	November 27, 2014	48-year-old male; immigrant from Bangladesh; in Canada since 2005 HIV negative, diabetes	Fever, weight loss, cough starting in August 2014	Bilateral upper lobe cavitations	INH/RMP/PZA/EMB × 2 months followed by INH/RMP × 7 months

Abbreviations: EMB, ethambutol; HIV, human immunodeficiency virus; INH, isoniazid; PZA, pyrazinamide; RMP, rifampicin; TB, tuberculosis.

[a]Isoniazid stopped after 14 days due to allergic reaction.

DISCUSSION

This study describes one of the few clusters of local transmission of *MAF* outside endemic African countries and, possibly, the first one in Canada. Genotyping of the isolates suggests local transmission in this cluster, with cases identified over almost 10 years. Of the 4 cases with identical MVTR signatures, 3 cases were enrolled in the same school at the same time, which offers epidemiological evidence of a link between cases.

A limitation of this work is that the genotyping method used in this study is the MIRU-VNTR typing method, the resolution of which is certainly lower than Whole Genome Sequencing for transmission analysis, and there is the possibility that these do not reflect true transmission events. However, we believe that in the limited context of this article, where the rareness of *MAF* disease outside endemic Africa is coupled with the occurrence of this rare disease in 2 cases born in Québec, with no other risk factors for *MAF* disease acquisition other than epidemiological links with our first case, an immigrant from an endemic country, during the period when this patient was contagious, an identical MIRU-VNTR pattern might be suggestive of transmission. The Supplementary Table shows genotypic profiles of 23 *MAF* isolates in Quebec province and allows us to remark on the diversity in MIRU-VNTR profiles in these isolates.

In terms of clinical presentation, *MAF* has been shown to follow a more indolent disease course with worse chest x-ray abnormalities compared with MTB disease [4, 8, 9]. Three of the 4 cases in our study presented with cavitary pulmonary disease. Our case initially presented with cutaneous involvement, a rare manifestation of *MAF* disease. Progression to miliary disease was almost certainly caused by steroid therapy initiated when an alternative diagnosis was considered. Our patient was not screened for latent TB at the time of contact tracing and in an evident lapse of recommendations of the Canadian Tuberculosis standards [10] was not screened before starting anti-TNF-α treatment. This is also against standardized practice in our institution where latent TB screening is incorporated into the workup before starting anti-TNF-α. Because the patient was referred to us from a different practice setup, we were unable to completely elucidate the reasons for this error.

CONCLUSIONS

Finally, our report emphasizes the importance of considering and reconsidering the diagnosis of TB in the immunosuppressed individual, even in the absence of an evident exposure.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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