Mycobacterium africanum Subtype II Is Associated with Two Distinct Genotypes and Is a Major Cause of Human Tuberculosis in Kampala, Uganda

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The population structure of 234 *Mycobacterium tuberculosis* complex strains obtained during 1995 and 1997 from tuberculosis patients living in Kampala, Uganda (East Africa), was analyzed by routine laboratory procedures, spoligotyping, and IS6110 restriction fragment length polymorphism (RFLP) typing. According to biochemical test results, 157 isolates (67%) were classified as *M. africanum* subtype II (resistant to thiophen-2-carboxylic acid hydrazide), 76 isolates (32%) were classified as *M. tuberculosis*, and 1 isolate was classified as classical *M. bovis*. Spoligotyping did not lead to clear differentiation of *M. tuberculosis* and *M. africanum*, but all *M. africanum* subtype II isolates lacked spacers 33 to 36, differentiating them from *M. africanum* subtype I. Moreover, spoligotyping was not sufficient for differentiation of isolates on the strain level, since 193 (82%) were grouped into clusters. In contrast, in the IS6110-based dendrogram, *M. africanum* strains were clustered into two closely related strain families (Uganda I and II) and clearly separated from the *M. tuberculosis* isolates. A further characteristic of both *M. africanum* subtype II families was the absence of spoligotype spacer 40. All strains of family I also lacked spacer 43. The clustering rate obtained by the combination of spoligotyping and RFLP IS6110 analysis was similar for *M. africanum* and *M. tuberculosis*, as 46% and 49% of the respective isolates were grouped into clusters. The results presented demonstrate that *M. africanum* subtype II isolates from Kampala, Uganda, belong to two closely related genotypes, which may represent unique phylogenetic branches within the *M. tuberculosis* complex. We conclude that *M. africanum* subtype II is the main cause of human tuberculosis in Kampala, Uganda.

Mycobacterium africanum is a member of the *Mycobacterium tuberculosis* complex, which also comprises the closely related species *M. tuberculosis*, *M. bovis*, *M. microti*, and *M. canettii* (21, 24). Since its first description in 1968 (3), *M. africanum* has been found in several regions of Africa, where it represents up to 60% of clinical strains obtained from patients with pulmonary tuberculosis (7, 18, 19, 23).

Recent surveys show highly variable prevalences of *M. africanum* in different African regions. For example, *M. africanum* was found in approximately 5% of patients with tuberculosis in the Ivory Coast and in at least 60% of patients in Guinea-Bissau (2, 10). Most of the studies presented so far have analyzed small numbers of strains from different regions, and systematic studies of prevalence and geographic distribution of *M. africanum* are still infrequent.

In contrast to *M. tuberculosis* and *M. bovis*, *M. africanum* strains show a higher variability of phenotypic attributes, comprising characteristics common to both *M. tuberculosis* and *M.*

bovis. This phenotypic heterogeneity of *M. africanum* complicates its unequivocal identification and may lead to misclassification of clinical strains. According to their biochemical characteristics, two major subgroups of *M. africanum* have been described, corresponding to their geographic origin in western (subtype I) or eastern (subtype II) Africa. Numerical analyses of biochemical characteristics revealed that *M. africanum* subtype I is more closely related to *M. bovis*, whereas subtype II more closely resembles *M. tuberculosis* (5).

In our recent work, we determined diagnostic criteria, including phenotypic and biochemical characteristics as well as results of the molecular spoligotyping technique, that permit the accurate differentiation of *M. africanum* subtypes I and II (15). Spoligotyping is a rapid molecular test based on the detection of various nonrepetitive spacer sequences located between small repetitive units (direct repeats) in the direct repeat locus of *M. tuberculosis* complex strains. However, spoligotyping does not allow differentiation of *M. africanum* subtypes from *M. tuberculosis* without additional routine laboratory procedures. This drawback led us to evaluate the usefulness of *gyrB* DNA sequence polymorphisms as a further molecular marker for differentiation of the species of the *M. tuberculosis* complex (16).

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We established a rapid PCR-restriction fragment length polymorphism (RFLP) assay that allows the differentiation of *M. bovis* subsp. *bovis*, *M. bovis* subsp. *caprae*, and *M. microti* as well as the clear identification of *M. africanum* subtype I strains. *M. africanum* subtype II and *M. tuberculosis*, however, displayed identical *gyrB* DNA sequences and were indistinguishable in this analysis. Thus, differentiation of *M. africanum* subtype II from *M. tuberculosis* continues to be based on phenotypic characteristics such as growth on bromocresol purple medium (16). This finding, in accordance with previous reports (5, 7), reiterates the close relationship between *M. africanum* subtype II and *M. tuberculosis* and questions the taxonomic status and phylogenetic position of *M. africanum* subtype II within the *M. tuberculosis* complex.

The present study investigated the population structure of *M. tuberculosis* complex strains obtained from patients with tuberculosis who were recruited at the Mulago Hospital in Kampala, Uganda, because the presence of *M. africanum* subtype II in limited study populations in Uganda has been reported (7, 15). The aim of this study was to assess the genetic relationship of *M. tuberculosis* and *M. africanum* subtype II in order to verify a genetic basis for this repeatedly described *M. africanum* subtype. Furthermore, the intention was to analyze the prevalence of *M. africanum* in a large study group of well-defined patients with tuberculosis. Based on the results obtained, we hypothesize that the majority of *M. tuberculosis* complex strains in Uganda belong to *M. africanum* subtype II and that this subtype contains two distinct genotypes (Uganda I and Uganda II) that may represent two closely related phylogenetic branches within the *M. tuberculosis* complex.

MATERIALS AND METHODS

Strains analyzed. A total of 234 *M. tuberculosis* complex strains isolated from sputum samples that had been collected between 1995 and 1997 were analyzed. Sputum samples were obtained from 234 adult patients with newly diagnosed initial episodes of sputum smear-positive pulmonary tuberculosis from the National Tuberculosis/Leprosy Program (NTLP) clinic (the largest tuberculosis clinic in Uganda) at Mulago Hospital. After decontamination (*N*-acetyl-L-cysteine-sodium hydroxide), the sediment was inoculated on Löwenstein-Jensen slants at 37°C as described elsewhere (12). Nearly all patients were ambulatory, and a few were hospitalized. The households of these patients were subsequently studied in the context of a household contact study. All strains were confirmed as *M. tuberculosis* complex by spoligotyping (11).

Biochemical tests and susceptibility testing. Biochemical analysis for differentiation within the *M. tuberculosis* complex included colony morphology, nitrate reduction on modified Dubos broth, niacin accumulation test (INH test strips; Difco, Detroit, Mich.), growth in the presence of thiophen-2-carboxylic acid hydrazide (TCH, 1 µg/ml), catalase activity at room temperature, and growth characteristics on Lebek and on bromocresol purple medium, as described previously (12, 15).

IS6110 RFLP and spoligotyping analysis. Extraction of DNA from mycobacterial strains and DNA fingerprinting with IS6110 as a probe was performed according to a standardized protocol described elsewhere (14, 20). Spoligotypes and IS6110 fingerprint patterns of mycobacterial strains were analyzed with the Gelcompar software (Windows NT, version 4.2; Applied Maths, Kortrijk, Belgium) as described previously (14, 20). Clusters were defined as groups of patients with bacterial strains showing identical spoligotype and/or IS6110 RFLP patterns. Spoligotyping of strains was performed as described by Kamerbeek et al. (11).

Quality control. The laboratory participated twice a year in external proficiency testing (national and international). For all test panels, negative and positive internal controls were included.

*Pvu*II-digested total DNA of reference strain Mt.14323 (obtained from the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) was used in each Southern blot experiment as an external size

standard and for quality control and quality assurance of IS6110 RFLP experiments. *M. tuberculosis* H37 (ATCC 27294) and *M. bovis* BCG (ATCC 27289) were used as control strains in each spoligotype experiment performed. The accuracy of each experiment and the normalization procedure performed were controlled by comparing the IS6110 fingerprint patterns or spoligotype patterns of reference strains present on each autoradiogram with those stored in the database.

RESULTS

In this study, *M. tuberculosis* complex strains from 234 patients with pulmonary tuberculosis were investigated by biochemical tests, spoligotyping, and IS6110 RFLP analysis.

Species differentiation. According to their phenotypic characteristics and biochemical test results, the 234 *M. tuberculosis* complex strains were classified as 157 *M. africanum* strains, 76 *M. tuberculosis* strains, and 1 *M. bovis* strain. *M. africanum* was identified on the basis of its colony morphology on Löwenstein-Jensen slants (dysgonic growth), microaerophilic growth on Lebek medium (Lebek is a semisolid medium which can be used to test the oxygen preference of mycobacterial strains), low catalase reaction, and lack of induction of a color change of bromocresol purple medium (pH-dependent change of color from blue to yellow, e.g., in the case of *M. tuberculosis* strains) (15). The biochemical characteristics of the strains analyzed and of the type strains *M. tuberculosis* H37 (ATCC 27294), *M. bovis* (ATCC 19210), and *M. africanum* (ATCC 25420) are summarized in Table 1. All strains classified as *M. africanum* showed resistance to TCH and were therefore differentiated as *M. africanum* subtype II, which more closely resembles *M. tuberculosis* (15).

Spoligotyping analysis. All strains were analyzed by spoligotyping, and the patterns obtained were digitized and analyzed for similarity with the Dice coefficient (position tolerance, 1.0%). A dendrogram was calculated with the unweighted pair group method with average linkage (UPGMA) for the *M. tuberculosis* and *M. africanum* strains.

The only *M. bovis* strain found showed the typical absence of spacers 39 to 43 and the presence of spacers 3 to 16. This strain could thus be identified as *M. bovis* subsp. *bovis* (pyrazinamide resistant [17]).

In accordance with previous results (15), all *M. africanum* subtype II strains showed no hybridization to the *M. bovis*-derived spacers 33 to 36. Although *M. africanum* strains were found mainly in two large groups of strains with similar spoligotype patterns, differentiation from *M. tuberculosis* could not be achieved in the dendrogram based on spoligotyping results (Fig. 1). Several *M. tuberculosis* and *M. africanum* strains showed only minor differences (one or two spacers) in the spoligotype patterns (see groups on top of dendrogram in Fig. 1 and 2), which resulted in adjacent positions in the dendrogram.

Considering the clustering rate among the strains analyzed, unique spoligotype patterns were found in only 40 (17%) of the 233 *M. tuberculosis* and *M. africanum* strains. The remaining 193 (83%) strains had a spoligotype pattern identical to that of one or more of the *M. tuberculosis* or the *M. africanum* strains. Among the *M. tuberculosis* strains, 57 (74%) were grouped into 17 clusters with identical spoligotype patterns. Each of the clusters contained between two and eight strains. Of the 157 *M. africanum* strains, 136 (87%) were grouped into 18 clusters,

TABLE 1. Biochemical characteristics of type strains *M. tuberculosis* H37 (ATCC 27294), *M. bovis* (ATCC 19210), and *M. africanum* (ATCC 25420) and the strains analyzed^a

Organism and group (no. of strains)	Test result (% of isolates)							Mean catalase activity ^b ± SD
	Niacin accumulation	Nitrate reduction	Growth in presence of:		Colony morphology	Change of color of bromocresol medium	Growth on Lebek medium	
			TCH	PZA				
<i>M. tuberculosis</i> H37 (ATCC 27294)	+	+	+	—	Eugonic	+	Aerophilic	6.0
<i>M. bovis</i> (ATCC 19210)	—	—	—	+	Dysgonic	—	Microaerophilic	0.1
<i>M. africanum</i> (ATCC 25420)	—	±	—	—	Dysgonic	—	Microaerophilic	0.2
<i>M. tuberculosis</i> (76)	+ (100)	+ (100)	+ (100)	— (97), + (3) ^c	Eugonic (100)	+ (100)	Aerophilic (100)	3.0 ± 1.2
<i>M. bovis</i> (1)	—	—	—	+	Dysgonic	—	Microaerophilic	0.1
<i>M. africanum</i> subtype II Uganda I (55)	+ (7), ± (87), — (6)	+ (100)	+ (100)	— (100)	Dysgonic (100)	— (100)	Microaerophilic (100)	0.5 ± 0.2
<i>M. africanum</i> subtype II Uganda II (102)	+ (5), ± (91), — (4)	+ (100)	+ (100)	— (100)	Dysgonic (100)	— (100)	Microaerophilic (100)	0.5 ± 0.2

^a Abbreviations and symbols: +, positive test result; —, negative test result; ±, weakly positive; PZA, pyrazinamide. One *M. tuberculosis* isolate, three *M. africanum* subtype II Uganda I, and two Uganda II isolates were resistant to isoniazid and cross-resistant to TCH.

^b Centimeters of foam production at room temperature.

^c Strains were resistant to isoniazid, streptomycin, and pyrazinamide.

with 2 to 37 strains per cluster. This indicated high genetic homogeneity among the *M. africanum* strains, an observation that was further supported by the inclusion of more than 50% of all *M. africanum* strains within the three largest clusters, which contained 17, 29, and 37 strains (Fig. 1).

IS6110 RFLP analysis. In order to further analyze the genetic relationship of the strains, DNA fingerprinting with IS6110 as a probe was performed. The IS6110 RFLP patterns of the *M. tuberculosis* and *M. africanum* subtype II strains were analyzed for similarity with the Dice coefficient (position tolerance, 1.3%), and a dendrogram was calculated, which is shown in Fig. 3.

In contrast to the dendrogram that was based on the spoligotyping results (Fig. 1), RFLP analysis grouped the *M. africanum* subtype II strains into two closely related genotype families (Uganda I [*n* = 55] and Uganda II [*n* = 102]). RFLP patterns among strains of these genotypes showed a similarity of at least 75% and were distinctly separated from the *M. tuberculosis* strains (Fig. 3). Even though *M. africanum* subtype II and *M. tuberculosis* strains showed very similar spoligotype patterns, they could be clearly distinguished by IS6110 RFLP typing (Fig. 2b). Overall, the *M. tuberculosis* IS6110 RFLP patterns were more variable than those of *M. africanum* strains, as was depicted by large differences in the IS6110 copy numbers, ranging from only 1 to 17 per strain (Fig. 3). In contrast, the IS6110 RFLP patterns among the *M. africanum* strains were more homogeneous, with copy numbers ranging between approximately 14 and 20 IS6110 bands per strain.

Separate evaluation of the spoligotype patterns of strains of the *M. africanum* subtype II genotypes Uganda I and II showed that the absence of spacer 40 is an obvious marker of both genotypes (Fig. 4). In addition, all strains of genotype Uganda I lack spacer 43. In contrast to these findings in *M. africanum* subtype II, most of the *M. tuberculosis* strains (44 of 57) showed hybridization to spacers 40 and 43 (data not shown). All of the *M. tuberculosis* strains, which lack one or both of spacers 40 and 43, were clearly distinguishable from the *M. africanum* strains by their IS6110 RFLP patterns. This further confirmed our species differentiation based on phenotypic and biochem-

ical characteristics. Thus, lack of spacers 40 and 43 is not an exclusive marker of *M. africanum* subtype II but might represent a useful additional criterion for *M. africanum* subtype identification in combination with biochemical test results.

When the results from spoligotyping and IS6110 RFLP analysis were combined, rates of strains in clusters with identical spoligotype and IS6110 RFLP patterns were reduced to 47% (110 of 234). Among the 76 *M. tuberculosis* strains in this study, 37 strains (49%) showed identical IS6110 and spoligotype patterns and were grouped into 13 clusters containing two to seven strains each. Among the 157 *M. africanum* strains, 72 (46%) were grouped in 28 such clusters with two to seven strains per cluster that consisted mainly (75%) of pairs of strains. Although the fingerprint polymorphism detected by spoligotyping was lower than that of IS6110 RFLP typing, an overall correlation between the two techniques was observed. All strains with identical IS6110 RFLP patterns also displayed identical or very similar spoligotype patterns (data not shown), confirming the genetic relationship of the strains determined by IS6110 RFLP typing. The accurate classification of the *M. africanum* subtype II genotypes Uganda I and II by IS6110 RFLP typing was further supported by the shared characteristic spoligotype features of the strains.

DISCUSSION

This study systematically analyzed the population structure of *M. tuberculosis* complex strains isolated between 1995 and 1997 from tuberculosis patients living in Kampala, Uganda.

Sixty-seven percent of the strains were *M. africanum* subtype II, suggesting that the main cause of human tuberculosis in Kampala is *M. africanum* subtype II. We further demonstrated that *M. africanum* subtype II strains from Kampala, Uganda, belong to two closely related genotypes (Uganda I and II) that share specific spoligotyping characteristics and are clustered into two IS6110 RFLP strain families.

Geographic variants of *M. africanum* had initially been described in studies by David et al. (5) and were more recently noted by Haas et al. (7). The results of these studies indicated

FIG. 1. Spoliotype patterns of the 233 *M. tuberculosis* (darker shading) and *M. africanum* subtype II (lighter shading) strains. Banding patterns are ordered by similarity in a dendrogram. The position of each spoliotyping hybridization spot is normalized so that banding patterns of all strains are mutually comparable. The scale depicts similarity of patterns calculated with the Dice coefficient and the UPGMA method.

FIG. 2. Spoligotype (a) and IS6110 RFLP (b) patterns of four pairs of *M. tuberculosis* and *M. africanum* subtype II strains. *M. tuberculosis* and *M. africanum* subtype II strains had very similar spoligotype patterns but were clearly separated by IS6110 RFLP typing.

that *M. africanum* subtype I predominantly originated from West African countries, whereas *M. africanum* subtype II was found predominantly in East Africa.

Systematic studies analyzing larger numbers of strains from one study region are still rare, and their interpretation is complicated by the lack of clear characteristics for the differentiation of *M. africanum* and its two subtypes. In our own studies (15, 16), we analyzed a collection of *M. africanum* strains from western and eastern African countries and found criteria which allowed the accurate differentiation of the two *M. africanum* subtypes in accordance with the geographic origin of the strains. The main criteria for the differentiation of the two *M. africanum* subtypes are susceptibility to TCH, hybridization to at least two of the *M. bovis*-derived spacers 33 to 36, and a specific *gyrB* DNA sequence for subtype I and resistance to TCH and lack of hybridization to spacers 33 to 36 for subtype II.

Recent studies in West African countries have shown *M. africanum* prevalence rates with high regional variability, ranging from approximately 5% in the Ivory Coast (2) to 61% (biovars 2, 3, and 4) in Guinea-Bissau (10). Because of their susceptibility to TCH, the *M. africanum* strains in these two studies were confirmed as *M. africanum* subtype I. Considering the IS6110 RFLP patterns obtained, an obvious characteristic of the *M. africanum* subtype I strains in both studies was the presence of an intermediate or small number of IS6110 bands, which has also been observed by Haas et al. (7) for *M. africanum* subtype I. In accordance with our previous results (15), the spoligotyping analysis performed by Källénus et al. (10) confirmed that *M. africanum* subtype I strains are characterized by a specific spoligotype pattern which is intermediate between those of *M. bovis* and *M. tuberculosis* (hybridization to spacers 33 to 36 as well as to spacers 39 to 43).

This typical genotype, the combination of an intermediate spoligotype pattern together with a small number of IS6110 bands, was further observed in a very recent study by Viana-Niero and coworkers (23), who analyzed a collection of *M. africanum* strains from several West African countries. All these studies verify the presence of *M. africanum* subtype I in West Africa, which is characterized by certain phenotypic properties as well as a characteristic spoligotype and IS6110 RFLP patterns. Our previous results indicate that this subtype may be identified by a specific *gyrB* DNA sequence, but this finding still remains to be analyzed for a larger number of *M. africanum* subtype I strains.

In accordance with our preliminary observations during a study of 49 *M. tuberculosis* complex strains from Kampala (18), the present study confirms that *M. africanum*, and particularly its subtype II, represents a major cause of human tuberculosis in this African region. This finding is in contrast to the results obtained in a study performed from 1992 to 1993 in the region of Buluba, Uganda, in which only 16% of the strains analyzed were differentiated as *M. africanum* (19). These contrasting results may be simply explained by a variable prevalence of *M. africanum* subtype II in different regions of Uganda or differences in the sampling procedures applied. A further possible reason is an increase in the prevalence of *M. africanum* subtype II in recent years, which might have resulted from other contributing factors, such as the increased rate of human immunodeficiency virus type I (HIV-1) in the Ugandan population.

In contrast to *M. africanum* subtype I, subtype II strains were resistant to TCH and showed no hybridization to spoligotype spacers 33 to 36. The most striking finding of this investigation is that the *M. africanum* subtype II strains from Kampala, Uganda, clustered in two closely related genotypes, which could be clearly separated from the *M. tuberculosis* strains

FIG. 3. IS6110 DNA fingerprint patterns of the 233 *M. tuberculosis* (darker shading) and *M. africanum* subtype II (lighter shading) strains. Banding patterns are ordered by similarity in a dendrogram. *M. africanum* subtype II strains were clustered in two closely related strain families (genotypes Uganda I and II) and were clearly separated from the *M. tuberculosis* strains.

FIG. 4. Representative spoligotype patterns of *M. africanum* subtype II strains of genotypes Uganda I and II (C and D) compared to spoligotype patterns of type strains *M. tuberculosis* H37 (ATCC 27294), *M. bovis* (ATCC 19210), *M. bovis* BCG (ATCC 27289), *M. africanum* (ATCC 25420), and a collection of *M. africanum* subtype I (A) and *M. africanum* subtype II (B) isolates from our previous work (15). In contrast to *M. bovis*, all *M. africanum* strains showed hybridization to several of the spacers 39 to 43 which were derived from the direct repeat (DR) region of *M. tuberculosis* H37. In the case of *M. africanum* subtype II, no hybridization was observed to the *M. bovis* BCG-derived spacers 33 to 36, whereas *M. africanum* subtype I isolates as well as the *M. africanum* type strain (ATCC 25420) showed hybridization to at least two of these spacers. All *M. africanum* subtype II strains showed a characteristic lack of hybridization to spacer 40. Strains of genotype Uganda I lack spacer 43 in addition (arrows). In contrast, *M. africanum* subtype I strains lack spacer 39.

analyzed by their RFLP pattern. Within both subtype II genotypes, the strains showed very homogenous IS6110 RFLP patterns, but with a large number of IS6110 copies per strain (approximately 16 to approximately 20), clearly differentiating these strains from *M. africanum* subtype I. A further characteristic of genotypes Uganda I and II is the absence of spoligotype spacer 40 and also the absence of spacer 43 in strains of Uganda I. These results indicate that the strains of these two genotypes are closely related and may have diverged from an *M. tuberculosis*-like ancestor.

In contrast to the homogenous IS6110 RFLP patterns observed for the *M. africanum* subtype II strains, *M. tuberculosis* strains from Kampala showed a high variability of IS6110 banding patterns as well of IS6110 copy numbers. One can speculate that the differences in homogeneity patterns between the *M. africanum* subtype II strains and the *M. tuberculosis* strains result from closely related indigenous mycobacterial populations in the region of Kampala and a high degree of influx from abroad resulting in highly diverse IS6110 RFLP patterns, respectively. In accordance, Daniel (4) presented an interesting study on the early history of tuberculosis in central Africa, which demonstrates that tuberculosis was present in central East Africa at the time of the earliest European entries in the region of Kampala.

The clustering rate obtained by the combination of spoligo-

typing and IS6110 RFLP analysis was similar for *M. tuberculosis* and *M. africanum* subtype II (46% and 49%, respectively) and indicates a high rate of recent human-to-human transmission for strains of both species. Similar clustering rates have recently been measured by IS6110 typing in other African countries such as Botswana (42% [13]), Namibia (47% [8]), and South Africa (45% [25]). Only slightly lower or comparable clustering rates have been reported from other areas of the world with a low incidence of tuberculosis, such as New York (37% [1]) and The Netherlands (47% [22]). This somewhat surprising observation may be due to short study periods or limited numbers of patients with pulmonary tuberculosis that were analyzed in these studies.

Considering the discriminatory power of both typing methods, the results in this study clearly indicate that spoligotyping alone is not well suited for differentiation of *M. tuberculosis* complex strains on the strain level in this high-incidence community. Also, spoligotyping did not facilitate an accurate analysis of the genetic relationship of the strains, as *M. tuberculosis* and *M. africanum* strains with similar spoligotype patterns were clearly separated by their IS6110 RFLP patterns and biochemical characteristics. In contrast to IS6110 RFLP patterns, for which modifications appear to occur by changes of single bands as a function of time (6), large alterations of spoligotype patterns seem to be possible in relatively short time periods.

Alterations of spoligotype patterns thus do not necessarily represent the overall rate of change of the genome. Hence, spoligotyping appears not to be a useful method for determination of the genomic relatedness of *M. tuberculosis* complex strains for phylogenetic purposes.

In conclusion, the results presented here and in earlier studies clearly confirm the existence of *M. africanum* subtype I (West Africa) and subtype II (East Africa, Uganda), which have been previously proposed by numerical analysis of the phenotypic characteristics. *M. africanum* subtype I and *M. africanum* subtype II represent two unique phylogenetic branches within the *M. tuberculosis* complex that originates in West and East Africa, respectively. Both *M. africanum* subtypes have been verified to represent a high portion of *M. tuberculosis* complex strains in certain regions of Africa, as we confirmed that more than 60% of the tuberculosis cases in Kampala are due to *M. africanum* subtype II and not to *M. tuberculosis*.

A high prevalence of *M. africanum* strains in human tuberculosis in Africa might have important implications for tuberculosis control, considering the enormous burden of tuberculosis and HIV-1/AIDS in Africa. Based on the clustering rates observed in our study, no difference in transmission patterns between *M. africanum* subtype II and *M. tuberculosis* could be verified. A preliminary result obtained by analyzing 13 patients indicated that presentations and responses to short-course chemotherapy are comparable for *M. africanum* and *M. tuberculosis* (9).

A more detailed analysis of the clinical presentation, therapy outcome, and epidemiological characteristics of more than 300 cases of *M. africanum*- and *M. tuberculosis*-induced tuberculosis that includes the strains presented in this study is in preparation. Further studies in larger study populations will be needed for more detailed analyses of the regional prevalence and transmission of *M. africanum*-induced tuberculosis, especially in the context of factors such as coinfection with HIV-1.

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