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Evidence that the Spread of *Mycobacterium tuberculosis* Strains with the Beijing Genotype Is Human Population Dependent[▽]

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This study describes a comparative analysis of the Beijing mycobacterial interspersed repetitive unit types of *Mycobacterium tuberculosis* isolates from Cape Town, South Africa, and East Asia. The results show a significant association between the frequency of occurrence of strains from defined Beijing sublineages and the human population from whom they were cultured ($P < 0.0001$).

Mycobacterium tuberculosis strains with the Beijing genotype have been shown to be globally widespread and are particularly prevalent in East Asia, where over 80% of strains from the Beijing, China, region are of this genotype (5). It has been hypothesized that Beijing strains have evolved unique properties, including the ability to evade the protective effect of *Mycobacterium bovis* BCG vaccination (19) and the ability to spread more efficiently than non-Beijing strains (2). However, the clinical presentations of patients with tuberculosis caused by a Beijing strain were found to vary between different geographical settings (3–5,16). Currently, it is not known whether the observed variability in clinical presentation is a function of the Beijing strain population found in particular geographical settings, is a function of the genetic composition of the human population, or is a function of a combination of these two variables.

This study aimed to test the hypothesis that host-pathogen compatibility determined the Beijing strain population structure in different host populations in different geographical settings. Cultures of *M. tuberculosis* isolates from patients of mixed ancestry (14) who were resident in Cape Town, South Africa (20), were classified as being of the Beijing genotype by spoligotyping (10). The Beijing strains were assigned to phylogenetic sublineages as described previously (8) and were genotyped by mycobacterial interspersed repetitive unit (MIRU) typing (17).

During the study period (January 1993 to December 2004), 25 MIRU types were identified among 321 tuberculosis cases infected with a Beijing strain (Table 1). A comparison of the MIRU type data for the Beijing strains from Cape Town and previously published MIRU type data for the Beijing strains from East Asia (1, 9, 12, 13, 15, 18) showed that nine of the Beijing MIRU types (types MT01, MT08, MT11, MT18, MT19, MT21, MT28, MT33, and MT54) were shared between these geographical settings (Table 1). This suggests that the nine shared Beijing MIRU types represent founder strains that were introduced into Cape Town from East Asia, as the latter is thought to be the evolutionary origin of strains with a Beijing genotype (5, 7, 12). The definition of founder MIRU types was supported by their disproportionately high number ($n = 267$) compared to the number with nonfounder MIRU types ($n = 54$) in tuberculosis patients from Cape Town (z test for the hypothesis that the proportion of types were founder MIRU types = 0.5; $P < 0.001$).

Superimposition of the Beijing MIRU type data onto the previously described phylogenetic tree of the Beijing strain family (8) provided a framework that could be used to predict the evolutionary order in which the 25 Beijing MIRU types had evolved (Fig. 1). From this prediction, the Beijing MIRU types could be partitioned into seven Beijing sublineages. The number of founder Beijing MIRU types was variable among the different Beijing sublineages (Fig. 1). Twenty-four of the Beijing MIRU types were unique to their respective sublineages, while the remaining Beijing MIRU type (MT11) was shared by three different sublineages (sublineages 2, 3, and 6) (Fig. 1 and Table 1), suggesting that MT11 was an ancestral Beijing MIRU type (12).

To determine the propensity of Beijing strains from the different sublineages to spread in the human population in Cape Town, the number of cases in circulation within each sublineage was compared to the number of founder strains for that sublineage (Table 1). The number of representatives of these founder strains was shown to be overrepresented in sublineage 7 ($n = 233$ cases from one founder strain) compared with the numbers in sublineages 1 to 6 ($n = 88$ cases from eight founder strains) (z test for the hypothesis that the proportion of sublineage 7 cases = 0.11; $P < 0.001$). In comparison, founder Beijing MIRU types MT01, MT08, MT11, MT18, MT19, MT21, MT33, and MT54 were overrepresented in the human population in East Asia (China, 73/130; Hong Kong, 108/211; Vietnam, 25/37; and Singapore, 45/56) compared to their representation in Cape Town, South Africa (79/321) (Fisher's exact test odds ratio [OR], 4.20; 95% confidence interval [CI], 3.06 to 5.77; $P < 0.0001$).

A significant association between the frequency of occur-

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TABLE 1. Geographical distribution of Beijing MIRU types from Asia and South Africa

MIRU type	Beijing sublineage(s)[b]	No. of copies in the following polymorphic MIRU loci:												No. (%) of strains from[h]:						
		2	4	10	16	20	23	24	26	27	31	39	40	RUS[c,d]	CHN[d]	HK[e]	VNM[d]	SGP[f]	BGD[g]	CT-SA
MT01[a]	4	2	2	3	3	2	5	1	7	3	4	3	3	1 (2.2)	5 (3.8)	7 (3.3)			2 (16.7)	1 (0.3)
MT02	NA[i]	2	2	3	3	2	5	1	5	3	5	3	3	27 (60.0)	2 (1.5)	4 (1.9)		2 (3.6)	7 (58.3)	
MT04	NA	2	2	3	2	2	5	1	5	3	5	3	3	1 (2.2)			1 (2.7)			
MT05	NA	2	2	3	3	2	5	1	4	3	5	3	3	1 (2.2)						
MT07	NA	2	2	3	3	2	5	1	5	3	6	3	3	2 (4.4)						
MT08[a]	6	2	2	3	2	2	5	1	7	3	5	3	3	1 (2.2)	2 (1.5)	3 (1.4)				2 (0.6)
MT09	NA	2	2	3	3	2	5	1	7	3	5	3	1	1 (2.2)			1 (2.7)			
MT11[a]	2, 3, 6	2	2	3	3	2	5	1	7	3	5	3	3	10 (22.2)	42 (32.3)	77 (36.5)	10 (27.0)	39 (69.6)		3 (0.9), 2 (0.6), 8(2.5)[j]
MT12	NA	2	2	1	3	2	5	1	7	3	5	3	3	1 (2.2)	1 (0.8)					
MT13	NA	2	2	3	3	2	6	1	7	1	5	3	1			8 (3.8)	1 (2.7)			
MT14	NA	2	2	3	3	2	5	1	7	3	6	3	3		2 (1.5)	3 (1.4)	1 (2.7)	2 (3.6)		
MT16	NA	2	2	3	3	2	5	1	7	3	5	3	2		5 (3.8)	12 (5.7)		5 (8.9)		
MT17	NA	2	2	3	4	2	5	1	7	3	5	3	3		1 (0.8)			1 (1.8)		
MT18[a]	6	2	2	3	3	2	5	1	7	3	5	4	3		2 (1.5)	5 (2.4)		2 (3.6)		7 (2.2)
MT19[a]	5	2	0	3	3	2	5	1	7	3	5	3	3			2 (0.9)	1 (2.7)			39 (12.1)
MT20	7	2	2	2	3	2	5	1	7	4	5	4	3							19 (5.9)
MT21[a]	6	2	2	3	3	2	5	1	8	3	5	3	3		4 (3.1)	6 (2.8)	2 (5.7)	4 (7.1)		13 (4.0)
MT25	7	2	2	2	3	2	5	1	7	3	3	4	3							18 (5.6)
MT26	6	2	2	3	3	2	5	1	7	3	5	4	4							1 (0.3)
MT27	7	2	2	2	3	2	5	1	3	3	5	4	3							1 (0.3)
MT28[a]	7	2	2	2	3	2	5	1	7	3	5	4	3		16 (12.3)	23 (10.9)	4 (10.8)			189 (58.9)
MT29	6	2	2	3	3	2	5	1	7	4	5	3	3							1 (0.3)
MT33[a]	6	NA	2	3	3	NA	NA	NA	6	3	5	3	3		11 (8.5)	4 (1.9)	12 (32.4)			3 (0.9)
MT37	NA	NA	2	3	3	NA	NA	NA	2	3	5	3	3		2 (1.5)				1 (8.3)	
MT43	NA	NA	2	3	3	NA	NA	NA	6	3	4	3	3						2 (16.7)	
MT44	NA	NA	2	2	3	NA	NA		7	3	5	3	3		6 (4.6)	9 (4.3)	1 (2.7)			
MT47	NA	NA	2	3	3	NA	NA	NA	7	1	5	3	1		3 (2.3)	6 (2.8)	3 (8.1)			
MT48	NA	NA	2	3	3	NA	NA	NA	4	3	4	3	3		2 (1.5)					
MT49	NA	NA	2	7	2	NA	NA	NA	7	3	4	3	3		3 (2.3)					
MT50	NA	NA	2	5	2	NA	NA	NA	7	3	4	3	3		2 (1.5)					
MT51	NA	NA	2	3	2	NA	NA	NA	5	1	5	3	3		2 (1.5)					
MT52	NA	NA	2	3	3	NA	NA	NA	6	3	5	3	2		2 (1.5)					
MT53	NA	NA	2	3	3	NA	NA	NA	7	3	5	1	3		2 (1.5)					
MT54[a]	6	NA	2	3	3	NA	NA	NA	7	3	5	2	3		7 (5.4)	4 (1.9)				1 (0.3)
MT55	NA	NA	2	2	3	NA	NA	NA	5	3	3	4	3		2 (1.5)					
MT56	NA	NA	2	2	3	NA	NA	NA	9	3	5	4	3		2 (1.5)					
MT57	NA	NA	2	2	3	NA	NA	NA	7	3	5	2	3		2 (1.5)					
MTSGP76	NA	2	2	6	2	2	5	1	7	3	4	3	3					1 (1.8)		
MTCT-SA1	1	2	2	3	3	2	5	1	6	3	5	3	1							1 (0.3)
MTCT-SA2	5	2	3	3	3	2	5	1	7	3	5	3	3							1 (0.3)
MTCT-SA3	6	2	2	3	3	2	5	1	7	2	5	3	3							3 (0.9)
MTCT-SA4	6	2	2	3	3	2	5	1	5	3	5	3	3							1 (0.3)
MTCT-SA5	6	2	2	4	3	2	6	1	5	3	3	2	3							1 (0.3)
MTCT-SA6	7	2	2	2	3	2	5	1	7	4	4	4	3							1 (0.3)
MTCT-SA7	7	2	2	2	3	2	5	1	8	4	5	4	3							1 (0.3)
MTCT-SA8	7	2	2	2	3	2	5	1	9	3	5	3	3							1 (0.3)
MTCT-SA9	7	2	2	2	3	2	5	1	7	3	4	4	3							1 (0.3)
MTCT-SA10	7	2	2	2	3	2	5	1	5	3	5	4	3							1 (0.3)
MTCT-SA11	7	2	2	4	3	2	5	1	5	3	3	2	3							1 (0.3)
MTHK1	NA	2	2	3	3	2	5	1	7	3	5	3	4			6 (2.8)				
MTHK2	NA	2	2	3	3	2	5	1	7	3	5	5	3			5 (2.4)				
MTHK3	NA	2	2	3	4	2	5	1	7	3	5	3	2			5 (2.4)				
MTHK4	NA	2	1	3	3	2	5	1	7	3	5	3	3			4 (1.9)				
MTHK5	NA	2	2	3	3	2	5	1	6	3	5	3	4			3 (1.4)				
MTHK6	NA	2	0	3	3	2	5	1	7	3	5	3	4			3 (1.4)				
MTHK7	NA	2	2	3	3	2	5	1	7	3	5	4	2			2 (0.9)				
MTHK8	NA	2	2	3	3	2	5	1	8	3	5	5	3			2 (0.9)				
MTHK9	NA	2	2	2	3	2	5	1	7	3	5	3	2			2 (0.9)				
MTHK10	NA	2	2	3	3	2	5	1	6	3	7	3	3			2 (0.9)				
MTHK11	NA	2	2	3	3	2	2	1	7	4	4	3	3			2 (0.9)				
MTHK12	NA	2	2	2	3	2	5	1	7	3	5	4	4			2 (0.9)				

[a] Founder MIRU types.
[b] According to reference 8.
[c] According to reference 13.
[d] According to reference 12.
[e] According to reference 9.
[f] According to reference 15.
[g] According to reference 1.
[h] RUS, Russia; CHN, China; HK, Hong Kong; VNM, Vietnam; SGP, Singapore; BGD, Bangladesh; CT-SA, Cape Town, South Africa.
[i] NA, not available.
[j] Data are for sublineages 2, 3, and 6, respectively.

rence of strains of defined Beijing sublineages and the human population from whom they were isolated was observed (for sublineages 1 to 6, $n = 88$ for Cape Town and $n = 253$ for East Asia; for sublineage 7, $n = 233$ for Cape Town and $n = 43$ for East Asia; Fisher's exact test OR, 15.58; 95% CI, 10.38 to 23.38; $P < 0.0001$).

It is unlikely that these findings can be explained by multiple importations of founder strains of sublineage 7 in preference

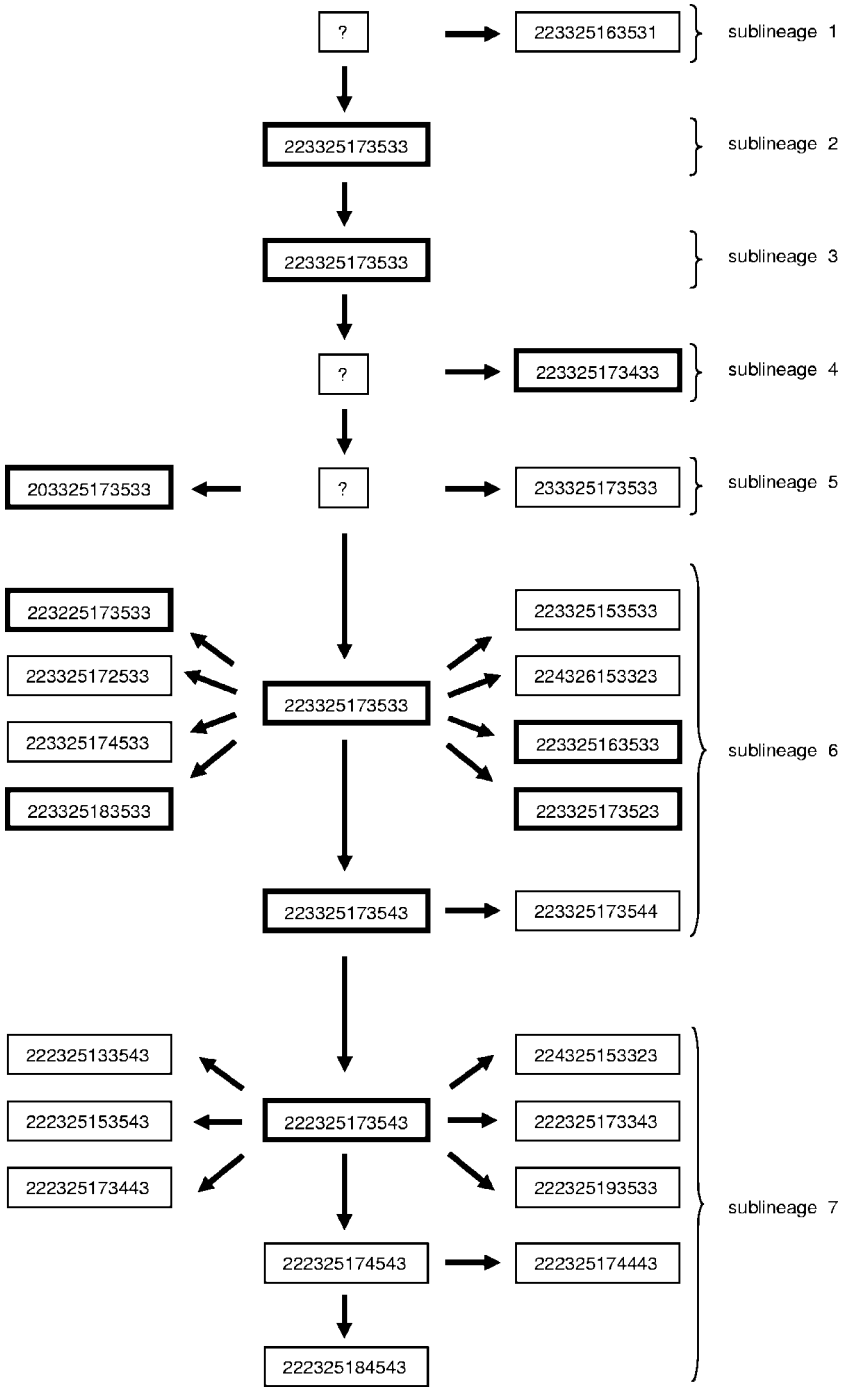

FIG. 1. Evolutionary scenario of Beijing MIRU types according to Beijing sublineage. Beijing MIRU types were grouped according to their respective Beijing sublineages (8), and the most parsimonious evolutionary order was proposed. The Beijing MIRU types are indicated within each box. Founder Beijing MIRU types are indicated by bold boxes. Unknown Beijing MIRU types are indicated by "?."

to founder strains of sublineages 1 to 6, given that immigrants to South Africa came from many different geographical regions in East Asia and that sublineage 7 founder strains are less frequently observed in East Asia. Accordingly, we propose that the situation in Cape Town represents an approximation to a common starting point for all the founder strains introduced, with those best adapted to the local population spreading most efficiently. This could be due to the innate characteristics of the strains within defined Beijing sublineages or the local host population. Susceptibility to *M. tuberculosis* per se has frequently been associated with the HLA genotype (11), and HLA allele frequencies are known to differ widely between human populations with different histories, with certain alleles totally absent in some populations. Our conclusion differs from

that of Gagneux et al. (6), as we demonstrate that strains from a defined sublineage (a subset of strains from an evolutionary lineage) may have been selected by a human population in a defined geographical setting.

In summary, the global success of the Beijing lineage may reflect either the selection of defined sublineages in different geographical settings by distinct human populations or the adaptation of strains in a defined sublineage to spread more readily in a distinct human population. We acknowledge that these contrasting conclusions cannot be easily distinguished with the available data. However, the emergence of a sublineage of Beijing strains with increased pathogenicity may have important implications for the Tuberculosis Control Program. Early diagnosis and contact tracing will be essential to curb the spread of these strains. Furthermore, it will be important to ensure that future vaccines protect against these strains.

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AUTHOR'S CORRECTION

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Volume 45, no. 7, p. 2263–2266, 2007. Pages 2264 and 2265: The MIRU patterns 224326153323 and 224325153323, associated with the *Mycobacterium tuberculosis* strains represented by MIRU types MTCT-SA5 and MTCT-SA11, respectively, in Table 1 and shown as members of sublineages 6 and 7, respectively, in Fig. 1 are not representative of the Beijing genotype but correspond to the LAM3 genotype and the T1 genotype, respectively. Exclusion of these isolates does not significantly alter the statistical analysis.