

Mycobacterial Interspersed Repetitive-Unit-Variable-Number Tandem-Repeat Analysis and Beijing/W Family of Mycobacterium tuberculosis

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Mycobacterial Interspersed Repetitive-Unit–Variable-Number Tandem-Repeat Analysis and Beijing/W Family of *Mycobacterium tuberculosis*

Hanekom et al. (1) highlighted that host-pathogen compatibility determines the Beijing strain population structure in different host populations. Hanekom's study was based on 321 *Mycobacterium tuberculosis* strains, classified as Beijing/W by spoligotyping (3). These strains were genotyped by 12-locus mycobacterial interspersed repetitive-unit (MIRU) analysis. We genotyped nearly 1,600 *M. tuberculosis* strains by spoligotyping (2) and 12-locus MIRU–variable-number tandem-repeat (VNTR) analysis (6), on the basis of the same protocols Hanekom followed. The isolates were collected from 1996 to 1998 and from 2005 to 2007 in the metropolitan area of Milan, Italy.

By doing this, we were able to observe that MIRU patterns 224325153323 and 224326153323 do not identify Beijing strains. MCTC-SA5 and MTCT-SA11 strains of Hanekom's study population were characterized by these MIRU genotypes, respectively, and were identified as part of the Beijing/W family of *M. tuberculosis*. None of the 13 strains of our population characterized by MIRU pattern 224325153323 was classified as Beijing/W by spoligotyping. Moreover, the analysis by 24-locus MIRU (5) showed differences in the genotype of these strains, which appeared to be identical by 12-locus MIRU typing (Table 1). Sourcing literature, we found that strains characterized by MIRU pattern 224325153323 are not classified as Beijing (4, 6). Furthermore, we genotyped by spoligotyping a strain characterized by MIRU pattern 224326153323, equal to Hanekom's strain MCTC-SA5. The spoligotyping genotype obtained was different from that of the Beijing family (Table 1).

In summary, our molecular analysis seems to show that Beijing/W strains are not characterized by MIRU patterns 224325153323 and 224326153323. The greater number of isolates (13 MIRU 224325153323 strains in our study versus only 1 in Hanekom's study) and the repeated analysis of the cases at issue strengthen our conclusions. These findings seem to be quite important, mainly for those who study *M. tuberculosis* phylogenesis and molecular epidemiology. If MIRU patterns 224325153323 and 224326153323 were part of the Beijing/W family, the Beijing/W genotype would be overestimated, giving

rise to a selection bias in epidemiological studies. If we had considered in our study 224325153323 and 224326153323 to be Beijing/W MIRU patterns, the percentage of events due to the *M. tuberculosis* Beijing/W family in native patients would have doubled and, on the other side, the drug resistance risk connected to Beijing/W strains would have reduced meaningfully. Indeed, in our study the majority of strains with MIRU patterns 224325153323 and 224326153323 were collected from Italian patients and weren't associated with drug resistance. Therefore, our hypothesis would have led to erroneous conclusions about Beijing/W family epidemiology and drug resistance.

Finally, our study confirms spoligotyping to be the most sensitive typing method for Beijing/W strain identification.

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TABLE 1. MIRU typing of *M. tuberculosis* isolates

Strain no. ^a	MIRU 12-locus pattern	MIRU 24-locus pattern	Spoligotyping pattern
SH1	224325153323		77777777760771
SH2	224325153323		77777764120771
SH3	224325153323		77777777720771
SH4	224325153323	2243251533232331034423373	77777774020771
SH5	224325153323		776177607000371
SH6	224325153323	22432515332323353442334	77777777720571
SH7	224325153323	224325153323233534423413	77777770000000
SH8	224325153323	224325153323233534422313	73777777520771
SH9	224325153323		776177607000371
SH10	224325153323		77777765720771
SH11	224325153323		77777770000000
SH12	224325153323	22432515332333334423_5_	77777770000000
SH13	224325153323	224325153323233534423_	77777770000000
SH14	224326153323		776177607760771

^a SH, Sacco Hospital strain.

Authors' Reply

We thank the authors Carugati et al. for their observation that mycobacterial interspersed repetitive-unit (MIRU) patterns 224325153323 and 224326153323 do not identify *Mycobacterium tuberculosis* strains of the Beijing genotype. After careful reevaluation of the two samples in question by spoligotyping and PCR analysis of regions of difference, we have found that the two strains were not members of the Beijing genotype. The strain (MTCT-SA11) with the MIRU pattern 224325153323 is a member of the LAM3 genotype, while the strain (MTCT-SA5) with the MIRU pattern 224326153323 is a member of the T1 genotype. We apologize for this error, which probably occurred as a result of a transcription error.

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Ed. Note: See associated Author's Correction on p. 2783.