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Molecular Typing of *Mycobacterium tuberculosis* by Mycobacterial Interspersed Repetitive Unit–Variable-Number Tandem Repeat Analysis, a More Accurate Method for Identifying Epidemiological Links between Patients with Tuberculosis

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IS*6110* fingerprinting of *Mycobacterium tuberculosis* is the standard identification method in studies on transmission of tuberculosis. However, intensive epidemiological investigation may fail to confirm transmission links between patients clustered by IS*6110*-restriction fragment length polymorphism (RFLP) typing. We applied typing based on variable numbers of tandem repeats (VNTRs) of mycobacterial interspersed repetitive units (MIRUs) to isolates from 125 patients in 42 IS*6110* clusters, for which thorough epidemiological data were available, to investigate the potential of this method in distinguishing epidemiologically linked from nonlinked patients. Of seven IS*6110* clusters without epidemiological links, five were split by MIRU-VNTR typing, while nearly all IS*6110* clusters with proven or likely links displayed conserved MIRU-VNTR types. These results provide molecular evidence that not all clusters determined on the basis of multibanded IS*6110* RFLP patterns necessarily reflect transmission of tuberculosis. They support the use of MIRU-VNTR typing as a more reliable and faster method for transmission analysis.

IS*6110* restriction fragment length polymorphism (RFLP) typing of *Mycobacterium tuberculosis* has been used extensively in studies on tuberculosis transmission and is one of the most widely applied and standardized molecular typing methods (1, 6, 10, 31, 34, 35). *M. tuberculosis* isolates from epidemiologically linked patients generally show identical IS*6110* RFLP patterns, thus comprising transmission clusters. Consequently, the finding that a substantial proportion of tuberculosis cases in industrialized countries is clustered by DNA fingerprinting is interpreted as a reflection of a high rate of recent transmission (2, 5, 8, 14, 22, 27, 28, 32, 39). However, IS*6110*-based RFLP fingerprints are not always reliable indicators of epidemiological linkage between tuberculosis patients (7, 33). Even the most meticulous analysis of all available data on possible contacts between clustered patients does not reveal epidemiological links in all cases (33). Furthermore, because in many settings tuberculosis often results from casual contacts, the majority of the links can be identified only after combining the genotyping of the *M. tuberculosis* isolates with intensive epidemiologic investigation. In addition, opportunities for early and thus more-efficient prevention and intervention are limited by the fact IS*6110* RFLP typing is labor intensive and requires weeks for culturing *M. tuberculosis*.

Typing methods based on mycobacterial interspersed repetitive unit (MIRU)-VNTR analysis offer a potential solution to the drawbacks faced using IS*6110* RFLP typing. MIRU-VNTR analysis determines the number of tandem repeats at multiple independent loci (12, 29). This PCR-based method is highly reproducible and much faster than IS*6110*-RFLP typing and displays a discriminatory power close to that of IS*6110*-RFLP, especially in low-incidence areas (9, 15, 18, 23, 25, 30). Previous studies have demonstrated the ability of MIRU-VNTR typing to split certain IS*6110* clusters, suggesting that the use of IS*6110* alone may overestimate the existence of transmission clusters (4, 9, 13, 15, 19, 21, 23). Therefore, MIRU-VNTR typing has been proposed as an efficient first-line typing method, to be followed by IS*6110*-RFLP subtyping of the resulting MIRU-VNTR clusters (9, 25). However, all but one of these observations were made on the basis of *M. tuberculosis* isolates harboring few copies of IS*6110*, for which IS*6110* RFLP typing is known to have limited discriminatory power. Furthermore, epidemiological information on the clustered cases was limited.

To further evaluate the usefulness of MIRU-VNTR typing for conducting population-based studies of recent transmission, we applied secondary MIRU-VNTR typing and spoligotyping (17) to isolates from patients in IS*6110* RFLP clusters selected from a previous population-based study, for whom extensive and well-

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TABLE 1. Characteristics of the TG classified in this study

TG	No. of patients	Description
1	44	Patients with a clear epidemiological link, confirmed by IS*6110*-PGRS typing
2	13	Patients with links as clear as those in TG1, confirmed by IS*6110*-PGRS typing, who should have been, but were not, detected by contact tracing
3	54	Patients with an epidemiological link which was initially unclear but became likely after IS*6110*-PGRS typing and the second interview
4	14	Patients for whom meticulous analysis of all available data ruled out an epidemiological link but whose IS*6110*-PGRS typing results suggested that they belonged to a certain cluster

structured epidemiological data were available (33). Our aim was to determine in a representative manner the potential of MIRU-VNTR typing to distinguish epidemiologically linked patients from unlinked patients in IS*6110* RFLP clusters.

MATERIALS AND METHODS

Study population. In a prospective, population-based study conducted in the province of North Holland, The Netherlands (population, 2,500,000), we previously investigated tuberculosis cases for which DNA fingerprint clustering was an indication of epidemiological linkage and therefore of recent transmission (33). Furthermore, we determined which of these patients could have been detected earlier by contact tracing. The study included all tuberculosis patients residing in the study area and reported to the Municipal Health Services, as is mandatory in The Netherlands. Of 664 patients included from 1 July 1998 to 1 July 2000, 483 (73%) had a positive *M. tuberculosis* culture. Patients who were part of an IS*6110* cluster with one or more other patients from North Holland and diagnosed within the 2-year period preceding or following such patients' diagnosis ($n = 155$) were assigned to transmission groups (TG) according to the likelihood of epidemiological linkage between patients. The assignments were based on the results of extensive interviews of the patients before and after the results of RFLP typing became available, combined with sociodemographic and clinical data.

For the present study, the isolates obtained from 114 of the 155 patients in IS*6110* clusters were subtyped using MIRU-VNTR. Isolates to be subtyped were chosen based on the availability of DNA at the mycobacteria laboratory at the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening of the National Institute of Public Health and the Environment; the 114 patients did not differ significantly from the other 41 patients with respect to age, sex, or Dutch versus non-Dutch ethnicity and nationality (*t* test and χ^2 test; $P > 0.05$). In addition, isolates from 11 patients from outside the above-described study period were included when a cluster was represented by only a single isolate within the study period. In the resulting sample of 125, assignments into the various transmission groups differed slightly between this study and the previous one because of the inclusion of these 11 isolates. The number of isolates subtyped by MIRU-VNTR per TG and the description of the epidemiological link belonging to each TG are depicted in Table 1.

***IS6110*/PGRS RFLP and spoligotyping.** All isolates were subjected to IS*6110* RFLP typing, as described previously (34). When strains harbored fewer than five IS*6110* copies, subtyping using the polymorphic GC-rich tandem repeat (PGRS) was performed as described earlier (36). Isolates were considered to belong to a cluster when no differences were found in IS*6110* or PGRS-banding patterns. Spoligotyping was performed according to a previously described method (17). Computer-assisted analysis of IS*6110*-PGRS RFLP patterns and spoligotyping patterns was done using Bionumerics software, version 3.5 for Windows (Applied Maths, Kortrijk, Belgium), as described previously (16, 37).

VNTR analysis. MIRU-VNTR typing relies on PCR amplification of different MIRU-VNTR regions by use of primers specific for the flanking regions of these MIRUs and on the determination of the sizes of the amplicons, which reflect the numbers of the amplified MIRU copies (12, 20, 25, 29). The 125 *M. tuberculosis* isolates were genotyped by multiplex PCR amplification as described previously (30). The MIRU loci used in this study correspond to a subset of 6 out of 12 previously defined MIRU loci containing VNTRs (29) and 6 additional loci containing VNTRs of other interspersed sequences (12, 20, 24). The VNTRs used in this study are shown in Table 2. They were selected from a wider set of loci on the basis of their variability in unrelated isolates, stability in clonally related isolates, and potential for robust PCR amplification and reliable size analysis (P. Supply, unpublished data). In this report, they are collectively designated MIRU-VNTRs. Briefly, the target genetic sequences were amplified using fluorescently labeled primers and 40 PCR cycles (26, 30). The samples were subjected to electrophoresis using a 96-well ABI 377 automated sequencer. Sizing of the PCR fragments and assignment of the various VNTR alleles in the 12 loci were done using the GeneScan and customized Genotyper software packages (PE Applied Biosystems). The genotypes are expressed as a numerical code representing the number of MIRU-VNTRs in each of the 12 loci.

RESULTS

The results of secondary MIRU-VNTR typing of the *M. tuberculosis* isolates from 125 patients, previously assigned to 42 IS*6110* RFLP clusters, are shown in Fig. 1 and Table 3.

Among the 24 IS*6110*-PGRS clusters comprising 57 patients with a proven epidemiological link (TG1 and TG2), only one (cluster 8), including two patients, was subdivided by MIRU-VNTR typing. Of the 23 IS*6110*-PGRS clusters comprising 54 patients with a likely epidemiological link (TG3), three (clusters 23, 32, and 34) were subdivided by MIRU-VNTR typing. In clusters 23 and 34, one isolate was distinguished from four and two other isolates, respectively, while cluster 32 included two isolates subdivided by MIRU-VNTR typing. In all cases where IS*6110*-PGRS clusters of TG1, TG2, or TG3 were split by MIRU-VNTR typing, the differences were minor and always concerned only one MIRU locus.

In contrast, of the seven IS*6110* clusters without established epidemiological links (TG4), five (71.4%) were split by MIRU-VNTR typing. This difference in the proportion of subdivided clusters is highly significant (TG4 versus TG1, TG2, and TG3, $P = 0.00097$ [Fishers' exact test]). MIRU-VNTR typing identified a total of 12 genotypes among isolates from 14 patients in these groups.

As an average, two MIRU-VNTR loci changed in each of the five TG4 clusters subdivided by MIRU-VNTR typing. This number was thus higher than the number of changes observed for the TG1, TG2, and TG3 clusters that were split by MIRU-VNTR typing (Fig. 1).

In total, 9 (21%) of the 42 IS*6110*-PGRS RFLP clusters were subdivided by MIRU-VNTR typing. The numbers of IS*6110* RFLP bands in these isolates ranged from 5 to 15.

Conversely, two examples (between clusters 9 and 17 and between cluster 16 and one isolate of cluster 32) were found in which isolates with distinct IS*6110*-PGRS RFLP patterns (59.4 and 64.1% similarity, respectively) exhibited identical VNTR patterns (Fig. 1). These isolates also exhibited distinct spoligotyping patterns. For these isolates the discriminative power of IS*6110* RFLP typing and spoligotyping was thus higher than that of the MIRU-VNTR typing.

In contrast, all isolates within the same IS*6110*-PGRS cluster exhibited identical spoligotyping patterns, except for isolates in cluster 38. One of the two TG4 isolates in cluster 38 contained one fewer direct repeat spacer than the other isolates of that

TABLE 2. Conditions for multiplex PCRs of 12 MIRU-VNTR loci

Multiplex	Locus[a]	VNTR length (bp)	$MgCl_2$ concn (mM)	PCR primer pairs (5′ to 3′, with labeling indicated)[b]	Reference
Mix A	VNTR 0580	77	3.0	GCGCGAGAGCCCGAACTGC (FAM) GCGCAGCAGAAACGTCAGC	30
	VNTR 2996	51	3.0	TAGGTCTACCGTCGAAATCTGTGAC CATAGGCGACCAGGCGAATAG (VIC)	30
	VNTR 0802	54	3.0	GGGTTGCTGGATGACAACGTGT (NED) GGGTGATCTCGGCGAAATCAGATA	30
Mix B	VNTR 0960	53	2.0	GTTCTTGACCAACTGCAGTCGTCC GCCACCTTGGTGATCAGCTACCT (FAM)	30
	VNTR 1644	53	2.0	TCGGTGATCGGGTCCAGTCCAAGTA CCCGTCGTGCAGCCCTGGTAC (VIC)	30
	VNTR 3192	53	2.0	ACTGATTGGCTTCATACGGCTTTA GTGCCGACGTGGTCTTGAT (NED)	30
Mix F	VNTR 0424	51	1.5	CTTGGCCGGCATCAAGCGCATTATT GGCAGCAGAGCCCGGGATTCTTC (FAM)	38
	VNTR 0577	58	1.5	CGAGAGTGGCAGTGGCGGTTATCT (VIC) AATGACTTGAACGCGCAAATTGTGA	38
Mix G	VNTR 2401	58	3.0	CTTGAAGCCCCGGTCTCATCTGT (FAM) ACTTGAACCCCCACGCCCATTAGTA	38
	VNTR 3690	58	3.0	CGGTGGAGGCGATGAACGTCTTC (VIC) TAGAGCGGCACGGGGGAAAGCTTAG	38
	VNTR 4156	59	3.0	TGACCACGGATTGCTCTAGT GCCGGCGTCCATGTT (NED)	38
Individual	VNTR 1982	78	1.5	CCGGAATCTGCAATGGCGGCAAATTAAAAG TGATCTGACTCTGCCGCCGCTGCAAATA (FAM)	26

[a] Locus designation according to the position (in kilobase pairs) on the *M. tuberculosis* H37Rv chromosome. VNTR 00580, 2996, 0802, 0960, 1644, and 3192 correspond to MIRU locus 4 (alias ETRD), 26, 40, 10, 16, and 31 (alias ETRE), respectively (12, 29). VNTR 577, 1982, and 4156 correspond to ETRC, QUB-18, and QUB 4156, respectively (12, 20, 24).

[b] FAM, 6-carboxyfluorescein.

IS*6110* cluster. This isolate had a MIRU-VNTR type identical to that of the two other isolates within the same IS*6110* cluster.

Comparison of the spoligotypes and IS*6110* fingerprints detected in this study with those in the international spoligotype and IS*6110*-RFLP databases revealed that the corresponding isolates belong to a wide variety of genotype families (11, 26). These families were distributed to identical degrees among the different "transmission groups." All IS*6110* clusters, except clusters 1, 15, 32, and 34, displayed IS*6110* profiles consisting of six or more bands.

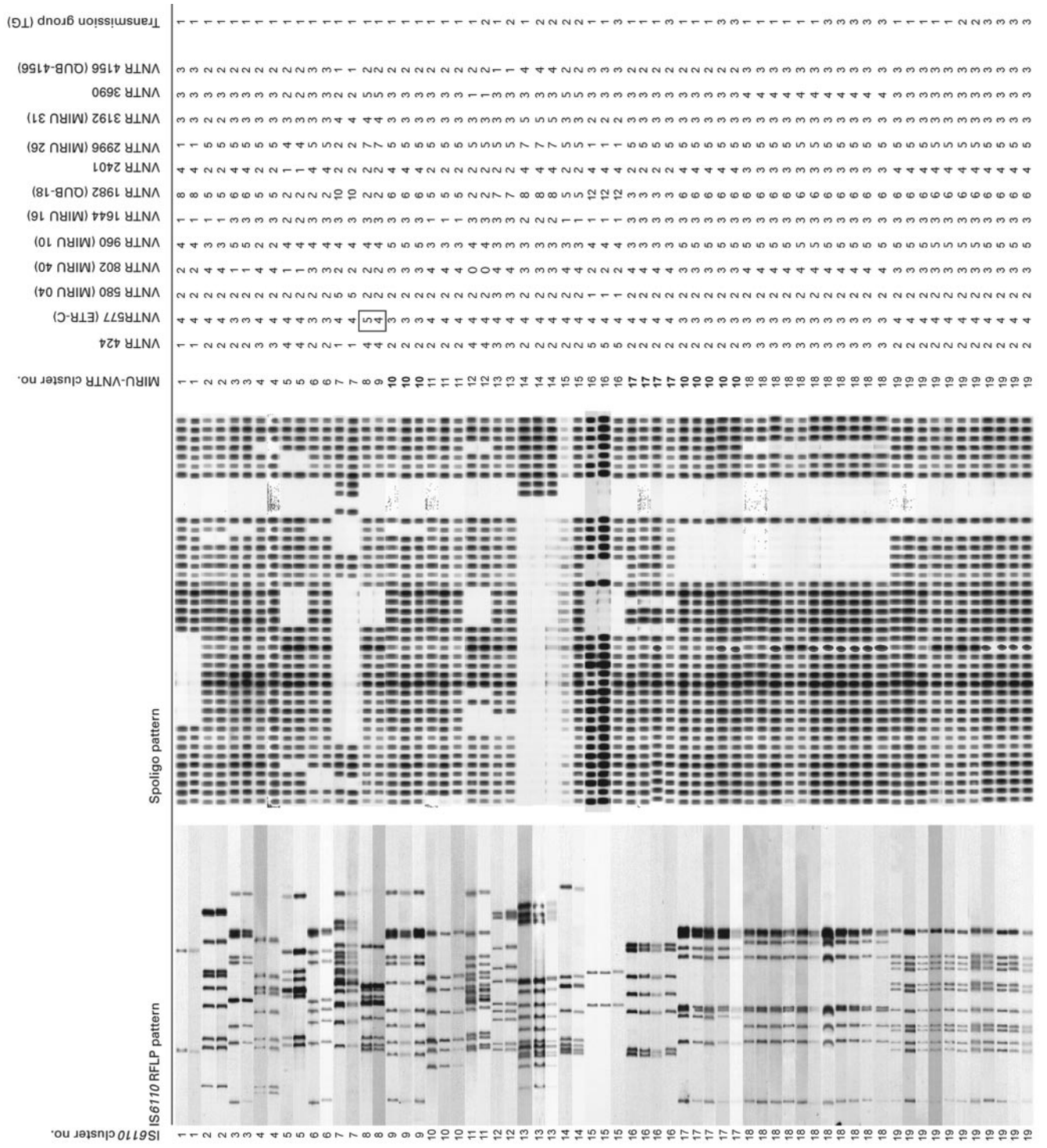
DISCUSSION

Molecular typing using IS*6110* has generally been used as the standard method in studies of the transmission of tuberculosis, on the assumption that IS*6110* RFLP-based clustering of cases is the result of recent transmission. However, even meticulous analysis of all epidemiological data fails to confirm transmission links among a number of patients within IS*6110*-PGRS RFLP clusters. For 14% of patients grouped into IS*6110*-PGRS clusters in our previous study, an epidemiological link was virtually ruled out after two consecutive series of extensive interviews of the patients (33).

Failures to detect recent transmission chains indicated by IS*6110*-PGRS clusters are classically attributed to the limited sensitivity of conventional contact investigation, but our findings suggest that some reflect limitations in the resolution of IS*6110*-PGRS RFLP. In the present study, MIRU-VNTR typing was found to be the most accurate first-line method to exclude epidemiological links.

In our study, 71.4% (5/7) of the IS*6110*-PGRS clusters comprising patients for whom an epidemiological link was not detected (TG4) and whose isolates showed RFLP fingerprints with high copy numbers of IS*6110* were subdivided by MIRU-VNTR typing. For these patients, meticulous analysis of all data, including the results of a second interview, indicated that they were unlikely to have met during the infectious period of the index patient. Examples include two immigrants from the same country, one with extrapulmonary tuberculosis diagnosed in 1998 after living in The Netherlands for 10 years and the other with infectious pulmonary tuberculosis that was diagnosed at immigration in 2000. Although a common but unidentified source for the two TG4 strains with matching MIRU-VNTRs cannot be ruled out completely, the thorough analysis of our epidemiological data makes this event less likely. The resolution of the other TG4 clusters by MIRU-VNTR typing fully corroborates the absence of a direct epidemiological link indicated by intensive contact investigation.

Conversely, in contrast to TG4, almost all patients in IS*6110*-PGRS clusters with an evident (TG1 and TG2) or likely (TG3) epidemiological link remained clustered after MIRU-VNTR typing. In addition, in the few IS*6110*-PGRS clusters in TG1, TG2, and TG3 that were split by MIRU-VNTR typing, the MIRU-VNTR types differed only by a single MIRU locus, compared to differences in up to three loci for the TG4 clusters split by MIRU-VNTR. TG1 and TG2 included relatives or close friends of an infectious index patient who were identified by contact tracing (TG1) or who should have been—but were not—identified by that intervention (TG2). The latter group includes persons in close contact with an index patient but not mentioned by him or her, persons not reached for the contact investigation by the Municipal Health Service, and persons not complying with that investigation. Only one IS*6110*-PGRS TG1 cluster, including two patients, was subdivided by MIRU-VNTR typing out of the 24 clusters comprising a total of 57 patients in TG1 and TG2. This latter observation most likely


IS6110 cluster no.
IS6110 RFLP pattern
Spoligo pattern
MIRU-VNTR cluster no.
VNTR 424
VNTR577 (ETR-C)
VNTR 580 (MIRU 04)
VNTR 802 (MIRU 40)
VNTR 960 (MIRU 10)
VNTR 1644 (MIRU 16)
VNTR 1982 (QUB-18)
VNTR 2401
VNTR 2996 (MIRU 26)
VNTR 3192 (MIRU 31)
VNTR 3690
VNTR 4156 (QUB-4156)
Transmission group (TG)

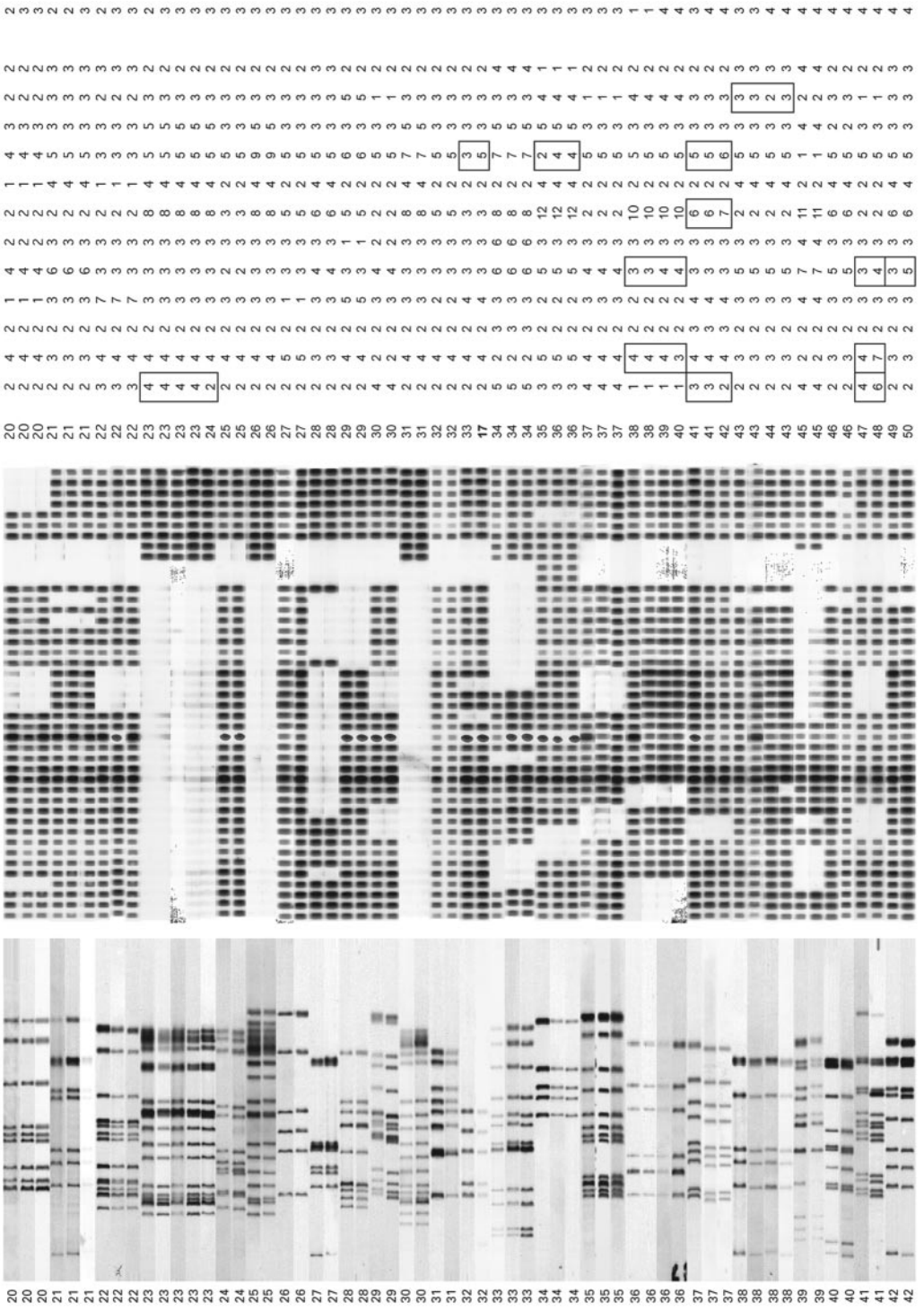


FIG. 1. IS*6110*-PGRS RFLP and MIRU-VNTR genotypes of the *M. tuberculosis* isolates from 125 patients in 42 IS*6110*-PGRS clusters, as assigned to four transmission groups (for explanation of transmission groups, see Table 1). Designations of MIRU-VNTR loci are given according to the position (in kilobase pairs) on the *M. tuberculosis* H37Rv chromosome. Alias designations are in parentheses. Spoligo, spoligotyping.

TABLE 3. Comparison of discriminatory power of MIRU-VNTR typing versus IS*6110*-PGRS RFLP typing in relation to epidemiological data

Epidemiological link between clustered patients (transmission group)	IS*6110* clusters involved[a]	No. of patients	No. of IS*6110*-PGRS clusters	No. (%) of IS*6110*-PGRS clusters split by MIRU-VNTR typing
Evident link (TG1 and TG2)	1–23, 36	57	24	1 (4.2)
Likely link (TG3)	15–35, 37, 38	54	23	3 (13)
No link established (TG4)	36–42	14	7	5 (71.4)

[a] See Fig. 1 for designated cluster numbers.
[b] Isolates from a total of 125 patients were examined.

reflects a rare and stochastic MIRU-VNTR mutation event, possibly DNA replication or homologous recombination dependent, in clonal populations originating from recent transmission (30). This hypothesis is consistent with the low proportion of changes in MIRU-VNTR profiles observed among serial isolates from chronically infected patients (25).

In TG3, including 54 patients in 23 IS*6110*-PGRS clusters, an epidemiological link which was initially unclear became likely after IS*6110* typing and a second interview. This group includes patients living in the same apartment complex or regularly using the same tram service as an index patient, as well as homeless persons who used housing facilities with an index patient before he or she was diagnosed and thereafter became untraceable. Only 3 of these 54 patients were not matched by identical MIRU-VNTR patterns within their three respective clusters. Notably, while one of these three clusters included isolates with 17 IS*6110* copies (cluster 23), the two others (clusters 32 and 34) included isolates with only 5 IS*6110* copies. Whereas the MIRU-VNTR difference in cluster 23 also probably reflects a rare MIRU-VNTR variation between clonally transmitted isolates (see above), another explanation may be possible for the differences observed in clusters 32 and 34. As noted earlier, a total of five IS*6110* copies is considered the upper limit in defining the low-copy-number isolates. For such isolates, IS*6110* RFLP is thought to be of limited specificity for detection of recent transmission even when complemented by spoligotyping and PGRS typing. Therefore, the possibility is not totally excluded that the patients whose low-copy-number isolates were distinguished by MIRU-VNTR typing were actually infected by as-yet-unidentified contacts, different from the contacts of the other patients of clusters 32 or 34.

A comparison of the overall discriminatory powers of MIRU-VNTR and IS*6110*-RFLP typing was beyond the scope of this study. It was designed to test the potential of MIRU-VNTR to split epiunlinked IS*6110*-PGRS clusters but not the potential of IS*6110*-PGRS to split epiunlinked MIRU-VNTR clusters. It is conceivable that if MIRU-VNTR typing had been used as initial typing method, a part of the MIRU-VNTR clusters would have been subdivided by IS*6110*-PGRS RFLP typing. In this study, two epidemiologically unlinked clusters with different IS*6110*-PGRS RFLP patterns had identical MIRU-VNTR patterns, consistent with the slightly lower resolution power of MIRU-VNTR typing compared to that of IS*6110*-RFLP typing (9, 25). Nevertheless, it is of considerable practical advantage to use MIRU-VNTR as the first-line method for transmission analysis, especially when combined with spoligotyping, as these two clusters had distinct spoligotypes, as has already been observed in previous studies (9).

In contrast to IS*6110*-PGRS-based RFLP typing, PCR-based MIRU-VNTR typing or spoligotyping can be applied with crude DNA extracts from heat-killed mycobacterial cultures as soon as they become positive, avoiding culture delays of several weeks. Depending upon the initial bacterial load, the total time needed to obtain the genotypes when starting from the clinical specimen can be reduced to a few days (3). Epidemiological investigations based on genotyping data can thus begin sooner, providing opportunities for the more rapid detection of secondary cases, latent tuberculosis infections, and sites of transmission (33). By combining MIRU-VNTR typing and spoligotyping, results can more easily be read and exchanged between laboratories than is the case with the complex IS*6110*-PGRS patterns. Therefore, the first-line use of MIRU-VNTR typing instead of IS*6110*-PGRS RFLP typing should provide a more rapid and more reliable insight into the dynamics of tuberculosis transmission both within and among communities or countries.

It can be concluded from this study that for a number of cases, estimated to be 14% in The Netherlands, the use of IS*6110*-PGRS RFLP typing will result in false clustering of tuberculosis patients, even if the isolates have high copy numbers of IS*6110*. The population-based study presented here included patients from both the general population and from tuberculosis risk groups in rural and urban areas representative of The Netherlands. Since the country's sociodemographic and disease incidence characteristics are similar to those of most developed countries, we predict that our observations can be widely generalized.

Taking into consideration the technical advantages and the accuracy gained for reliable exclusion of epidemiological links, a combination of MIRU-VNTR typing and spoligotyping instead of RFLP-based methods would thus be recommendable in many settings for first-line screening of potential tuberculosis case clusters.

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REFERENCES

1. **Agerton, T., S. Valway, B. Gore, C. Pozsik, B. Plikaytis, C. Woodley, and I. M. Onorato.** 1997. Transmission of a highly drug-resistant strain of *Mycobacterium tuberculosis*. Community outbreak and nosocomial transmission via a contaminated bronchoscope. JAMA **278:**1073–1077.

2. **Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Drucker, and B. R. Bloom.** 1994. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiological methods. N. Engl. J. Med. **330:**1710–1716.
3. **Allix, C., P. Supply, and M. Fauville-Dufaux.** 2004. Utility of fast mycobacterial interspersed repetitive unit-variable number tandem repeat genotyping in clinical mycobacteriological analysis. Clin. Infect. Dis. **39:**783–789.
4. **Barlow, R. E., D. M. Gascoyne-Binzi, S. H. Gillespie, A. Dickens, S. Qamer, and P. M. Hawkey.** 2001. Comparison of variable number tandem repeat and IS*6110*-restriction fragment length polymorphism analyses for discrimination of high- and low-copy-number IS*6110 Mycobacterium tuberculosis* isolates. J. Clin. Microbiol. **39:**2453–2457.
5. **Barnes, P. F., and M. D. Cave.** 2003. Molecular epidemiology of tuberculosis. N. Engl. J. Med. **349:**1149–1156.
6. **Bock, N. N., J. P. Mallory, N. Mobley, B. DeVoe, and B. Brooks Taylor.** 1998. Outbreak of tuberculosis associated with a floating card game in the rural south: lessons for tuberculosis contact investigations. Clin. Infect. Dis. **27:** 1221–1226.
7. **Braden, C. R., G. L. Templeton, M. D. Cave, S. Valway, I. M. Onorato, K. G. Castro, D. Moers, Z. Yang, W. W. Stead, and J. H. Bates.** 1997. Interpretation of restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates from a state with a large rural population. J. Infect. Dis. **175:**1446–1452.
8. **Caminero, J. A., M. J. Pena, M. I. Campos-Herrero, J. C. Rodriguez, I. Garcia, P. Cabrera, C. Lafoz, S. Samper, H. Takiff, O. Alfonso, J. M. Pavon, M. J. Torres, D. van Soolingen, D. E. Enarson, and C. Martin.** 2001. Epidemiological evidence of the spread of a *Mycobacterium tuberculosis* strain of the Beijing genotype on Gran Canaria Island. Am. J. Respir. Crit. Care Med. **164:**1165–1170.
9. **Cowan, L. S., L. Mosher, L. Diem, J. P. Massey, and J. T. Crawford.** 2002. Variable-number tandem repeat typing of *Mycobacterium tuberculosis* isolates with low copy numbers of IS*6110* by using mycobacterial interspersed repetitive units. J. Clin. Microbiol. **40:**1592–1602.
10. **Daley, C. L., P. M. Small, G. F. Schecter, G. K. Schoolnik, R. A. McAdam, W. R. Jacobs, Jr., and P. C. Hopewell.** 1992. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus: an analysis using restriction fragment length polymorphisms. N. Engl. J. Med. **326:**231–235.
11. **Filliol, I., J. R. Driscoll, D. van Soolingen, B. N. Kreiswirth, K. Kremer, G. Valétudie, D. D. Anh, R. Barlow, D. Banerjee, P. J. Bifani, K. Brudey, A. Cataldi, R. C. Cooksey, D. V. Cousins, J. W. Dale, O. A. Dellagostin, F. Drobniewski, G. Engelmann, S. Ferdinand, D. Gascoyne-Binzi, M. Gordon, C. Gutierrez, W. H. Haas, H. Heersma, E. Kassa-Kalembo, H. M. Li, A. Makristathis, C. Mammina, G. Martin, P. Moström, I. Mokrousov, V. Narbonne, O. Narvskaya, A. Nastasi, S. N. Niobe-Eyangoh, J. W. Pape, V. Rasolofo-Razanamparany, M. Ridell, M. L. Rossetti, F. Stauffer, P. N. Suffys, H. Takiff, J. Texier-Maugein, V. Vincent, J. H. de Waard, C. Sola, and N. Rastogi.** 2003. Snapshot of moving and expanding clones of *Mycobacterium tuberculosis* and their global distribution assessed by spoligotyping in an international study. J. Clin. Microbiol. **41:**1963–1970.
12. **Frothingham, R., and W. A. Meeker-O'Connell.** 1998. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology **144:**1189–1196.
13. **Gascoyne-Binzi, D. M., R. E. Barlow, R. Frothingham, G. Robinson, T. A. Collyns, R. Gelletlie, and P. M. Hawkey.** 2001. Rapid identification of laboratory contamination with *Mycobacterium tuberculosis* using variable number tandem repeat analysis. J. Clin. Microbiol. **39:**69–74.
14. **Geng, E., B. Kreiswirth, C. Driver, J. Li, J. Burzynski, P. DellaLatta, A. LaPaz, and N. W. Schluger.** 2002. Changes in the transmission of tuberculosis in New York City from 1990 to 1999. N. Engl. J. Med. **346:**1453–1458.
15. **Hawkey, P. M., E. G. Smith, J. T. Evans, P. Monk, G. Bryan, H. H. Mohammed, M. Bardhan, and R. N. Pugh.** 2003. Mycobacterial interspersed repetitive unit typing of *Mycobacterium tuberculosis* compared to IS*6110*-based restriction fragment length polymorphism analysis for investigation of apparently clustered cases of tuberculosis. J. Clin. Microbiol. **41:**3514–3520.
16. **Hermans, P. W. M., F. Messadi, H. Guebrexabher, D. van Soolingen, P. E. W. de Haas, H. Heersma, H. de Neeling, A. Ayoub, F. Portaels, D. Frommel, M. Zribi, and J. D. A. van Embden.** 1995. Analysis of the population structure of *Mycobacterium tuberculosis* in Ethiopia, Tunisia and the Netherlands: usefulness of DNA typing for global tuberculosis epidemiology. J. Infect. Dis. **171:**1504–1513.
17. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
18. **Kremer, K., D. van Soolingen, R. Frothingham, W. H. Haas, P. W. M. Hermans, C. Martin, P. Palittapongarnpim, B. B. Plikaytis, L. W. Riley, M. A. Yakrus, J. M. Musser, and J. D. A. van Embden.** 1999. Comparison of methods based on different molecular epidemiological markers for typing *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. J. Clin. Microbiol. **37:**2607–2618.
19. **Kwara, A., R. Schiro, L. S. Cowan, N. E. Hyslop, M. F. Wiser, S. R. Harrison, P. Kissinger, L. Diem, and J. T. Crawford.** 2003. Evaluation of the epidemiologic utility of secondary typing methods for differentiation of *Mycobacterium tuberculosis* isolates. J. Clin. Microbiol. **41:**2683–2685.
20. **Le Fleche, P., M. Fabre, F. Denoeud, J. L. Koeck, and G. Vergnaud.** 2002. High resolution, on-line identification of strains from the *Mycobacterium tuberculosis* complex based on tandem repeat typing. B. M. C. Microbiol. **2:**37.
21. **Lok, K. H., W. H. Benjamin, M. E. Kimerling, V. Pruitt, D. Mulcahy, N. Robinson, N. B. Keenan, and N. E. Dunlap.** 2002. Molecular typing of *Mycobacterium tuberculosis* strains with a common two-band IS*6110* pattern. Emerg. Infect. Dis. **8:**1303–1305.
22. **Maguire, H., J. W. Dale, T. D. McHugh, P. D. Butcher, S. H. Gillespie, A. Costetsos, H. Al-Ghusein, R. Holland, A. Dickens, L. Marston, P. Wilson, R. Pitman, D. Strachan, F. A. Drobniewski, and D. K. Banerjee.** 2002. Molecular epidemiology of tuberculosis in London 1995–7 showing low rate of active transmission. Thorax **57:**617–622.
23. **Mazars, E., S. Lesjean, A. L. Banuls, M. Gilbert, V. Vincent, B. Gicquel, M. Tibayrenc, C. Locht, and P. Supply.** 2001. High-resolution minisatellite-based typing as a portable approach to global analysis of *Mycobacterium tuberculosis* molecular epidemiology. Proc. Natl. Acad. Sci. USA **98:**1901–1906.
24. **Roring, S., A. Scott, D. Brittain, I. Walker, G. Hewinson, S. Neill, and R. Skuce.** 2002. Development of variable-number tandem repeat typing of *Mycobacterium bovis*: comparison of results with those obtained by using existing exact tandem repeats and spoligotyping. J. Clin. Microbiol. **40:**1–8.
25. **Savine, E., W. M. Warren, G. D. van der Spuy, N. Beyers, P. D. van Helden, C. Locht, and P. Supply.** 2002. Stability of variable-number tandem repeats of mycobacterial interspersed repetitive units from 12 loci in serial isolates of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **40:**4561–4566.
26. **Shamputa, I. C., L. Rigouts, L. A. Eyongeta, N. A. El Aila, A. van Deun, A. H. Salim, E. Willery, C. Locht, P. Supply, and F. Portaels.** 2004. Genotypic and phenotypic heterogeneity among *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients. J. Clin. Microbiol. **42:**5528–5536.
27. **Small, P. M., P. C. Hopewell, S. P. Singh, A. Paz, J. Parsonnet, D. C. Ruston, G. F. Schecter, C. L. Daley, and G. K. Schoolnik.** 1994. The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. N. Engl. J. Med. **330:**1703–1709.
28. **Solsona, J., J. A. Caylà, E. Verdú, M. P. Estrada, S. Garcia, D. Roca, B. Miquel, P. Coll, F. March, and the Cooperative Group for Contact Study of Tuberculosis Patients in Ciutat Vella.** Molecular and conventional epidemiology of tuberculosis in an inner city district. Int. J. Tuber. Lung Dis. **5:**724–731.
29. **Supply, P., E. Mazars, S. Lesjean, V. Vincent, B. Gicquel, and C. Locht.** 2000. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. Mol. Microbiol. **36:**762–771.
30. **Supply, P., S. Lesjean, E. Savine, K. Kremer, D. van Soolingen, and C. Locht.** 2001. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. J. Clin. Microbiol. **39:**3563–3571.
31. **Valway, S. E., M. P. C. Sanchez, T. F. Shinnick, I. Orme, T. Agerton, D. Hoy, J. Scott Jones, H. Westmoreland, and I. M. Onorato.** 1998. An outbreak involving extensive transmission of a virulent strain of *Mycobacterium tuberculosis*. N. Engl. J. Med. **338:**633–639.
32. **Van Deutekom, H., J. J. J. Gerritsen, D. van Soolingen, E. J. C. van Ameijden, J. D. A van Embden, and R. A. Coutinho.** 1997. A molecular epidemiological approach to studying the transmission of tuberculosis in Amsterdam. Clin. Infect. Dis. **25:**1071–1077.
33. **Van Deutekom, H., S. P. Hoijng, P. E. W. de Haas, M. W. Langendam, A. Horsman, D. van Soolingen, and R. A. Coutinho.** 2004. Clustered tuberculosis cases: do they represent recent transmission and can they be detected earlier? Am. J. Respir. Crit. Care Med. **169:**806–810.
34. **Van Embden, J. D. A., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. W. M. Hermans, C. Martin, R. McAdam, and T. M. Shinnick.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
35. **Van Soolingen, D.** 2001. Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements. J. Intern. Med. **249:**1–26.
36. **Van Soolingen, D., P. E. W. de Haas, P. W. M. Hermans, P. M. A. Groenen, and J. D. A. van Embden.** 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **31:**1987–1995.
37. **Van Soolingen, D., L. Qian, P. E. W. de Haas, J. T. Douglas, H. Traore, F. Portaels, H. Z. Qing, D. Enkhsaikan, P. Nyamadawa, and J. D. A. van Embden.** 1995. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. J. Clin. Microbiol. **33:**3234–3238.
38. **Warren, R. M., T. C. Victor, E. M. Streicher, M. Richardson, G. D. van der Spuy, R. Johnson, V. N. Chihota, C. Locht, P. Supply, and P. D. van Helden.** 2004. Clonal expansion of a globally disseminated lineage of *Mycobacterium tuberculosis* with low IS*6110* copy numbers. J. Clin. Microbiol. **42:**5774–5782.
39. **Weis, S. E., J. M. Pogoda, Z. Yang, M. D. Cave, C. Wallace, M. Kelley, and P. F. Barnes.** 2002. Transmission dynamics of tuberculosis in Tarrant county, Texas. Am. J. Respir. Crit. Care Med. **166:**36–42.