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Original Article

Evaluation of the Selected 12-locus MIRU for Genotyping Beijing Family *Mycobacterium Tuberculosis* in Korea

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Background: Mycobacterial Interspersed Repetitive Units (MIRUs) that are located mainly in intergenic regions dispersed throughout the *Mycobacterium tuberculosis* genome. The selected MIRU loci, which were composed of a 12-locus set, demonstrated a high power for discrimination of *Mycobacterium tuberculosis* isolates collected from Kangwon province of Korea. To evaluate its ability to discriminate the *M. tuberculosis* strains, 45 clinical isolates were genotyped using the methods IS*6110* RFLP and MIRU.
Methods: All the samples were collected during the period from January 2007 to December 2007 from TB patients, who were residents and registered to a public health center of Kangwon Province in Korea. A total of 45 DNAs were extracted from clinical isolated mycobacterial strains and genotyped using IS*6110* RFLP, the MIRU method.
Results: We compared the 12-MIRU with IS*6110* RFLP in the 45 samples, the 12-locus version offered less discriminatory power (Hunter-Gaston discriminatory index [HGDI]: 0.959 vs 0.998; 57.78% of clustered cases vs 8.89%).
Conclusion: This 12-locus MIRU can be useful when additional combinations of other loci for genotyping *M. tuberculosis* in Korea where the Beijing family strains are dominant.

Key Words: Genotype; Korea; Interspersed Repetitive Sequences; *Mycobacterium tuberculosis*

Introduction

The molecular typing of *Mycobacterium tuberculosis* has been extensively applied to analyze, improve the knowledge and control of tuberculosis (TB) by allowing the detection of transmission and the distinction between re-infection and relapse[1]. IS*6110*-restriction fragment length polymorphism (RFLP) is the reference technique for genotyping *M. tuberculosis* because of its high discriminatory power[2]. However, the need for well-grown cultures and purified DNA to obtain RFLP data means that it takes a long time to produce results. All the more, RFLP is limited when analyzing MTB strains with a low number of IS*6110* copies.

Different PCR-based genotyping approaches targeting the variable number of tandem repeats (VNTR) have been developed to compensate for the limitations of RFLP. These include VNTR analysis based on mycobacterial interspersed repetitive units (MIRU) that are located mainly in intergenic regions dispersed throughout the *Mycobacterium tuberculosis* genome[3-5].

And which has been considered a good alternative method and has proven to be faster and easier to perform. MIRU-VNTR genotyping based on a 12-loci set (MIRU-12) has been evaluated in several studies in different settings. Some authors have found it to show a discriminatory power equivalent to that of RFLP and have considered it an alternative to IS*6110*-RFLP for epidemiological purposes[4-7].

However, other authors have found limitations in its discriminatory power and incomplete correlation with the RFLP analysis[8-10], indicating that MIRU analysis should be combined with an additional genotyping method[10-13].

We compared the MIRU-12 version and IS*6110*-RFLP in independent samples in the context of molecular epi-

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demiology studies. These data could serve to clarify the final application of this procedure in our setting.

Materials and Methods

1. Study population

All the samples were collected during the period from January 2007 to December 2007 from TB patients who were resident and registered to public health center of Kangwon Province in Korea. Total 45 DNAs were extracted from mycobacterial strains and DNA fingerprinted.

2. IS*6110* RFLP genotyping

M. tuberculosis isolates were cultured on Löwenstein-Jensen medium, and DNA was extracted as previously described[14]. Each isolate was classified by IS*6110* RFLP genotyping using internationally standardized protocols. IS*6110* RFLP patterns were analyzed using BioNumerics ver. 5.1 (Applied-Maths, Sint-Martens-Latem, Belgium) with position tolerance settings allowing a 0.5%, band based dice setting and dendrogram type was UPGMA.

3. MIRU typing

Twelve MIRU-loci were amplified by PCR as described previously[5,15-17]. The number of repeats at each genomic locus was calculated according to the electrophoretic mobility of the corresponding PCR product[4].

Alleles were assigned numerical values according to the number of repeats present in that genomic locus. Isolates were genotyped using selected 12 loci among the 24-locus set (Table 1). Combinations of MIRU loci were the following combination of MIRUs; MIRU 4 (ETR D), MIRU 26, MIRU 40, MIRU 10, MIRU 16, MIRU 31 (ETR E), MIRU 2, MIRU 23, MIRU 39, MIRU 20, MIRU 24, MIRU 27 (QUB-5).

The MIRU typing was performed using Ex Taq with GC PCR buffer I (Takara Bio, kindly provided by Dr. Shinji Maeda at Research Institute of Tuberculosis in Japan). The PCR mixture was prepared in a 20 μL volume with 2× GC PCR buffer I, 0.5 U Ex Taq, 200 μM each of four dNTPs, 0.5 μM each of the primer set and 10 ng template DNA. Then PCR was carried out for all 12-loci under the following conditions: initial denaturation at 94°C for 5 min, and then 35 cycles of 94°C for 30 s, 57～63°C for 30s and 72°C for 3 min, followed by a final extension at 72°C for 10 min[17].

4. Allelic diversity, discrimination

The allelic diversity (h) at each MIRU locus was calculated using the index $h=1-\sum x_i^2$, where x_i is the frequency of the ith allele at the locus, as used in other studies[18,19]. The Hunter-Gaston discriminatory index (HGDI) was calculated in accordance with a method ex-

Table 1. Locus designations and PCR primer sequences used in this study for the 12-locus set

No.	Locus	Alias (es)	Repeat unit length (bp)	PCR primer pairs (5' to 3')	
1	154	MIRU 2	53	TGGACTTGCAGCAATGGACCAACT	TACTCGGACGCCGGCTCAAAAT
2	580	MIRU 4; ETR D	77	GCGCGAGAGCCCGAACTGC	GCGCAGCAGAAACGCCAGC
3	960	MIRU 10	53	GTTCTTGACCAACTGCAGTCGTCC	GCCACCTTGGTGATCAGCTACCT
4	1644	MIRU 16	53	TCGGTGATCGGGTCCAGTCCAAGTA	CCCGTCGTGCAGCCCTGGTAC
5	2059	MIRU 20	77	TCGGAGAGATGCCCTTCGAGTTAG	GGAGACCGCGACCAGGTACTTGTA
6	2531	MIRU 23	53	CTGTCGATGGCCGCAACAAAACG	AGCTCAACGGGTTCGCCCTTTTGTC
7	2687	MIRU 24	54	CGACCAAGATGTGCAGGAATACAT	GGGCGAGTTGAGCTCACAGAA
8	2996	MIRU 26	51	TAGGTCTACCGTCGAAATCTGTGAC	CATAGGCGACCAGGCGAATAG
9	3007	MIRU 27; QUB-5	53	TCGAAAGCCTCTGCGTGCCAGTAA	GCGATGTGAGCGTGCCACTCAA
10	3192	MIRU 31; ETR E	53	ACTGATTGGCTTCATACGGCTTTA	GTGCCGACGTGGTCTTGAT
11	4348	MIRU 39	53	CGCATCGACAAACTGGAGCCAAAC	CGGAAACGTCTACGCCCCACACAT
12	802	MIRU 40	54	GGGTTGCTGGATGACAACGTGT	GGGTGATCTCGGCGAAATCAGATA

plained in another paper to evaluate the combination of some MIRU loci[20].

Results

From 45 collected isolates were analyzed using IS*6110* RFLP. The percentage of clustered isolates was 8.89% (4/45). The two clusters were composed of the Beijing genotype strains. The total number of Beijing genotype strains used in this study was 32 (71.11%). The Beijing family strains were already confirmed to be dominant in Korean. The American CDC has recommended the standard 12-locus MIRU for TB analyses[13,21]. Each isolate was subsequently genotyped with 12 MIRU-loci and analyzed (Tables 2, 3). As a main approach to comparing the efficiency of MIRU-12 with IS*6110* RFLP, we used the same collected samples to test the efficiency of the genotyping method. RFLP clustered 8.89% of the isolates in 2 clusters (2 to 4 representatives), whereas MIRU-12 clustered 57.78% in 9 clusters (9 to 26 representatives). Therefore, the Hunter-Gaston discriminatory index (HDGI) was higher for IS*6110* RFLP (0.998) than for MIRU-12 (0.959). Consequently, these 12-locus combinations overestimated clustering. The loci with the highest HGDIs were MIRU26 (0.696), MIRU31 (0.595), and MIRU39 (0.503) (Table 4). The performance of these locus combinations, in relation to the IS*6110* RFLP genotyping method was compared using figure (Figure 1).

So, the 12-locus MIRU version offered less discriminatory power (Hunter-Gaston discriminatory index [HDGI]: 0.959 vs. 0.998; 57.78% of clustered cases vs. 8.89%).

To determine whether MIRU genotyping could be used as a method to epidemiologically group strains with the Beijing genotype, the correlation between MIRU genotype and IS*6110* RFLP genotyping was quantified. Selected 12 MIRU- locus combinations correctly genotyped 65.12% of the isolates, in comparison to 100% with IS*6110* RFLP genotyping as a gold standard.

Discussion

The search for an alternative to IS*6110* RFLP, has led to the development of a PCR-based techniques. These techniques have proven to be fast and easy to perform, and it allows the direct exchange of data between laboratories. Different combinations of MIRU and other VNTR loci have been introduced, but most of the studies have focused on a set of 12-locus MIRU, which the American CDC has recommended. One purpose of this study is to evaluate the MIRU system with equal discriminatory power to that of IS*6110* RFLP in Korea. This format offers a higher discriminatory power (close to the

Table 2. Allelic profiles of 12 MIRU loci in 45 *M. tuberculosis* isolates

MIRU locus	Code of repetitive unit (s)								
	0	1	2	3	4	5	6	7	8
MIRU 2	402 bp	455 bp	508 bp	561 bp	614 bp	667 bp	720 bp	773 bp	
MIRU 4 (ETR D)	184 bp	261 bp	338 bp	352 bp	415 bp	492 bp	569 bp		
MIRU 10	482 bp	536 bp	589 bp	642 bp	695 bp	748 bp	802 bp	855 bp	907 bp
MIRU 16	565 bp	618 bp	671 bp	724 bp	777 bp	830 bp			
MIRU 20	210 bp	287 bp	364 bp	441 bp					
MIRU 23	150 bp	200 bp	253 bp	306 bp	359 bp	412 bp	465 bp	518 bp	571 bp
MIRU 24	395 bp	447 bp	501 bp	555 bp					
MIRU 26	285 bp	336 bp	387 bp	438 bp	489 bp	540 bp	591 bp	642 bp	693 bp
MIRU 27 (QUB-5)	498 bp	551 bp	604 bp	657 bp	710 bp				
MIRU 31 (ETR E)	492 bp	545 bp	598 bp	651 bp	704 bp	757 bp	810 bp	862 bp	
MIRU 39	540 bp	591 bp	642 bp	693 bp	744 bp	795 bp	846 bp	897 bp	
MIRU 40	354 bp	408 bp	462 bp	516 bp	570 bp	624 bp	678 bp	732 bp	

Table 3. Selected 12 MIRU code numbers of the 45 strains

Samples	M2	M4	M10	M16	M20	M23	M24	M26	M27	M31	M39	M40
1	2	2	3	3	2	5	1	7	3	5	3	5
2	2	2	2	3	2	5	1	5	3	3	2	5
3	2	2	2	2	2	5	1	4	3	3	2	7
4	2	2	3	3	2	5	1	7	3	6	3	5
5	2	2	3	3	2	5	1	7	3	6	3	4
6	2	2	3	3	2	5	1	3	3	5	3	5
7	2	2	3	3	2	5	1	7	3	5	3	5
8	2	2	3	3	2	5	1	5	3	5	3	5
9	2	2	3	3	2	5	1	3	3	3	2	5
10	2	2	5	1	2	5	1	1	3	3	2	4
11	2	2	2	3	2	5	1	5	3	3	2	5
12	2	2	3	3	2	5	1	8	3	6	3	5
13	2	2	3	3	2	5	1	7	3	5	3	5
14	2	2	2	4	2	5	1	5	3	3	2	5
15	2	2	3	3	2	5	1	7	3	6	3	4
16	2	2	3	3	2	5	1	3	3	3	2	5
17	2	3	2	1	2	5	1	5	3	3	2	9
18	2	2	3	3	2	5	1	3	3	5	3	5
19	2	2	3	3	2	5	1	7	3	5	3	5
20	2	2	3	3	2	5	1	5	3	5	3	5
21	2	2	3	4	2	5	1	7	3	5	6	5
22	2	2	3	3	2	5	1	7	3	5	3	5
23	2	2	3	3	1	5	1	7	4	5	3	5
24	2	2	2	3	2	5	1	6	3	5	3	5
25	2	2	3	3	2	5	1	5	3	5	3	5
26	2	2	3	3	2	5	1	7	4	5	3	5
27	2	2	3	3	2	5	1	3	3	5	3	5
28	2	2	3	3	2	5	1	7	3	5	8	5
29	2	2	3	3	2	5	1	2	3	3	2	5
30	2	2	3	3	2	5	1	7	3	4	3	4
31	2	2	3	3	2	5	1	3	4	4	3	5
32	2	2	3	3	2	5	1	7	4	5	3	4
33	2	2	3	3	2	5	1	6	3	5	3	4
34	2	2	3	4	2	5	1	7	3	5	3	5
35	2	2	2	3	2	5	1	4	3	3	2	3
36	2	2	3	3	2	5	1	7	3	5	3	5
37	2	2	2	3	2	5	1	6	3	5	3	5
38	2	2	1	3	2	5	1	7	3	5	3	5
39	2	2	3	3	2	5	1	7	3	5	8	5
40	2	4	2	3	2	5	1	5	1	3	2	5
41	2	2	3	3	2	5	1	7	3	5	3	5
42	2	2	3	3	2	5	1	7	3	5	3	5
43	2	2	3	4	2	5	1	7	3	5	6	5
44	2	2	3	3	2	5	1	7	3	5	3	5
45	2	2	3	3	2	5	1	7	3	4	3	5

gold standard IS*6110* RFLP) than other combinations[19,22].

However other studies, have revealed lower discriminatory power and a low correlation with IS*6110* RFLP data[13,23].

In our study that, compared with IS*6110* RFLP, 12-locus MIRU overestimated by defining a higher num-

Table 4. The *h* and HGDI values for each locus of the MIRU loci selected for analysis

No.	Locus	Alias	*h* (n=45)	HGDI (n=45)
1	154	MIRU 2	0	0
2	580	MIRU 4	0.086	0.087
3	960	MIRU 10	0.389	0.396
4	1644	MIRU 16	0.277	0.282
5	2059	MIRU 20	0.044	0.044
6	2531	MIRU 23	0	0
7	2687	MIRU 24	0	0
8	2996	MIRU 26	0.682	0.696
9	3007	MIRU 27	0.208	0.206
10	3192	MIRU 31	0.583	0.595
11	4348	MIRU 39	0.492	0.503
12	802	MIRU 40	0.341	0.348

ber of clusters. Furthermore, 57.78% of MIRU-12 clusters showed a low or no correlation with RFLP data. These data require caution when considering substituting RFLP with 12-locus MIRU-12.

Despite the finding that MIRU typing did not provide sufficient resolution for the genotyping of *M. tuberculosis* strains compared with IS*6110* RFLP, the advantages of molecular method typing over RFLP are apparent. PCR based genotyping methods are easy to perform, and give data that are easily interpretable and exchangeable between laboratories. Therefore, further efforts should be expanded to improve its discriminatory power for the Beijing family strains are dominant area like

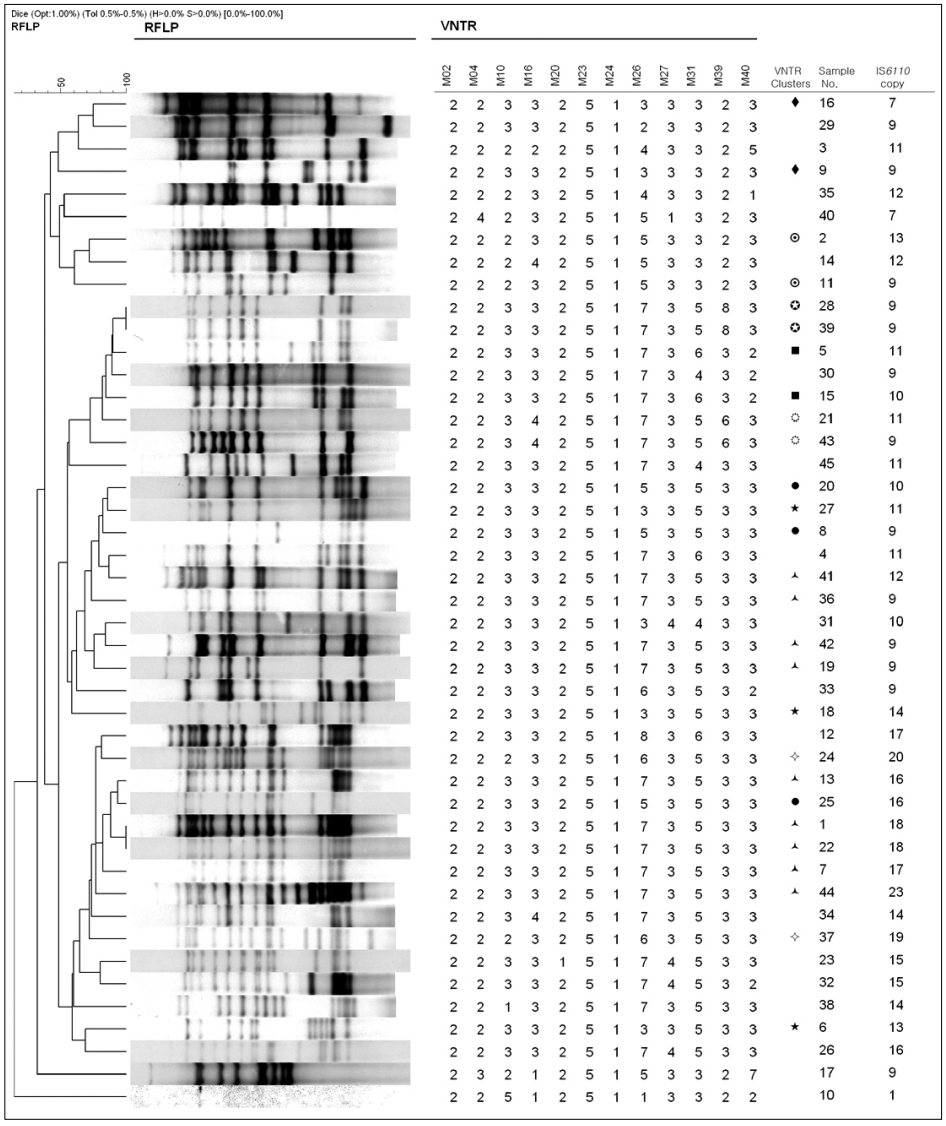

Figure 1. IS*6110* RFLP and MIRU patterns of the *M. tuberculosis* isolates from 45 patients.

Korea.

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