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Improved Molecular Epidemiological Analysis of *Mycobacterium tuberculosis* Strains Using Multi-Locus Variable Number of Tandem Repeats Typing

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Tuberculosis is one of the major bacterial diseases in Japan (1). Advances in the molecular epidemiological analysis of this disease have greatly improved tuberculosis prevention; e.g., by providing confirmation of tuberculosis infection among epidemiologically linked patients (2) and determining risk factors for tuberculosis infection (3). However, only a limited number of population-based studies by epidemiological analysis have been carried out in Japan (4,5); therefore, the risk factors for tuberculosis infection have not been well analyzed.

Restriction fragment length polymorphism using an IS*6110* probe (RFLP analysis) has been the "gold standard" method for the molecular epidemiological analysis of *Mycobacterium tuberculosis*, although RFLP analysis has disadvantages such as being time-consuming (3) and having difficulty in comparing large numbers of analyzed data (6). Variable number of tandem repeats (VNTR) typing is an alternative method for the molecular epidemiological analysis of tuberculosis, and standardization of this method for a global analysis of tuberculosis has been proposed (7). However, recent studies have shown that the global VNTR typing method cannot adequately differentiate *M. tuberculosis* Beijing genotype strains, and that several VNTR loci must be added to obtain sufficient differentiation of these strains (8,9). Beijing genotype strains are highly prevalent in Japan (10,11); therefore, VNTR typing should be done with the additional VNTR loci in Japan.

A total of 177 *M. tuberculosis* strains, isolated from epidemiologically unlinked patients in Chiba Prefecture, Japan, during 2003 - 2005, were investigated using RFLP and VNTR analysis. RFLP analysis was performed as previously described and similarities of RFLP patterns were calculated using the Dice coefficient (12). In these studies, a RFLP-identical cluster was defined as a group of strains with 100% similarity as determined by RFLP analysis, and 55 of the 177 strains were grouped in 18 RFLP-identical clusters (Table 1). A RFLP-similar cluster was defined as a group of strains with at least 90% similarity by RFLP analysis, and 110 of the 177 strains were grouped in 27 RFLP-similar clusters (Table 1).

The 177 strains were also analyzed by VNTR typing using both the 15 VNTR loci proposed as a standard set for global typing (7) and 8 VNTR loci proposed as the "Beijing option" to differentiate Beijing genotype strains (9). A VNTR cluster was defined as a group of strains with at least 95% similarity calculating using the Pearson products-moment correlation coefficient, and 22 of the 177 strains were grouped in 10 VNTR clusters (Table 1). All of the 22 strains were also grouped in RFLP-identical clusters (data not shown).

The proportion of tuberculosis cases due to recent infection was estimated as the percent transmission calculated using the "n-1 method" (13). The percent transmission was 20.9% for RFLP-identical cluster strains, 46.9% for RFLP-similar cluster strains, and 6.8% for VNTR cluster strains (Table 1). The different estimates of recent tuberculosis infection were due to the lower degree of strain differentiation by RFLP analysis, which was seen in the larger sizes of Beijing genotype RFLP clusters compared to non-Beijing genotype RFLP clusters (Table 2).

The results of this study indicate that the method used for molecular epidemiological analysis is a critical factor for the accurate estimation of recent tuberculosis infection. Although this was not a population-based study, the results could be misinterpreted as indicating that many inapparent transmissions of tuberculosis occurred if RFLP data alone were used to estimate recent tuberculosis infection. In that case, traditional epidemiological investigations are mistaken to be ineffective for tuberculosis prevention. In contrast, if VNTR data were used for the estimation, the results indicate that inapparent transmission of tuberculosis is relatively rare, suggesting that traditional epidemiological investigations can

Table 1. *M. tuberculosis* clusters and transmission determined by VNTR and RFLP analysis

Analytical method	No. of strains		Percent strains in clusters	No. of clusters	Percent transmission
	Clustered	Unclustered			
RFLP-identical	55	122	31.1	18	20.9
RFLP-similar	110	67	62.1	27	46.9
VNTR	22	155	12.4	10	6.8

VNTR, variable number of tandem repeats; RFLP, restriction fragment length polymorphism.

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Table 2. Number of *M. tuberculosis* strains in clusters formed by RFLP and VNTR analysis

Analytical method	Genotype	Cluster size						
		2	3	4	5	8	12	24
RFLP-identical	non-Beijing	5	–	–	–	–	–	–
	Beijing	8	3	–	–	1	1	–
RFLP-similar	non-Beijing	2	–	–	2	–	–	–
	Beijing	11	4	4	2	–	1	1
VNTR	non-Beijing	–	–	–	–	–	–	–
	Beijing	8	2	–	–	–	–	–

Abbreviations are in Table 1.

reveal almost all transmission of tuberculosis. However, the annual rate of tuberculosis infection in Japan is higher than in other developed countries (1), indicating that existing measures for tuberculosis prevention are insufficient to reduce the prevalence of tuberculosis. Therefore, population-based studies using molecular epidemiological methods able to differentiate *M. tuberculosis* strains prevalent in Japan (e.g., multi-locus VNTR typing) are needed to analyze the epidemiology of tuberculosis infections and to implement specific effective measures for tuberculosis prevention.

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