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# VNTR typing studies of *Mycobacterium leprae* in China: Assessment of methods and stability of markers during treatment

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*Summary*

*Objective* To evaluate the reliability and feasibility of two methods of multilocus variable number of tandem repeat analysis (MLVA) for strain typing of *M. leprae*, and to study whether short tandem repeat loci are stable and suitable for epidemiological study of leprosy.

*Methods* Total DNA was extracted from skin biopsies of 20 new multibacillary (MB) patients from China diagnosed in 2006. To determine the copy numbers of short tandem repeats (STRs) for 13 loci, we amplified each locus individually by PCR, followed by sequence analysis of the amplicons. Separately, the same loci, plus four others were amplified by Multiplex PCRs (MP) using fluorescent primers and the copy number was identified by fragment length analysis (MP-FLA). MLVA was also performed at different times during treatment for a subset of the patients.

*Results and conclusions* Genetic variability of *M. leprae* in China can be assessed in microsatellite loci. (GTA)9 and (TTC)21 loci are hypervariable, with array sizes of 25 repeat units or more. The expansion of the (GTA)9 locus is a characteristic of some *M. leprae* isolates in China. A high level of allele concordance was observed between PCR-sequencing and MP-FLA methods. However, MP-FLA method was cost-effective, rapid, high throughput and suitable for strain typing. Five of the 20 isolates of *M. leprae* were from patients residing in the same township in Qiubei County, Yunnan, and matched closely by MLVA. Three of these patients are family contacts of previously diagnosed patients, with intra-familial strain types being similar, suggesting infections from common sources and transmission chain(s). The VNTR

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patterns were highly similar in biopsy and slit skin smears (SSS) before treatment, and in the SSS collected at various time points during treatment. Taken together, VNTR strain typing is a useful tool for study of short range transmission in leprosy.

## Introduction

Leprosy control strategies are designed to stop transmission through early case detection and treatment with Multidrug therapy (MDT) recommended by WHO[1] but the incidence of leprosy in South-West China has not dramatically decreased after 20 years of MDT implementation.[2] According to the 2008 WER report, there were 1526 cases in China during 2007.[3]

Strain typing of *M. leprae* based on Variable Number-Tandem Repeats (VNTRs) has been performed in Qiubei County, a high endemic County, China since 2003.[4] The study has already identified several clusters of patients whose *M. leprae* specimens shared similar VNTR profiles and has uncovered characteristic features of leprosy transmission in this region. However, MLVA strain typing using direct sequencing of single PCR products for each locus is laborious and time-consuming, and requires an adequate amount of *M. leprae* DNA. Multiplex PCR, which extends the number of targets that can be amplified with the available template DNA, followed by capillary electrophoresis for fragment length analysis (FLA) is simple, rapid, and reproducible, and can be adapted to a large set of isolates. For the development of a reliable genotyping technique, the present study attempts to compare the two typing techniques based on 20 *M. leprae* isolates and to further understand the genotypic variants of *M. leprae* in China.

Short tandem repeat (STR) loci are prone to variation in copy number, and therefore offer genetic variability to otherwise highly stable and clonal genomes of *M. leprae*. To date, 32 STR loci with a spectrum of allelic diversities have been reported for *M. leprae*.[5–8] For tracing transmission of leprosy in endemic regions, discriminatory yet stable loci are required. In general, it is accepted that shared DNA patterns represent recent transmission events and demonstrate that the source of *M. leprae* is common in chains of transmission. However, whether VNTR typing is suitable for tracing transmission of leprosy is dependent on the stability of VNTR loci. If the evolution of a marker is too rapid during the epidemiological investigation, linked cases will likely be missed. In contrast, a marker with low levels of variation, and long–term stability may lead to an overestimation of recent transmission. Therefore, the study of stability and evolutionary characteristics of VNTR loci in *M. leprae* is necessary for the correct interpretation of molecular epidemiological data in leprosy. In this study we assess the stability of STR loci in slit skin smear samples at diagnosis and during treatment. In addition, we compared alleles in skin biopsy and skin slit scrapings collected before treatment.

## Materials and Methods

### PATIENTS' ENROLLMENT AND SPECIMEN COLLECTION

Twenty newly detected multibacillary (MB) leprosy cases were enrolled in the IDEAL study during 2006 from three field sites in South-West provinces of China [Qiubei, Yunnan (9), Xinyi, Guizhou (6), Xichang, Sichuan (2) and Beijing Tropical Medicine Research Institute (3)]. After informed consent was obtained from the patients, skin biopsies were collected by the punch excision method.

### DNA EXTRACTION

DNA was extracted from skin biopsies by DNeasy tissue Kit (QIAgen), according to the manufacturer's recommendation.

### VNTR GENOTYPING

The genotyping of 13 loci was performed in BTMRI by single PCR-sequencing using the specific primers recommended in the IDEAL Standard operating procedure[9] using AmpliTaq Gold (Applied Biosystems) or other polymerase enzymes (Promega) requiring the use of nested PCR in some loci. However, Qiagen Multiplex PCR enzyme was more sensitive, and could overcome the need for nested PCR. The allelic number was determined by DNA sequencing of amplicons (Shanghai Sangon Biological Engineering Technology and Services Co. Ltd., Beijing). The genotyping of 17 loci, including (AT)15, (TA)18 and 6-3a (*rpoT*) was performed by Multiplex-PCR (MP) to amplify four or five VNTR loci simultaneously in four combinations. Multiplex PCR products were subjected to fragment length analysis (FLA) for determining the allele number for each VNTR locus.[10] Multiplex-PCR and fragment length analysis (MP-FLA) was performed in Colorado State University (CSU, Proteomics and Metabolomics Facility).[10,11]

## Results

### COMPARISON OF THE REPEAT UNITS ON EACH VNTR LOCUS DETERMINED BY PCR-SEQUENCING AND MULTIPLEX-PCR FOR FLA

The repeat units, or copy number of the 13 and 17 loci are listed in Table 1 for each of the 20 DNA samples.

The genotyping of 13 loci were performed by both single PCR-sequencing and MP-FLA. Analysis based on the 13 loci in 20 isolates, shows considerable concordance between the two methods in the present study, with three differences in the 260 data points. The differences were at two loci [(GTA)9 and (AC)8a] for patient (#296) and (GTA)9 for patient (#306). (GTA)9 is a highly divergent VNTR locus of *M. leprae* in China and to identify the exact repeat units is difficult by both direct sequencing and MP-FLA when copy number is over 25, as in the case of sample from patient #306. Interestingly, in the biopsy for patient #296 there were two bands and two peaks of (GTA)9 on agarose gel electrophoresis and FLA chromatograms respectively, corresponding to 9 and 25 copy alleles.

For some of the microsatellites, which typically yield a cluster of stutter products,[12] we report two possible alleles (± one copy) when it is not clear which is the dominant product.

### THE FEATURES OF VNTR GENOTYPES IN 20 ISOLATES OF *M. LEPRAE* COLLECTED FROM CHINA

The diversity or allelic numbers of the 17 VNTR loci is similar to that reported before. *M. leprae* isolates collected from China lack polymorphism in several minisatellite loci, 21-3, 27-5, 18-8, 23-3, 12-5, *rpo*T and one microsatellite locus, (GGT)5. However, in this collection, we observed new alleles in the patients that came to BTMRI in Beijing (originally from Tibet region, Sichuang Province); a four copy 12-5 allele and a 4 copy *rpoT* allele,

**Table 1.** The copy numbers of VNTR loci determined by PCR- sequencing and MP-FLA

| | | Combination 1 | | | | | Combination 2 | | | | Combination 3 | | | | Combination 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code# | Field site | (AC)8b | (GTA)9 | (GGT)5 | (AT)17 | rpoT | 21-3 | (AC)9 | (AT)15 | (AC)8a | 27-5 | 6-7 | (TA)18 | (GAA)21 | 18-8 | 23-3 | 12-5 | (TA)10 |
| 287* | YN-QB | 8 | 9 | 4 | 13 | 3 | 2 | 9 | 16 | 12 | 5 | 8 | 23 | 19 | 7 | 2 | 3 | 10 |
| 287** | | 8 | 9 | 4 | 13 | ND | 2 | 9 | ND | 12 | 5 | 8 | ND | 19 | ND | 2 | 3* | 10 |
| 298* | YN-QB | 8 | 9 | 4 | 13 | 3 | 2 | 9 | 16 | 11 | 5 | 8 | 20 | 22 | 7 | 2 | 3 | 10 |
| 298** | | 8 | 9 | 4 | 13 | ND | 2 | 9 | ND | 11 | Neg | 8 | ND | 22 | ND | 2 | 3 | 10 |
| 299* | YN-QB | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 299** | | 8 | 9 | 4 | 14 | ND | 2 | 9 | ND | 11 | 5 | 8 | ND | 22 | ND | 2 | 3 | 10 |
| 302* | YN-QB | 8 | 9 | 4 | 13 | 3 | 2 | 9 | 18 | 10 | 5 | 8 | 20 | 28 | 7 | 2 | 3 | 10 |
| 302** | | 8 | 9 | 4 | 13 | ND | 2 | 9 | ND | 10 | 5 | 8 | ND | 28? | ND | 2 | 3 | 10 |
| 312* | YN-QB | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 21 | 22 | 7 | 2 | 3 | 10 |
| 312** | | 8 | 9 | 4 | 14 | ND | 2 | 9 | ND | 11 | 5 | 8 | ND | 22 | ND | 2 | 3 | 10 |
| 296* | YN-QB | 8 | 9/25† | 4 | 13 | 3 | 2 | 8 | 15/16 | 10† | 5 | 8 | 14 | 13 | 7 | 2 | 3 | 10 |
| 296** | | 8 | 9 | 4 | 13 | ND | Neg | 8 | ND | 11† | Neg | 8 | ND | 13 | ND | 2 | Neg | 10 |
| 289* | YN-QB | 7 | 10 | 4 | 18 | 3 | 2 | 9 | 14 | 8 | 5 | 7 | 16 | 16 | 7 | 2 | 4 | 12 |
| 289** | | 7 | 10 | 4 | 18 | ND | 2 | 9 | ND | 8 | 5 | 7 | ND | 16 | ND | 2 | 4* | 12 |
| 300* | YN-QB | 8 | 25 | 4 | 15 | 3 | 2 | 8 | 15 | 10 | 5 | 8 | 14 | 13 | 7 | 2 | 3 | 12 |
| 300** | | 8 | 25 | 4 | 15 | ND | Neg | 8 | ND | 10 | 5 | 8 | ND | 13 | ND | 2 | Neg | 12 |
| 301* | YN-QB | 8 | 15 | 4 | 13 | 3 | 2 | 8 | 14 | 9 | 5 | 9 | 21/20 | 22 | 7 | 2 | 3 | 13 |
| 301** | | 8 | 15 | 4 | 13 | ND | 2 | 8 | ND | 9 | 5 | 9 | ND | 22 | ND | 2 | 3 | 13 |
| 303* | GZ-XY | 8 | 12 | 4 | 15 | 3 | 2 | 8 | 22 | 10 | 5 | 8 | 12 | 20 | 7 | 2 | 3 | 13 |
| 303** | | 8 | 12 | 4 | 15 | ND | 2 | 8 | ND | 10 | 5 | 8 | ND | 20 | ND | 2 | 3 | 13 |
| 304* | GZ-XY | 8 | 24 | 4 | 14 | 3 | 2 | 8 | 13 | 10 | 5 | 7 | 21 | 23 | 7 | 2 | 3 | 10 |
| 304** | | 8 | 24 | 4 | 14 | ND | 2 | 8 | ND | 10 | 5 | 7 | ND | 23 | ND | 2 | 3 | 10 |
| 305* | GZ-XY | 7 | 13 | 4 | 11 | 3 | 2 | 9 | 11 | 9 | 5 | 8 | 11 | 22/23 | 7 | 2 | 3 | 18 |
| 305** | | 7 | 13 | 4 | 11 | ND | 2 | 9 | ND | 9 | 5 | 8 | ND | 22? | ND | 2 | 3 | 18? |
| 306* | GZ-XY | 9 | 56† | 4 | 17 | 3 | 2 | 9 | 13 | 12 | 5 | 8 | 15 | 17 | 7 | 2 | 3 | 11 |
| 306** | | 9 | 34/40?† | 4 | 17 | ND | 2 | 9 | ND | 12 | 5 | 8 | ND | 17 | ND | Neg | 3 | 11 |
| 307* | GZ-XY | 8 | 24 | 4 | 14 | 3 | 2 | 8 | 13 | 10 | 5 | 7 | 21 | 23 | 7 | 2 | 3 | 10 |
| 307** | | 8 | 24 | 4 | 14 | ND | 2 | 8 | ND | 10 | Neg | 7 | ND | 23 | ND | Neg | Neg | 10 |
| 314* | GZ-XY | 8 | 47 | 4 | 17 | 3 | 2 | 9 | 13 | 7 | 5 | 7 | 19 | 20 | 7 | 2 | 3 | 11 |
| 314** | | 8 | 47 | 4 | 17 | ND | 2 | 9 | ND | 7 | 5 | 7 | ND | 20 | ND | 2 | 3 | 11 |
| 293* | Beijing | 9 | 19 | 4 | 13 | 3 | 2 | 8 | 17 | 12 | 5 | 9 | 15 | 15 | 7 | 2 | 3 | 18 |
| 293** | | 9 | 19 | 4 | 13 | ND | 2 | 8 | ND | 12 | 5 | 9 | ND | 15 | ND | 2 | 3* | 18 |
| 294* | Beijing | 7 | 12 | 4 | 13 | 3 | 2 | 8 | 10 | 9 | 5 | 6 | 15 | 12 | 8 | 2 | 4 | 9 |
| 294** | | 7 | 12 | 4 | 13 | ND | 2 | 8 | ND | 9 | 5 | 6 | ND | 12 | ND | 2 | 4* | 9 |

**Table 1.** *continued*

| | | Combination 1 | | | | | Combination 2 | | | | Combination 3 | | | | Combination 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code# | Field site | (AC)8b | (GTA)9 | (GGT)5 | (AT)17 | rpoT | 21-3 | (AC)9 | (AT)15 | (AC)8a | 27-5 | 6-7 | (TA)18 | (GAA)21 | 18-8 | 23-3 | 12-5 | (TA)10 |
| 308* | Beijing | 7 | 26 | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 308** | | 7 | 26 | 4 | 9 | ND | 2 | 7 | ND | 8 | 5 | 9 | ND | 12 | ND | 2 | 3 | 9 |
| 309* | CS-XC | 10 | 25 | 4 | 14 | 3 | 2 | 7 | 20 | 9 | 5 | 8 | 16 | 18 | 7 | 2 | 3 | 16 |
| 309** | | 10 | 25 | 4 | 14 | ND | 2 | 7 | ND | 9 | 5 | 8 | ND | 18 | ND | 2 | 3 | 16 |
| 310* | CS-XC | 7 | 17 | 4 | 12 | 3 | 2 | 9 | 16 | 10 | 5 | 10 | 14 | 11 | 7 | 3 | 3 | 21 |
| 310** | | 7 | 17 | 4 | 12 | ND | 2 | 9 | ND | 10 | 5 | 10 | ND | 11 | ND | 2 | 3 | 21 |

*: MP-FLA result; **: PCR sequencing result; ND, Not determined; Neg, No PCR product, MP-FLA signal or sequence was unreadable; Shaded boxes indicate that two alleles are reported when it is not clear which is the dominant product: † allele differences were noted between two methods: ? difficult to call the allele.

and another 4 copy 12-5 allele in a Yunnan patient. The non-polymorphic loci including the *rpo*T gene may be more important when comparing these with isolates from other countries.

### THE IMPACT OF VNTR LOCI ON THE STRAIN TYPING OF *M. LEPRAE*

Of the nine from patients from Qiubei County, Yunnan, six are from the same township (Table 1). Except for patient #296, five of these six patients have *M. leprae* with highly similar VNTR patterns in (AC)8b, (GTA)9, (AT)17, (AC)9, 6-7 and (TA)10, but varying in the (TTC)21, (TA)18, and (AT)15 loci. These may be variants of a common group of strains, denoted as B.[4] Isolate from #296 differs from the other five by one repeat unit at the (AC)9 locus, and may belong to group A, although it has fewer copies at (TA)18 and (TTC)21. Interestingly, isolates with the nine repeat units in (GTA)9 locus have not been found in GZ-XY and CS-XC.

### VNTR PROFILES OF PATIENTS FROM MULTI-CASE FAMILIES

By collecting only 20 isolates in a one year study period, and performing contact surveys on these cases, it is difficult to reveal the real transmission dynamics of leprosy in the present study. However, since three of these cases (#296, #287, #302), were actually detected because of contact surveys of previously diagnosed patients in the same township (GZ) in Qiubei County, it was possible to search for shared genotypes among these existing or newly constituted multicase families, family 1, 12 and 13 (Table 2).[4] Although not identical, all of these strains except from #296, appear to be related, belonging to the Group B classification of Qiubei strain types.[4]

### COMPARISON OF THE *M. LEPRAE* VNTR PROFILES BETWEEN SKIN BIOPSY AND SSS AT DIAGNOSIS, AND SSSS COLLECTED AT SUBSEQUENT TIME POINTS DURING TREATMENT

In four patients, allelic variation based on the repeat units of the 17 VNTR loci was monitored (Table 3A).

The VNTR profiles based on sampling of biopsy and SSS at diagnosis was concordant in three patients for whom paired samples were available. One copy changes in (GTA)9, (TTC)21 and (TA)10, were noted. In serial SSS specimen collected during the treatment, in 2 month intervals. For seven other patients Combination 1 MP-FLA that includes (AC)8b, (GTA)9, (GGT)5, 6-3a (*rpoT*) and (AT)17 was performed. Variation was also seen in (AT)17 locus. (GGT)5 and 6-3a (*rpoT*) alleles show no variation in this patient population. The alleles for the other three loci are listed in Table 3B. In most cases, the alleles for biopsy and SSS before treatment were the same. Inconsistencies in repeat numbers or PCR negative amplification were more common in the SSSs collected after four or six months of treatment.

## Discussion

The present results demonstrated that Multiplex PCR followed by capillary electrophoresis is an efficient, economical, and accurate means for high-throughput and large-scale genotyping of *M. leprae*. The study further confirms that the genotypes of *M. leprae* isolates collected

**Table 2.** VNTR profiles of patients included in the current study identified by household contact survey in 2006 of previously known multicase families in Qiubei

| Family No. | Code No. | Type | Relation | Date collection | (AC)9 | 6-7 | (GTA)9 | (AT)17 | (AC)8a | (TA)10 | (AT)15 | (TA)18 | (GAA)21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1(GZ) | 81 | BL | Son | 2002.03 | 9 | 7 | 9 | Neg | Neg | ND | ND | ND | ND |
| | 135 | BL | Son | 2004.05 | 9 | 8 | 9 | 13 | 11 | 10 | 16 | 24 | 22 |
| | 237 | BL | Father | 2005.04 | 9 | 8 | 9 | 13 | 12 | 10 | 16 | 21 | 23 |
| | 287* | BL | Son | 2006.03 | 9 | 8 | 9 | 13 | 12 | 10 | 16 | 24 | 19 |
| | 317 | BT | Aunt | 2006.10 | 9 | 8 | 9 | 13 | 11 | 10 | 16 | 24 | 25 |
| 13(GZ) | 239 | BL | Father | 2005.04 | 9 | 8 | 9 | 13 | 11 | 10 | 16 | 24 | 21 |
| | 302* | BL | Son-in-law | 2006.08 | 9 | 8 | 9 | 13 | 10 | 10 | 18 | 20 | 28 |
| 12(GZ) | 296* | BL | Brother | 2006.05 | 8 | 8 | 9 | 13/14 | 11 | 10 | 16 | 14 | 13 |
| | 297 | BT | Brother | 2006.06 | 9 | 8 | 9 | 13 | 10 | 10 | 16 | 14 | 48? |

* The samples enrolled into the present study. The others are from cases in other studies including those previously reported.[4] Difficult to call the allele; BT, Borderline Tuberculoid; BL, Borderline Lepromatous.

**Table 3A.** Comparison of variation in copy numbers of VNTRs in *M. leprae* collected from biopsy and serial SSS specimen: 17 loci mapped by MP-FLA for samples from four leprosy patients

| | | Combination 1 | | | | | Combination 2 | | | | Combination 3 | | | | Combination 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code# | MDT (months) | (AC)8b | (GTA)9 | (GGT)5 | (AT)17 | rpoT | 21-3 | (AC)9 | (AT)15 | (AC)8a | 27-5 | 6-7 | (TA)18 | (GAA)21 | 18-8 | 23-3 | 12-5 | (TA)10 |
| 299-B | 0 | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 299-SSS1 | 0 | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 299-SSS2 | 2 | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 299-SSS3 | 4 | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 299-SSS4 | 6 | 8 | 9 | 4 | 14 | 3 | 2 | 9 | 15 | 11 | 5 | 8 | 22 | 22 | 7 | 2 | 3 | 10 |
| 308-B | 0 | 7 | 26 | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 308-SSS1 | 0 | 7 | Neg | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 308-SSS2 | 2 | 7 | Neg | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 308-SSS3 | 4 | Neg | Neg | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 308-SSS4 | 6 | 7 | Neg | 4 | 9 | 4 | 2 | 7 | 12 | 8 | 5 | 9 | 11 | 12 | 7 | 2 | 3 | 9 |
| 309-B | 0 | 10 | 25 | 4 | 14 | 3 | 2 | 7 | 20 | 9 | 5 | 8 | 16 | 18 | 7 | 2 | 3 | 16 |
| 309-SSS1 | 0 | 10 | 25 | 4 | 14 | 3 | 2 | 7 | Neg | 9 | 5 | 8 | 16 | 19 | 7 | 2 | 3 | 16 |
| 309-SSS2 | 2 | 10 | 24 | 4 | 14 | 3 | 2 | 7 | Neg | 9 | 5 | 8 | 16 | 19 | 7 | 2 | 3 | 17 |
| 309-SSS3 | 4 | 10 | 24 | 4 | 14 | 3 | 2 | 7 | Neg | 9 | Neg | Neg | Neg | Neg | 7 | 2 | 3 | Neg |
| 309-SSS4 | 6 | 10 | 24 | 4 | 14 | 3 | 2 | 7 | Neg | 9 | 5 | 8 | 16 | 19 | 7 | 2 | 3 | 17 |
| 305-B | 0 | 7 | 13 | 4 | 11 | 3 | 2 | 9 | 11 | 9 | 5 | 8 | 11 | 22 | 7 | 2 | 3 | 18 |
| 305-SSS2 | 2 | 7 | 13 | 4 | 11 | 3 | 2 | 9 | 11 | 9 | 5 | 8 | 11 | 22 | 7 | 2 | 3 | 17 |
| 305-SSS3 | 4 | 7 | 13 | 4 | 11 | 3 | 2 | 9 | 11 | 9 | Neg | Neg | Neg | Neg | 7 | 2 | 3 | 17 |

**Table 3B.** Comparison of variation in copy numbers of VNTRs in *M. leprae* collected from biopsy and serial SSS specimen: 5 loci (Combination 1) mapped for samples from 7 seven patients

| | | Combination1 | | | | |
|---|---|---|---|---|---|---|
| Code# | MDT (months) | (AC)8b | (GTA)9 | (GGT)5 | (AT)17 | rpoT |
| 287-B | 0 | 8 | 9 | 4 | 13 | 3 |
| 287-SSS1 | 0 | 8 | 9 | 4 | 13 | 3 |
| 287-SSS2 | 2 | 8 | 9 | 4 | 13 | 3 |
| 287-SSS3 | 4 | 8 | 9 | 4 | 16 | 3 |
| 294-B | 0 | 7 | 12 | 4 | 13 | 3 |
| 294-SSS1 | 0 | 7 | 12 | 4 | 13 | 3 |
| 294-SSS2 | 2 | 7 | 12 | 4 | 13 | 3 |
| 294-SSS3 | 4 | 7 | 12 | 4 | 13 | 3 |
| 293-B | 0 | 9 | 19 | 4 | 13 | 3 |
| 293-SSS-1 | 0 | 9 | 19 | 4 | 13 | 3 |
| 293-SSS2 | 2 | 9 | 19 | 4 | 13 | 3 |
| 293-SSS4 | 6 | Neg | Neg | 4 | Neg | 3 |
| 298-B | 0 | 8 | 9 | 4 | 13 | 3 |
| 298-SSS1 | 0 | 8 | 9 | 4 | 13 | 3 |
| 298-SSS2 | 2 | 8 | 9 | 4 | 12 | 3 |
| 298-SSS3 | 6 | 8 | 9 | 4 | NS | 3 |
| 302-B | 0 | 8 | 9 | 4 | 13 | 3 |
| 302-SSS1 | 0 | 8 | 9 | 4 | 13 | 3 |
| 302-SSS3 | 4 | Neg | Neg | Neg | Neg | 3 |
| 302-SSS4 | 6 | Neg | 9 | 4 | 13 | 3 |
| 296-B | 0 | 8 | 9/25 | 4 | 13 | 3 |
| 296-SSS1 | 0 | Neg | Neg | Neg | 11 | 3 |
| 296-SSS4 | 6 | Neg | Neg | Neg | 13 | 3 |
| 289-B | 0 | 7 | 10 | 4 | 18 | 3 |
| 289-SSS1 | 0 | 7 | 10 | 4 | Neg | 3 |
| 289-SSS2 | 2 | 7 | 10 | 4 | 16 | 3 |
| 289-SSS3 | 4 | 7 | 10 | 4 | 16 | 3 |
| 300-B | 0 | 8 | 25 | 4 | 15 | 3 |
| 300-SSS2 | 2 | 8 | 26 | 4 | 15 | 3 |
| 300-SSS3 | 4 | 8 | Neg | 4 | Neg | 3 |
| 300-SSS4 | 6 | 8 | 26 | 4 | 16 | 3 |
| 310-B | 0 | 7 | 17 | 4 | 12 | 3 |
| 310-SSS1 | 0 | 7 | 19 | 4 | 12 | 3 |
| 310-SSS2 | 2 | Neg | Neg | Neg | Neg | 3 |

B: Biopsy; SSS 1–4: Slit Skin Smear collected at 0, 2, 4 or 6 months of MDT; Neg: No PCR product or MP-FLA signal.

from China are from a distinct closely related lineage, with little diversity even when typed across 17 short tandem repeat loci, and that the ongoing transmission is revealed primarily by variations at six loci including the more stutter prone (AT) type di-nucleotide loci, and the trinucleotide repeat loci such as (GTA)9 and (GAA)21. By continuing the study in a region studied since 2003, it was possible to identify three new cases by contact survey of previous patients, and demonstrate infection by a common isolate. In addition, finding five of six new cases in the same township with closely matching *M. leprae* indicates that localised transmission of leprosy is occurring and is identifiable by MLVA.

*M. leprae* is not cultivable; therefore, the study of stability of STRs at various loci is limited to examining the allele profiles after successive passage in mouse or armadillo infection models. In a study of nine loci in *M. leprae* passaged in foot pads of nude mice, the copy number remained stable.[6] Regarding the stability of VNTR loci during the treatment, one study has compared alleles in biopsy in five loci [(AT)14, (AT)15, (AT)17, (GAA)21, and (GTA)9], before and after one month of treatment,[13] and found that alleles in some loci shift by 1–3 copies. Because of this finding by Monot *et al.*,[13] concerns over the usefulness of VNTRs for epidemiological purposes in tracing transmission was raised. In this present context, we examined many loci, at multiple time points before and during treatment, and concluded that while allelic shifts at the highly variable microsatellites occur, considerable overall stability is maintained. Moreover, SSS as collected in this study is a mixture of skin scrapings from multiples sites, including that of the biopsy site. When biopsy or SSS were compared, the results were nearly congruent, suggesting that in clinics that don't collect biopsies, SSSs would be an alternative source of DNA before treatment. In Qiubei County, Yunnan we had noted and described previously strain types that we termed A and B, which were similar in VNTR profile and geographic distribution, that differed by one copy at the (AC)9 locus (either 9 or 8 copies).[4]

Thus these data, along with those from the Philippines and India suggest that certain, but not all, STR loci are prone to stuttering during incubation, treatment and transmission.[11,14] The allelic shifts tend to occur in loci with the highest allelic diversities. However, in China, where all the strains belong to SNP type 3, VNTR strain typing with the current panel of 17 markers remains as a useful discriminatory tool for tracing regional, community and familial *M. leprae* genotypes, their transmission, distribution and evolution of recent variants.[4] When additional markers are discovered, they will be included in strain typing.

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