Various genotypes of *Mycobacterium leprae* from Mexico reveal distinct geographic distribution

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Summary

**Objective** To classify *Mycobacterium leprae* isolates from multiple areas in Mexico based on variable number of tandem repeats of 6 base within the *rpoT* gene and three single nucleotide polymorphism (SNP), and to analyse their geographic distribution in the context of the origin of leprosy in Mexico.

**Results** Analysis for *rpoT* genotyping of 64 samples collected in the west and southwestern areas revealed that 46 isolates were of the 4 copy type and 18 isolates were of the 3 copy type. All samples from the eastern coastal area (n = 24) and from the Yucatan peninsula (n = 12) were of the 3 copy type. Six isolates from the west and southwestern area were SNP-type 1, 13 isolates were SNP-type 2 and 45 isolates were SNP-type 3. Nineteen of 24 isolates from the eastern coastal area were SNP-type 3 and one was SNP-type 4. Seven isolates from the Yucatan peninsula were SNP-type 3 and one was SNP-type 4.

**Conclusion** The difference of the proportion of each genotype between the western areas and the eastern areas indicated the expansion of leprosy through different paths in Mexico.

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Introduction

Genotype of *Mycobacterium leprae* classified by variation of 6 base tandem repeat copy in the *rpoT* gene shows characteristic biased geographical distribution in the world.\(^1-^3\) Prevalence of four genotypes (SNP type 1–4) defined by three single nucleotide polymorphisms (SNP) of *M. leprae* also varies globally.\(^7\) Geographical distribution of isolates distinguished by either genotyping approach is considered to be useful for tracing the global spreading of leprosy. Previous studies have shown that the dominant *Mycobacterium leprae* *rpoT* genotype in Japan, Korea and Mexico is the 4 copy tandem repeat of 6 base tandem repeat in the *rpoT* gene (4 copy type). In contrast, the majority of *M. leprae* isolates tested globally are isolates with 3 copy tandem repeat of 6 base tandem repeat (3 copy type).\(^1-^3\) To expand the analysis of geographic distribution of *M. leprae* classified by the *rpoT* gene polymorphism and SNP, isolates from multiple areas in Mexico were analysed. Samples were previously collected in the west and southwestern regions of Mexico.\(^2\) More samples were collected from the eastern coastal area and the Yucatan peninsula because the origin of the people in this area is different from the origin of the people in the west and southwestern regions of Mexico.

Materials and Methods

In west and southwest regions of Mexico, 64 samples were collected including previously reported 27 samples.\(^2\) Two samples are from Sinaloa state, 52 samples are from Jalisco state, and 10 samples are from Guanajuato state in West and Southwest region. Twelve samples

![Figure 1. Sample collection sites.](image-url)
were from the Yucatan peninsula. Eight samples are from Yucatan state, two samples from
Quinatanaroo state, and two samples are from Campeche state. Twenty four samples were
from the eastern coastal area. Ten samples are from Nuevoleon state, 12 samples are from
Tamaulipas state, and two samples are from Veracrus state (Figure 1).

Bacillary materials were obtained from the skin lesion by the slit skin method as same as
for the bacterial index examination after obtaining the patient’s informed consent. Samples
were treated for preparing template DNA as described elsewhere. Polymorphism in the $rpoT$
genotype and SNP type of isolates were determined by the same method as previously described.

Results

Analysis for $rpoT$ genotyping of 64 samples collected in the west and southwestern areas
revealed that 46 isolates out of 64 isolates were 4 copy type and 18 isolates were 3 copy type
(Table 1).

On the contrary, all samples from the eastern coastal area ($n = 24$) and from the Yucatan
peninsula ($n = 12$) were of the 3 copy type. Six isolates from the west and southwestern area
were SNP-type 1, 13 isolates were SNP-type 2, and 45 isolates were SNP-type 3 respectively.
SNP-type 4 was not detected in the west and or southwestern areas (see the Table).

Table 1. Prevalence of each $rpoT$ genotype and SNP type in different areas of Mexico

<table>
<thead>
<tr>
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<th>West and Southwest</th>
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<tbody>
<tr>
<td></td>
<td>Jalisco state*</td>
<td>Sinaloa state*</td>
<td>Guanajuato state*</td>
</tr>
<tr>
<td>3 copy type</td>
<td>18 isolates</td>
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<td>0</td>
</tr>
<tr>
<td>SNP type 1: 3 isolates</td>
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<tr>
<td>SNP type 2: 5 isolates</td>
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<td>SNP type 3: 10 isolates</td>
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<td></td>
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</tr>
<tr>
<td>4 copy type</td>
<td>34 isolates</td>
<td>2 isolates</td>
<td>10 isolates</td>
</tr>
<tr>
<td>SNP type 1: 3 isolates</td>
<td></td>
<td>SNP type 3: 2 isolates</td>
<td>SNP type 2: 1 isolates</td>
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<tr>
<td>SNP type 2: 7 isolates</td>
<td></td>
<td></td>
<td>SNP type 3: 9 isolates</td>
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<tr>
<td>SNP type 3: 24 isolates</td>
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<tr>
<td>Yucatan peninsula**</td>
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<tr>
<td></td>
<td>Yucatan state</td>
<td>Campeche state</td>
<td>Quinatanaroo state</td>
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<tr>
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<td>8</td>
<td>2</td>
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<tr>
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<td>SNP type 4: 1 isolates</td>
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<td>Eastern coast**</td>
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<tr>
<td></td>
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<td>Tamaulipas state</td>
<td>Vearcruz state</td>
</tr>
<tr>
<td>3 copy type</td>
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<td>SNP type 3: 10 isolates</td>
<td>SNP type 3: 1 isolates</td>
</tr>
<tr>
<td>4 copy type</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

* Fifteen samples of Jalisco state, two samples of Sinaloa state, and 10 samples of Guanajuato state are same with previously reported.

** Four samples each from Yucatan peninsula and the Eastern coastal area could not be analysed for SNP.
SNP typing for 20 of 24 isolates from the eastern coastal area and eight samples of 12 samples from the Yucatan area were determined. Nineteen of 20 isolates from the eastern coastal area were SNP-type 3 and one was SNP-type 4. Of the eight samples from the Yucatan, seven isolates were SNP-type 3 and one was SNP-type 4. Strains with 4 copy type of the rpoT gene were predominant among isolates obtained from west and southwestern areas, however no 4 copy type strain was detected from the eastern coastal area and the Yucatan area.

Discussion

The global spreading of M. leprae with different genotypes was indicated in previous studies as for other infectious diseases. The predominance of the rpoT gene 4 copy type in the Japan mainland and the Korean peninsula is attributed to movement of Mongoloid people who migrated to Japan through the Korean peninsula. The results of SNP genotyping also implied the spread of M. leprae with SNP-type 3 from Africa to Latin American countries. The current population of Mexico consists of various ethnic groups and the origins of the majority race in the western area and eastern coast is different. No isolates were detected with the 4 copy type from either the eastern coastal area or the Yucatan area. Moreover, the predominance of the 4 copy type in the western area suggests the expansion of leprosy through different paths in Mexico.

SNP-type 4 isolates were detected only in Africa, the French West Indies and Brazil as well as in Japanese Brazilians thought to have been infected in Brazil. The origin of SNP-type 4 isolates in Latin American countries was assumed to be in Africa. The existence of two SNP-type 4 isolates out of the 28 that were typed in the eastern coastal area and the Yucatan area suggests that leprosy in this area was carried by slave trade as proposed previously. Globally, the proportion of SNP-type 2 is lower than SNP-type 1 in many areas except Ethiopia, Malawi, and mainland Japan. The results of the Mexican samples showed a higher prevalence of SNP-type 2 in the west and southwest areas of Mexico. Our previous study suggested that the spread of leprosy in this area by the movement of Mongoloid migration from Asia through the Bering Strait on the basis of the high prevalence of rpoT 4 copy type in East Asian countries. It is of interest that the prevalence of each SNP type in these areas is also similar to that of mainland Japan. It is assumed that high prevalence of rpoT 4 copy type and SNP-type 2 in the west and southwest areas is related to the migration of mongoloid people from East Asia to American continent through the Bering Strait.

Geographical distribution of M. leprae classified with rpoT gene polymorphism and SNP is useful for analysing the historic spread of leprosy, but it is also applicable for the investigation of current intercontinental transfer as shown for Japanese Brazilian cases. Application of this kind of genotyping to specific geographical areas may be useful for the dissection of leprosy infection among immigrants. A panel of more SNP type might be useful for precise analysis of the regional spread of the disease in a country which shows different dominant genotype in each area.

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References


