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Single nucleotide polymorphisms typing of *Mycobacterium leprae* reveals focal transmission of leprosy in high endemic regions of India

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Abstract

Earlier studies indicate that genotyping of *Mycobacterium leprae* based on single-nucleotide polymorphisms (SNPs) is useful for analysis of the global spread of leprosy. In the present study, we investigated the diversity of *M. leprae* at eight SNP loci using 180 clinical isolates obtained from patients with leprosy residing mainly in Delhi and Purulia (West Bengal) regions. It was observed that the frequency of SNP type 1 and subtype D was most predominant in the Indian population. Further, the SNP type 2 subtype E was noted only from East Delhi region and SNP type 2 subtype G was noted only from the nearby areas of Hoogly district of West Bengal. These results indicate the occurrence of focal transmission of *M. leprae* infection and demonstrate that analysis by SNP typing has great potential to help researchers in understanding the transmission of *M. leprae* infection in the community.

Keywords: Delhi, *Mycobacterium leprae*, Purulia, single-nucleotide polymorphisms, transmission

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Introduction

Leprosy, caused by *Mycobacterium leprae*, is a chronic infectious disease affecting primarily the peripheral nerves, skin and mucous membranes. It is one of the oldest recorded diseases of humankind. Even today, leprosy remains a major health problem in 130 countries of the world, excluding the small number of cases in Europe [1]. In India, a total of 127 000 new cases were detected during the year 2011–12 with an annual new case detection rate of 10.35 per 100 000 population and a prevalence of 0.68 per 10 000 of the population. Till now 32 States/Union Territories have attained leprosy elimination. Even now, after two decades of multi-drug therapy there are 11 districts with annual new case detection rates > 50/100 000 population: in Chhattisgarh, Gujarat, Maharashtra, West Bengal, Dadra & Nagar Haveli, Orissa and Delhi [4]. At this juncture of elimination, there is a need to develop suitable techniques and tools for understanding the epidemiology of leprosy and identify sources as well as reasons for the persisting load of infection. Even after years of global campaigns on elimination of leprosy and rigorous case finding along with the availability of multi-drug therapy regimens [5–7], its continued occurrence strongly implies the existence of subclinical human and environmental reservoirs of the pathogen [8,9].

In recent years, molecular strain-typing methodologies have added to our understanding and deciphering of complicated conventional infectious disease epidemiology. With the
discovery of the complete genome sequence for *M. leprae* isolated from Tamil Nadu, India—called the TN strain [10]—selection of potential polymorphic genomic markers for strain typing has become feasible. The first genetic markers that showed polymorphism were short tandem repeats in the *M. leprae* genome. One was a 6-bp intragenic sequence in the *rpoT* gene, and the second, a trinucleotide (TTC) repeat element—upstream of a pseudogene [11,12]. These sequences exhibit variable numbers of tandem repeats when sequenced from different isolates. Many genetic fingerprinting methods have been applied for *M. leprae* characterization, including insertion elements like pol(A) [13], restriction fragment length polymorphism analysis of the heatshock protein 65 gene [14], variable numbers of tandem repeats analysis [15–17] and single-nucleotide polymorphisms (SNPs) [18–21]. Among these, SNP and variable numbers of tandem repeats typing were the only methods to reveal any genetic diversity among *M. leprae* strains. In the present study we carried out SNP subtyping of the clinical isolates from different regions of India.

**Material and Methods**

In the present study, *M. leprae* strains were obtained from patients with a clinical diagnosis of leprosy. Ethical approval to use the diagnostic specimens for research was obtained from the ethics review board of The Leprosy Mission Trust (TLM), India.

**Collection of specimens**

A total of 180 slit skin scrapings obtained from leprosy patients who attended the Outpatient Departments of the TLM Community Hospitals of Shahdara (Delhi), Naini (UP), Purulia (West Bengal) and Miraj (Maharashtra) during 2007–10, were included in the study. Slit skin scrapings were obtained from these patients for the SNP typing analysis (Table 1). All 180 multibacillary cases were diagnosed and classified by standard clinical criteria (NLEP guidelines: http://nlep.nic.in/).

**Extraction of genomic DNA**

NHDP63 and Thai 53 DNA, obtained from Colorado State University (Fort Collins, CO, USA) were used as reference strains. Genomic DNA was isolated by cell wall disruption with proteinase K and 0.1 M Tris–HCl as described previously [22]. The reaction was terminated at 97°C for 15 min. This lysate preparation was then used for PCR.

**SNP typing and subtyping**

The *M. leprae* SNP loci 1, 2 and 3 (nucleotide positions 14676, 1642875 and 2935685, respectively, on the sequenced TN strain) were amplified using previously reported primer sequences and protocol [18,23].

Subtyping of SNP type 1 (nucleotide positions 8453, 313361, 61425 and 1642879, respectively) and type 2 (nucleotide positions 310278, 1104235, 2751790 and 2935693) was carried out using previously reported primer sequences and amplification conditions [19].

Sequencing of PCR products was outsourced to a commercial company (Xplorigen Technologies Pvt Limited; Delhi, India). Sequence data were analysed further by using CODEON-CODE ALIGNER 4.0.3.

**Results**

**Distribution of SNP *M. leprae* genotypes**

The SNP types detected were limited to SNP type 1, CGA and SNP type 2, TAC. In this study, the SNP type 1 was identified in 166 samples, whereas the SNP type 2 was detected in only 15 samples (Table 2, Supplementary files).

The SNP subtyping of samples from leprosy endemic areas in Delhi and UP showed similar patterns (2E) in eight strains. SNP subtype 2G was observed from Purulia, West Bengal (Fig. 1).

**Clustering of SNP subtype 2E from a local area of East Delhi**

We analysed 65 samples from Delhi and UP and found that the D subtype was predominant in this area. We observed that subtype 2E was clustered in a local area of north-east Delhi where old leprosy patients were residing (Table 3a, Fig. 2).

**TABLE 1. Number of samples from each area under study**

<table>
<thead>
<tr>
<th>Area</th>
<th>TLM Shahdara (Delhi NCR+UP)</th>
<th>TLM Purulia, (West Bengal, Jharkhand and Bihar)</th>
<th>TLM Miraj (Maharashtra, Karnataka)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>65</td>
<td>100</td>
<td>15</td>
<td>180</td>
</tr>
</tbody>
</table>

TLM, The Leprosy Mission Trust.

**TABLE 2. Distribution of genotypes in different areas of India**

<table>
<thead>
<tr>
<th>Area</th>
<th>SNP type 1</th>
<th>SNP type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
</tr>
<tr>
<td></td>
<td>(C)</td>
<td>(D)</td>
</tr>
<tr>
<td></td>
<td>(E)</td>
<td>(F)</td>
</tr>
<tr>
<td></td>
<td>(G)</td>
<td>(H)</td>
</tr>
<tr>
<td>Purulia (100)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Delhi (41)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>UP (24)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miraj (15)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (180)</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism.
We analysed 100 samples from West Bengal and found that the D subtype was predominant in this area. Subtype 2G was clustered in a coastal part of West Bengal (Hoogly) where old leprosy patients were residing (Table 3b, Fig. 1).

**Discussion**

Leprosy is almost exclusively a disease of the developing world, affecting mainly areas of Asia, Africa, Latin America and the Pacific. Africa and Asia have the maximum disease burden. Within endemic locales the distribution of leprosy is not uniform, and areas of high prevalence sometimes border areas of very low prevalence with little or no disease. The global registered prevalence of leprosy was 192,246 at the beginning of 2011 [1]. Despite the administration of multi-drug therapy for over two decades, new cases are being reported from endemic regions in several countries without any reduction in the annual new case detection rate.

India is a country in South Asia bounded by the Indian Ocean on the south, the Arabian Sea on the south-west, and the Bay of Bengal on the south-east. It is surrounded by leprosy endemic countries such as Nepal, China, Bangladesh, Sri Lanka and India’s Andaman and Nicobar Islands. In this study, the samples were taken from different parts of India—northern, eastern and a few samples from the western
part of India. These M. leprae genotypes based on SNP subtyping were from different geographical areas of India that showed the presence of M. leprae with both type 1 and type 2 SNPs. We found that most (92%) of the strains had SNP type 1.

In some studies, it has been suggested that in endemic countries >50% of patients with leprosy may have a history of intimate contact with an infected person (often a household member) [24]. Widespread exposure, as evidenced by nasal presence of M. leprae in the endemic population [25], could play an important role in maintaining endemicity of the disease in certain areas. Transmission of leprosy can be by direct or indirect means involving fomites but is thought to occur most frequently through long-term direct contact with an infected host [26]. The present study was therefore conducted to understand the transmission and epidemiology of leprosy. Several reports have published the usefulness of polymorphic markers for M. leprae as an epidemiological tool in the differentiation of strains of M. leprae [15,19,21, 23,27,28]. However, the polymorphism for each of these genomic markers should be experimentally evaluated in the patient populations of interest. The SNP types were examined based on polymorphisms of nucleotides at positions 14676, 164275 and 2935685 of M. leprae genomic DNA. Four types of SNP—type 1, CGA; type 2, CTA; type 3, CTC; and type 4, TTC—were reported previously [18]. Our present study showed that the M. leprae type belonged to SNP type 1 and 2 at a ratio of 92 : 8. This is in accordance with what has been reported by previous studies in South Asia [19].

On further subtyping of these strains, we found the 1D subtype to be predominant in our area (Table 2). Type 2G was found only from strains from the coastal region of West Bengal. Subtype 2G was also reported by Monot et al. [19] from the population of Nepal and northern India. We analysed subtype 2E from the samples of East Delhi where old leprosy colonies were situated. Type 2E was also reported from Malawi [19]. India was a home to the ancient Indus Valley Civilization and a region of historic trade routes and vast empires; the Indian subcontinent has been identified with commercial and cultural wealth since ancient times. From the Stone Age, people from different regions have migrated to India and made it their home. Therefore, the population comprises descendants of groups of people belonging to almost all the ‘racial stocks’ of mankind and their admixtures, who settled in India. A large slave trade took place in the 18th and 19th centuries in Malawi, so at that time some M. leprae ancestral lineages appear to have disseminated and become established among the people of neighbouring and proximal South-east Asian countries. Considering the above, the findings of subtypes of M. leprae like that of Malawi were not unusual. On the whole, our study showed a distribution of M. leprae with the 1D SNP subtype being dominant, followed by 1C, 1A and 2G from India, which is similar to that reported from Nepal and Bangladesh [19]. One of the previous studies from Western India in a rural and urban area near Mumbai reported mainly subtype D, followed by B [29].

Data analysed from this study indicate that future epidemiological studies involving SNP typing along with subtyping of strains may give deeper insight into the distribution and transmission patterns of M. leprae.

Acknowledgements

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Author Contribution

ML and RSJ conceived and designed the experiments. ML, RT and MS performed the experiments; ML and SC analysed the data; and ML, RSJ and US wrote the paper.

Transparency Declaration

All authors declare that there are no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Sequence alignment for individual subtype.

References


