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 *Mycobaterium 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 spite of considerable research efforts made during the past few years, the nature of transmission and the possibility of existence in environmental reservoirs other than humans of *M. leprae* [2,3] have eluded the researchers investigating the occurrence of continuous transmission of disease in endemic regions. In 130 countries worldwide, at the beginning of 2010 the total number of new cases were 192 246 and during the year 2011 a total of 228 474 new cases of leprosy were detected [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 Outatient 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.

TABLE 1. Number of samples from each area under study

Area	TLM Shahdara (Delhi NCR+UP)	TLM Purulia, (West Bengal, Jharkhand and Bihar)	TLM Miraj (Maharashtra, Karnataka)	Total
Number of samples	65	100	15	180

TLM, The Leprosy Mission Trust.

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 CodonCode 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 2. Distribution of genotypes in different areas of India

Area	SNP type 1				SNP type 2			
Subtype loci	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)
Purulia (100)	2	0	18	75	0	0	5	0
Delhi (41)	1	0	2	31	7	0	0	0
UP (24)	0	0	7	15	1	0	1	0
Miraj (15)	0	0	2	13	0	0	0	0
Total (180)	3	0	29	134	8	0	6	0

SNP, single-nucleotide polymorphism.