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Single Nucleotide Polymorphisms (SNPs) in *Mycobacterium leprae* and their role in strain typing of leprosy bacilli

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Abstract

Leprosy is a chronic infectious disease caused by the bacterium *Mycobacterium leprae*. It is a disease of public health concern mainly because of its potential to cause disability in a small proportion of those affected and is a cause for social stigma and discrimination. Strain differentiation by Single Nucleotide Polymorphisms (SNP) could be useful in tracing origins and routes of infection, general leprosy surveillance and prevalence. The current study focuses on strain typing of *M. leprae* through the use of SNPs to differentiate between strains located all over the world. Strain typing methods like surface antigen typing, multi-locus enzyme electrophoresis (MLEE), phage typing, fragment length polymorphism (RFLP), variable umber tandem repeats (VNTR) and SNP are now available. Among all typing procedures, SNPis very accurate for molecular strain typing of *M. leprae*. The current review article clearly monitored the predominance of SNPs in different geographical areas. Based on the SNPs types, it explains how *M. leprae* strains are distributed globally and analyses which SNPs are located in the different countries. Along with this, it explains how strains are transmitted and migrate from one area to other.

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Leprosy is a debilitating and chronic disease caused by *Mycobacterium leprae (M. leprae)* and it is associated with the disability, stigma and discrimination to the affected individuals (Scollard., *et al.* 2004). Leprosy is a disease generally associated with poverty and related factors like overcrowding; however, it may affect persons of any socioeconomic group. Dapsone worked wonderfully at first, but later, *M. leprae* eventually began developing resistance to dapsone. There was no treatment for this disease until the introduction of the drug Dapsone in 1940s. *M. leprae*, mostly found in warm tropical countries. Norway was the first person to identify the germ that causes leprosy

under the microscope. Hansen's discovery of *M. leprae* proved that leprosy was caused by a germ, and thus was not hereditary, from a curse or from a sin. *M. leprae* is mainly survived in the coldest parts of the body like hands, feet, nose, etc. and it is an obligate intracellular pathogen which mainly infects macrophages and schwan cells and peripheral nerves (Races., *et al.* 1994). It is a slow growing bacillus and one leprosy bacillus takes 12–14 days to divide into two. Optical microscopy shows *M. leprae* in clumps, rounded masses, or in groups of bacilli side by side, and ranging from 1-8 μM in length and 0.2-0.5 μM in diameter. The cell wall has high molecular weight mycolic acids which renders the bacilli resist to decolourization using Zeil Neilsen-Carbol Fuschin method and hence its acid fast nature. The main hosts for *M. leprae* are humans and armadillos. Like any other communicable disease, transmission of leprosy from source of infection to susceptible host is determined by a number of factors related to agent, host and environment. The present study aims to analyse the strain typing pattern of *M. leprae* in terms of Single Nucleotide Polymorphisms (SNPs) globally, thereby explaining short chain transmission, strain migration and strain variation.

Global efforts of leprosy have been successfully eradicated in controlling of its spread. Over the past 20 years, more than 14 million leprosy patients have been cured, about 4 million since 2000. According to official reports were received from 105 countries and territories, the globally 0.23 million leprosy cases were registered in the year 2012 (WHO report 2012). The number of cases detected during 2011 was 0.21 million compared with 0.22 million in 2010. Leprosy has been eliminated from 119 countries out of 122 countries where the disease was considered as a public health problem in 1985 (WHO report 2012). The prevalence rate of the disease has dropped in the year 2000 by 90% – from 21.1 per 10 000 inhabitants to less than 1 per 10 000 inhabitants. Dramatic decrease in the global disease burden from 5.2 million in 1985 to 0.81million in 1995 to 0.75 million at the end of 1999 to 0.18 million cases at the end of 2011.

In India, a total of 0.12 million new cases were detected during the year 2011-12, which gave Annual New Case Detection Rate (ANCDR) of 10.35 per 100,000 populations. This showed 1.24% marginal reduction in ANCDR of from 2010-11 (10.48%). A total of 0.08 million cases were on record as on 1st April 2012, gave a Prevalence rate (PR) of 0.68 per 10,000 populations. A total of 3865 Gr. II disability detected in the new leprosy cases during 2011-12, which gave the Gr. II Disability Rate of 3.14/million population. In addition 4817 Gr. I cases were recorded which was 3.78% of the new cases detected during the year. A total of 0.01 million new child cases were recorded, which gave the Child Case rate of 1.0/100,000 population (NLEP 2012). The detection new cases and other complicated leprosy in each year and the national prevalence reflect the continuing spread of leprosy. In such areas the strain typing and strain differentiation are very helpful to identify the source of infection, transmission of infection and spreading of disease (Linder., *et al.* 2008 and Shetty., *et al.* 2005).

For the detection as well as strain typing of *M. leprae*, numerous techniques have been developed which are directly or indirectly based on amplification of specific DNA fragment from the whole genome of the leprosy bacilli. This amplification of DNA by PCR intern depends on the selection of specific primers from data base (Cole., *et al*. 2001). The strain typing method very helpful to identify global distribution of *M. leprae*, and to trace out source of leprosy bacteria. Strain typing of *M. leprae* has associated with not only for identification of leprosy bacilli distribution but also identification of correlation between leprosy bacilli (Monot., *et al*. 2005). So strain typing of leprosy bacilli is very much interesting in upcoming days and present strain typing methods include surface antigen typing (Lancefield., *et al*. 1928), multi-locus enzyme electrophoresis (MLEE) (Milkman., *et al*. 1973) and phage typing (Ahmed., *et al*. 1987) were used for strain typing. DNA based strain typing methods were started from 1990 and were fragment length polymorphism (RFLP) typing at locus such as rRNA operons (Low., *et al*. 1988) fingerprinting by randomly primed PCR (Welsh., *et al*. 1990) pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST) (Maiden., *et al*. 1998), variable umber tandem repeats (VNTR) and SNPs. Among all typing procedure SNPs was very accurate for molecular strain typing of *M. leprae*.

Errors in DNA replication have associated with changing in single nucleotides and it is considered as evolutionary marker. SNP polymorphism is very lower than other members of *Mycobacterium tuberculosis* complex (Fleischmann., *et al.* 2002). The molecular epidemiology and population biology of many pathogens have based on SNP analysis only (Maiden., *et al*. 1998-27). Single base changes in *M. leprae* are due to absence of genes that are involved in DNA repair. The large number of non functional pseudogenes would be expected to reduce selective pressure against fixation of these changes (Dawes., *et al*. 2001-24). Low GC content is one of the reason where

identification of higher genetic diversity in *M. leprae*. In *M. leprae,* deamination of C to U is failed which is possible reason for the low GC content in *M. leprae* resulted in SNP generation. Initial studies of SNPs of *M. leprae* revealed that very less polymorphism in leprosy but later studies, explained higher polymorphism in SNPs (Clark-Curtiss., *et al*. 1989-29). SNPs have different types which were distinct one bacterium to other bacteria. These SNPs were classified into 4 types based on global distribution pattern (Monot., *et al*. 2005). This 4 types were further divided into 16 subtypes designated as 1A to -D, SNP 2E to -H, SNP 3I to -M, and SNP 4N to -P (Monot., *et al*. 2009).

In the year 2008, the SNP analysis was carried out by taking 45 biopsy samples from Mali. Three polymorphic positions were analysed and all are found to be same SNP typing that is SNP 4. This SNP typing results is characterized by the nucleotides T, T, and C at genome positions 14676, 1642875, and 2935685 respectively. This type of SNP mainly found in the region of West Africa which indicates this leprosy bacilli have been came from West Africa during the era of the slave trade (Monot., *et al*. 2008). During the year 2009, one report from Philippines was identified in which the total population of leprosy patients were 228 samples. The samples types were frozen biopsies and ethanol fixed biopsies which were stored from the year 1980 to 2007. DNA was isolated from the all biopsy samples by kit method and subjected to PCR and SNP analysis. For this SNP analysis PCR-RFLP method was employed. All population were associated with the stable SNP typing pattern. Most samples showed SNP-1 type lineage which is equal to Asian samples and rest of the samples linked with the SNP-3 type which were belongs to putative postcolonial lineage (Sakamuri., *et al*. 2009).

Leprosy bacilli can also persist in bones so bones samples can also helpful for the molecular study of *M. leprae*. Strains typing of *M. leprae* from bone samples have been carried by taking 10 samples from Europe region. The 10 samples of bone showed signs like osteomylitis, including resorption of the anterior nasal spine, rounding and widening of the nasal aperture, erosion of the alveolar margin and pitting of the hard palate. From all the 10 samples, 6 samples were associated with positive PCR amplification and those samples were subjected to SNP analysis by sequencing method. SNP analysis by sequencing method described that only single SNP type was seen in Europe region. The SNP type includes in this region is SNP-3 (Watson., *et al*. 2009). A single *M. leprae* which was collected from chimpanzee has been analysed for SNP analysis for 3 locus in Japan. In this study DNA isolated from that animal and DNA was isolated by kit method. This DNA molecule was subjected to PCR amplification followed by DNA direct sequence for the positions 14,676, 1,642,875, and 2,935,685 of *M. leprae* DNA were identified as T, T, and C, respectively. The SNP analysis described that, this strain have associated with SNP-4 which was equal to SNP type of Caribbean and South America, probably via the slave trade, but not from Japan or other Asian countries (Suzuki., *et al*. 2010).

Like Europe area, strain typing of *M. leprae* from skeletal material has been performed. This study selected 7 groups of skeletal materials from different areas from the Japan mainly the Hatanai site (N40u229, E141u299) in Aomori prefecture, in the northeastern part of Honshu Island in Japan. DNA isolated by kit method from those material and DNA materials was subjected to PCR amplification and then direct sequence of PCR products. PCR amplification and the sequence identified at 3 specific loci positions 14,676, 1,642,875 and 2,935,685 of DNA which were identified at C, G and A respectively. This type SNP typing results were denoted as SNP type 1. This type SNP-1 is mainly seen in the India and South Asia (Suzuki., *et al*. 2010). In the year 2011, one report was addressed for strain typing of *M. leprae* by SNP. This study was conducted in rural and urban area of Mumbai, India, included 199 new cases of leprosy. From all the samples DNA was isolated by kit method and those DNA material were subjected PCR. The positive PCR amplicons were again subjected to RFLP for the analysis of SNP. In the SNP analysis, these samples were found to be SNP-1 type. This SNP-1 was sub divided into A, B, C and D at positions 383599, 313361, 61425, and 1642875, respectively. Most of the patients with SNP type 1D followed by SNP type 1B was identified (Sanjana., *et al*. 2011).

Standard M. leprae strains like NHDP63, Br4923, Thai-53, and 3039/210 have been obtained from mouse footpad (MFP) systems and in armadillo animal systems were utilized for the strain typing of *M. leprae* in Japan. In this study 18 MFP strains were obtained from Leprosy Research Centre, National Institute of Infection Diseases, Tokyo, Japan. Among these strains SNP type -3 identified as predominant (Wei., *et al*. 2011). The study from Brazil also carried out strain typing of *M. leprae* by taking 51 skin biopsy samples. The clinical diagnosis of those 51 patients was mainly by skin lesions, nerve damage and AFB analysis. The wgenomic DNA were subjected to PCR.

The PCR amplicons were again performed for RFLP to analyse SNP types. Among 4 types of SNPs, 3 types SNPs were observed in Brazil with SNP-4 as predominant followed by SNP-3 then SNP-1 (Aanda., *et al*. 2012).

The lepromatous leprosy samples were identified in burials in the Magdalen Hill Archaeological Research Project (MHARP) in Winchester, UK. From that are 9 burials samples were collected and DNA was isolated from those samples and PCR amplicons was done by using specific primers. PCR products again send to SNP analysis and identified as 3 samples with SNP type 3I-1 and remaining samples were identified as SNP type 2F. This study concluded that SNP type BI-1 is due to migration of bacteria from America to Europe where as SNP type 2F is due to migration bacteria from Middle East to India and South-East Asia to Europe (Taylor., *et a*l. 2013). Recently Indian leprosy patients also strain typed mainly in TLM Community Hospitals of Shahdara (Delhi), Naini (UP), Purulia (West Bengal) and Miraj (Maharashtra) by taking 180 Slit Skin Smear samples (SSS). Genomic DNA was isolated from the all 180 leprosy bacilli by standard procedure. PCR amplification was carried out by taking whole those samples. Then SNP analysis was done. The SNP results include, SNP type 1D was most predominant in the Indian population, SNP type 2E was noted only from East Delhi region and SNP type 2G was identified only from the nearby areas of Hoogly district of West Bengal (Lavania., *et al*. 2013).

Strain typing of *M. leprae* from multi cases families and surround areas like water has been carried out in India to find out possible link between strain and surroundings. The study conducted in TLM by taking samples from multicases families and surrounding non environment. In these strains SNP type- 1D was identified as predominant (Turankar., *et al*. 2014). For the strain typing of *M. leprae* SNP and VNTR have been identified as good techniques in the universe. Attention must to be made to increase to potentiality of these two techniques to increase higher resolution in strain typing procedure.

The current article clearly explained the predominance of SNPs in different geographical areas. Based on the SNPs types it monitored how *M. leprae* strains are distributed globally and explains which SNPs were located at the different countries. In addition, it explains how strains were transmitted and migrated from one area to another. To find out some novel markers in leprosy is helpful to increase the strength in the SNP techniques. We hope future research will focus on tracing out novel markers for the strain typing of *M. leprae* by SNPs.

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