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Genotyping of *Mycobacterium leprae* on the Basis of the Polymorphism of TTC Repeats for Analysis of Leprosy Transmission

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The polymorphism of TTC repeats in *Mycobacterium leprae* was examined using the bacilli obtained from residents in villages at North Maluku where *M. leprae* infections are highly endemic (as well as from patients at North Sulawesi of Indonesia) to elucidate the possible mode of leprosy transmission. TTC genotypes are stable for several generations of passages in nude mice footpads and, hence, are feasible for the genotyping of isolates and epidemiological analysis of leprosy transmission. It was found that bacilli with different TTC genotypes were distributed among residents at the same dwelling in villages in which leprosy is endemic and that some household contacts harbored bacilli with a different genotype from that harbored by the patient. Investigations of a father-and-son pair of patients indicated that infections of bacilli with 10 and 18 copies, respectively, had occurred. Genotypes of TTC repeats were found to differ between a son under treatment and two brothers. These results reveal the possibility that in addition to exposure via the presence of a leprosy patient with a multibacillary infection who was living with family members, there might have been some infectious sources to which the residents had been commonly exposed outside the dwellings. A limited discriminative capacity of the TTC polymorphism in the epidemiological analysis implies the need of searching other useful polymorphic loci for detailed subdivision of clinical isolates.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* infection. It has long being believed that the source of infection is untreated leprosy patients. It has also been predicted that multidrug therapy (MDT) with strong bactericidal antibiotics (such as rifampin) would reduce the source of infection and consequently interrupt further transmission to others. The number of new cases, however, has shown no substantial decline even though MDT with strong bactericidal drugs has been used in the past two decades. It is reported that about 600,000 to 700,000 new cases are continuously found in the world every year (20), which suggests that the transmission of leprosy bacilli still occurs, especially in countries of endemicity. Elucidation of the mode of transmission would be essential to help prevent new infection. The differentiation of strains of leprosy bacilli by genomic polymorphism might be of great value in efforts to understand the mode of transmission of the disease.

The range of molecular techniques for epidemiological analysis has expanded in recent years, and there are now many genotypic methods that allow a high level of discrimination between bacterial strains. Restriction fragment length polymorphism analysis, which is the method most widely used for molecular epidemiology of tuberculosis, is not applicable for leprosy, since *M. leprae* cannot be grown in artificial medium, and almost no divergence was found by this fingerprinting assay (19). Shin et al. discovered a genomic divergence of *M. leprae* by the variation of TTC repeats (17) and subdivided 34 isolates into 15 subtypes. Genotyping according to the TTC repeats for fragments amplified by PCR seemed to be feasible for molecular epidemiological analysis of leprosy transmission.

A previous study by Saeki et al. revealed that *M. leprae* existed on the surface of nasal cavities of residents in areas of endemicity (16). Here, we report the distribution of different TTC genotypes of *M. leprae* among family members of each dwelling in the villages with high-level leprosy prevalence and inconsistent genotypes obtained from patients and their family members in the same dwelling. The results strongly supported previously proposed hypotheses on the existence of an infectious source(s) other than that of patients living with family members.

MATERIALS AND METHODS

Nasal swab samples from residents in villages where leprosy is endemic. Nasal swab samples which showed positive PCR results in a previous study by Saeki et al. that was designed to elucidate the distribution of *M. leprae* in the places where leprosy is endemic (16) were used in this study for the comparison of TTC genotypes among family members. These samples originated from residents in two Indonesian villages in North Maluku with a high-level prevalence of leprosy, Gamtala and Lolori. In Gamtala village, 353 nasal swab samples and 277 samples in Lolori village were collected. The population of Gamtala village was 509 (median age, 25) and that of Lolori was 434 (median age, 20); the numbers of houses were 105 and 77, respectively. The prevalence of leprosy in Gamtala was 4.0% (14/353), and the paucibacillary/multibacillary (PB/MB) case ratio was 10/4. In Lolori, the prevalence was 3.3% (9/277) and the PB/MB ratio was 9/0 (16). MDT recommended by the World Health Organization was introduced in the middle of the 1990s in these areas. No tap water was supplied, and several families shared one well for bathing, washing, and cooking. Living surroundings were almost the same in both villages. The surface of the nasal cavity was swabbed with sterilized wet cotton swabs (Mentip1P-75, Nippon Menbo, Tokyo, Japan), which were then frozen until use. The cotton was washed in 500 μl of phosphate-buffered saline containing 0.05% Tween 80 to release the sample from the cotton swab, and the aliquot was centrifuged at 10,000 × *g* for 20 min. The sediment was then digested with lysis buffer to prepare the template DNA (5) and subjected for sequencing as described below.

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TABLE 1. Stability of TTC repeats of *M. leprae* strains during serial passage

Strain	Origin	Generations examined[a]	Copy numbers of repeats
Ky-1	Japanese new MB case	3, 5, 7, 8	9, 9, 9, 9
Ky-2	Japanese relapse MB case	4, 5, 6	11, 11, 11
Th-53	Thailander new MB case	3, 7, 11	14, 14, 14
Ze-2	Japanese relapse MB case	1, 3	11, 11
Ho-4	Japanese relapse MB case	Biopsy, 1, 2, 4	10, 10, 10, 10
Ko3-2	Korean new MB case	Biopsy, 1, 2, 4	13, 13, 13, 13
Ku-3	Japanese relapse MB case	Biopsy, 1, 2	10, 10, 10
Ku-6	Japanese relapse MB case	Biopsy, 1, 3	16, 16, 16
Ts-1	Japanese new MB case	Biopsy, 1, 2	9, 9, 9
Ze-4	Japanese relapse MB case	Biopsy, 1, 2, 3, 4	10, 10, 10, 10, 10
Ze-5	Japanese relapse MB case	Biopsy, 1, 2, 3	11, 11, 11, 11, 11

[a] Biopsy, bacilli from clinical material; generation 1 to 11, passage time in nude mouse footpad.

Samples from patients. To clarify whether the TTC genotype in one patient varies or not, genotypes of the bacilli obtained from various lesions of one patient were compared. Slit-skin smear samples from 49 lesions of 22 patients in North Maluku and North Sulawesi were obtained. Those patients in North Sulawesi included many refugees from North Maluku. Samples were collected in the same manner as is used for routine slit-skin smear testing for bacterial index examination. The sample on the disposable surgical blade was soaked in 70% ethanol and kept at room temperature until use. The sample was removed from the blade and collected as a pellet by centrifugation at 10,000 × *g* for 20 min in 70% ethanol and then washed with phosphate-buffered saline. The template was prepared by treatment with lysis buffer as mentioned above, and then the TTC genotype was examined.

Samples from household patients. TTC genotypes of the bacilli from patients living in the same dwelling were examined. Samples of five groups were collected as follows. Cases 1 and 2 (a pair of patients with household MB cases) consisted of one son and his father. Case 3 was a household case consisting of one son treated for 2 months, a new case of infection of his father, and three new cases of infection of his sister and brothers. Case 4 (a household case) consisted of one son treated for 4 months and one new case of infection of his brother. Case 5 consisted of one patient treated for 9 months and two new cases of infection of his brothers (see Table 4). Samples were collected from at least two lesions of each patient. The genotype of each isolate was examined as described below.

Samples from *M. leprae* inoculated in nude mice. To clarify the stability of the locus, we used various resources, including clinical material and different generations of serial passages in nude mice (13, 14), for comparisons of the numbers of TTC repeats within 11 strains of *M. leprae*. Template DNAs of each sample were prepared from the bacillary suspensions (which were preserved at −84°C). Each strain was maintained in nude mice footpads, with serial passages every 12 months (Table 1).

Preparation of template DNA and sequencing analysis. Templates from nasal swab materials, slit-skin samples, and bacillary suspensions were prepared by treatment with lysis buffer at 60°C overnight as described previously (5), and TTC repeat regions were amplified by PCR with the primers indicated by Shin et al. (17). Copy numbers of TTC repeats were examined by the direct sequencing of the PCR products. Briefly, the regions flanking TTC repeats were amplified using a G mixture and a FailSafe PCR system (EPICENTRE, Madison, Wis.). DNA samples for sequencing were recovered with a MinElute gel extraction kit (QIAGEN GmbH, Hilden, Germany) after electrophoresis of PCR products. Samples were sequenced with a BigDye terminator cycle sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Norwalk, Conn.) and an ABI Prism 310 genetic analyzer (Perkin-Elmer). The nucleotide sequences obtained were analyzed using DNASIS software (Hitachi Software Engineering, Yokohama, Japan). The forward primer was used in all sequencing reactions, since (according to the results of our preliminary study) the nucleotide sequences of interest detected by the reverse primer were deduced to be identical with those detected by the forward primer (data not shown).

Ethical approval. Informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee of National Institute of Infectious Diseases, Tokyo, Japan. Bacillary samples of nasal mucus and slit-skin smears were collected when informed consent was obtained.

RESULTS

Stability of TTC copies. Laboratory-maintained isolates showed various TTC genotypes. The copy numbers of TTC repeats in each isolate from the nude mice showed no changes and remained stable after serial passages in nude mice footpads; they also remained unchanged between the isolates from bacilli from clinical material and the corresponding isolates obtained from nude mice footpads (Table 1). Th-53 isolate was transferred with nine passages in nude mice footpads, but the number of the repeats did not change at all.

Genotype of the bacilli on the nasal mucus. The results of a previous study by Saeki et al. indicated that 28.2% (92/326) of Gamtala's villagers and 25.2% (68/270) of Lolori's villagers carried leprosy bacilli on the surface of their nasal cavities (16). *M. leprae*-positive PCR products for TTC genotyping were obtained from 48 samples out of 92 previous *M. leprae*-positive samples from Gamtala and 49 samples out of 68 previous *M. leprae*-positive Lolori's samples. Of 105 dwellings, there were 8 houses in Gamtala and 12 houses in Lolori in which more than two PCR-positive individuals carried the bacilli on the surface of their nasal cavities (Table 2). Residents in five houses in Gamtala showed different TTC genotypes of 10, 11, 12, and 13 copies. Residents in 11 houses in Lolori harbored different TTC genotypes from each other; their TTC genotypes were 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, and 24 repeats. The TTC repeats of the bacilli from the new MB case in Gamtala consisted of 10 copies, but the bacilli from his family contacts showed 11 and 13 copies of TTC repeats. The bacilli from a patient who had had contact with a PB case in house number 68 of Lolori showed 12 copies, but the genotype of the strain from this PB patient showed 14 copies (Table 2). In this area, the cases with 10 copies of TTC repeats were predominant; cases with 13 copies were the second populous group (Table 3).

Genotype of the bacilli in the lesions. For all patients examined, samples from different lesions of each patient showed identical genotypes. The TTC genotype with 10 copies was also dominant in the patients from North Maluku and North Sulawesi. The smallest number of repeats was 8; the largest was 29. In addition, other genotypes (such as 9, 11, 12, 13, 14, 15, 16, and 18 TTC copies) were detected. The frequency of each TTC genotype observed in samples from the nasal cavities of the residents and lesions of the patients is shown in Table 3.

TABLE 2. TTC genotypes of *M. leprae* detected from the surfaces of nasal mucosa of residents living in the same house

Village	House	TTC genotypes
Gamtala	9	10,[a] 11, 13, 13
Gamtala	15	13, 13
Gamtala	25	13, 13
Gamtala	78	10, 10
Gamtala	82	10, 13[b]
Gamtala	97	10,[c] 10, 13, 13, 13
Gamtala	99	10,[b] 10,[b] 10, 10, 13
Gamtala	100	10,[c] 10,[c] 10, 10, 12
Lolori	2	10, 18
Lolori	4	18, 18
Lolori	7	10, 12,[b] 18
Lolori	19	9, 12
Lolori	21	10, 14
Lolori	41	10, 13, 13
Lolori	49	8, 13
Lolori	54	13, 16
Lolori	62	12, 13
Lolori	66	11, 12
Lolori	68	12, 14[d]
Lolori	71	10, 24

[a] Newly detected MB case.
[b] Nerve enlargement without skin lesion.
[c] MB case (released from registration).
[d] PB case (registered).

Comparison of TTC genotypes among patients in a dwelling. The TTC genotypes of *M. leprae* in household leprosy cases were compared. The genotypes of one pair (the son and his father; case 2) indicated clearly independent genotypes from each other (10 copies in the son and 18 copies in the father). Bacilli of one MB case (the patient treated for 9 months; case 5) showed 12 copies of TTC repeats, while his brother harbored bacilli with 14 TTC repeats and the bacilli from another brother showed 12 TTC repeats. Another three groups of household cases (cases 1, 3, and 4) showed identical TTC genotypes within the family (Table 4).

TABLE 3. Frequency of each genotype observed in residents and patients

No. of repeats	Genotype frequency		
	Nasal mucus	Patient lesion[a]	Total
7	3		3
8	4	2	6
9	3	2	5
10	35	15	50
11	6	1	7
12	7	4	11
13	26	5	31
14	6	2	8
15		2	2
16	1	1	2
17	1		1
18	3	1	4
24	2		2
29		1	1
Total	97	36	133

[a] Includes 14 patients of household cases listed in Table 4.

TABLE 4. TTC genotypes of *M. leprae* obtained from household leprosy cases[a]

Case	Patient (TTC genotype) in supposed index case	Patient(s) (TTC genotype) in other household cases
1	Father (10)	Son (10)
2	Father (18)	Son (10)
3	Son (13)	Father (13), daughter (13), son (13) Son (13)
4	Son (8)	Son (8)
5	Son (12)	Son (12), son (14)

[a] At least two samples from different lesions were examined from each patient.

DISCUSSION

Elucidation and understanding of the source and the routes of transmission of *M. leprae* are essential in developing measures to prevent an infection. Previous seroepidemiological studies indicated widespread *M. leprae* infections within a population (1, 2, 10, 18), and studies by PCR on the distribution of the bacilli also found that many individuals in areas in which leprosy is endemic carried *M. leprae* on the surface of their nasal cavities (11, 16, 18). These studies suggested the presence of an infectious source other than that of a patient within the same dwelling. The aim of this study was to clarify microbiologically whether or not MB cases in the same dwelling represent the main source of infection.

Establishing a methodology to discriminate the isolates of *M. leprae* is fundamental for these purposes. Although many attempts have been made to subtype *M. leprae* isolates by genomic divergence (4, 9, 19), no useful methods for epidemiological analysis have been developed. Recently two genomic polymorphisms successfully discriminated isolates of *M. leprae* (12, 17). One of the authors (M. Matuoka) discovered that *M. leprae* isolates could be divided into two subtypes on the basis of the polymorphism in the *rpoT* gene. The geographical distribution of each genotype in the world was biased and seemed to be related to prehistoric movement of the human race (12). Nevertheless, the genomic diversity of the *rpoT* cannot be used for epidemiological tracing of the transmission of leprosy bacilli. Genotyping to compare diversity of short-tandem-repeat loci on the basis of PCR is feasible for molecular epidemiological analysis, since *M. leprae* is not cultivable and shows very low levels of diversion in genomic DNA (6). Variety in the copy numbers of TTC repeats can be used to classify *M. leprae* into a considerable number of subtypes and discriminate isolates for each leprosy case.

For subtyping the organisms, genetic polymorphisms must remain stable during a few rounds of infection. All isolates transferred in 1 to 11 passages in nude mice retained the same TTC genotype. Because of the wide variation and stability of the TTC repeats, genotyping of the bacilli by the TTC polymorphism proved to be satisfactory for epidemiological analysis of leprosy and discrimination of *M. leprae* distributed in areas of endemicity.

It is reasonable to assume that if the index case in the same dwelling is the source of infection, the genotypes detected in the house should be identical among the household members. In this study, various types of TTC genotypes were detected from nasal mucosa of the villagers in areas of endemicity.

However, our results clearly demonstrated that there were families with different TTC genotypes of *M. leprae* on the surface of nasal cavities among the residents in the same dwelling. Therefore, the results of the investigation suggest that these residents are contaminated by bacilli with different genotypes. No variations in genotype among the isolates obtained from various lesions in the same patient were shown. This result consequently enables comparisons of the genotypes of bacilli obtained from different patients. We had identified the existence of TTC genotypes of *M. leprae* that differed between the newly detected family contacts and the supposed index case patient. These results strongly suggest that the bacilli did not originate from a single patient in the dwelling and also indicate the exposure of the family members to infectious sources out of the dwelling. Previous seroepidemiological studies suggested that for the majority of cases, the possible source of infection might be in the environment rather than in direct contact with leprosy patients (1, 2, 10). The findings by PCR, which revealed the wide distribution of the bacilli among the residents in areas of endemicity, also indicated that the transmission of the bacilli was not only from the leprosy patients (11, 16, 18). The present study strongly supports these assumptions respecting the infectious source(s). Although many epidemiological observations indicated that the household contact was the risk factor for the development of leprosy (7, 15), on the other hand, many new cases among people without any known household contacts with patients were detected (7). It is therefore not necessarily the case that the household patient is the only source of infection of leprosy bacilli. The tendency seen of the accumulation of patients in some families might be attributed to other conditions such as susceptibility to leprosy infection, which is related to genetic predisposition as well as to acquired factors (3).

Two groups of the household leprosy cases showed apparently different TTC genotypes between a father and his son and among brothers. The inconsistency of the genotypes between *M. leprae* isolates obtained from household cases of patients living in the same dwelling clearly indicates that these patients are not always the source in infections of the other family members. Though the members of the other groups of leprosy cases showed the same genotype, whether those people were truly infected by the patient in the house was unclear. The presence of the same genotype in two cases doesn't necessarily imply the infection was transmitted from a patient to family contacts, for some TTC genotypes, such as those of 10 and 13 repeats, were widely distributed in the areas. Other polymorphisms which can discriminate within a given TTC genotype are needed to elucidate this problem. Better epidemiological analysis could be done by the combination of various genotyping techniques. However, TTC genotyping enabled the subtyping of *M. leprae* into more types than *rpoT* genotyping. It is expected that other short-polymorphic-tandem-repeat loci exist in *M. leprae* genome, in similarity to those observed in investigations of *M. tuberculosis* (8). A combination with genotyping using other polymorphisms might be a useful tool for precise epidemiological analysis. Other genotyping measures depending on other short-polymorphic-tandem-repeat loci are required. The frequency of 24 or 25 TTC repeats was the highest in the previous study, which examined *M. leprae* isolates obtained in Cebu, Philippines (17). Bacilli with 10 copies of TTC repeats were most frequently isolated in the present study, and the bacilli with large numbers (such as 37) of TTC repeats were not detected (Table 3). It is of interest to compare the frequencies of each genotype in different areas, since the results of a previous study indicated that the spread of the bacilli with specific genotypes was consistent with migration of some human groups (12).

The evidence resulting from the present molecular epidemiological study indicated the existence of an infectious source other than patients in the same dwelling. Wide distribution of the bacilli among residents (11, 16, 18) and a high positive ratio of anti-PGL-1 antibody among healthy residents (2, 10) suggested that the bacilli existed in certain sources to which people were commonly exposed. Taking these results into consideration, the environment seems to be the most likely infectious source. However, exactly what the infectious source is has not been elucidated so far.

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REFERENCES

1. **Abe, M., T. Ozawa, F. Minagawa, and Y. Yoshino.** 1990. Immunoepidemiological studies on subclinical infection in leprosy. II. Geographical distribution of seropositive responders with special reference to their possible source of infection. Jpn. J. Lepr. **59:**162–168.
2. **Cho, S. N., S. H. Kim, R. V. Cellona, G. P. Chan, T. T. Fajardo, G. P. Walsh, and J. D. Kim.** 1992. Prevalence of IgM antibodies to phenolic glycolipid I among household contacts and controls in Korea and the Philippines. Lepr. Rev. **63:**12–20.
3. **de Vries, R. R. P., and T. H. M. Ottenhoff.** 1994. Immunogenetics of leprosy, 113–121. *In* R. C. Hastings (ed.), Leprosy. Churchill Livingstone, New York, N.Y.
4. **de Wit, M. Y. L., and P. R. Klatser.** 1994. *Mycobacterium leprae* isolates from different sources have identical sequences of the spacer region between the 16S and 23S ribosomal RNA genes. Microbiology **140:**1983–1987.
5. **de Wit, M. Y. L., W. R. Faber, S. R. Krieg, J. T. Douglas, S. B. Lucas, N. Montreewasuwat, S. R. Pattyn, R. Hussain, J. M. Ponnighaus, R. A. Hartskeerl, and P. R. Klatser.** 1991. Application of a polymerase chain reaction for the detection of *Mycobacterium leprae* in skin tissues. J. Clin. Microbiol. **29:**906–910.
6. **Douglas, Y.** 2003. Proposal for molecular epidemiology of leprosy. Lepr. Rev. **74:**11–17.
7. **Fine, P. E., J. M. Sterne, J. M. Ponnighaus, L. Bliss, J. Saui, A. Chihana, M. Munthali, and D. K. Warandorff.** 1997. Household and dwelling contacts as risk factors for leprosy in northern Malawi. Am. J. Epidemiol. **146:**91–102.
8. **Frothingham, R., and W. A. Meeker-O'Connell.** 1998. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. Microbiology **114:**1189–1196.
9. **Fsihi, H., and S. T. Cole.** 1995. The *Mycobacterium leprae* genome: systematic sequence analysis identifies key catabolic enzymes, ATP-dependent transport systems and novel *polA* locus associated with genomic variability. Mol. Microbiol. **16:**909–919.
10. **Izumi, S., T. Budiawan, K. Saeki, M. Matsuoka, and K. Kawatsu.** 1999. An epidemiological study on *Mycobacterium leprae* infection and prevalence of leprosy in endemic villages by molecular biological technique. Ind. J. Lepr. **71:**37–43.
11. **Klatser, P. R., S. M. van Beers, B. Madjid, R. Day, and M. Y. L. de Wit.** 1993. Detection of *Mycobacterium leprae* nasal carriers in populations for which leprosy is endemic. J. Clin. Microbiol. **31:**2947–2951.
12. **Matsuoka, M., S. Maeda, M. Kai, N. Nakata, G. T. Chae, T. P. Gillis, K. Kobayashi, S. Izumi, and Y. Kashiwabara.** 2000. *Mycobacterium leprae* typing by genomic diversity and global distribution of genotypes. Int. J. Lepr. **68:**121–128.
13. **Matsuoka, M., Y. Kashiwabara, and Y. Namisato.** 2000. A *Mycobacterium*

leprae isolate resistant to dapsone, rifampin, ofloxacin and sparfloxacin. Int. J. Lepr. **68:**452–455.
14. **Nakamura, M.** 1994. Elimination of contaminants in a homogenate of nude-mouse footpad experimentally infected with *Mycobacterium leprae.* Jpn. J. Lepr. **64:**47–50. (In Japanese.)
15. **Noordeen, S. K.** 1994. The epidemiology of leprosy, p. 29–45. *In* R. C. Hastings (ed.) Leprosy. Churchill Livingstone, New York, N.Y.
16. **Saeki, K., T. Budiawan, M. Matsuoka, and S. Izumi.** 2000. Epidemiological significance of *M. leprae* in the residential environment: detection of *Mycobacterium leprae* on the surface of nasal cavity of inhabitants in a leprosy endemic area using the polymerase chain reaction. Jpn. J. Dermatol. **110:** 153–160. (In Japanese.)
17. **Shin, Y.-C., H. Lee, H. Lee, G. P. Walsh, J.-D. Kim, and S.-N. Cho.** 2000. Variable numbers of TTC repeats in *Mycobacterium leprae* DNA from leprosy patients and use in strain differentiation. J. Clin. Microbiol. **38:**4535–4538.
18. **van Beers, S., M., S. Izumi, B. Madjid, Y. Maeda, R. Day, and P. R. Klatser.** 1994. An epidemiological study of leprosy infection by serology and polymerase chain reaction. Int. J. Lepr. **62:**1–9.
19. **Williams, D. L., T. P. Gillis, and F. Portaels.** 1990. Geographically distinct isolates of *Mycobacterium leprae* exhibit no genotypic diversity by restriction fragment length polymorphism analysis. Mol. Microbiol. **41:**1653–1659.
20. **World Health Organization.** 2002. Leprosy global situation. Wkly. Epidemiol. Rec. **77:**1–8.