Microsatellite Mapping of *Mycobacterium leprae* Populations in Infected Humans

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To investigate genetic diversity in a bacterial population, we measured the copy numbers of simple sequence repeats, or microsatellites, in *Mycobacterium leprae* from patients living in and around Hyderabad, India. Three microsatellite loci containing trinucleotide or dinucleotide repeats were amplified from infected tissues, and the copy numbers were established by sequence analysis. Extensive diversity was observed in a cross-sectional survey of 33 patients, but closely related profiles were found for members of a multicase family likely to share a common transmission source. Sampling of multiple tissues from single individuals demonstrated identical microsatellite profiles in the skin, nasal cavity, and bloodstream but revealed differences at one or more loci for *M. leprae* present in nerves. Microsatellite mapping of *M. leprae* represents a useful tool for tracking short transmission chains. Comparison of skin and nerve lesions suggests that the evolution of disease within an individual involves the expansion of multiple distinct subpopulations of *M. leprae*.

Leprosy is a chronic and debilitating disease caused by infection with *Mycobacterium leprae*. The bacteria are often found in skin lesions, but their abilities to invade the Schwann cells of the peripheral nervous system and to induce a tissue-damaging immune response account for the deformities characteristic of the disease. Once diagnosed, leprosy can be cured by an effective and inexpensive drug regimen, and the global implementation of standardized multidrug therapy for leprosy represents one of the great advances in public health in the last decades of the 20th century. The number of leprosy patients registered worldwide has fallen from a peak of 10 to 15 million to a current total of less than 1 million. However, the transmission of leprosy continues unabated in high-burden countries, with the number of new leprosy cases registered each year remaining relatively constant (17). In India, which bears the highest global burden of leprosy, more than half a million new cases are diagnosed every year (26). It is possible that effective treatment fails to interrupt the transmission cycle as a result either of significant transmission prior to diagnosis or of some source of infection other than patients with active disease. An understanding of the transmission of leprosy will be important in planning further progress toward a world that is ultimately free of leprosy.

An understanding of the epidemiology of tuberculosis has advanced significantly with the availability of tools for screening the genetic diversity of the related mycobacterial pathogen *M. tuberculosis*. Strain typing systems have been used to monitor outbreaks (1), to distinguish between reactivation and reinfection as a cause of disease (16, 25), to estimate the extent of recent transmission (21), and to study mycobacterial population biology from local and global perspectives (3, 22). A

similar approach may be useful in advancing an understanding of the epidemiology of leprosy, although there are two important limitations. First, highly fastidious *M. leprae* cannot be cultured in the laboratory, and genetic analysis therefore must be done directly with infected tissues. This type of analysis is dependent upon the availability of samples containing detectable bacterial DNA, thus excluding a significant subset of patients who have the tuberculoid form of the disease, characterized by the presence of very few bacilli in skin lesions. By analogy with *M. tuberculosis*, PCR-based typing systems for polymorphic loci could be applied to leprosy cases at the multibacillary end of the disease spectrum. The *M. tuberculosis* genome contains a number of variable minisatellite loci, short sequences of 10 to 100 bp which are often present as tandem repeats. The development of minisatellite genotyping methods based on mycobacterial interspersed repetitive unit and variable nucleotide tandem repeat analysis schemes has resulted in highly discriminatory typing systems (23). However, for leprosy, the second limitation is that initial studies demonstrated very little evidence of any genetic diversity among *M. leprae* isolates (4, 6). An exception is a report by Shin et al. (20) documenting variation associated with a region of the *M. leprae* genome containing a TTC trinucleotide repeat. Diversity at the TTC locus was further confirmed in a report by Matsuoka et al. (18). That study also highlighted the need for identifying additional polymorphic loci for subtyping leprosy strains. In a recent study of four clinical isolates passaged in armadillos, Groat-house and colleagues (10) began to evaluate several additional short tandem repeat loci in the leprosy genome for their potential as epidemiological tools in tracking routes of transmission.

Simple sequence repeats of this nature, referred to as microsatellites (2 to 5 bp), are susceptible to replication slippage, or “slipped-strand mispairing,” which can result in an increase or a decrease in the copy number of the repeat element during cell division. Microsatellites have been used extensively for

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TABLE 1. Microsatellite loci and PCR primers

Repeat motif	Genome location ^a	Adjacent genes ^b	Primer	Sequence	Amplicon (bp) ^c
TTC ^d	2785432–2785494	ML2344 (pseudo) (2785364–2784593)	TTC-F ₁	GGACCTAAACCATCCCGTTT	201
			TTC-R ₁	CTACAGGGGGGCACTTAGCTC	
		ML2345 (pseudo) (2786390–2786807)	TTC-F ₂	CGTTGGGTTTCGATCGAATCGA	131
			TTC-R ₂	GCACGCCGACGGGAATAAGT	
AGT ^d	2583816–2583839	ML2172 (hyp) (2583247–2583603)	AGT-F ₁	TGCCACCTTCGGTTATAGAAGCAAGT	257
			AGT-R ₁	GGCAGGTCCAGTGCCTTTGCT	
		ML2173 (pseudo) (2584381–2584584)	AGT-F ₂	ATCAACGCTGCGGTTTCGCAG	151
			AGT-R ₂	ATATGCATGCCGGTGGTGTGCT	
AT	948935–948964	ML0798 (hyp) (948302–946524)	AT-F ₁	CAATATGCGGGTTGGCGCTTCTG	168
			AT-R ₁	CCGTCTGGCTCGATGGCTGGATTC	
		ML0799 (hyp) (949448–949041)	AT-F ₂	GAAAAGCTAGGGTGATGGGCGCTC	127
			AT-R ₂		

^a Genome coordinates are those established for the sequenced isolate of *M. leprae* (5).

^b Pseudo, pseudogene; hyp, hypothetical protein product gene.

^c The amplicon size is that predicted for the *M. leprae* sequenced isolate.

^d The TTC and AGT microsatellites are synonymous with the AGA and GTA loci, respectively, both studied recently in leprosy strains propagated in armadillo hosts (10).

mapping the genomes of higher eukaryotes (7), and several have been linked directly to human disease (8). There has been more limited use of microsatellites for bacterial typing. Metzgar et al. (19) concluded that the high frequency of variation made such loci unsuitable for phylogenetic analysis of *Escherichia coli*, although they noted a potential use in distinguishing closely related strains, and simple sequence repeats have been used for typing of the highly clonal pathogens *Bacillus anthracis* (12) and *Yersinia pestis* (15). A special case of microsatellite diversity is that of the “contingency loci” exploited by some bacterial pathogens (2). By making the expression of key surface antigens dependent on variations in the copy numbers of simple sequence repeats, these bacteria take advantage of replication slippage to generate subpopulations that are capable of undergoing selective expansion in an appropriate environment, such as that of host immune pressure during infection.

Screening of the genome sequence of *M. leprae* (5) identified a series of microsatellite regions, and we reasoned that these were the most likely sites for genetic diversity. In the present study, we used three of these regions to probe the extent of genetic diversity of *M. leprae* in a group of leprosy patients and also in different tissues from the same patient.

MATERIALS AND METHODS

Patients and clinical criteria. All of the patients included in the study were recruited through the leprosy clinic at the LEPRO India Blue Peter Research Centre (BPRC), Hyderabad, India. Patients were graded clinically and histologically on the leprosy spectrum according to the standard Ridley-Jopling classification as having paucibacillary tuberculoid leprosy, borderline tuberculoid leprosy, or multibacillary states of borderline lepromatous leprosy and lepromatous leprosy. A subset of patients was diagnosed as undergoing a reversal reaction on the basis of reactive skin changes (erythema and/or edema of existing lesions, new skin lesions that were not relapsing leprosy, or erythema nodosum leprosum) or experiencing acute neuritis (peripheral nerve tenderness, new sensory symptoms or signs, or new motor symptoms or signs). Permission was obtained for this study from the local ethics committee for BPRC and from the London School of Hygiene and Tropical Medicine (LSHTM) ethics committee. Informed consent was obtained from all of the subjects involved in the study.

Punch biopsy specimens 6 mm in diameter were taken for diagnostic purposes from the active edge of skin lesions of new patients with untreated leprosy ($n = 33$). Sections from each biopsy specimen were stained with hematoxylin-eosin and a modified Fite-Faraco stain for *M. leprae*. The bacterial load was assessed by microscopy and expressed on a logarithmic scale as a bacillary index. A portion of each biopsy specimen was snap-frozen and transported on dry ice to LSHTM, where it was stored in liquid nitrogen until studied. Additional clinical

samples were taken from seven patients with untreated lepromatous leprosy. Skin slit smears were prepared from left and right earlobes, and the resulting material was pooled for DNA extraction. The nasal cavity was sampled by using a sterile cotton swab, and this material was similarly processed for DNA extraction. Finally, DNA was prepared from mononuclear cells isolated from 10 ml of peripheral blood. Partial-thickness nerve biopsy specimens had been taken from an additional subset of eight patients undergoing a reversal reaction or experiencing neuritis for an earlier research and diagnostic study (14). These biopsy specimens (2 to 4 mm) had been taken from the radial cutaneous nerve at the wrist with local anesthetic (1% lidocaine). At the same time, a biopsy specimen was also taken from a skin lesion of each patient. To study microsatellite repeats in a multicase family, DNA was extracted from stored paraffin blocks prepared from skin biopsy specimens.

Amplification and analysis of *M. leprae* DNA. A BLASTN search of the genome sequence of *M. leprae* (5) accessed from the Leproma World Wide Web Server (<http://genolist.pasteur.fr/Leproma/>) was performed to identify consecutive di- and trinucleotide repetitive elements. Once a locus was identified, primer pairs were designed to amplify the region around the marker. The sequences of the primers and genome coordinates are provided in Table 1. All of the repeat loci were located in noncoding regions between two genes or pseudogenes.

For the extraction of *M. leprae* DNA, finely chopped skin or nerve biopsy specimen, material scraped from skin slit smears, or a peripheral blood mononuclear cell pellet was added to 0.9 ml of guanidinium lysis buffer, part of the NucliSens commercial kit (Biomerieux). The samples were homogenized with a disposable pestle, and then partially purified DNA was prepared according to the manufacturer's instructions. Paraffin-embedded sections were dewaxed twice for 5 min each time with 1 ml of xylene and centrifuged ($14,000 \times g$, 5 min). The supernatant was discarded, and traces of solvent were removed by washing the pellet twice for 5 min each time with 1 ml of 100% ethanol. After centrifugation ($14,000 \times g$, 5 min), the pellet was air dried. Samples were digested with 90 μ l of Tris-EDTA buffer containing proteinase K (Qiagen) for 30 min at 56°C. Mycobacteria were lysed in a final volume of 0.9 ml of guanidinium lysis buffer, and DNA was purified as described above. DNA purified from armadillo-grown *M. leprae* and supplied by Patrick Brennan through the NIH Leprosy Contract (<http://www.cvmb.colostate.edu/mip/leprosy/index.html>) was used as a positive control.

Hot-start PCR was performed with a final volume of 25 μ l by using a Perkin-Elmer 9700 thermal cycler and an Excite Core kit (BioGene) according to the manufacturer's instructions. After an initial denaturation step (10 min at 95°C), 43 cycles of amplification were performed as follows: denaturation at 95°C for 10 s, annealing at 58 or 62°C for 30 s, and extension at 72°C for 30 s. A final extension was performed at 72°C for 2 min. Two primer sets were used to amplify the TTC locus: TTC-F₁ and TTC-R₁ amplified the ~200-bp product described by Shin et al. (20), and TTC-F₂ and TTC-R₂ amplified a shorter, ~131-bp product. The same repeat numbers were obtained with either pair, although the shorter product was more reproducibly amplified from samples (such as paraffin-embedded tissues) that contained partially degraded DNA. Similarly, primer pairs generating long or short products were used for the AGT locus. A heminested PCR was used for the AT locus; 43 cycles were carried out with primers AT-F₁ and AT-R₁, and then 25 cycles were carried out with primers AT-F₂ and AT-R₂ (Table 1). A concentration of 2 mM MgCl₂ was used in the long- and short-

product PCRs for the TTC locus and in the heminested PCR for the AT locus; 1.5 mM MgCl₂ was used in the PCRs for the AGT locus. The annealing temperature was 58°C for all reactions, except for that with primers AGT-F₁ and AGT-R₁, for which 62°C was used.

PCR products were initially screened by electrophoresis on 3% (wt/vol) agarose gels. When sequencing was undertaken, products were separated on 2 or 3% (wt/vol) low-melting-point agarose (Invitrogen), and bands were excised with a sterile scalpel blade and purified by using a NucleiClean DNA isolation kit (Sigma-Aldrich). Negative controls, consisting of extraction reagents without clinical material, were used throughout the isolation procedures and included in PCR assays along with several template (water) blanks to ensure the absence of contamination in typing experiments.

Cycle sequencing was performed by using a Perkin-Elmer 2400 PCR system with an ABI Dye Terminator Ready Reaction kit (Perkin-Elmer Applied Systems) according to the manufacturer's protocol; subsequent analysis was carried out by using an ABI 310 genetic analyzer. The majority of the samples were analyzed on more than one occasion by independent PCR amplification, often with alternative primer pairs. In each instance, repeat analyses demonstrated identical copy numbers.

RESULTS

Genetic diversity within a geographically defined population. Three microsatellite loci were selected for study on the basis of their reproducible amplification by PCR and evidence of polymorphisms in preliminary studies. They included the TTC repeat originally described by Shin et al. (20), an AGT repeat, and an AT dinucleotide repeat (Table 1). Each locus was amplified from biopsy specimens taken from skin lesions of 33 patients attending the leprosy clinic at BPRC, and the copy numbers of the repeat elements were determined by sequence analysis (Table 2). For this study, we used a series of skin biopsy specimens collected mainly from patients with untreated multibacillary disease between 1997 and 1999. These provided a relatively rich source of *M. leprae* DNA, suitable for multiple sampling to confirm the reproducibility of the assay. The sensitivity of the amplification procedure for microsatellite loci was similar to that obtained with other single-gene PCR assays for leprosy, consistently generating products from multibacillary lesions and from about half of the paucibacillary lesions tested. Considerable variations were seen at each of the loci, with copy numbers ranging from 10 to 25 for TTC, 6 to 13 for AGT, and 8 to 19 for AT. Eight pairs of individuals and two trios of individuals had matching repeat copy numbers at two loci, but only two patients—17 and 33—had identical genotypes at all three loci (Table 2). Analysis of the geographic distribution of the patient population on the basis of residential address did not reveal any obvious clustering of the patients with some matching genotypes (Fig. 1); we were unable to identify any epidemiological link between the two patients with matching genotypes.

Lack of diversity in a multicase household. One family attending the BPRC leprosy clinic included four cases of leprosy, affecting a mother and her son, his wife, and his wife's father (Fig. 2). We were able to generate PCR products from paraffin-embedded tissue samples from the mother and her son (both with multibacillary disease) and from the father, who had paucibacillary leprosy, but not from the son's wife. All three samples matched at the TTC and AGT loci, while one of the three differed by one copy number at the AT dinucleotide repeat. The homogeneity observed in the multicase family (Fig. 2) is in contrast to the heterogeneity observed with random sampling (Table 2).

TABLE 2. Diversity of *M. leprae* microsatellite profiles in patient population in Hyderabad, India

Patient	Diagnosis ^a	Bacillary index ^b	Copy number for:		
			TTC	AGT	AT
1	BT	1+	10	7	13
2	BT	0	14	6	— ^c
3	BL	2+	15	8	—
4	BL	3+	14	6	10
5	LL	5+	14	9	19
6	LL	5+	15	11	11
7	BT	0	13	9	15
8	LL	5+	14	7	17
9	LL	5+	13	9	9
10	LL	5+	12	9	12
11	BL	5+	14	13	11
12	BL	5+	10	6	8
13	BL	5+	17	9	14
14	BL	5+	10	8	11
15	LL	5+	13	11	15
16	LL	5+	14	10	16
17	LL	5+	18	9	13
18	BL	4+	—	8	13
19	BL	4+	17	12	17
20	BL	0	10	10	11
21	LL	5+	13	13	11
22	BL	2+	13	12	13
23	BL	3+	15	10	12
24	BL	2+	14	7	—
25	BL	4+	22	9	17
26	BL	5+	11	12	13
27	LL	5+	15	11	12
28	BL	3+	25	8	15
29	LL	6+	13	12	15
30	LL	5+	15	10	14
31	LL	5+	11	6	17
32	BL	4+	13	9	17
33	LL	5+	18	9	13
<i>M. leprae</i> standard ^d			13	10	17

^a BT, borderline tuberculoid leprosy; BL, borderline lepromatous leprosy; LL, polar lepromatous leprosy.

^b The bacillary index is the bacterial load in the biopsy sample assessed by microscopy.

^c —, absence of data as a result of insufficient material or failure to amplify a PCR product.

^d The *M. leprae* standard is a DNA preparation from armadillo-grown *M. leprae*; the copy numbers for repeat elements in this sample differ from those in the sequenced isolate (TTC, 21; AGT, 8; AT, 15) (5).

Comparison of *M. leprae* isolates from different clinical sites. We next investigated the possibility of genetic diversity of *M. leprae* populations within a single individual. For seven patients with lepromatous leprosy in 2003, we were able to compare the results for a skin biopsy specimen taken from the site of a lesion with those for skin smear specimens taken from the earlobes, a nasal swab specimen, and a specimen from a preparation of peripheral blood mononuclear cells. While we were not able to obtain results for all loci from all of the sites, where data were available, there was an exact match in copy number for each of the specimens from the same patient (Table 3). For an additional eight patients, we were able to compare microsatellite profiles for skin lesion specimens with those for peripheral nerve biopsy specimens taken from patients experiencing adverse neurological reactions as part of a research and diagnostic study. In contrast to the multisite comparisons shown in Table 3, differences at one or more loci were

FIG. 1. Geographic distribution of leprosy patients. Patient numbers (Table 2) are used to indicate the place of residence of each patient in the area surrounding Hyderabad and, in the grey-shaded inset, in an expanded map of the city of Hyderabad. Circled numbers indicate two patients with identical copy numbers at repeat loci.

found for seven of the eight patients in the comparison of skin and nerve biopsy specimens (Table 4).

DISCUSSION

Comparison of the genome sequences of *M. leprae* and *M. tuberculosis* shows that the genetic complement of the leprosy bacillus has been extensively eroded as a result of deletion events and formation of pseudogenes (5). In spite of this evidence of a turbulent evolutionary history, previous studies showed that contemporary *M. leprae* is remarkably free of genetic diversity (4, 6). Our results demonstrate that simple sequence repeats are exempt from this overall conservation. It is possible that *M. leprae* has lost some of the genes that would normally function to ensure the fidelity of replication at such loci (e.g., *mut* genes or *dnaQ*) (9, 11). Consistent with the variability observed at the TTC and other short tandem repeat loci (10, 18, 21), mapping of three *M. leprae* microsatellites revealed extensive diversity in a cross-sectional survey of the patient population in Hyderabad, India. Although the limited numbers preclude a detailed statistical analysis, the absence of

any obvious linkage disequilibrium among the three loci suggests a highly dynamic population structure. Targeted analysis of a multicaser family demonstrated that the microsatellite profile was conserved in the context of a presumed transmission link, and the pattern observed for the overall patient population suggests that the continuing incidence of leprosy in this community was the result of a complex series of transmission events, rather than an outbreak caused by the recent transmission of one or more dominant stable clones. Further studies of genetic diversity in samples with known epidemiological links will be important in establishing the extent to which microsatellite mapping can be used as a reliable marker for longer transmission chains. In light of the very high degree of diversity, it may be useful to extend the panel of polymorphic loci to develop relationship models that incorporate partial mismatches. Compared to the *M. tuberculosis* genome, the *M. leprae* genome is rich in simple sequence repeats (2 to 5 bp) that could be used in the development of such a multilocus typing scheme (10, 24). Whereas we used sequence analysis as a highly reliable approach in the present study, the approach of

FIG. 2. A family with multiple cases of leprosy. Clinical diagnosis (LL, lepromatous leprosy; BT, borderline tuberculoid leprosy), bacillary index (BI), and date (month/year) of commencement of treatment are shown for four family members with leprosy, along with the repeat copy numbers for the three microsatellite loci. We were unable to amplify any *M. leprae* DNA from the skin biopsy sample from the son's wife.

microsatellite mapping can be readily adapted to a multiplex assay with gel- or column-based size fractionation formats suitable for epidemiological studies (10).

For a conventional cultivable bacterium, it would be of interest to quantify the frequency of replication slippage by screening multiple colonies for variations in copy numbers and

by testing stability during serial passage. This microbiological approach was not possible for *M. leprae*, but we were able to compare microsatellite profiles for samples taken from different anatomic sites from the same individual. While skin, blood, and nasal cavity samples consistently generated matching profiles, frequent mismatches were observed when bacteria in skin and nerves were compared. These results indicate the presence of two subpopulations of bacteria that have different dominant genotypes and presumably have expanded independently of each other. Two models can be proposed to account for these observations. The first model assumes that the unique se-

TABLE 3. Conservation of microsatellite profiles in samples from various peripheral sites from the same patient^a

Patient	Repeat locus	No. of samples from the following site:			
		Skin biopsy	Skin smear	Nasal swab	Peripheral blood mononuclear cells
101	TTC	17	17	17	17
	AGT	10	10	10	10
	AT	15	15	15	15
102	TTC	14	14	14	14
	AGT	7	7	7	7
	AT	19	19	19	— ^b
103	TTC	17	17	17	17
	AGT	8	8	8	8
	AT	14	14	14	14
104	TTC	13	13	13	13
	AGT	13	13	—	13
	AT	18	18	18	18
105	TTC	15	15	15	15
	AGT	9	9	9	9
	AT	13	13	13	13
106	TTC	15	15	15	15
	AGT	8	8	8	8
	AT	16	16	—	16
107	TTC	16	16	16	16
	AGT	12	12	12	12
	AT	21	21	—	21

^a All patients had lepromatous leprosy.

^b —, absence of data as a result of insufficient material or failure to amplify a product.

TABLE 4. Differences in microsatellite profiles in nerve and skin lesion samples from the same patient

Patient	Diagnosis ^a	Tissue	Bacillary index	Copy number for:		
				TTC	AGT	AT
201	LL neu	Skin	5+	14	8	13
		Nerve	5+	15	8	13
202	LL	Skin	5+	15	11	11
		Nerve	5+	15	11	14
203	BL-RR	Skin	3+	19	7	11
		Nerve	5+	19	7	11
204	BL-RR	Skin	5+	14	12	14
		Nerve	5+	14	14	15
205	BL-RR	Skin	3+	20	8	17
		Nerve	3+	20	10	18
206	BL neu	Skin	2+	13	11	13
		Nerve	5+	14	11	13
207	LL neu	Skin	3+	13	11	14
		Nerve	3+	13	11	15
208	BT neu	Skin	0	11	8	13
		Nerve	0	11	11	13

^a LL, lepromatous leprosy; neu, neuritic complications during treatment; BL, borderline lepromatous leprosy; RR, reversal reaction; BT, borderline tuberculoid leprosy.

quence revealed by PCR amplification corresponds to the dominant genotype within a heterogeneous population. Entry into the nerve provides an opportunity for a limited number of bacteria to undergo clonal expansion in a relatively isolated new niche. This situation could allow the emergence of an initially subdominant population, either through a purely stochastic process or as the result of some selective advantage. The intergenic or interpsudogenic location of the microsatellite loci makes it unlikely that a difference in repeat copy number would itself confer any biological advantage (in a manner comparable to that of a contingency locus, for example), but it is possible that it acts as a marker for some biologically distinct subpopulation of bacteria and that it is carried along as a passive "hitchhiker" during selective expansion of this subpopulation. In the second model, some feature of *M. leprae* replication in the nerve—such as an accelerated doubling time—promotes a concerted change in the copy number at microsatellite loci in a way that results in an alteration of the dominant overall genotype. It is interesting that each of the nine mismatches identified in the present study involved an increased copy number in the nerve samples, reminiscent of the microsatellite expansion associated with a series of human diseases (8, 13). Whatever the underlying mechanism, the finding of a difference in *M. leprae* subpopulations infecting the nerves and the skin is novel and unexpected. An improved understanding of the factors involved in the invasion of neural tissues by *M. leprae* may reveal new ways of preventing the pathological sequelae of leprosy even in the absence of a complete block in transmission.

This study demonstrates that it is feasible to apply a molecular epidemiology approach to the study of leprosy in a clinical setting. The inclusion of more loci in typing schemes is likely to improve the discrimination of this approach (10). In addition to further exploration of microsatellite diversity, it will be important to search for other forms of genetic variation suitable for strain typing; a systematic screen for single nucleotide polymorphisms may be useful, for example. Important goals will be to identify typing systems capable of providing reliable information about the transmission dynamics of *M. leprae* and to use these to assist in the search for interventions that will reduce the number of new cases of leprosy.

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REFERENCES

- Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Drucker, and B. R. Bloom. 1994. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods. *N. Engl. J. Med.* **330**:1710–1716.
- Bayliss, C. D., D. Field, and E. R. Moxon. 2001. The simple sequence contingency loci of *Haemophilus influenzae* and *Neisseria meningitidis*. *J. Clin. Invest.* **107**:657–662.
- Bifani, P. J., B. Mathema, N. E. Kurepina, and B. N. Kreiswirth. 2002. Global dissemination of the *Mycobacterium tuberculosis* W-Beijing family strains. *Trends Microbiol.* **10**:45–52.
- Clark-Curtiss, J. E., and G. P. Walsh. 1989. Conservation of genomic sequences among isolates of *Mycobacterium leprae*. *J. Bacteriol.* **171**:4844–4851.
- Cole, S. T., K. Eiglmeier, J. Parkhill, K. D. James, N. R. Thomson, P. R. Wheeler, N. Honore, T. Garnier, C. Churcher, D. Harris, K. Mungall, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. M. Davies, K. Devlin, S. Duthoy, T. Feltwell, A. Fraser, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, C. Lacroix, J. Maclean, S. Moule, L. Murphy, K. Oliver, M. A. Quail, M. A. Rajandream, K. M. Rutherford, S. Rutter, K. Seeger, S. Simon, M. Simmonds, J. Skelton, R. Squares, S. Stevens, K. Taylor, S. Whitehead, J. R. Woodward, and B. G. Barrell. 2001. Massive gene decay in the leprosy bacillus. *Nature* **409**:1007–1011.
- Cole, S. T., P. Supply, and N. Honore. 2001. Repetitive sequences in *Mycobacterium leprae* and their impact on genome plasticity. *Lepr. Rev.* **72**:449–461.
- Collins, J. R., R. M. Stephens, B. Gold, B. Long, M. Dean, and S. K. Burt. 2003. An exhaustive DNA micro-satellite map of the human genome using high performance computing. *Genomics* **82**:10–19.
- Cummings, C. J., and H. Y. Zoghbi. 2000. Fourteen and counting: unraveling trinucleotide repeat diseases. *Hum. Mol. Genet.* **9**:909–916.
- Dawes, S. S., and V. Mizrahi. 2001. DNA metabolism in *Mycobacterium leprae*. *Lepr. Rev.* **72**:408–414.
- Grothouse, N. A., B. Rivoire, H. Kim, H. Lee, S.-N. Cho, P. J. Brennan, and V. D. Vissa. 2004. Multiple polymorphic loci for molecular typing of *Mycobacterium leprae*. *J. Clin. Microbiol.* **42**:1666–1672.
- Iyer, R. R., A. Pluciennik, W. A. Rosche, R. R. Sinden, and R. D. Wells. 2000. DNA polymerase III proofreading mutants enhance the expansion and deletion of triplet repeat sequences in *Escherichia coli*. *J. Biol. Chem.* **275**:2174–2184.
- Keim, P., L. B. Price, A. M. Klevytska, K. L. Smith, J. M. Schupp, R. Okinaka, P. J. Jackson, and M. E. Hugh-Jones. 2000. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriol.* **182**:2928–2936.
- Kennedy, L., E. Evans, C. M. Chen, L. Craven, P. J. Detloff, M. Ennis, and P. F. Shelbourne. 2003. Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis. *Hum. Mol. Genet.* **12**:3359–3367.
- Khanolkar-Young, S., D. B. Young, M. J. Colston, J. N. Stanley, and D. N. Lockwood. 1994. Nerve and skin damage in leprosy is associated with increased intralosomal heat shock protein. *Clin. Exp. Immunol.* **96**:208–213.
- Klevytska, A. M., L. B. Price, J. M. Schupp, P. L. Worsham, J. Wong, and P. Keim. 2001. Identification and characterization of variable-number tandem repeats in the *Yersinia pestis* genome. *J. Clin. Microbiol.* **39**:3179–3185.
- Lillebaek, T., A. Dirksen, I. Baess, B. Strunge, V. O. Thomsen, and A. B. Andersen. 2002. Molecular evidence of endogenous reactivation of *Mycobacterium tuberculosis* after 33 years of latent infection. *J. Infect. Dis.* **185**:401–404.
- Lockwood, D. N. 2002. Leprosy elimination—a virtual phenomenon or a reality? *Br. Med. J.* **324**:1516–1518.
- Matsuoka, M., L. Zhang, T. Budiawan, K. Saeki, and S. Izumi. 2004. Genotyping of *Mycobacterium leprae* on the basis of the polymorphism of TTC repeats for analysis of leprosy transmission. *J. Clin. Microbiol.* **42**:741–745.
- Metzgar, D., E. Thomas, C. Davis, D. Field, and C. Wills. 2001. The microsatellites of *Escherichia coli*: rapidly evolving repetitive DNAs in a non-pathogenic prokaryote. *Mol. Microbiol.* **39**:183–190.
- Shin, Y. C., 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.
- Small, P. M., P. C. Hopewell, S. P. Singh, A. Paz, J. Parsonnet, D. C. Ruston, G. F. Schecter, C. L. Daley, and G. K. Schoolnik. 1994. The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. *N. Engl. J. Med.* **330**:1703–1709.
- Smith, N. H., J. Dale, J. Inwald, S. Palmer, S. V. Gordon, R. G. Hewinson, and J. M. Smith. 2003. The population structure of *Mycobacterium bovis* in Great Britain: clonal expansion. *Proc. Natl. Acad. Sci. USA* **100**:15271–15275.
- Sola, C., I. Filliol, E. Legrand, S. Lesjean, C. Locht, P. Supply, and N. Rastogi. 2003. Genotyping of *Mycobacterium tuberculosis* complex using MIRUs: association with VNTR and spoligotyping for molecular epidemiology and evolutionary genetics. *Infect. Genet. Evol.* **3**:125–133.
- Spurgies, R. S., T. N. Quitgwa, K. L. Smith, J. Schupp, E. G. Palmer, R. A. Cox, and P. Keim. 2003. Molecular typing of *Mycobacterium tuberculosis* by using nine novel variable-number tandem repeats across the Beijing family and low-copy-number IS6110 isolates. *J. Clin. Microbiol.* **41**:4224–4230.
- van Rie, A., R. Warren, M. Richardson, T. C. Victor, R. P. Gie, D. A. Enarson, N. Beyers, and P. D. van Helden. 1999. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N. Engl. J. Med.* **341**:1174–1179.
- Weekly Epidemiology Record. 2002. Leprosy. Global situation. *Wkly. Epidemiol. Rec.* **77**:1–8.