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566

567 **FIGURE LEGENDS**

568 **Figure 1: The *M. leprae* targets of dapsone (ML0224, *folP1*), rifampicin (ML1891c, *rpoB*),**
569 **and fluoroquinolones (ML0006, *gyrA*).** The partial nucleotide (upper) and corresponding amino
570 acid (lower) sequences containing the drug resistance determining regions (DRDR) of the target
571 genes are presented. The nucleotides and the amino acid numbers are with reference to the open
572 reading frames of the genes for the *M. leprae* TN strain as found in the Leproma website
573 (http://genolist.pasteur.fr/Leproma/). The codons/amino acids implicated in drug resistance are
574 shown within boxes. The primer sequences selected for real-time PCR- HRM are underlined.

575 **Figure 2: Real-time PCR-HRM analysis for detection of mutations in *M. leprae* drug**
576 **resistance determining regions (DRDR assays).**

577 Representative real-time-PCR normalized melt curves (A) and the differential curves (B) for *M.*
578 *leprae* strains analyzed at the DRDRs of *folP1*, *rpoB* and *gyrA*. The green color was assigned to
579 NHDP63 strain serving as the wild type for each DRDR. The mutants or mixed genotype strains
580 are shown in red, orange and blue. The genotypes of the mutants are indicated within parentheses
581 next to the sample name.

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582 **Figure 3: Sequence chromatograms of samples depicting multiple alleles in *gyrA* and *folP1***
583 **DRDR**. Arrows indicate nucleotide positions where mixed alleles were detected for samples
584 named in the chromatograms.

585 **Figure 4: Real-time PCR-HRM analysis for SNP detection for *M. leprae* typing (SNP typing**
586 **assays).** Representative real-time-PCR normalized melt curves (A) and the differential curves
587 (B) for *M. leprae* strains analyzed at three SNP loci as indicated beside the panels. The green
588 color was assigned to NHDP63, and the corresponding alleles for each locus are indicated (this is
589 referred to as Cluster 1 in Tables 6 and 7). The red curves indicate strains with the alternative
590 allele at each locus (this is referred to as Cluster 2 in Tables 6 and 7).

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Partial sequence of *M. leprae* |ML0224|*folP1*

```
 91 - gct gtc cag cac ggc ctg gca atg gtc gcg gaa ggc gcg gcg att gtc gac gtc ggt ggc
 31 -  A   V   Q   H   G   L   A   M   V   A   E   G   A   A   I   V   D   V   G   G
151 - gaa tcg acc cgg ccc ggt gcc att agg acc gat cct cga gtt gaa ctc tct cgt atc gtt
 51 -  E   S   T   R   P   G   A   I   R   T   D   P   R   V   E   L   S   R   I   V
```

Partial sequence of *M. leprae* |ML1891c|*rpoB*

```
1261 - cgt ccg gtg gtc gcc gct atc aag gaa ttc ttc ggc acc agc cag ctg tcg cag ttc atg
 421 -  R   P   V   V   A   A   I   K   E   F   F   G   T   S   Q   L   S   Q   F   M
1321 - gat cag aac aac cct ctg tcg ggc ctg acc cac aag cgc cgg ctg tcg gcg ctg ggc ccg
 441 -  D   Q   N   N   P   L   S   G   L   T   H   K   R   R   L   S   A   L   G   P
1381 - ggt ggt ttg tcg cgt gag cgt gcc ggg cta gag gtc cgt gac gtg cac cct tcg cac tac
 461 -  G   G   L   S   R   E   R   A   G   L   E   V   R   D   V   H   P   S   H   Y
```

Partial sequence of *M. leprae* |ML0006|*gyrA*

```
181 - tta gac tcc ggt ttc cgc ccg gac cgt agc cac gct aag tca gca cgg tca gtc gct gag
 61 -  L   D   S   G   F   R   P   D   R   S   H   A   K   S   A   R   S   V   A   E
241 - acg atg ggc aat tac cat ccg cac ggc gac gca tcg att tat gac acg tta gtg cgc atg
 81 -  T   M   G   N   Y   H   P   H   G   D   A   S   I   Y   D   T   L   V   R   M
```

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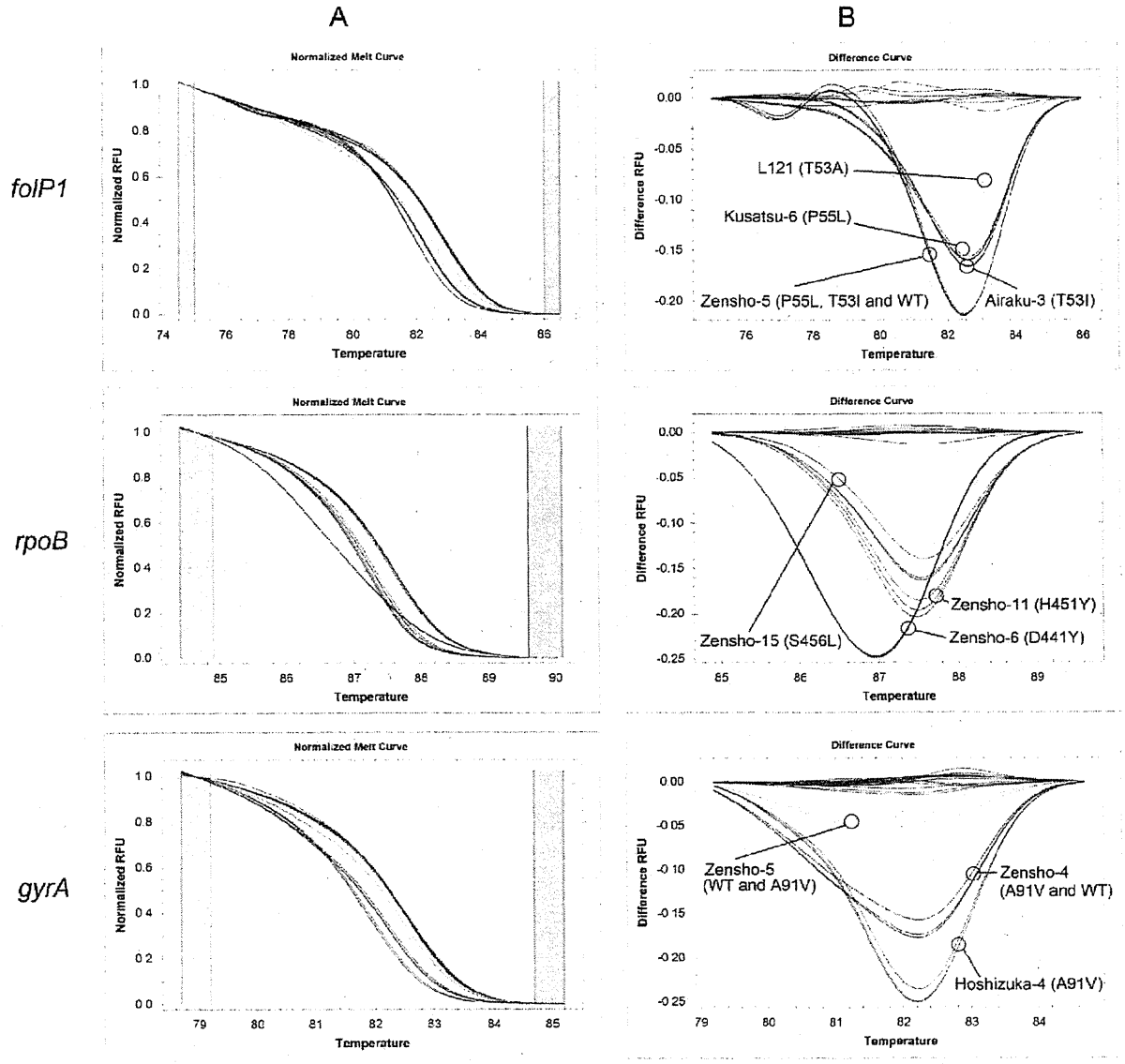

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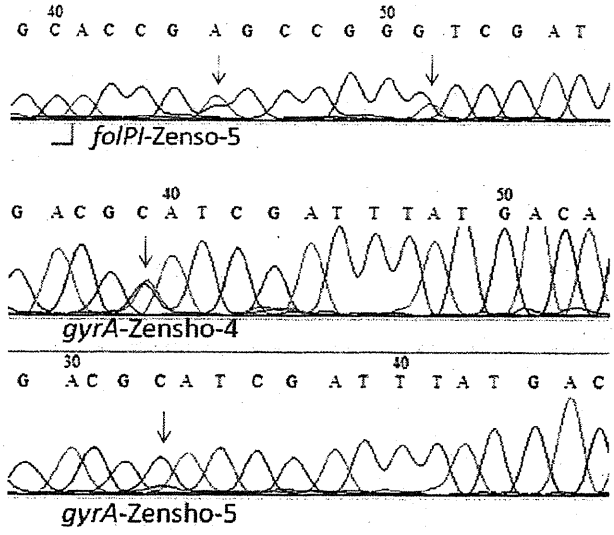

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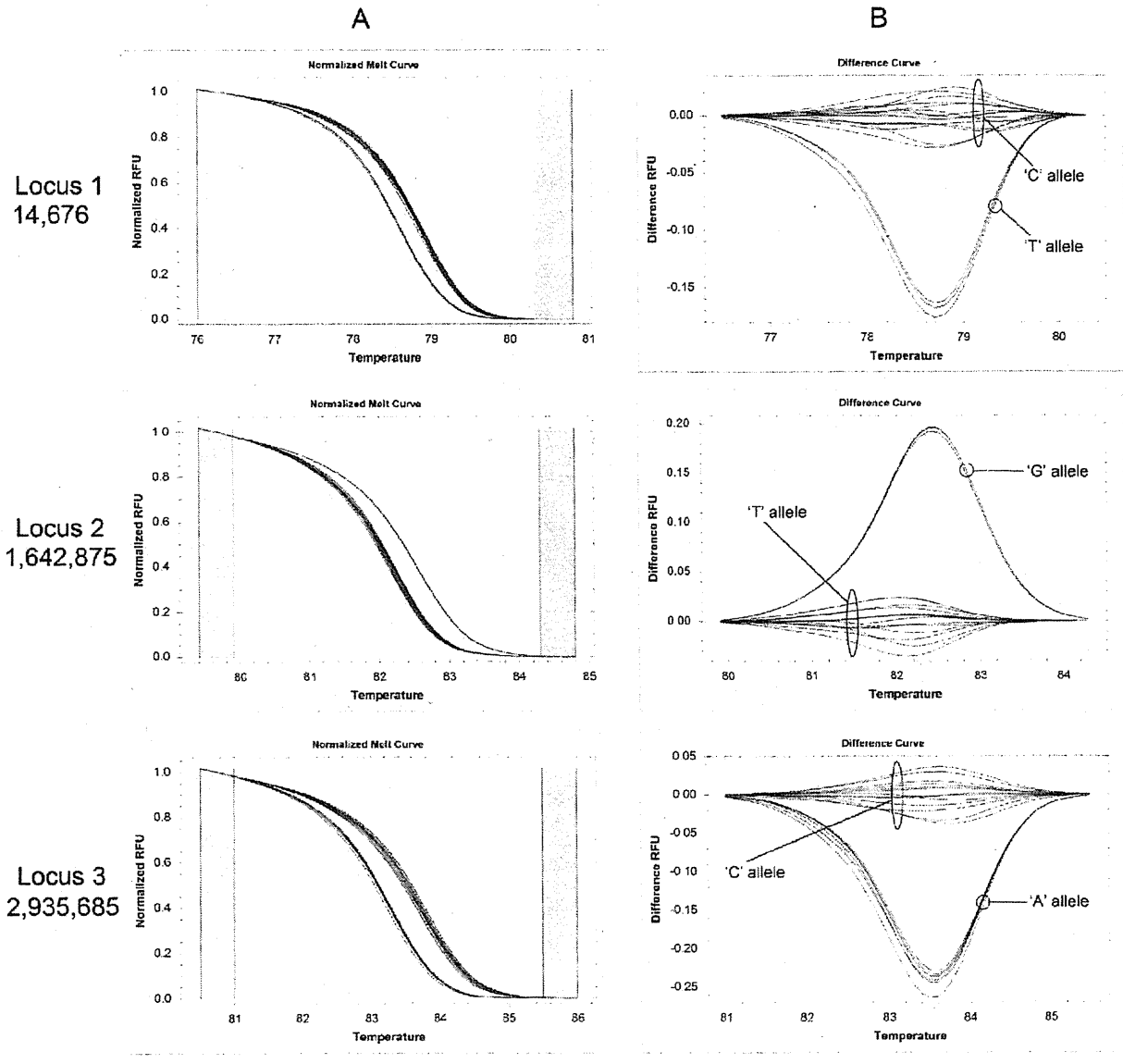

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Table 1: Primer sequences for SNP typing by RT-PCR-HRM analysis

SNP Target: Location [a,b]	Primer name	Primer Location [b]	Primer sequence (5'-3')
Locus1: 14,676	HRM14F	14601-14621	TGAACAGTCTCGTAACCGTG
	HRMM14R [c]	14721-14701	CAATGCATGCTAGCCTTAATG
Locus2: 1,642,875	HRM16F	1642813-1642836	CTCGTCACAAATCCGAGTTTGAAT
	HRM16R	1642925-1642902	GTAGTAGTCTTCCAAGTTGTGGTG
Locus3: 2,935,685	HRM29F	2935599-2935616	TGGTGTCGGTCTCCATCC
	HRM29R[d]	2935716-2935699	ACCGGTGAGCGCACTAAG

[a, c, d] per Monot *et al* (28)

[b] per *M. leprae* TN genome sequence (http://genolist.pasteur.fr/Leproma/)

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Table 2: Comparison of RT-PCR Cycle threshold C(t) values and estimates of starting quantity

Sample type	Strains	DRDRs					
		folP1[a]		*rpoB*[b]		*gyrA*[c]	
		C(t)	SQ(pg)[d]	C(t)	SQ(pg)	C(t)	SQ(pg)
ADML[e]	NHDP63(0.1pg)	22.02	1000.00	23.61	1000.00	22.87	1000.00
	NHDP63 (1pg)	25.36	100.00	27.41	100.00	26.41	100.00
	NHDP63 (10pg)	28.83	10.00	31.37	10.00	30.28	10.00
	NHDP63 (100pg)	32.29	1.00	35.25	1.00	33.95	1.00
	NHDP63 (1ng)	35.11	0.10	39.1	0.10	37.3	0.10
MFP[f]	Airaku-2	26.17	59.97	28.66	51.13	27.22	64.48
	Airaku-3	26.63	43.98	28.99	41.29	28.12	36.43
	Amami	25.54	92.43	28.25	66.69	26.81	83.70
	Hoshizuka-4	29.20	7.60	31.58	7.84	30.55	7.81
	Kusatsu-3	28.61	11.36	31.26	9.60	29.96	11.42
	Kusatsu-6	26.64	43.60	29.13	37.81	28.07	37.61
	Ryukyu-6	27.44	25.24	29.56	28.59	28.53	28.11
	Zensho-2	26.49	48.24	28.85	45.26	27.56	51.82
	Zensho-4	26.84	38.04	29.44	30.91	28.12	36.44
	Zensho-5	27.40	25.95	29.97	22.06	29.05	20.28
	Zensho-9	26.72	41.14	29.39	31.85	28.25	33.60
	Zensho-15	28.03	16.92	30.69	13.80	29.35	16.75
	Gushiken	25.64	86.33	27.75	91.88	26.47	103.63
	Hoshizuka-5	27.74	20.50	29.92	22.67	28.72	24.99
	Indonesia-1	26.89	37.00	29.47	30.27	27.96	40.34
	Korea-3-2	27.48	24.63	29.44	30.96	28.51	28.56
	Thai-53	27.29	27.89	29.84	23.92	28.22	34.26
	Thai-311	25.80	77.56	28.18	69.74	26.77	85.79

[a,b,c] The % efficiency, correlation of coefficient of determination R^2 and slope are 95.4%, 0.997 and 3.373 for *folP1*; 83%, 0.998 and 3.811 for *rpoB* and 91.3%, 0.997 and 3.549 for *gyrA*

[d] Starting quantity (SQ); all DNA templates were tested in triplicate for each target and quantitated according to the NHDP63 DNA standard curve.

[e] ADML: Armadillo derived *M. leprae*.

[f] MFP: Mouse foot-pad derived *M. leprae*.

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Table 3: RT-PCR-HRM assay for *M. leprae* DRDR mutation detection (DRDR assays)

Sample type	Template	*folP1*			*rpoB*			*gyrA*		
		Reported DRDR genotype[a]	HRM Cluster[b]	HRM Cluster verification by sequencing[c]	Reported DRDR genotype	HRM Cluster	HRM Cluster verification by sequencing	Reported DRDR genotype	HRM Cluster	HRM Cluster verification by sequencing
ADML[d]	NHDP63	No mutation	WT		No mutation	WT		No mutation	WT	No mutation
	Airaku-2[e]	P(CCC)55L(CTC)	WT	No mutation	S(TCG)456L(TTG)	WT	No mutation	No mutation	WT	No mutation
	Airaku-3	T(ACC)53I(ATC)	V		No mutation	WT		No mutation	WT	
	Amami	P(CCC)55L(CTC)	V		No mutation	WT		No mutation	WT	
	Hoshizuka-4	P(CCC)55S(CTC)	V		S(TCG)456L(TTG)	V		A(GCA)91V(GTA)	V	
	Kusatsu-3	T(ACC)53I(ATC)	V		No mutation	WT		No mutation	WT	
	Kusatsu-6	P(CCC)55L(CTC)	V		D(GAT)441Y(TAT)	V		No mutation	WT	
	Ryukyu-6	No mutation	WT		No mutation	WT		A(GCA)91V(GTA)	V	
	Zensho-2	P(CCC)55L(CTC)	V		No mutation	WT		No mutation	WT	
	Zensho-4[f]	T(ACC)53I(ATC)	V		S(TCG)456L(TTG)	V		A(GCA)91V(GTA)	V	A(GCA)91V(GTA) No mutation
MFP[g]	Zensho-5[h]	P(CCC)55L(CTC)	V	Pro(CCC)55Leu(CTC) Thr(ACC)53Ile(ATC) No mutation	S(TCG)456L(TTG)	V	S(TCG)456L(TTG)	No mutation	WT	No mutation A(GCA)91V(GTA)
	Zensho-9	No mutation	V	Pro(CCC)55Leu(CTC)	H(CAC)451Y(TAC)	V	H(CAC)451Y(TAC)	No mutation	WT	
	Zensho-15	Unknown	V	Pro(CCC)55Leu(CTC)	Unknown	V	S(TCG)456L(TTG)	Unknown	V	A(GCA)91V(GTA)
	Gushiken	No mutation	WT		No mutation	WT		No mutation	WT	
	Hoshizuka-5	No mutation	WT		No mutation	WT		No mutation	WT	
	Indonesia-1	No mutation	WT	No mutation	No mutation	WT	WT	No mutation	WT	No mutation
	Korea-3-2	No mutation	WT		No mutation	WT		No mutation	WT	
	Thai-53	No mutation	WT		No mutation	WT		No mutation	WT	
	Thai-311	No mutation	WT		No mutation	WT		No mutation	WT	

[a]per Matsuoka, M. (22) , sequenced verified
[b]HRM Cluster is designated as WT for wild type or V for variant target sequence. NHDP63 with same sequences as in TN strain was considered as WT.
[c]Representative samples of each of the clusters were verified by PCR product sequencing and the genotypes detected are indicated.
[d]ADML: Armadillo derived *M. leprae*.
[e]The HRM clustering results were not concordant with expected genotypes for both *rpoB* and *folP1 genes* for Airaku-2 (22). VNTR strain typing was performed for this strain which confirmed that it was indeed not Airaku 2 (44). However, the designation Airaku-2 was retained during the course of the study and in all Tables in this report.
[f]HRM assay for *gyrA* DRDR separated this strain from wild type and other mutants; DNA sequencing results showed C and T mixed allele (See **Figure 2**, *gyrA*, orange curves and **Figure 3**).
[g]MFP: Mouse foot-pad derived *M. leprae*
[h]HRM assay for *folP1* and *gyrA* DRDRs separated this strain from wild type and other mutants which share the same genotype, DNA sequencing show minor mixed alleles at codon 53 and 55 in *folP1* and codon 91 in *gyrA* (See **Figure 2**, blue curve in *folP1*-panel B and orange curve in *gyrA*-panel B and **Figure 3**).

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Table 4: Sensitivity and specificity of HRM detection of DRDR mutations in clinical biopsy DNA samples

Target	Classification	Number of samples	Number of samples[a]				
			<0.1 pg	0.1-1 pg	1-10 pg	10-100 pg	100-1000 pg
folP1	True wild type	112	1	16	37	50	8
	True mutant	5	2	2	1	0	0
	False wild type	0	0	0	0	0	0
	False mutant	4	2	1	1	0	0
	Total	121	5	19	39	50	8
	Sensitivity[b]	100%	100%	100	100	na[d]	na
	Specificity[c]	96.50%	33%	94.10%	97.40%	100%	100%
rpoB	True wild type	115	2	12	34	59	8
	True mutant	0	0	0	0	0	0
	False wild type	0	0	0	0	0	0
	False mutant	6	3	3	0	0	0
	Total	121	5	15	34	59	8
	Sensitivity	na	na	na	na	na	na
	Specificity	95.04%	40%	80%	100%	100%	100%
gyrA	True wild type	115	2	16	36	55	6
	True mutant	0	0	0	0	0	0
	False wild type	0	0	0	0	0	0
	False mutant	6	4	2	0	0	0
	Total	121	5	18	36	55	6
	Sensitivity	na	na	na	na	na	na
	Specificity	95%	20%	88.90%	100%	100%	100%

[a] Number of samples grouped according to the starting concentration SQ (pg)

[b] Sensitivity is defined as number of true mutants/(number of true mutants + number of false wild types)

[c] Specificity is defined as number of true wild type/(number of true wild types + number of false mutants)

[d] 'na': not applicable as no true mutants were present in the samples set.

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Table 5: Comparison of RT-PCR Cycle threshold C(t) values and estimates of starting quantity

Sample type	Strains	SNP					
		Locus1[a]		Locus2[b]		Locus3[c]	
		C(t)	SQ(pg)[d]	C(t)	SQ(pg)	C(t)	SQ(pg)
ADML[e]	NHDP63(1ng)	21.95	1000.00	21.97	1000.00	21.01	1000.00
	NHDP63 (100pg)	25.40	100.00	25.34	100.00	24.24	100.00
	NHDP63 (10pg)	29.09	10.00	28.73	10.00	27.76	10.00
	NHDP63 (1pg)	32.70	1.00	32.24	1.00	31.14	1.00
	NHDP63 (0.1ng)	36.50	0.10	35.75	0.10	34.43	0.10
	Thai-53	27.14	26.40	27.42	20.71	26.09	29.48
	3039	27.16	25.97	27.15	24.60	26.16	28.16
	BR4923	27.25	25.36	27.18	24.36	26.24	26.79
MFP[f]	Airaku-2	26.35	58.92	26.30	53.48	25.25	53.64
	Airaku-3	27.08	37.60	27.14	30.74	25.90	34.52
	Amami	26.13	67.50	25.85	72.29	25.01	63.49
	Hoshizuka-4	29.95	6.47	29.52	6.19	28.73	5.00
	Kusatsu-3	29.17	10.40	29.32	7.11	28.00	8.22
	Kusatsu-6	27.30	32.85	27.16	30.02	26.08	30.61
	Ryukyu-6	28.17	19.35	27.90	18.30	26.58	21.74
	Zensho-2	27.28	33.35	27.73	20.67	25.62	41.90
	Zensho-4	27.70	25.73	27.26	28.14	26.41	24.31
	Zensho-5	27.82	23.87	27.65	21.76	27.02	16.04
	Zensho-9	27.38	31.21	27.12	30.92	26.35	25.33
	Zensho-15	28.68	14.11	28.45	12.79	27.64	10.52
	Gushiken	25.90	77.88	25.96	67.15	24.39	97.04
	Hoshizuka-5	28.04	20.87	28.03	16.79	26.59	21.60
	Indonesia-1	27.86	23.70	27.40	25.64	25.93	33.77
	Korea-3-2	27.93	22.40	28.05	16.58	26.46	23.56
	Thai-53	27.42	30.50	27.32	26.91	26.19	28.33
	Thai-311	25.95	75.25	25.88	70.67	24.84	71.41

[a,b,c] The % efficiency, correlation of coefficient of determination R^2 and slope are 90.3%, 0.994 and 3.579 for Locus 1; 91.7%, 0.998 and 3.538 for Locus 2 and 91.7%, 0.999 and 3.539 for Locus 3

[d] Starting quantity (SQ); All DNA templates were tested in triplicate for each target and quantitated according to the NHDP63 DNA standard curve.

[e] ADML: Armadillo derived *M. leprae*.

[f] MFP: Mouse foot-pad derived *M. leprae*.

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Table 6: RT-PCR-HRM assay for *M. leprae* SNP typing. A: The expected RT-PCR-HRM cluster patterns for the three loci which generate four SNP types. **B**: SNP typing of MFP-LRC and armadillo derived reference strains based on the cluster pattern defined in **A**.

A

SNP type	Locus1	Locus2	Locus3
	Allele[a]/HRM Cluster[b]		
Type 1	C/1	G/2	A/2
Type 2	C/1	T/1	A/2
Type 3	C/1	T/1	C/1
Type 4	T/2	T/1	C/1

B

Sample type	Strain	HRM Cluster			SNP type
		Locus1	Locus2	Locus3	
MFP	Airaku-2	1	2	2	1
	Airaku-3	1[d]	2[d]	2	1[c]
	Amami	1	1	1	3
	Hoshizuka-4	1	1	1	3
	Kusatsu-3	1	1	1	3
	Kusatsu-6	1	1	1	3
	Ryukyu-6	1	1	1	3
	Zensho-2	1	1	1	3
	Zensho-4	1	1	1	3
	Zensho-5	1	1	1	3
	Zensho-9	1	1	1	3
	Zensho-15	1	1	1	3
	Gushiken	1	2	2	1
	Hoshizuka-5	1	1	1	3
	Indonesia-1	1[d]	2[d]	2	1[c]
	Korea-3-2	1	1	1	3
	Thai-53	1	2	2	1
	Thai-311	1[d]	2[d]	2	1[c]
ADML	Thai-53	1	2	2	1
	3039	1	1	2	2
	BR4923	2	1	1	4
	NHDP63	1	1	1	3

[a] The SNP alleles are indicated (28).
[b] NHDP63 allele is assigned to Cluster 1 and the alternative allele to Cluster 2.
[c] The SNP types are different from previous reports (22).
[d] Amplicons sequence verified

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Table 7: Concordance of PCR-RFLP and RT-PCR-HRM methods for *M. leprae* SNP typing of clinical isolates.

Sample	Sample type	PCR-RFLP[a]		SNP type	HRM Cluster[b]		
		Locus 2/*CviKI*	Locus3/*BstUI*		Locus1	Locus2	Locus3
NP101	Clinical	-	-	1	1	2	2
NP103	Clinical	-	-	1	1	2	2
NP108	Clinical	-	-	1	1	2	2
NP109	Clinical	-	-	1	1	2	2
NP110	Clinical	-	-	1	1	2	2
NP111	Clinical	-	-	1	1	2	2
NP112	Clinical	-	-	1	1	2	2
NP114	Clinical	-	-	1	1	2	2
NP116	Clinical	-	-	1	1	2	2
NP117	Clinical	-	-	1	1	2	2
NP118	Clinical	-	-	1	1	2	2
NP119	Clinical	-	-	1	1	2	2
NP120	Clinical	-	-	1	1	2	2
NP123	Clinical	-	-	1	1	2	2
NP106	Clinical	-	-	1	V[c]/1	2	2
NP113	Clinical	-	-	1	V[c]/1	2	2
NP102	Clinical	+	-	2	1	1	2
NP104	Clinical	+	-	2	1	1	2
NP115	Clinical	+	-	2	1	1	2
NP122	Clinical	+	-	2	1	1	2
NP124	Clinical	+	-	2	1	1	2
NP105	Clinical	+	-	2	1	1	2
NP121	Clinical	+	-	2	1	1	2
NP125	Clinical	nd[d]	-	2	1	V[c]/1	2
NP107	Clinical	nd[d]	-	1	1	2	2
NHDP63	ADML	+	+	3	1	1	1
Thai53	ADML	-	+	1	1	2	2
BR4923	ADML	+	+	4	2	1	1

[a] PCR-RFLP assay (31)
[b] NHDP63 allele is assigned to Cluster 1 and the alternative allele to Cluster 2.
[c] V: HRM automatically called these three strains NP106, NP113 and 125 into a different cluster (variant). When the melting curves were manually examined, NP113 and NP125 were in the same cluster as that of NHDP63, while NP106 appeared to belong to a different cluster. Locus1 amplicons of NP106 was sequenced, and the SNP allele, C was same as that of NHDP63 and TN strains.
[d] not determined due to low amount of amplicon

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REVIEW ARTICLE

Current status of leprosy: Epidemiology, basic science and clinical perspectives

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ABSTRACT

Leprosy has affected humans for millennia and remains an important health problem worldwide, as evidenced by nearly 250 000 new cases detected every year. It is a chronic infectious disorder, caused by *Mycobacterium leprae*, that primarily affects the skin and peripheral nerves. Recent advances in basic science have improved our knowledge of the disease. Variation in the cellular immune response is the basis of a range of clinical manifestations. The introduction of multidrug therapy has significantly contributed to a decrease in the prevalence of the disease. However, leprosy control activities, including monitoring and prevention programs, must be maintained.

Key words: diagnosis, disability, leprosy, *Mycobacterium leprae*, social stigma.

INTRODUCTION

Leprosy, or Hansen's disease, is a chronic infectious disease caused by the acid-fast bacterium *Mycobacterium leprae*. Norwegian physician Gerhard Armauer Hansen identified the bacillus in the patients in 1873, making leprosy the first disease ascribed to a bacterial origin. Leprosy usually affects the dermis of the skin and peripheral nerves, but has a wide range of clinical manifestations. It can be progressive and cause permanent damage if left without treatment. Divided into paucibacillary (TB; tuberculoid pole) or multibacillary (MB; lepromatous pole), depending on the bacillary load, the disease manifests first in discoloration of the skin and then in rashes and nodules. The introduction of dapsone (diphenyl sulfone, DDS) in 1941 brought the first effective therapy, and multidrug therapy (MDT) was introduced by the World Health Organization (WHO) in 1981 to limit the development of drug resistance. Endemic leprosy has declined markedly and the disease is now rare in most industrialized countries. It is still a major public health problem in developing countries, where hundreds of thousands of new cases are diagnosed each year. In many of these countries, social stigmatization is an additional burden. Therefore, it is important that control activities continue if the disease burden and damaging impacts of leprosy are to be reduced. Dermatologists should be familiar with leprosy and other diseases needed for differential diagnosis.

EPIDEMIOLOGY

The WHO publishes an annual report on the worldwide incidence of leprosy, including the number of new cases, prevalence and disabilities.[1] The detection of new cases by the WHO has declined from 514 718 in 2003 to 244 796 in 2009, but the rate of decrease is getting smaller each year. Among 244 796 new cases in 2009, 16 countries that reported 1000 or more new cases accounted for 93% of the total. These countries and the number of cases detected in 2009 are: India (133 717 cases), Brazil (37 610 cases), Indonesia (17 260 cases), Bangladesh (5239 cases), the Democratic Republic of the Congo (5062 cases), Ethiopia (4417 cases), Nepal (4394 cases), Nigeria (4219 cases), Myanmar (3147 cases), the United Republic of Tanzania (2654 cases), Sudan (2100 cases), Sri Lanka (1875 cases), the Philippines (1795 cases), China (1597 cases), Madagascar (1572 cases) and Mozambique (1191 cases).

The proportion of new cases with multibacillary leprosy ranged from 32.70% in the Comoros in Africa to 95.04% in the Philippines. The proportion of females among newly detected cases ranged from 6.50% in Ethiopia to 59.11% in the Central African Republic. The proportion of children among new cases ranged from 0.60% in Argentina to 30.30% in Papua New Guinea. Grade 2 disabilities in new cases ranged from 1.45% in Liberia to 22.8% in China. As the number of new cases declines, the damaging

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impact of the disease on the physical, social and economic well-being of individuals and families affected by leprosy are also expected to decline.

Very few new leprosy patients are registered in developed countries. When leprosy is detected, it is primarily found among immigrants from countries where the disease is still endemic. There is an association between the incidence of leprosy and socioeconomic factors such as gross national product (GNP), personal housing expenditures and the number of persons per household, suggesting that improvements in socioeconomic conditions greatly contribute to the reduction of leprosy.[2] The proportion of children under the age of 15 years among newly detected cases would be a good indicator of the situation in a country/region. Similarly, the proportion of cases with grade 2 and visible disabilities among newly detected cases would be a reflection of early detection and treatment.

BACTERIOLOGY AND GENOMICS

Mycobacterium leprae is an obligate intracellular parasite that cannot be cultivated *in vitro*. It grows very slowly with an approximate generation time of 12–14 days. The inability to cultivate *in vitro* and the lack of animal models have been major disadvantages for leprosy research. However, the availability of the *M. leprae* genome sequence has contributed to knowledge of the disease. The first genome sequence of *M. leprae*, completed in 2001,[3] revealed that only half of the small genome contains protein-coding genes, while the remainder consists of pseudogenes and non-coding regions (Fig. 1). The number of pseudogenes is much larger in the *M. leprae* genome than in other mycobacteria,[4] and the number and proportion are exceptionally large in comparison with other pathogenic and non-pathogenic bacteria and archaea.[5,6] Many of the *M. leprae* pseudogenes are the result of stop codon insertions thought to be caused by the dysfunction of sigma factors or the insertion of repetitive sequences derived from transposons.[7–9] Despite this genetic damage, a specialized intracellular environment free from evolutionary competition has allowed the organism to survive.[3,10,11] It has been speculated that *M. leprae* has lost over 1500 genes from its genome and that non-coding regions are functionally silent and useless.[12] However, analyses have demonstrated that some of the pseudogenes and non-coding regions are highly expressed at the RNA level, and that expression of these RNA in clinical samples shows varying patterns among patients, suggesting as yet unknown functions.[13–16]

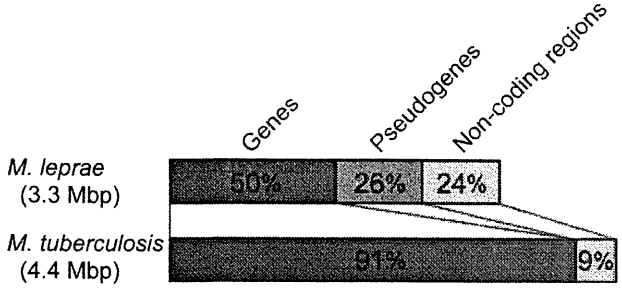

Figure 1. Only half of the *Mycobacterium leprae* genome contains functional genes. The percentage of functional genes, pseudogenes and non-coding regions are illustrated for *M. leprae* and *Mycobacterium tuberculosis* genomes.

Single nucleotide polymorphisms (SNP) and short or variable number tandem repeats have been used for *M. leprae* genotyping. SNP analysis revealed four primitive subtypes of *M. leprae* and the number is increasing as the analysis progresses.[17–19] Some reports have also presented the possibility of dual infections or phenotypically distinct strains of *M. leprae*; however, these situations are still somewhat obscure.[20,21]

TRANSMISSION AND PATHOLOGY

It is evident that humans are the major reservoir of *M. leprae* infection, while naturally occurring infection has been reported in wild animals, including the nine-banded armadillo and several species of primates.[22–32] A recent study found that the same genotypic strain of *M. leprae* was detected at high incidence in wild armadillos and leprosy patients in the southern USA, suggesting that leprosy may be a zoonosis in regions in which armadillos serve as a reservoir.[33]

Although transmission of *M. leprae* is not entirely understood, it is thought that long-term exposure of the respiratory system to airborne droplets is the main route of infection.[34,35] *M. leprae* is not very virulent, meaning that most people affected with leprosy are non-infectious, probably because the bacilli remain within the infected cells. Multibacillary patients, however, excrete *M. leprae* from their nasal mucosa and skin.[36] Close and repeated contact with these patients is also a source of transmission. Upon MDT treatment, however, the patients rapidly lose infectivity.

Even if infected, a long incubation period is required before clinical manifestation. The long incubation period of leprosy was demonstrated by an SNP analysis of an *M. leprae* genome derived from one of four spontaneous leprosy cases in chimpanzees. The chimpanzee was infected with *M. leprae* during infancy in West Africa, but the pathogenic signs of leprosy did not appear for at least 30 years.[30]

Mycobacterium leprae primarily infects histiocytes (or tissue macrophages) in the dermis and Schwann cells in the peripheral nerves. The unique tropism for peripheral nerves can lead to deformities even after the pathogen is successfully treated. The outcome of infection and clinical manifestation depend on the cellular immunity of the host, which is the first line of defense against *M. leprae* infection. There is a relationship between clinical manifestation and cytokine profiles within the skin lesions. T-helper cell (Th)1 cytokines, such as interleukin (IL)-2 and γ-interferon, play important roles in cellular immune responses in paucibacillary leprosy. Th2 cytokines, including IL-4, IL-5 and IL-10, augment humoral immune responses and predominate in multibacillary leprosy. Thus, there is an inverse correlation in the cytokine profiles that create the basis of paucibacillary and multibacillary leprosy.

Mycobacterium leprae should be recognized by the innate immune system and phagocytized by host macrophages. Toll-like receptor (TLR)2, in conjunction with TLR1, recognizes the cell wall lipids of *M. leprae* and subsequently activates innate immune responses.[37,38] However, some bacilli escape this initial attack of innate immunity and successfully parasitize the phagosome of macrophages. CORO1A, an actin-binding scaffold protein in the cell membrane of host cells, inhibits the phagosome/lysosome fusion, thereby helping the pathogen escape digestion.[38–40]

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Mycobacterium leprae parasitization of macrophages occurs in a foamy or enlarged phagosome filled with lipids.[40,41] Because it is aerobic, it may survive in a granuloma environment with a relatively low oxygen tension gradient using lipids and fatty acids as carbon sources.[42] *M. leprae* creates a lipid-rich phagosome environment that is favorable for its survival.[43] Adipose differentiation-related protein (ADRP) and perilipin expression, which contribute to lipid intake, significantly increase following *M. leprae* infection. Infection also has a pronounced effect on Schwann cell lipid homeostasis via regulation of lipid droplet biogenesis and traffic, which favors *M. leprae* intracellular survival.[44]

It was long thought that leprosy might have a strong host genetic component. With the use of gene expression profiling, gene expression patterns associated with host immune response in lesions of human leprosy have been clarified.[45] Genes belonging to the leukocyte immunoglobulin-like receptor (LIR) family were significantly upregulated in lesions of lepromatous patients suffering from the disseminated form of the infection.[45] A genome-wide search for loci affecting the susceptibility to leprosy mapped a susceptibility locus to chromosome 6q25-q26.[46] There is a close relationship between leprosy susceptibility and SNP in the genes encoding tumor necrosis factor (TNF)-α and IL-10.[47]

CLINICAL FEATURES

Leprosy is a systemic disease that primarily affects the skin, nerves and eyes. *M. leprae* infection induces diverse clinical manifestations depending on the host immune responses. Paucibacillary leprosy is a milder disease characterized by few (≤5) hypopigmented, anesthetic skin lesions. The multibacillary form is associated with multiple (>5) skin lesions, nodules, plaques, thickened dermis or skin infiltration, and in some instances, involvement of the nasal mucosa, resulting in nasal congestion and epistaxis. The involvement of certain peripheral nerves may also be noted. In most cases of both paucibacillary and multibacillary disease, the diagnosis is straightforward. However, the small proportion of suspected cases that do not exhibit anesthetic patches require examination by a specialist to find other cardinal signs of the disease, including nerve involvement and a positive laboratory test for acid-fast bacilli.

Patients commonly present with weakness or numbness as the result of a peripheral-nerve lesion, or a burn or ulcer in an anesthetic hand or foot. In typical multibacillary leprosy, diffuse infiltration of the skin is evident. There may be many lesions that are not hypoaesthetic, while only a few hypopigmented lesions with reduced sensation are seen in paucibacillary patients. Careful inspection of the entire body is important. The great auricular nerve, ulnar nerve, median nerve, radial-cutaneous nerve, posterior tibial nerve and lateral popliteal nerve are frequently involved with enlargement, with or without tenderness, and standard regional patterns of sensory and motor loss.[48] Neuritic leprosy in India and Nepal is characterized by asymmetrical involvement of peripheral nerve trunks without visible skin lesions.[49–51]

The Ridley–Jopling classification system,[52] based on the *M. leprae*-specific immunological resistance status of the host, is clinically relevant and widely used, although the WHO only distinguishes between paucibacillary and multibacillary for simplicity of use in endemic countries. Ridley–Jopling divided the disease into six categories based on dermatological, neurological and histopathological findings: indeterminate (I), tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL) and lepromatous (LL) (Fig. 2). TT leprosy can be associated with rapid and severe nerve damage, whereas LL is associated with chronicity and long-term complications. Borderline disease is unstable and can be complicated by lepra reactions as described in the "Lepra Reactions" section.

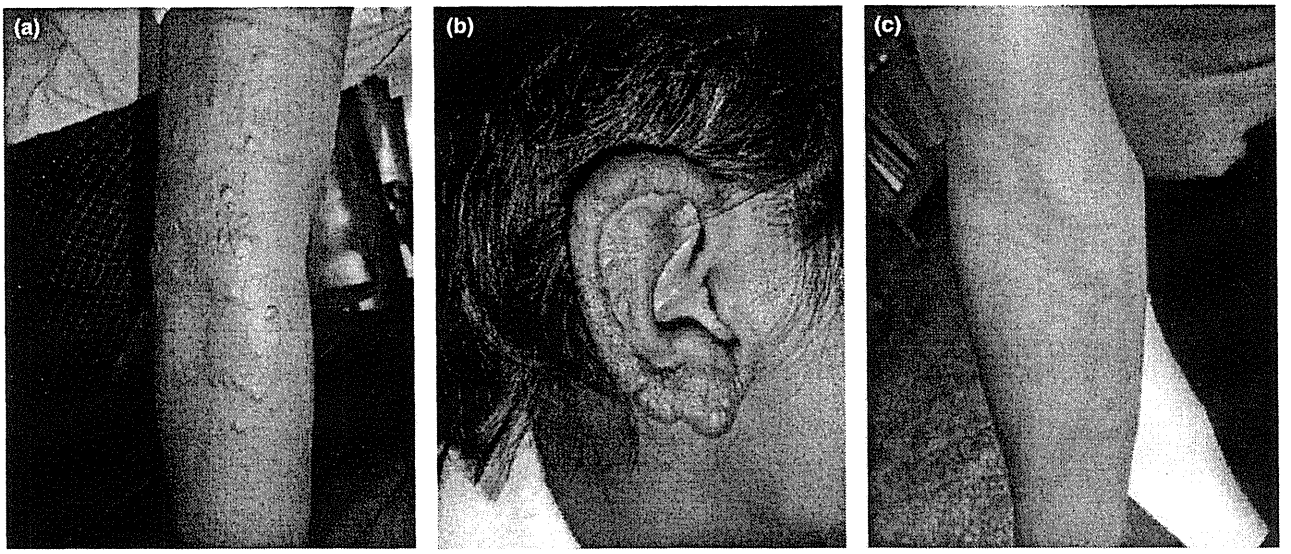

Figure 2. Typical dermatological views of leprosy patients. A multibacillary case (lepromatous) showing multiple nodules in the arms (a) and ears (b), and a paucibacillary case (borderline tuberculoid) with large erythema annulare, with discoloration in the middle of the lesion accompanied by loss of sensation (c).

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