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Molecular epidemiology of leprosy based on VNTR typing in Thailand

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Summary Recently about 500 new cases of leprosy have been reported each year in Thailand. In addition to a steady rate of new case detection, Thailand is in Southeast Asia where leprosy is endemic in neighbouring countries; therefore, strain differentiation could be useful in tracing origins and routes of infection, and general leprosy surveillance. To identify suitable markers for differentiation of *M. leprae* strains in different global geographic regions and to determine the applicability of a systematic genotyping method for tracing leprosy transmission, variable nucleotide tandem repeats (VNTRs) of 14 loci were evaluated using DNA extracts from a total of 97 skin biopsies and slit skin smear samples. The alleles per locus ranged from 2–26 providing adequate strain differentiation. Microsatellite loci (GAA)21, (AT)17 are highly polymorphic followed by (GTA)9, (AC)8a, (AC)8b, and (AC)9. The minisatellites 6-7, 21-3 and 27-5 exhibited a limited number of alleles. The repeat of 23-3 showed no polymorphism. Overall, the strain types can be divided into two distinct Thai groups, according to the alleles at the (GGT)5 and 21-3 loci. However, there are no obvious geographical patterns of distribution of VNTR strain types. Closely matched VNTR profiles found in household members of two multi-case families suggested infection through a common source.

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Introduction

The number of new cases of leprosy reported in Thailand had fallen to 638 in 2005 and to 508 in 2007, and a current national prevalence rate of 0·17 per 10 000 population.[1] Although the national prevalence rate is considerably less than 1·0 per 10 000 population, allowing Thailand to have achieved the leprosy elimination goal as defined by WHO, there are some regions, notably in the Northeast, where the actual prevalence is still high. In addition, the new case detection rate although in decline, is sufficiently high to suggest continuing leprosy transmission. The new epidemiological tools being developed for strain typing of *M. leprae* should be useful in national leprosy surveillance/control efforts towards true reduction in incidence, and in epidemiological investigation. Many variable nucleotide tandem repeat (VNTR) loci in *Mycobacterium leprae* genome have been reported to be polymorphic with a potential to serve as genetic markers to differentiate strains of *M. leprae*.[2–12] However, the characteristics of polymorphism vary depending on the population, and can be a reflection of that population at the national and local level. We therefore measured the copy numbers of VNTRs known as microsatellites (repeat length < 6 bases) and minisatellites (repeat length ≥ 6 bases) in Thai clinical isolates of *M. leprae* and evaluated their usefulness as a means of strain typing for tracing of leprosy transmission and investigation of the distribution of *M. leprae* genotypes in Thailand.

Materials and Methods

CLINICAL SPECIMENS

The study was approved by the Ethical Committee of the Ministry of Public Health, Thailand. Leprosy patients mainly from the Northeastern region ($N = 73$) and a few from the Central ($N = 17$), West ($N = 1$), Northern ($N = 2$) and Southern regions ($N = 3$) were enrolled for the study. The information for the province of one patient was not available. Of 97 leprosy patients 93 were diagnosed as multibacillary (MB) and four as paucibacillary (PB).[1] Leprosy subtypes[13] were identified as follows: lepromatous leprosy (LL) ($N = 32$), borderline leprosy (BL) ($N = 55$), borderline borderline (BB) ($N = 2$), borderline tuberculoid with positive bacterial indices (BT +) ($N = 3$), borderline tuberculoid with a negative bacterial index (BT-) ($N = 1$) and tuberculoid leprosy (TT) ($N = 2$). Two cases of unclassified leprosy types were included. Sixty seven cases were male and 30 were female. The ages of patients were in the range of 7–97 years with a mean of 45 years. A total of 150 household contacts of 52 leprosy patients were also clinically examined.

Clinical details and bacteriological index (BI)[1] were recorded. Slit smears and skin biopsies were collected and kept frozen at $-20\,^{\circ}C$ until they were processed for DNA extraction. Slit skin smears were stored in 70% ethanol and skin biopsies were kept as such in dry tubes.

DNA EXTRACTION

DNA extraction was conducted at the National Institute of Health (NIH), Thailand. Particulate material from the slit skin smear was sedimented by centrifugation at 12 000 × g for 20 min at room temperature, washed and subjected to lysis in 200 μl buffer (1 mg/ml proteinase K in 0·05% Tween 20, 0·1 M Tris-HCl, pH 8) overnight at 60 °C in a water bath.

The next day proteinase K was inactivated by incubation of the lysate at 97 °C for 10 min. Two aliquots of the lysate, 100 μl each were stored at − 20 °C until further use.[14] A portion of each biopsy was cut and manually ground in glass tissue homogenizers in a total volume of 300 μl buffer.[12] DNA was extracted from 100 μl of skin homogenate using the DNeasy tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

POLYMERASE CHAIN REACTION

Multiplex PCR[11] and sequencing analysis was utilised for 57 samples. Single PCR for each locus was performed as described by Gillis *et al.*[14] for 52 samples coded as TIDEAL, 12 of which were also previously tested by multiplex PCR and sequence analysis. Amplicons were detected by agarose gel electrophoresis.

DETERMINING VNTR ALLELES

Copy numbers of repeats in the 12-5, 21-3, 23-3 and 27-5 minisatellites were identified by agarose gel electrophoresis and comparing product sizes against DNA size standards. Alleles of all microsatellites [(AC)8a, (AC)8b, (AC)9, (TA)10, (AT)17, (GGT)9, (GTA)9, (GAA)21 (originally referred to as TTC repeat) and some minisatellites (6-7 and some of 12-5) were determined by sequencing of the amplicons (ABI 3130 XL Genetic Analyzer at National Institute of Health (NIH), Thailand or ABI 3130 at Proteomics and Metabolomics Facility at Colorado State University). The electropherograms were analysed by two readers.

Results and Discussion

Skin specimens were obtained from a total of 97 leprosy patients. PCR products were obtained from most samples using the single PCR method with Amplitaq gold enzyme and the primers described in the standard protocol.[14] The loci that gave low yields in amplification were (TA)10, (AT)17 and 6-7. In most cases PCR, sequencing results were readable, although interference with dye blobs and complications from mixed signals, stutter or unidentifiable end points of repeats were concerns. Difficulties in reading sequences due to unidentified exact end points of repeats were found more often for targets with di-nucleotide and GAA repeats. Multiplex PCR followed by fluorescent fragment length analysis[11] was reported to be cheaper and facile for multilocus VNTR analysis (MLVA) strain typing of *M. leprae*, with potential for high throughput and addition of new loci, and will be the preferred means in our continuing molecular epidemiology studies towards interpreting the transmission of leprosy in Thailand. The VNTR profiles of the DNA from Thai specimens, classified according to the provincial origin of patients are shown in Figure 1.

The VNTR allele patterns are listed in Table 1.

The VNTR data reveals a range of allelic diversities across the loci: monomorphic, bi-allelic, tri-allelic and polymorphic (greater than 4 alleles). The frequencies are listed in Table 2.

The (GAA)21 locus is obviously highly polymorphic. We have examined this locus previously in Thailand, and found many isolates with the same allele, but whether they were genetically related was not clear.[12]

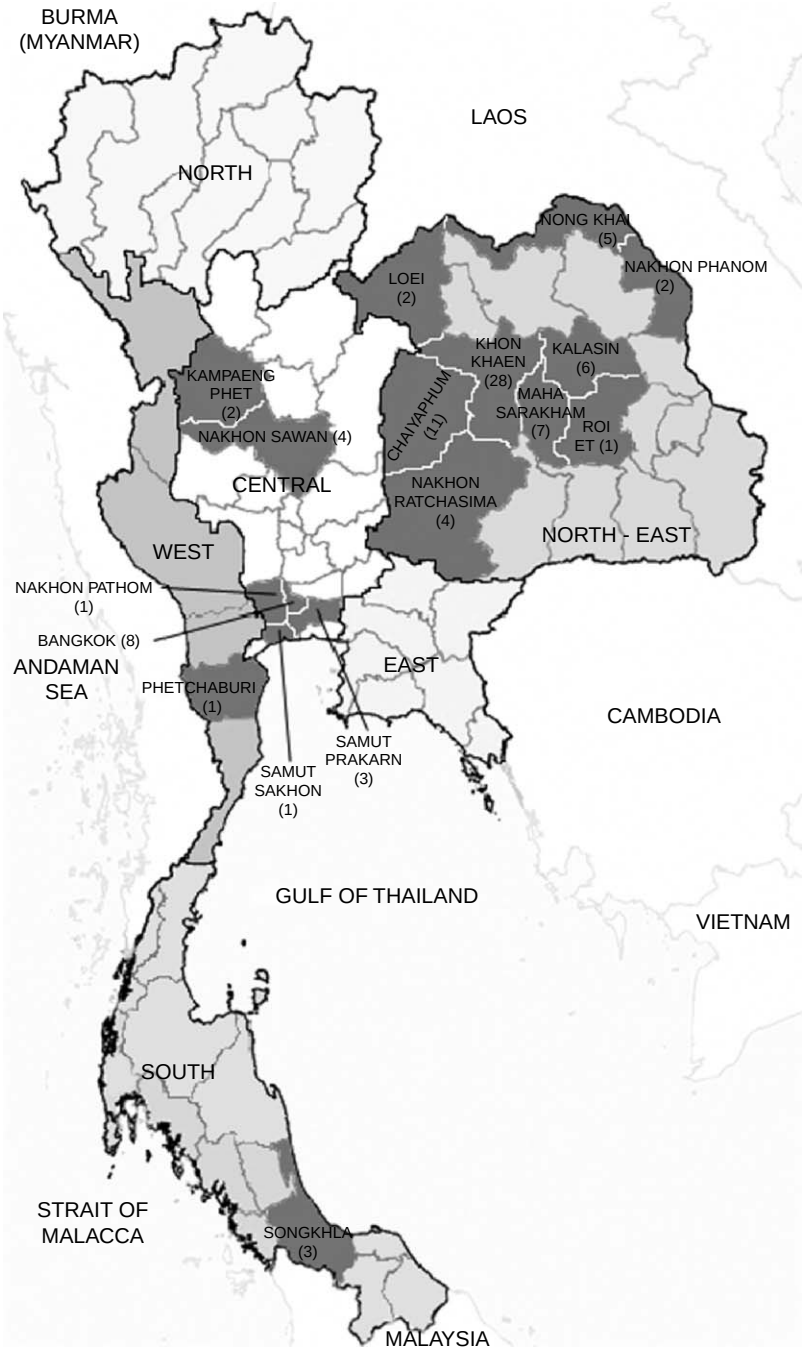

Figure 1. The Provincial division of Thailand and origin of Thai patients according to the Provinces: North, North East, Central, West, and South divisions of Thailand.

Table 1. VNTR profiles of Thai isolates grouped according to provinces and the correlation between (GGT)5 and 21-3 loci

ID	Dx	Sample	BI	(AC)8b	(GTA)9	(GGT)5	(AT)17	21-3	(AC)9	(AT)15	(AC)8a	27-5	6-7	(TA)18	(GAA)21	12-5	23-3	Province
NIH1	MB/BB	B	5	8	9	4	21	2	9	20	10	5	7	11	12	4	2	Loei
TIDEAL1	MB/LL	B	6	8	10	5	10	2	9	NA	10	5	6	NA	21	5	2	Loei
TIDEAL2	MB/BL	B	2	6	10	4	12	2	9	NA	8	5	6	NA	17	4	2	SamutPrakarn
TIDEAL3	MB/BL	B	2	6	10	4	17	2	8	NA	7	5	6	NA	14	4	2	SamutPrakarn
NIH2	MB/BL	B	2	8	8	4	10	2	9	13	10	5	5	21	23	4	2	SamutPrakarn
TIDEAL4	MB/BT	B	1	7	9	5	14	3	7	NA	10	5	6	NA	18	4	2	UdonThani
TIDEAL5	MB/LL	B	5	7	9	5	14	3	7	NA	9	5	6	NA	18	4	2	UdonThani
NIH3	MB/LL	B	5	6	10	4	11	2	7	13	10	5	6	14	16	4	2	UdonThani
NIH4	MB/LL	B	5	8	9	4	11	2	7	18	7	5	6	21	26	4	2	UdonThani
NIH5	MB/BL	B	5	7	10	4	14	3	9	13	9	5	5	13	17	4	2	UdonThani
NIH6	MB/BL	B	1	7	9	5	13	3	7	14	10	5	6	13	19	4	2	UdonThani
NIH7	MB/BL	B	4	8	8	6	9	2	8	15	10	5	6	16	42	4	2	UdonThani
TIDEAL6	MB/LL	SS	1	7	8	5	14	3	7	NA	10	5	6	NA	24	4	2	NA
TIDEAL10	MB	B		7	7	4	14	2	8	NA	10	4	6	NA	14	3	2	Bangkok
TIDEAL12	MB/LL	SS	3	6	9	4	11	2	9	NA	9	5	6	NA	17	5	2	Bangkok
NIH8	MB/BL	B	4	7	10	5	13	3	7	11	9	5	6	12	18	4	2	Bangkok
TIDEAL7	**MB/BL**	**B**	**4**	**7**	**10**	**5**	**16**	**3**	**7**	**NA**	**9**	**5**	**5**	**NA**	**20**	**4**	**2**	**Bangkok**
TIDEAL8	**MB/BT**	**B**	**1**	**7**	**10**	**4**	**16**	**3**	**7**	**NA**	**9**	**5**	**5**	**NA**	**20**	**4**	**2**	**Bangkok**
TIDEAL9	**MB/Bl**	**B**	+	**7**	**10**	**5**	**15**	**3**	**7**	**NA**	**9**	**5**	**5**	**NA**	**20**	**4**	**2**	**Bangkok**
NIH9	MB/LL	B	4	7	8	5	13	3	7	13	9	5	6	11	18	4	2	Bangkok
TIDEAL11	PB	B	0	6	9	NA	13	2	8	NA	9	NA	5	NA	6	4	2	Bangkok
NIH10	MB/BL	B	5	6	10	4	12	2	9	15	7	5	6	14	10	4	2	Chaiyapum
NIH11	MB/BL	B	+	6	9	4	12	2	8	20	9	5	6	20	17	4	2	Chaiyapum
TIDEAL14	MB/BL	B	5	7	15	4	16	2	9	NA	9	5	6	NA	15	4	2	Chaiyapum
NIH12	MB/BL	B	3	8	12	4	10	2	9	13	10	5	6	18	26	4	2	Chaiyapum
TIDEAL15	MB/BL	B	4	8	12	4	8	2	8	NA	9	5	6	NA	20	4	2	Chaiyapum
TIDEAL13	MB/LL	SS	6	7	9	4	13	3	10	NA	10	5	6	NA	16	4	2	Chaiyapum
NIH13	MB/LL	B	6	8	9	4	11	2	8	21	11	5	6	18	28	4	2	Chaiyapum
NIH14	MB/BL	B		7	9	5	14	3	10	14	10	5	5	12	16	4	2	Chaiyapum
NIH15	MB/BL	B	4	7	9	5	14	3	7	14	10	5	6	11	17	4	2	Chaiyapum
NIH16	MB/LL	B	4	7	9	5	10	3	7	12	10	5	6	11	10	4	2	Chaiyapum
NIH17	MB/LL	B	4	7	7	5	12	2	8	15	10	5	6	11	14	3	2	Chaiyapum
TIDEAL18	MB/BL	B	4	7	10	4	10	2	8	NA	9	5	6	NA	21	4	2	Kalasin
NIH18	MB/LL	B	6	7	9	5	18	3	7	11	11	5	6	15	19	4	2	Kalasin
TIDEAL16	MB/LL	B	3	7	9	5	18	3	8	NA	9	5	6	NA	22	4	2	Kalasin
TIDEAL17	MB/LL	SS	6	7	9	5	15	3	7	NA	11	5	5	NA	19	4	2	Kalasin

Table 1. *continued*

ID	Dx	Sample	BI	(AC)8b	(GTA)9	(GGT)5	(AT)17	21-3	(AC)9	(AT)15	(AC)8a	27-5	6-7	(TA)18	(GAA)21	12-5	23-3	Province
TIDEAL19	MB/LL	SS	6	7	9	5	11	3	7	NA	9	5	6	NA	12	4	2	Kalasin
NIH19	MB/BL	B	6	7	9	4	13	2	7	17	10	5	6	11	21	4	2	Kalasin
TIDEAL47	MB/LL	B	6	6	14	4	12	2	8	NA	6	5	6	NA	15	4	2	Kampaengphet
TIDEAL20	MB/BL	B	1	6	8	5	11	2	8	NA	8	5	6	NA	18	4	2	Kampaengphet
NIH20	MB/BL	B	2	8	10	4	16	2	8	14	11	4	6	12	18	4	2	KhonKaen
NIH21	MB/BL	B	2	6	11	4	15	2	8	16	9	5	6	13	12	4	2	KhonKaen
TIDEAL23	MB/BL	B	3	6	11	4	10	2	9	NA	9	5	6	NA	30	4	2	KhonKaen
TIDEAL27	MB/BL	B	2	7	9	4	16	2	8	NA	9	5	6	NA	14	4	2	KhonKaen
NIH22	MB/BL	B	4	8	12	4	8	2	9	16	6	5	6	19	19	4	2	KhonKaen
NIH23	MB/BL	B	4	8	11	4	10	2	8	23	10	5	6	15	25	4	2	KhonKaen
TIDEAL21	MB/BL	B	3	8	11	4	13	2	9	NA	11	5	6	NA	11	4	2	KhonKaen
TIDEAL30	MB/BL	B	3	8	8	4	9	2	8	NA	9	5	6	NA	28	4	2	KhonKaen
TIDEAL31	MB/BL	B	3	8	8	4	9	2	8	NA	10	5	6	NA	24	4	2	KhonKaen
TIDEAL32	MB/BL	B	4	8	10	4	10	2	8	NA	10	5	6	NA	35	4	2	KhonKaen
NIH24	MB/BL	B	1	9	8	4	9	2	8	23	10	5	6	16	34	4	2	KhonKaen
TIDEAL26	MB/LL	B	6	6	11	4	10	2	9	NA	10	5	7	NA	24	4	2	KhonKaen
TIDEAL29	MB/LL	B	4	8	9	4	11	2	8	NA	10	5	7	NA	28	4	2	KhonKaen
NIH25	MB/LL	B	5	9	10	4	10	2	8	26	11	5	6	24	25	4	2	KhonKaen
TIDEAL22	PB/TT	B	0	6	9	4	11	2	7	NA	10	5	6	NA	19	4	2	KhonKaen
TIDEAL25	MB/BL	B	1	7	9	5	13	3	7	NA	12	5	5	NA	16	4	2	KhonKaen
TIDEAL28	MB/BL	B	2	7	9	5	13	3	7	NA	10	5	6	NA	23	4	2	KhonKaen
TIDEAL33	MB/BL	B	4	7	10	5	15	3	7	NA	9	5	6	NA	18	4	2	KhonKaen
TIDEAL34	MB/BL	B	5	7	9	5	18	3	7	NA	9	5	6	NA	21	4	2	KhonKaen
TIDEAL35	MB/BL	B	3	7	10	5	13	3	7	NA	9	5	6	NA	16	4	2	KhonKaen
NIH26	MB/BL	B	5	8	9	5	10	2	8	23	10	5	6	20	30	4	2	KhonKaen
NIH27	MB/LL	B	5	7	8	5	13	3	7	12	10	5	6	13	17	4	2	KhonKaen
NIH28	MB/LL	B	4	7	9	6	13	3	7	15	7	5	6	14	18	4	2	KhonKaen
NIH29	MB/LL	B	6	7	9	5	13	3	8	13	11	5	6	11	21	4	2	KhonKaen
TIDEAL24	MB/LL	B	5	7	9	6	15	3	7	NA	10	5	6	NA	21	4	2	KhonKaen
NIH30	MB/BL	B	6	6	12	4	11	2	8	15	12	5	6	18	19	4	2	KhonKaen
NIH31	MB/BT	B	0	7	10	4	14	3	8	13	10	5	6	11	18	4	2	KhonKaen
NIH32	MB/BL	B	2	6	11	5	12	3	8	15	9	5	6	12	12	4	2	KhonKaen
NIH33	MB/BL	B	+	6	11	4	13	2	8	15	7	5	6	23	30	4	2	MahaSarakham
NIH34	MB/BL	B	3	8	10	4	12	2	8	20	8	5	7	16	26	4	2	MahaSarakham
TIDEAL36	MB/BL	B	3	8	8	4	10	2	8	NA	10	5	6	NA	36	4	2	MahaSarakham
TIDEAL38	MB/LL	B	5	8	9	4	10	2	7	NA	10	5	6	NA	18	4	2	MahaSarakham
NIH35	MB/LL	B	4	7	9	5	17	3	7	15	10	5	6	11	29	4	2	MahaSarakham

Table 1. *continued*

ID	Dx	Sample	BI	(AC)8b	(GTA)9	(GGT)5	(AT)17	21-3	(AC)9	(AT)15	(AC)8a	27-5	6-7	(TA)18	(GAA)21	12-5	23-3	Province
NIH36	MB/BL	B	3	7	9	5	13	3	7	14	8	5	6	9	23	4	2	MahaSarakham
TIDEAL37	MB/BL	B	4	7	9	5	12	3	7	NA	8	5	6	NA	13	4	2	MahaSarakham
TIDEAL39	MB/BL	B	3	7	10	5	13	3	9	NA	12	5	7	NA	17	4	2	NakhonPanom
TIDEAL42	MB/BL	B	5	7	9	5	16	3	6	NA	9	5	6	10	26	4	2	NakhonPanom
TIDEAL40	MB/LL	B	5	6	10	5	11	2	9	NA	9	5	7	NA	18	4	2	NakhonPathom
NIH37	MB/BL	B	4	7	9	5	14	3	7	13	10	5	6	11	19	4	2	NakhonRatchasima
TIDEAL43	MB/BL	B	4	7	9	5	15	3	7	NA	10	5	6	NA	27	4	2	NakhonRatchasima
NIH38	MB/LL	B	+	7	10	5	11	3	10	22	9	5	5	12	15	4	2	NakhonRatchasima
TIDEAL41	MB/LL	B	6	7	9	5	12	3	7	NA	9	5	5	NA	20	4	2	NakhonRatchasima
NIH39	**MB/BL**	**B**	**0**	**7**	**9**	**5**	**19**	**3**	**7**	**12**	**10**	**5**	**6**	**12**	**16**	**3**	**2**	**NakhonSawan**
NIH40	**MB/BL**	**B**	**2**	**7**	**9**	**5**	**23**	**3**	**8**	**11**	**10**	**5**	**6**	**12**	**15**	**3**	**2**	**NakhonSawan**
NIH41	**MB/BL**	**B**	**2**	**7**	**9**	**5**	**16**	**3**	**7**	**12**	**10**	**5**	**6**	**12**	**16**	**3**	**2**	**NakhonSawan**
NIH42	**MB/LL**	**B**	**3**	**7**	**9**	**5**	**16**	**3**	**7**	**11**	**10**	**5**	**6**	**12**	**15**	**3**	**2**	**NakhonSawan**
NIH43	MB/BL	B	5	6	12	4	20	2	8	18	8	5	6	17	16	4	2	NongKhai
NIH44	MB/LL	B	4	8	10	4	19	2	8	14	10	5	6	13	14	4	2	NongKhai
TIDEAL44	MB/LL	B	6	8	9	4	13	2	8	NA	10	5	6	NA	30	4	2	NongKhai
TIDEAL45	MB/BL	B	3	6	10	5	10	2	8	NA	10	4	NA	NA	12	4	2	NongKhai
TIDEAL46	PB/BT	B	0	6	10	5	12	2	8	NA	10	5	NA	NA	21	4	2	NongKhai
TIDEAL48	MB/BL	B	3	6	9	4	9	2	8	NA	8	5	6	NA	17	4	2	Phetburi
TIDEAL49	MB/BL	B	3	6	10	5	13	2	8	NA	8	5	6	NA	14	4	2	Roi-et
TIDEAL50	MB/LL	B	4	5	8	5	12	3	8	NA	9	5	6	NA	15	5	2	SamutSakhon
TIDEAL51	MB/BL	B	–	6	8	4	11	2	8	NA	7	5	5	NA	20	4	2	Songkla
TIDEAL52	PB/TT	B	0	6	10	5	12	2	8	NA	8	5	6	NA	NA	NA	NA	Songkla
NIH45	MB/BB	B	3	6	9	5	10	2	9	14	10	4	6	17	15	4	2	Songkla

NA or -; Not available. TIDEAL samples were not typed at (AT)15 and (TA)18 loci.

Dx: Leprosy subtypes: lepromatous leprosy (LL), borderline leprosy (BL), borderline borderline (BB), borderline tuberculoid with positive bacterial indices (BT +), borderline tuberculoid with a negative bacterial index (BT-) and tuberculoid leprosy (TT).

Sample: B = biopsy, SS = slit skin smear.

BI: Bacteriological Index.

Shaded columns indicate the relationship between the alleles of (GGT)5 and 21-3 loci; the 4-2 and 5-3 allele pair motifs are frequent compared to other combinations.

M. leprae VNTR patterns in two multi-case families (MCFs) one in Bangkok and another in Nakhon Sawan are shown in bold letters.

Skin sample; B: Biopsy, SS: slit skin smear.

Samples with the prefix NIH and TIDEAL in the ID were analysed by multiplex PCR and single PCR respectively.

Table 2. VNTR alleles and the number of occurrences in the Thailand population

(AC)8b		(GTA)9		(GGT)5		(AT)17		21-3		(AC)9		(AC)8a		27-5		6-7		(GAA)21		12-5		23-3	
5	1	7	2	4	46	8	2	2	54	6	1	6	2	4	4	5	12	6	1	3	6	2	97
6	25	8	12	5	47	9	5	3	43	7	36	7	6	5	92	6	77	10	2	4	87		
7	47	9	43	6	3	10	15			8	41	8	9			7	6	11	1	5	3		
8	22	10	26			11	12			9	16	9	28					12	5				
9	2	11	7			12	12			10	3	10	42					13	1				
		12	5			13	18					11	7					14	6				
		14	1			14	9					12	3					15	7				
		15	1			15	6											16	8				
						16	8											17	8				
						17	2											18	11				
						18	3											19	7				
						19	2											20	6				
						20	1											21	7				
						21	1											22	1				
						23	1											23	3				
																		24	3				
																		25	2				
																		26	4				
																		27	1				
																		28	3				
																		29	1				
																		30	4				
																		34	1				
																		35	1				
																		36	1				
																		42	1				

The (AT)15 and (TA)18 loci are not shown because they were not typed for TIDEAL samples.

The first column per locus listed in the header row shows the allele, and the second column has the number of isolates in which the particular allele was found.

In general, the mutation rates of VNTRs correspond approximately to their allelic diversities, and number of alleles and array sizes.[15] Therefore, it is possible to divide the population into major groups based on the markers that have few alleles; further strain differentiation within these groups occurs due to variants found at the more polymorphic loci.

Firstly, the analysis highlights the observation that the loci 27-5, 12-5 and 23-3 are almost monomorphic, with the predominant alleles being 5, 4 and 2 respectively. This is a conserved pattern in *M. leprae* from most of Asia[16,17] and sub-populations in America. Secondly, either the (GGT)5 or the 21-3 VNTR locus can split the Thai *M. leprae* population, due to their predominantly bi-allelic properties. Furthermore, a striking association between the 5 copy allele of (GGT)5 with the 3 copy allele of 21-3, and the 4 copy (GGT)5 allele with the 2 copy allele of 21-3, supports genetic linkage within these two groups. The 4-2 pattern of alleles for (GGT)5-21-3 loci is found in the majority of the *M. leprae* global populations that we have studied. The local strains within Thailand are differentiated from the others due to a combination of the variations at loci 6-7, (AC)8b, (AC)9, (GTA)9 and (AC)8a of intermediate allelic diversities, and the loci (AT)17, (AT)15, (TA)18 and (GAA)21 which are more diverse. The *rpoT* gene carries the 3 copy allele of a hexamer repeat (locus 6-3a) (data not shown).

SNP typing according to the Monot *et al.*[18] scheme is ongoing and may shed further insight into the ecology of the Thai *M. leprae* isolates proposed herein, and their phylogenetic relationships to Asian and global isolates.

The genetic relationships amongst Thai isolates are not apparent, without formal consideration of additional epidemiological data from patient histories and information about the incidence of leprosy and the time-space distribution in the villages and communities where they reside. These data are not included in the current study, and will be expanded in the future. Leprosy is well-controlled in Thailand, and the problem areas are well defined and confined. These factors should enable the selection of suitable study sites and populations for the identification of transmission of leprosy using molecular techniques.

However, in the present study there were seven samples from patients belonging to two families, one in Bangkok and another in Nakhon Sawan (shown in bold in Table 1). Intra-familial strain type conservation is evident, on the background of local strain type variants, supporting a role and utility of VNTR mapping for community based *M. leprae* transmission studies.

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