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Identification and comparison of *Mycobacterium leprae* genotypes in two geographical regions of Colombia

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Summary

Objective To evaluate and establish genomic strain typing markers suitable for the identification of transmission patterns of leprosy in different regions of Colombia.

Design Patients from Agua de Dios, Barranquilla and Cartagena cities and neighbouring towns were enrolled during 2006–2007. Slit skin smears or biopsies were obtained from newly detected untreated patients, and those undergoing multidrug therapy. DNA was extracted from the clinical samples and tested using 15 different short tandem repeat and three SNP polymorphic markers.

Results and Conclusion Differences or similarities between strain types from the northeast ($n = 20$) and central regions of Colombia ($n = 18$) were noted. The alleles at two loci, 27-5 and 12-5 were different in the *M. leprae* in the two regions. The other microsatellite loci may be useful for further intra-population differentiation. There was strong association of 27-5 and 12-5 alleles with the SNP types. The 4-5 combination of alleles was associated with SNP type 3, while the 5-4 combination was mostly associated with SNP type 1, 2 or 4. The SNP type 4 *M. leprae* isolates were seen in patients in the northeast, but not in the central part.

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Introduction

Multidrug therapy (MDT) had contributed to the dramatic reduction of prevalence of leprosy worldwide. However, MDT has not influenced the control of incidence as was expected.[1] This situation is evident in Colombia, where the prevalence is $<1/10{\cdot}000$ inhabitants, data that places the country in the post elimination phase. Nevertheless, the number of new cases per year (400–500) continues to be similar to the number found before MDT was implemented during the 1980s (SIVIGILA www.minsalud.gov.co). Two strain typing methods for identification of *M. leprae* genotypes giving circulating isolates in two endemic regions and a comparison of the genotypes were applied with the purpose of generating information on underlying transmission patterns, and thus persistent incidence of leprosy.

Materials and Methods

During 2006–2007 we studied volunteer patients registered in the leprosy control programme of Cartagena and Barranquilla cities located at Bolívar and Atlántico states respectively, and in Agua de Dios city, Cundinamarca state, where a leprosarium is situated.

Information about leprosy patients was obtained from each local Leprosy Control Programme. Permanent contact with the health personnel in charge of the programme provided us with information about the location of new and in-treatment patients. The study outline was evaluated and approved by each local Ethical Committee. Leprosy patients were enrolled in Barranquilla, Cartagena and Agua de Dios cities. After explaining the study details, an informed consent form was signed by each participant. As described elsewhere in this issue,[2,3] slit skin smears or biopsies were obtained and stored in 70% ethanol until further processing in the laboratory at Instituto Colombiano de Medicina Tropical (ICMT) or Colorado State University (CSU). Total DNA extracted by Qiagen DNeasy kit was submitted to multiplex PCR–fragment length analysis (MP-FLA) as described in an accompanying paper[2] based on methodology developed by Kimura *et al.*[3] FLA was performed on the ABI 3130 Applied Biosystems Genetic Analyzer at the Proteomics and Metabolomics facility at CSU. SNP typing, based on three SNPs[4] was performed using a PCR-RFLP technique,[5] and direct sequencing for confirmation.

Results

Regions where this study was performed showed a leprosy incidence of 3-4/100,000 and a cumulative prevalence $>1/10\,000$. Therefore these geographical regions are ideal for the study of leprosy transmission in a country considered in the post-elimination phase. Figure 1 is a map of the regions of Colombia where the study was performed.

The study sites belong to the two different geographical regions of Colombia, the Atlantic Coast and the Central regions.

Although samples from more than 100 patients were obtained, the majority did not yield adequate VNTR data as they were of low bacillary load (BI $<$ 2·0) or due to the patients being in active treatment. Strain types from a total of 18 patients enrolled in Agua de Dios (AD) and 20 patients in Cartagena-Barranquilla (CB), most of them already under MDT

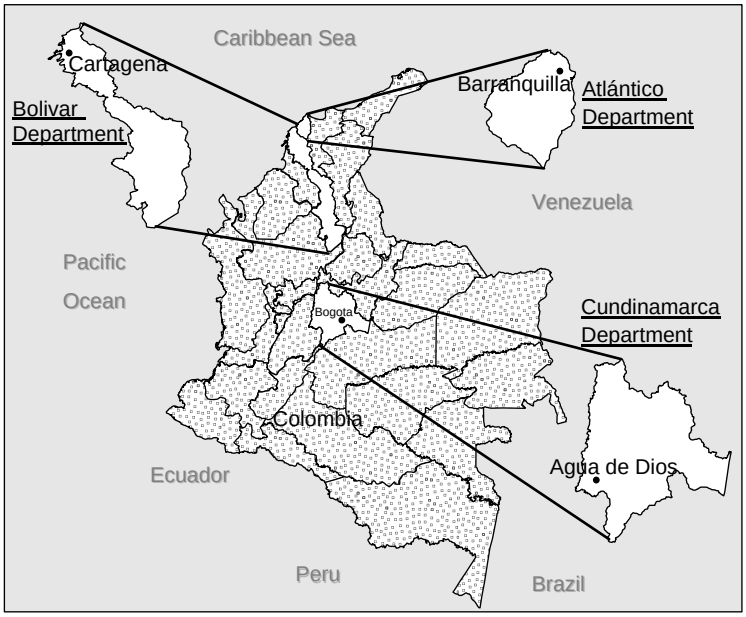

Figure 1. A map of Colombia indicating the three states (or departments) Bolivar, Atlantico, and Cundinamarca from where patients were recruited for the studies.

treatment, but still having a positive Bacillary Index (BI), were compiled. The available VNTR and SNP genotypes are listed in Table 1.

There were no significant allelic differences in these two populations as shown in Table 2, aside for the 27-5 and 12-5 loci (highlighted in Table 1).

There were two patients in the CB population that carried infrequent alleles 4 and 5 respectively. However, in the AD group, this 4-5 allele combination at these loci is more frequent. This inverse VNTR signature prompted us to search for a secondary marker indicative of higher level of genetic linkage. Therefore SNP typing according to the Monot *et al.* scheme was performed.[4] In Agua de Dios, 2/18 strains were SNP type 2 and 9/18 were SNP type 3; in seven cases it was not possible to get conclusive results. A differential distribution pattern was observed in Barranquilla – Cartagena, with 10/20 isolates being of SNP type 4, 5/20 were SNP type 3 and 2/20 were SNP type 1. In three cases, it was not possible to get all three SNP locus amplicons for final SNP type designation (Table 1).

Discussion

This study revealed the ability of VNTR loci to distinguish strain types. In addition it does highlight evidence of geographical clustering. Agua de Dios is located in the centre of Colombia and was colonised by people from Spain. Cartagena and Barranquilla received African population as slaves, so it could be possible that the isolates from this region have other geographic origins. The finding of SNP 4 types in the North East is consistent with the model of Monot *et al.* However, the direct ancestors of the American SNP type 3 isolates which carry the 4-5 signature is not clear (also refer to study from Brazil in this series).[6]

Table 1. VNTR and SNP genotypes of patients from Agua de Dios (AD) and Cartagena-Barranquilla (CB)

No	Code	BI	MDT (mo)	(AC)8b	(GTA)9	(GGT)5	(AT)17	21-3	(AC)9	(AT)15	(AC)8a	27-5	6-7	(TA)18	(GAA)21	18-8	12-5	23-3	SNP
1	AD-6	1·8	2	7	11	4	10	2	8	25	11	**4**	6	17	10	(–)	**5**	2	2
2	AD-11	2·4	31	7	8	4	12	2	8	22	8	**4**	6	(–)	11	(–)	**5**	2	3
3	AD-28	2·4	9	7	8	4	13	2	8	17	8	**4**	6	14	11	(–)	**5**	2	1/2
4	AD-29	2·0	14	7	10	4	12	2	8	15	8	**4**	6	19	10	(–)	**5**	2	3
5	AD-41	2·4	8	7	10	4	12	2	8	15	8	**4**	6	18	10	6	**5**	2	1/2
6	AD-254	1·6	23	7	8	4	(–)	(–)	(–)	(–)	(–)	**4**	6	14	15	(–)	(–)	(–)	(–)
7	AD-380	2·6	0	7	9	4	(–)	2	8	18	9	**4**	6	17	10	9	**5**	2	3
8	AD-431	2·2	0	7	10	4	(–)	(–)	8	(–)	10	**4**	5	17	11	8	**5**	2	2
9	AD-125	0·6	1	7	11	4	12	2	7	(–)	9	**4**	6	19	10	8	**4**	2	3
10	AD-128	1·8	0	7	11	4	13	(–)	8	(–)	9	**4**	6	14	11	8	**5**	2	3
11	AD-222	na	4	7	8	4	12	2	8	(–)	8	**4**	6	17	11	9	**5**	2	3
12	AD-126	0	4	(–)	10	4	14	(–)	8	(–)	(–)	**4**	6	15	11	8	**5**	2	3
13	AD-229	1·4	na	7	(–)	(–)	(–)	(–)	8	(–)	(–)	**4**	6	(–)	11	(–)	(–)	2	(–)
14	AD-232	1·0	na	7	12	4	14	2	8	15	9	**4**	5	18	10	8	**5**	2	3
15	AD-114	2·4	13	7	10	4	10	2	9	16	9	**4**	7	(–)	11	(–)	**5**	2	2/3
16	AD-130	2	6	7	11	4	14	2	8	18	9	**4**	7	14	10	(–)	**6**	2	3
17	AD-197	2·4	0	(–)	(–)	(–)	(–)	(–)	(–)	17	(–)	(–)	7	13	12	(–)	(–)	(–)	(–)
18	AD-204	2·4	24	(–)	10	4	13	2	8	17	10	(–)	7	18	11	(–)	(–)	2	(–)
19	CB-1	3·0	4	7	6	4	14	2	8	14	9	**5**	9	18	12	8	**4**	1	1
20	CB-3	3·0	2	7	9	4	12	2	7	21	10	**5**	6	21	21	(–)	**4**	3	4
21	CB-5	1·8	7	(–)	(–)	4	(–)	(–)	8	15	8	(–)	(–)	(–)	(–)	(–)	**4**	2	(–)
22	CB-6	3·0	7	7	8	4	15	2	6	14	10	**5**	6	21	15	(–)	**4**	2	2/3/4
23	CB-8	2·6	3	7	9	4	13	2	8	15	9	**6**	6	15	17	8	**4**	1	1/2
24	CB-9	1·8	7	(–)	8	4	12	2	8	14	9	**5**	6	20	17	(–)	**4**	2	3
25	CB-11	1·2	4	7	8	4	(–)	2	8	14	8	**5**	6	17	16	(–)	**4**	2	4
26	CB-14	1·8	7	6	9	4	10	2	8	(–)	9	**5**	6	13	13	8	**4**	2	1
27	CB-15	na	10	7	10	4	14	(–)	7	(–)	(–)	(–)	(–)	(–)	(–)	8	(–)	2	4
28	CB-16	2·0	17	7	7	4	12	2	8	(–)	9	**4**	6	13	11	8	(–)	2	3
29	CB-28	2·2	10	(–)	(–)	4	(–)	(–)	(–)	13	9	**5**	(–)	11	14	8	(–)	2	4
30	CB-30	3·0	4	7	8	4	13	2	8	13	8	**5**	6	14	16	(–)	**4**	2	4
31	CB-48	3·0	2	7	9	4	12	2	8	16	9	**4**	5	14	11	8	**5**	2	3
32	CB-49	2·6	na	7	8	4	14	2	8	15	8	**5**	6	16	14	(–)	**4**	2	4
33	CB-52	2·6	0	7	8	4	12	2	8	13	8	**5**	6	11	15	(–)	**4**	2	4
34	CB-53	3·0	0	7	8	4	16	2	6	15	10	**5**	6	21	13	(–)	**4**	2	4
35	CB-54	2·6	0	7	9	4	13	2	8	14	9	**4**	5	16	11	8	**5**	2	3
36	CB-55	2·6	0	7	12	4	12	2	6	14	8	**5**	8	17	(–)	(–)	**3**	2	3
37	CB-57	2·8	0	7	11	4	15	2	7	(–)	9	**5**	8	20	15	(–)	**5**	2	4
38	CB-58	2·8	0	7	8	4	13	2	8	14	8	**5**	6	(–)	18	(–)	**4**	2	4

na: not available, (–): No PCR product or FLA signal or sequence not clear.

Table 2. The *M. leprae* STR alleles and their frequencies in two populations of leprosy patients in Colombia

	Combination # 1				Combination # 2				Combination # 3				Combination # 4		
	(AC)8b	(GTA)9	(GGT)5	(AT)17	21-3	(AC)9	(AT)15	(AC)8a	27-5	6-7	(TA)18	(GAA)21	18-8	12-5	23-3
AD (18)	**7 (15)**	8 (4) 9 (1) **10 (6)** 11 (4) 12 (1)	**4 (16)**	10 (2) **12 (5)** 13 (3) 14 (3)	**2 (12)**	7 (1) **8 (14)** 9 (1)	**15 (3)** 16 (1) **17 (3)** 18 (2) 22 (1) 25 (1)	8 (5) **9 (6)** 10 (2) 11 (1)	**4 (16)**	5 (2) **6 (12)** 7 (4)	13 (1) **14 (4)** 15 (1) **17 (4)** 18 (3) 19 (2)	10 (7) **11 (9)** 12 (1) 15 (1)	6 (1) **8 (5)** 9 (2)	4 (1) **5 (12)** 6 (1)	**2 (16)**
C-B (20)	Neg (3) 6 (1) **7 (16)**	Neg = 2 6 (1) 7 (1) **8 (8)** 9 (5) 10 (1) 11 (1) 12 (1)	Neg = 2 **4 (20)**	Neg = 5 10 (1) **12 (6)** 13 (4) 14 (3) 15 (2) 16 (1)	Neg = 6 **2 (17)**	Neg = 2 6 (3) 7 (3) **8 (13)**	Neg = 7 13 (3) **14 (7)** 15 (4) 16 (1) 21 (1)	Neg = 4 8 (7) **9 (9)** 10 (3)	Neg = 2 4 (3) **5 (14)** 6 (1)	Neg = 0 5 (2) **6 (12)** 8 (2) 9 (1)	Neg = 3 11 (2) 13 (2) 14 (2) 15 (1) 16 (2) 17 (2) 18 (1) 20 (2) **21 (3)**	Neg = 0 **11 (3)** 12 (1) 13 (2) 14 (2) **15 (3)** 16 (2) 17 (2) 18 (1) 21 (1)	Neg = 10 **8 (8)**	Neg = 4 3 (1) **4 (13)** 5 (3)	Neg = 2 1 (2) **2 (17)** 3 (1)
	Neg = 3	Neg = 2	Neg = 0	Neg = 3	Neg = 3	Neg = 1	Neg = 4	Neg = 1	Neg = 2	Neg = 3	Neg = 3	Neg = 3	Neg = 12	Neg = 3	Neg = 0

Eighteen samples from the AD and 20 samples from the C-B patient Group were analyzed by MLVA. For each locus, the allele type(s) found followed by the corresponding number of samples (in parenthesis) in which they were detected are listed. The loci for which PCR or sequence data were not obtained are indicated as Neg. The most frequent alleles are shown in bold and underlined type.

VNTR data from Asia, where SNP type 3 isolates are found in China,[7] Japan,[8,9] and to a smaller percent in the Philippines[5] do not have the 4-5 signature, but have 5-4 instead. There are also implications that the SNP type 4 are not directly descended from the American SNP type 3s were derived, but from a larger group that gave rise to SNP type 1, SNP type 2 and SNP type 3.

Further and deeper epidemiology, including strain typing of *M. leprae* in these endemic states and in other states in Colombia, will add to the knowledge of strain types and their origins. These procedures will provide tools for better tracing of isolates to prevent transmission.

Acknowledgements

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