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Article *in* Tropical Medicine & International Health · March 2010

DOI: 10.1111/j.1365-3156.2010.02491.x · Source: PubMed

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Tropical Medicine and International Health doi:10.1111/j.1365-3156.2010.02491.x

VOLUME 15 NO 5 PP 632–638 MAY 2010

Candidate gene study of susceptibility to cutaneous leishmaniasis in Sri Lanka

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Summary

OBJECTIVES To investigate the association between selected single nucleotide polymorphisms (SNPs) in *TNF, LTA* and *SLC11A1* genes and risk of endemic cutaneous leishmaniasis (CL) in Sri Lanka through a case–control disease association study.

METHODS An anonymized DNA resource representative of the Sri Lankan population was genotyped initially to establish baseline parameters. This was followed up by genotyping 200 patients and 200 matched controls. Published or modified PCR/RFLP methods were employed for genotyping.

RESULTS Comparison of the different ethnic groups showed the distribution of alleles of *LTA +252 A>G* to differ significantly in Tamils and Moors when compared with Sinhalese. The differences seen at allele level were also reflected in the haplotypes defined by these SNPs at the TNF locus. The case–control analysis did not show an association between the SNPs or the haplotypes investigated and CL. The distribution of these variant alleles in other populations, where they are positively associated with leishmaniasis, differed significantly from the Sri Lankan study cohort.

CONCLUSIONS The selected polymorphisms do not predispose to CL in the Sri Lankan population. The study of extended haplotypes at these loci using a sufficiently powered sample collection would elaborate the findings of this study. In the face of an evolving disease pattern in the country with other forms of leishmaniasis now being reported, prevalence of polymorphisms predisposing to these forms calls for heightened surveillance and preparedness.

keywords cutaneous leishmaniasis, genetic susceptibility, *TNF* locus, *SLC11A1,* Sri Lanka

Introduction

Leishmaniasis is a group of parasitic diseases of global proportions with 12 million people affected and an estimated two million new cases occurring every year (WHO 2009). The localized cutaneous form of leishmaniasis (CL) is endemic in Sri Lanka. The parasite in the country has been identified as *Leishmania donovani* MON 37 (Karunaweera *et al.* 2003). This species commonly causes visceral leishmaniasis (VL) in many Asian and East African foci. More than 2000 patients have been diagnosed since 2001 at the Department of Parasitology, Faculty of Medicine, Colombo alone, which is a tertiary referral centre for diagnosis of leishmaniasis in Sri Lanka. Even though not potentially fatal or debilitating as the visceral or the mucocutaneous forms, cutaneous leishmaniasis (CL) can be personally and socially disruptive (Herwaldt 1999) and has been recognized in its own right as a disease entity requiring intense control measures (WHO 2007).

As with many other parasitic diseases, the clinical manifestations of leishmaniasis are a result of the interplay between parasite, host and environmental factors (Akilov *et al.* 2007). The finding that the three species of *Leishmania* responsible for different disease phenotypes in fact shows very little genetic diversity (Peacock *et al.* 2007) which further highlights the contribution of host factors in determining the outcome of infection. In Sri Lanka, our previous epidemiological findings that point towards clustering of the condition among Sinhalese suggest the possibility of genetically determined host factors contributing to susceptibility to CL (Samaranayake *et al.* 2008).

The variability in the immune response and the ensuing pathology confer susceptibility to leishmaniasis and also contribute to its varied clinical outcomes (Blackwell 1999; Handman *et al.* 2005). Thus, genes implicated in the immune responses have been the focus of many studies examining host susceptibility to leishmaniasis. Three candidate genes:-tumour necrosis factor (*TNF;* previously called tumour necrosis factor alpha), lymphotoxin alpha (*LTA;* previously called tumour necrosis factor beta) and solute carrier family 11A member 1 (*SLC11A1;* previously called NRAMP1*)* – which have received such particular

attention were selected by us for our candidate gene disease association studies.

T helper 1-(Th1) and Th2-type immune responses with their associated profile of cytokines have been associated with resistance or susceptibility to leishmaniasis respectively (Roberts *et al.* 2000). TNF, a Th1-type cytokine with a broad spectrum of biological effects, is one of the initial factors to be secreted in the body's defense response against infection and inflammation. Its role in activating macrophages is especially important in controlling infections with *Leishmania*; where the parasite is adapted to survive in the hostile environment of the lysosomal compartment of macrophages (Murphy *et al.* 2007). However, high levels of activity of TNF, as defined by an exaggerated and over-aggressive response, have been implicated in clinical manifestations of leishmaniasis as well as their severity (Castes *et al.* 1993). A single nucleotide polymorphism (SNP) in the promoter region of *TNF* (−308G>A) has been widely linked to the magnitude of this secretory response and confers susceptibility to mucocutaneous and visceral forms of leishmaniasis (Cabrera *et al.* 1995; Blackwell 1999; Karplus *et al.* 2002).

LTA which lies in tandem with *TNF* in HLA class111 region of chromosome 6 encodes lymphotoxin alpha, a proinflammatory cytokine with a similar but distinct role to that of TNF. A similar correlation has been made between the +252A>G SNP in intron 1 of *LTA* which predisposes to leishmaniasis, with the variant allele being associated with elevated cytokine levels (Messer *et al.* 1991). Furthermore, various studies have suggested that polymorphisms in genes encoding these two cytokines mutually influence their production (Pociot *et al.* 1993; Bouma *et al.* 1996). These two SNPs define four haplotypes and those constituting one or both variant alleles which have been significantly associated with different manifestations of CL in some populations (Cabrera *et al.* 1995).

SLC11A1 is considered to play a key role in innate host resistance to intra macrophage pathogens. It encodes a proton/divalent cation (Fe2+, Zn2+ and Mn2+) antiporter which localizes to the late endosomal/lysosomal compartment of the macrophage. Its functionality is associated with an enhanced activity of proinflammatory immune pathways, including the formation of nitric oxide (NO) via transcriptional stimulation of inducible nitric oxide synthase (iNOS) expression. Several polymorphisms of this gene in both 5′and 3′ regions have been implicated in VL. In Sudan, the 274C>T polymorphism in exon 3 and 469+14G>C polymorphism in intron 4 were associated with susceptibility to VL caused by *L. donovani* (Mohamed *et al.* 2003) -the same species causing CL in Sri Lanka. We hypothesized that some of these candidate markers may also predispose patients to the cutaneous form of the disease, caused by a dermotropic variant of the same parasite species.

There is only limited data on genetic variants predisposing to CL (Lara *et al.* 1991; Cabrera *et al.* 1995; Kamali-Sarvestani *et al.* 2006). Furthermore, there are no reports of such studies in South Asian populations. The objectives of this study therefore were: (i) to establish frequencies of the variant alleles of the *TNF −308G>A, LTA +252 A>G* and *SLC11A1* 274 C>T, *SLC11A1* 469+14G>C, *SLC11A1* D543N polymorphisms in the three major ethnic groups in the Sri Lankan population; – Sinhalese, Sri Lankan Tamils, and Moors (ii) to establish the frequencies of the haplotypes defined by these SNPs in the three major ethnic groups in the Sri Lankan population (iii) to carry out a case–control study to examine the association of these SNPs and the haplotypes defined by them with CL in the Sri Lankan population.

Materials and methods

Subjects

These investigations constituted an initial population genetic study, followed by a case–control study. Samples for the population genetic study were from an anonymized population-based sample collection maintained in the Human Genetics Unit of the Faculty of Medicine, University of Colombo (FMUC). This collection had been made for studies of this nature with the approval of the Ethics Review Committee of FMUC (Dissanayake *et al.* 2009). Thirty each of Sinhalese, Sri Lankan Tamil and Moor samples (50% men) were randomly selected from this collection and genotyped.

Subjects for the case–control studies were recruited prospectively according to a protocol approved by the Ethics Review Committee of FMUC. All subjects gave written informed consent to participate in the study. Cutaneous leishmaniasis was confirmed in those with clinically suggestive skin lesions by direct smear and/or culture.

The initial case–control collection consisted of 200 patients with CL and 200 controls matched for age, sex, ethnicity and area of residence. A detailed discussion of recruitment of this cohort and their phenotypic features has already been published (Samaranayake *et al.* 2008).

Genotyping

DNA was extracted from peripheral venous blood using the Wizard genomic DNA purification kit (Promega Corp,

USA). Genotyping was performed using PCR followed by restriction digestion, as described previously (Liu *et al.* 1995; Wattavidanage *et al.* 1999) or by using modified PCR primers to introduce restriction sites. Detailed genotyping methods are provided as supplementary information (Table S1).

Statistical analysis

The Chi-squared test was used to test the genotypes at each polymorphic locus for Hardy–Weinberg equilibrium (HWE) in the three populations and in cases and controls separately; and to compare differences in allele frequencies between cases and controls. Estimation of haplotype frequencies, measurement of pairwise linkage disequilibrium (*D'* and r^2) and hypothesis testing were carried out using Haploview (Barrett *et al.* 2005). Rare haplotypes occurring at combined frequencies of <0.05 were omitted from the analysis. All reported *P*-values are two tailed. QUANTO version1.2.3 (Gauderman & Morrison 2007) was used for power calculations assuming an additive model for the variant alleles.

Results

Tables 1 & 2 show the results of the population genetic study. The genotype and variant allele frequencies of the SNPs in the candidate genes are in Table 1. All SNPs were in HWE. The distribution of alleles of *LTA +252 A>G* in Tamils and Moors differed significantly from that of Sinhalese.

The haplotypes defined by the two SNPs in the *TNF* and *LTA* genes were considered together as the two genes are located next to each other on chromosome 6. The differences seen at SNP level were also reflected in the haplotypes defined by these SNPs. The haplotypes on chromosome 2 defined by the SNPs in the *SLC11A1* gene were considered separately. The results are in Table 2.

A summary of the demographic and phenotypic features of the initial collection of 200 cases with CL is given in Table 3. The number of samples which had to be excluded from the analysis because of the inability to determine genotype did not exceed 1% (4/400) for any of the SNPs. All polymorphisms in cases and controls were in HWE.

Table 1 Genotype and variant allele frequencies of polymorphisms in the *TNF*, *LTA* and *SLC11A1* genes in the Sri Lankan population

	Sinhalese (S)	Tamils (T)	Moors (M)	Significance (*P*)		
Genotype/Variant allele	*N* (%)	*N* (%)	*N* (%)	SvsT	SvsM	TvsM
TNF G-308A (rs1800629)						
GG	17 (56.7)	13 (43.3)	19 (63.3)	0.10	0.82	0.07
GA	13 (43.3)	13 (43.3)	10 (33.3)			
AA	0 (0)	4 (13.3)	1 (3.3)			
rs1800629A	13 (21.7)	21 (35.0)	12 (20.0)			
LTA A252G (rs909253)						
GG	0 (0)	2 (6.7)	2 (6.7)	0.02	0.01	0.85
GA	10 (33.3)	17 (56.7)	18 (60.0)			
AA	20 (66.7)	11 (36.7)	10 (33.3)			
rs909253G	10 (16.7)	21 (35.0)	22 (36.7)			
SLC C274 T (rs2276631)						
CC	23 (76.7)	25 (86.2)	23 (76.7)	0.34	0.89	0.27
CT	7 (23.3)	4 (13.8)	6 (20.0)			
TT	0 (0.0)	0 (0.0)	1 (3.3)			
rs2276631T	7 (11.7)	4 (6.9)	8 (13.3)			
SLC G469 + 14C (rs3731865)						
GG	26 (92.9)	24 (80.0)	24 (80.0)	0.14	0.17	0.90
GC	2 (7.1)	6 (20.0)	6 (20.0)			
CC	0 (0.0)	0 (0.0)	0 (0.0)			
rs3731865C	2 (3.6)	6 (10.0)	6 (10.0)			
SLC D543N (rs17235409)						
GG	27 (93.1)	25 (86.2)	25 (83.3)	0.40	0.28	0.81
GA	2 (6.9)	4 (13.8)	5 (16.7)			
AA	0 (0.0)	0 (0.0)	0 (0.0)			
rs17235409A	2 (3.4)	4 (6.9)	5 (8.3)			

Table 2 Haplotypes defined by SNPs on chromosome 6 (of *LTA* and *TNF*) and on chromosome 2 (of *SLC11A1*) in the Sri Lankan population

Locus/Haplotype			Frequency			Significance (*P*-value)		
			Sinhalese (S)	Tamils (T)	Moors(M)	SvT	SvM	TvM
LTA 252	*TNF*-308							
A	G		0.69	0.47	0.58	0.01	0.17	0.29
A	A		0.14	0.18	0.05	0.58	0.13	0.06
G	G		0.09	0.18	0.22	0.16	0.04	0.62
G	A		0.08	0.17	0.15	0.11	0.27	0.75
SLC11A1	*SLC11A1*	*SLC11A1*						
274	469+14	1703						
C	G	G	0.83	0.79	0.74	0.58	0.20	0.50
T	G	G	0.11	0.03	0.07	0.10	0.62	0.24
C	G	A	0.02	0.06	0.07	0.17	NA	0.78
C	C	G	0.01	0.07	0.05	NA	NA	0.63

NA indicates those haplotypes with a combined frequency of <5.0%. These were excluded from the analysis.

Table 3 Summary of demographic and phenotypic features of patients with CL

Characteristic	No. and (%) of patients
Gender	
Male	143 (71.5)
Female	57 (28.5)
Age group (years)	
0–9	3 (1.5)
10–19	28 (14.0)
20–29	48 (24.0)
30–39	58 (29.0)
40–49	27 (13.5)
50–59	15 (7.5)
60–69	14 (7.0)
70–79	6 (3.0)
80–89	1 (0.5)
Ethnic group	
Sinhalese	200 (100.0)
No. of lesions	
Single	180 (90.0)
Multiple (1–4)	20 (10.0)
Multiple affected family members	11 (5.5)

The results of the case–control analysis at single SNP level are in Table 4. All SNPs were in HWE. None of the SNPs or haplotypes (results not shown) was associated with CL.

In an attempt to understand the differences in results obtained in this study and previously published studies, we compared the allele frequencies and haplotype frequencies we had observed with those reported in previous studies, where a significant association for these polymorphisms with leishmaniasis was documented (Table 5). All variant alleles of *TNF*, *LTA* and SLC11A1 and most haplotype frequencies (results not shown) at the TNF locus in these populations differed significantly from those of Sinhalese. A haplotype comparison at *SLC11A1* was not possible as the relevant frequencies in the Sudanese have not been reported (Mohamed *et al.* 2003).

Discussion

In this study, we set out to investigate the association of selected genes with susceptibility to CL, using SNPs and haplotypes defined by these SNPs as genetic markers. The analysis of these genetic markers in the cases and controls was preceded by a population genetic study to establish the background population frequencies.

TNF has been associated with many infectious and inflammatory conditions. Similarly, *SLC11A1* has been associated with mycobacterial diseases such as tuberculosis (Shaw *et al.* 1997), leprosy (Abel *et al.* 1998) and also a range of auto immune diseases (Rodríguez *et al.* 2002, Esposito *et al.* 1998, Kotze *et al.* 2001). Thus, the baseline data on the frequency of the variations in these genes in the Sri Lankan population that we report here would help in designing genetic association studies of sufficient power for these conditions in the Sri Lankan population.

The population genetic study showed significant differences in allele and haplotype distribution at the TNF locus among the main ethnic groups which constitute the Sri Lankan population, with Sinhalese differing significantly from Tamils and Moors. This finding was interesting in view of our observation during recruitment of cases, where the condition was confined almost exclusively to Sinhalese (Samaranayake *et al.* 2008).

Table 4 Genotype and variant allele frequencies of *TNF, LTA* and *SLC11A1* polymorphisms among cases and controls and their association with CL

SNP/Gene	Genotype/Variant allele	Cases *N* (%)	Controls *N* (%)	OR	95% CI	*P*-value
rs1800629	GG	121 (60.5)	114 (57.0)	1.00		
TNF	GA	67 (33.5)	73 (36.5)	0.865	0.569–1.314	0.5
	AA	10 (5.0)	11 (5.5)	0.856	0.35–2.094	0.73
	rs1800629A	87 (21.96)	95 (23.98)			
rs909253	AA	97 (48.5)	89 (44.5)	1.00		
LTA	GA	91 (45.5)	97 (48.5)	0.861	0.574–1.291	0.47
	GG	11 (5.5)	14 (7.0)	0.721	0.31–1.671	0.44
	rs909253G	113 (28.3)	125 (31.25)			
rs2276631	CC	151 (75.5)	147 (73.5)	1.00		
SLC11A1	CT	42 (21.0)	48 (24.0)	0.852	0.531–1.366	0.51
	TT	5 (2.5)	4 (2.0)	1.217	0.320–4.621	0.77
	rs2276631T	52 (13.3)	56 (14.5)			
rs3731865	GG	175 (87.5)	176 (88.0)	1.00		
SLC11A1	GC	22 (11.0)	22 (11.0)	1.006	0.537–1.882	0.97
	CC	0 (0.0)	0 (0.0)			
	rs3731865T	22 (5.6)	22 (5.6)			
rs17235409	GG	164 (82.0)	168 (84.0)	1.00		
SLC11A1	GA	34 (17.0)	27 (13.5)	1.29	0.745–2.234	0.36
	AA	1 (0.5)	1 (0.5)	1.024	0.064–16.515	0.97
	rs17235409A	36 (9.2)	29 (7.3)			

Table 5 Distribution (%) of variant alleles predisposing to leishmaniasis in other study populations

		Population (phenotype)			
Gene	Allele	Sinhalese (CL)	Venezuelans (MCL)	Sudanese (VL)	Significance (*P*-value)
TNF	rs1800629A	21.7	7.0		<0.05
LTA	rs909253G	16.7	69.0		<0.05
SLC11A1	rs2276631T	11.7		79.9	<0.05
	rs3731865C	3.6		12.0	0.018
	rs17235409A	3.4		88.0	<0.05

MCL, muco cutaneous leishmaniasis; VL, visceral leishmaniasis.

However, the case–control analysis provided no evidence for an association of the SNPs and haplotypes defined by these SNPs with predisposition to CL. To the best of our knowledge, this is one of the largest studies on the association of genes with CL to be conducted and the first such study to be conducted in the South Asian region. In this study, recruitment of patients was subjected to stringent criteria to avoid population stratification as mentioned previously. The sample size of this case–control study provided 80% power to detect a genotype relative risk between 1.5 and 2.1 depending on variant allele frequencies, at a significance level of 0.05. The power to detect smaller genetic effects, which may be the case in a multifactorial disease of this nature, is limited by sample size. This may explain our finding of lack of predisposition of the selected markers and CL. Conversely, considering polymorphisms at the TNF locus, our results are in agreement with other studies (Cabrera *et al.* 1995; Kamali-Sarvestani *et al.* 2006) where no association was detected between these SNPs and CL. Interestingly, the studies which found significant associations of *TNF* locus with muco cutaneous leishmaniasis (Cabrera *et al.* 1995) caused by *Leishmania braziliensis* or symptomatic VL caused by *Leishmania chagasi* (Karplus *et al.* 2002) had 80% power to detect a genotype relative risk of 6.8 or 2.1 respectively, depending on variant allele frequencies assuming a baseline population risk of 50% and an additive model of inheritance. These observations, taken together with the

observation that frequencies of variations in the candidate genes in Sinhalese differed significantly from other populations with positive associations, support the conclusion that these variations are not associated with CL in the Sinhalese.

We noted several interesting trends in our dataset even though statistically significant conclusions could not be drawn. The variant alleles of *TNF* and *LTA* which has been associated with susceptibility to CL in other studies (−308A and +252G respectively) showed a negative risk when the heterozygous and the rare homozygous states of patients were compared against the controls (OR<1). Further, in *SLC11A1 469+14G>C*, the risk was almost equivalent (OR = 1.006) in the heterozygous state.

The previous observations reiterate the fact that the final clinical outcome in leishmaniasis is dependent upon the diversity of the human host, parasite and less well defined other local factors. The contradictory results regarding the contribution of genotypes and alleles highlight the variable genetic effect across populations as well as the possibility of other polymorphisms in linkage disequilibrium which influence the outcome of disease. Thus, the importance of focusing on complete genes defined by their haplotypes rather than on individual polymorphisms in assessing genetic risk needs to be emphasized. Possible differences in the underlying mechanisms of pathophysiology should also be considered in interpreting these differences in findings. Furthermore, investigating the polymorphisms of a cytokine and association of disease in a population will not be complete without an understanding of the polymorphisms in the genes coding for its receptors as demonstrated at the TNF cluster (Hajeer & Hutchinson 2000).

In conclusion, the data of our study showed that the selected polymorphisms did not influence susceptibility to CL in the Sri Lankan population. The study of extended haplotypes at these loci using a sufficiently powered sample collection would elaborate the findings of this study. The allele and haplotype frequencies reported here would be useful in accurate design of such an investigation. The recent reports from Sri Lanka of patients with mucosal localization as well as the visceral form of disease acquired locally indicate an evolving pattern of disease. The prevalence of polymorphisms linked to susceptibility of these forms as demonstrated by this study, calls for heightened measures of surveillance and preparedness in the island.

Acknowledgements

This study was supported by National Health Research Council (ETR/G/NHRC/RP-17/2005) University Grants Commission of Sri Lanka (UGC/ICD/045) and IRQUE Project, FMUC (CIA2/2006/01). We thank consultant dermatologists Dr. P.H. Chandrawansa and Dr. J. Akarawita for the support extended in sample collection and R. L. Ihalamulla, S. Jayasinghe and K. H. Weerasena for technical assistance.

References

Abel L, Sanchez FO, Oberti J *et al.* (1998) Susceptibility to leprosy is linked to the human NRAMP1 gene. *The Journal of Infectious Diseases* **177**, 133–145.

Akilov OE, Amor K & Tayyaba H (2007) Clinical manifestations and classification of Old World cutaneous leishmaniasis. *International Journal of Dermatology* **46**, 132–142.

Barrett JC, Fry B, Maller J & Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265.

Blackwell JM (1999) Tumour Necrosis Factor alpha and Mucocutaneous Leishmaniasis. *Parasitology Today* **15**, 73–75.

Bouma G, Crusius JBA, Pool MO *et al.* (1996) Secretion of Tumour Necrosis Factor α and Lymphotoxin α in Relation to Polymorphisms in the TNF Genes and HLA-DR Alleles. Relevance for Inflammatory Bowel Disease. *Scandinavian Journal of Immunology* **43**, 456–463.

Cabrera M, Shaw MA, Sharples C *et al.* (1995) Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *Journal of Experimental Medicine* **182**, 1259–1264.

Castes M, Trujillo D, Rojas ME *et al.* (1993) Serum levels of tumor necrosis factor in patients with American cutaneous leishmaniasis. *Biological Research* **26**, 233–238.

Dissanayake VHW, Weerasekera LY, Gammulla CG & Jayasekara RW (2009) Prevalence of genetic thrombophilic polymorphisms in the Sri Lankan population – implications for association study design and clinical genetic testing services. *Experimental and Molecular Pathology* **87**, 159–162.

Esposito L, Hill NJ, Pritchard LE *et al.* (1998) Genetic analysis of chromosome 2 in type 1 diabetes: analysis of putative loci IDDM7, IDDM12, and IDDM13 and candidate genes NRAMP1 and IA-2 and the interleukin-1 gene cluster. *Diabetes* **47**, 1797–1799.

Gauderman WJ & Morrison JM (2007) Quanto 1.2.3: a computer program for power and sample size calculations for genetic-epidemiology studies Available at: http://hydra.usc.edu/gxe (accessed 1 September 2009).

Hajeer AH & Hutchinson IV (2000) TNF-alpha gene polymorphism: clinical and biological implications. *Microscopy Research and Technique* **50**, 216–228.

Handman E, Elso C, Foote S, Baker JR, Muller R & Rollinson D (2005) Genes and Susceptibility to Leishmaniasis. *Advances in Parasitology* **59**, 1–75.

Herwaldt BL (1999) Leishmaniasis. *The Lancet* **354**, 1191–1199.

Kamali-Sarvestani E, Rasouli M, Mortazavi H & Gharesi-Fard B (2006) Cytokine gene polymorphisms and susceptibility to cutaneous leishmaniasis in Iranian patients. *Cytokine* **35**, 159–165.

Karplus TM, Jeronimo SMB, Chang H *et al.* (2002) Association between the tumor necrosis factor locus and the clinical outcome of leishmania chagasi infection. *Infection and Immunity* **70**, 6919–6925.

Karunaweera ND, Pratlong F, Siriwardane HVYD, Ihalamulla RL & Dedet JP (2003) Sri Lankan cutaneous leishmaniasis is caused by Leishmania donovani zymodeme MON-37. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **97**, 380–381.

Kotze MJ, de Villiers JNP, Rooney RN *et al.* (2001) Analysis of the NRAMP1 gene implicated in iron transport: association with multiple sclerosis and age effects. *Blood Cells, Molecules, and Diseases* **27**, 44–53.

Lara ML, Layrisse Z, Scorza JV *et al.* (1991) Immunogenetics of human american Cutaneous Leishmaniasis: study of HLA haplotypes in 24 families from Venezuela. *Human Immunology* **30**, 129–135.

Liu J, Fujiwara TM, Buu NT *et al.* (1995) Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. *American Journal of Human Genetics* **56**, 845–853.

Messer G, Spengler U, Jung MC *et al.* (1991) Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF- beta production. *Journal of Experimental Medicine* **173**, 209–219.

Mohamed HS, Ibrahim ME, Miller EN *et al.* (2003) SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. *European Journal of Human Genetics* **12**, 66–74.

Murphy KM, Travers P & Walport M (2007) Innate immunity. In: *Janeway's Immunobiology*, 7th edn. Garland Science, London, pp. 39–103.

Peacock CS, Seeger K, Harris D *et al.* (2007) Comparative genomic analysis of three Leishmania species that cause diverse human disease. *Nature Genetics* **39**, 839–847.

Pociot F, Briant L, Jongeneel CV *et al.* (1993) Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-α and TNF-β by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *European journal of Immunology* **23**, 224–231.

Roberts LJ, Handman E & Foote SJ (2000) Science, medicine, and the future: leishmaniasis. *British Medical Journal* **321**, 801–804.

Rodríguez MR, González-Escribano MF, Aguilar F, Valenzuela A, García A & Núñez-Roldán A (2002) Association of NRAMP1 promoter gene polymorphism with the susceptibility and radiological severity of rheumatoid arthritis. *Tissue Antigens* **59**, 311–315.

Samaranayake TN, Dissanayake VHW & Fernando SD (2008) Clinical manifestations of cutaneous leishmaniasis in Sri Lanka possible evidence for genetic susceptibility among the Sinhalese. *Annals of Tropical Medicine and Parasitology* **102**, 383–390.

Shaw MA, Collins A, Peacock CS *et al.* (1997) Evidence that genetic susceptibility to *Mycobacterium tuberculosis* in a Brazilian population is under oligogenic control: linkage study of the candidate genes NRAMP1 and TNFA. *Tubercle and Lung Disease* **78**, 35–45.

Wattavidanage J, Carter R, Perera KLRL *et al.* (1999) TNFa*2 marks high risk of severe disease during Plasmodium falciparum malaria and other infections in Sri Lankans. *Clinical & Experimental Immunology* **115**, 350–355.

WHO (2007) *Report of the Consultative Meeting on Cutaneous Leishmaniasis*. Geneva, WHO Headquarters.

WHO (2009) *Leishmaniasis, Burden of Disease*. URL: Available at: http://www.who.int/leishmaniasis/burden/en/ (accessed 1 October 2009).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. PCR conditions used for determination of the selected gene polymorphisms.

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