Genetic Predisposition to Self-Curing Infection with the Protozoan *Leishmania chagasi*: A Genomewide Scan


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The protozoan *Leishmania chagasi* can cause disseminated, fatal visceral leishmaniasis (VL) or asymptomatic infection in humans. We hypothesized that host genetic factors contribute to this variable response to infection.

A family study was performed in neighborhoods of endemicity for *L. chagasi* near Natal in northeastern Brazil. Study subjects were assessed for the presence of VL or asymptomatic infection, which was defined by a positive delayed-type hypersensitivity (DTH) skin test response to *Leishmania* antigen without disease symptoms. A genomewide panel of 385 autosomal microsatellite markers in 1254 subjects from 191 families was analyzed to identify regions of linkage. Regions with potential linkage to the DTH response on chromosomes 15 and 19, as well as a novel region on chromosome 9 with potential linkage to VL, were identified.

Understanding the genetic factors that determine whether an individual will develop symptomatic or asymptomatic infection with *L. chagasi* may identify proteins essential for immune protection against this parasitic disease and reveal strategies for immunotherapy or prevention.

Leishmaniasis refers to a spectrum of vectorborne diseases caused by *Leishmania* species protozoa. Although there is considerable overlap, each *Leishmania* species most often causes a specific human disease syndrome or syndromes. Visceral leishmaniasis (VL), which is caused by *Leishmania donovani*, *Leishmania infantum*, or *Leishmania chagasi*, is the cause of most *Leishmania*-induced deaths. However, even among individuals infected with the same *Leishmania* species, there is considerable variability in clinical outcome. Environmental factors contribute but cannot wholly account for differences in the outcome of infection. These observations have led scientists to examine host factors that might influence the clinical consequences of infection.

The present study focuses on infection with *L. chagasi*, which is the cause of VL in a region in northeastern Brazil where the disease is highly endemic. Infection with this protozoan can result in either asymptomatic...
infection, which may be detected by a positive skin test reaction to parasite antigen, or disseminated disease with hepatosplenomegaly, fever, cachexia, and immunocompromise [1, 2]. The mortality rate among individuals with symptomatic VL in most areas of endemicity is 5%–10%, even when treatment is available. Most L. chagasi infections resolve spontaneously. Indeed, only ~1 in 6 infected children or 1 in 16–18 adults develops symptomatic disease [3–5]. A consistent marker of healed infection and protection against subsequent reinfection is the delayed-type hypersensitivity (DTH) skin test (a Montenegro test) response to Leishmania antigen [5, 6]. Factors that determine whether a person will have spontaneous resolution of L. chagasi infection and develop a DTH response or whether he or she will experience progression to symptomatic and potentially fatal disease are incompletely understood.

VL was previously more commonly found in rural areas of Brazil, but, since the 1980s, human migration from rural to peri-urban areas has resulted in outbreaks in major cities in northeastern Brazil [7–10]. The existence of these regions where high exposure is prevalent has enabled studies of host genetic factors that put individuals at risk for developing particularly severe cases of infection.

An asymptomatic outcome of L. chagasi infection is particularly important to understand, because individuals with this outcome develop an appropriate type-1 immune response to the parasite without developing disease symptoms, and they are likely to be immune to subsequent reinfection. The goal of the present study was to locate regions in the human genome that show evidence of linkage to genes controlling the development of asymptomatic infection with L. chagasi in families living in areas of endemicity. Asymptomatic infection was measured by the induration size determined by the DTH test, which provides a quantitative measurement of a type-1 DTH immune response to the parasite. Our data indicated that distinct chromosomal regions may contain genes controlling the DTH response as opposed to progressive VL. This finding supports the hypothesis that these phenotypes represent differentially programmed immune responses occurring after infection with the same organism.

**MATERIALS AND METHODS**

**Study Area**

The study site is in the eastern region of the state of Rio Grande do Norte in the peri-metropolitan area surrounding Natal, a city of ~700,000 people in northeastern Brazil. VL has been endemic in Natal and its surrounding regions since the mid-1980s [8], accounting for 70% of all reported VL cases in the state of Rio Grande do Norte between 1990 and 2004. VL concentrates in small geographic foci, and infection rates vary over time in each neighborhood where the disease is endemic. We identified neighborhoods in which transmission of L. cha-

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191 families were available for genetic analyses. Relatives. After exclusion of these subjects, 1254 subjects from with VL who had underlying HIV infection and 4 of their were either living in a household with a person who had VL a negative result of serological testing for anti-Leishmania antibodies, and response to therapy [7]. Subjects with VL had either active VL at the time of study or a history of VL validated through review of either hospital charts or the family’s copies of their medical records.

**Phenotype Definitions**

**VL.** A diagnosis of VL was verified by clinical history, laboratory validation (a positive bone marrow smear finding or a positive result of serological testing for anti-Leishmania antibodies), and response to therapy [7]. Subjects with VL had either active VL at the time of study or a history of VL.

**DTH** (Positive DTH skin test response). All subjects underwent DTH skin testing. Because it can take 6 months to several years to develop a DTH response after receiving successful treatment for VL [14], the DTH+ group included only those subjects who had a history negative for active VL and were found to have a DTH+ phenotype. Successive DTH skin tests were performed ≥1 year apart, to avoid boosting [15]. Induration size, as determined by the DTH skin test, varied from 0 to 15 mm. For this genetic study, the exact number of millimeters of induration was analyzed as a quantitative trait. Although induration ≥5 mm is considered to denote a positive reaction clinically, we analyzed the actual size of the induration, because our aim is to identify genes that control the host type-1 immune responses to parasite antigens. We hypothesize that individuals at either end of the spectrum (large vs. small induration) may have different genetic profiles.

**Ab** (anti-Leishmania antibody positive). This group included 56 of the 107 individuals who were found to be Ab+ by serological testing and who also had a negative DTH skin test response (induration, <5 mm). For genetic analysis, Ab+ individuals were coded as having an unknown phenotype, because positive serological test results denote the presence of acute infection [13], which can progress to the VL, DTH+, or DTH- (defined as a negative DTH skin test response and a negative result of serological testing for anti-Leishmania antibodies) phenotype [5, 12, 16].

**DTH-.** This category included individuals with a negative result of serological testing for anti-Leishmania antibodies and a negative DTH skin test response (induration, <5 mm) who were either living in a household with a person who had VL or living in the area of endemicity near a VL case family.

Subjects excluded from the genetic study were 3 individuals with VL who had underlying HIV infection and 4 of their relatives. After exclusion of these subjects, 1254 subjects from 191 families were available for genetic analyses.

**Genotyping**

Genotyping was performed at the Center for Inherited Disease Research (CIDR; Johns Hopkins University, Baltimore, MD), with permission from the Brazilian National Research Council (protocol RMX 18/04). Short tandem repeat (STR) autosomal markers across the human genome (n = 385), a modified version of the Marshfield Genetics screening set (version 8; Marshfield Clinic Research Foundation), were genotyped for each individual. The average spacing between markers was 9 cM, with no gaps >20 cM. The overall rate of missing genotypes was 5.54%.

**Data Quality Control**

Data were released from the CIDR after the Genetic Analysis System (GAS; version 2.0 [December 1995]; available at: http://users.ox.ac.uk/~ayoung/gas/html) was run, to identify any Mendelian inconsistencies and/or systematic laboratory or binning problems. Relationship errors were further identified using Relcheck software (version 0.67 [August 1995]; available at: http://www.biostat.wisc.edu/~kbroman/software/) [17], which examines extended pedigrees for consistent patterns of allele sharing between classes of relatives across the genome. We also identified any residual Mendelian errors by use of the “error” option in Merlin (Multipoint Endpoint for Rapid Likelihood Inference) software (version 1.01; available at: http://www.sph.umich.edu/csg/abecasis/Merlin/). All errors were corrected before analyses were performed. Marker allele frequencies were calculated for founders only. No significant deviations from Hardy-Weinberg equilibrium (P<.001) were identified using an exact test.

**Statistical Analyses**

**Heritability and quantitative linkage analysis.** The size of skin test induration (in millimeters) was log-transformed to normalize data for analysis of the quantitative DTH trait. The log-transformed DTH phenotype approximated a normal distribution as assessed using a quantile-quantile normal plot. All individuals classified as having VL were coded as “missing” for DTH, because the DTH size varies depending on the length of time after disease.

Familial correlations were estimated using the FCOR program of the SAGE (Statistical Analysis for Genetic Epidemiology) software package (version 4.8 [1987]; available at: http://darwin.cwru.edu/sage/). Heritability (h²), or the proportion of variation attributable to additive genetic factors, was estimated from the sibling correlations r by use of the equation $h^2 = 2r$

We performed 2 linkage analyses on the continuous DTH phenotype by use of the Merlin software program (version 1.0-α). First, we performed nonparametric quantitative regression analysis to test for allele sharing among individuals with similar DTH skin test responses. The sample mean was used to estimate the phenotypic mean, and the degree of identity-by-descent (IBD) allele sharing was estimated using multipoint methods in which all markers contributed information. Second, we per-
formed variance components analysis using all members of all the families in the Merlin program, including age and sex, which were used as covariates [18]. The Merlin program performs variance components linkage analysis under the assumption of no dominance and calculates marker-specific heritability for each phenotypic trait [19].

**Qualitative linkage analysis.** For the dichotomous disease phenotype VL, we performed nonparametric linkage analysis with use of the Merlin program. We tested for excess IBD allele sharing among affected relatives by use of the S-all statistic originally described by Whittemore and Halpern [20]. Nonparametric allele-sharing logarithm of the odds ratio (LOD) scores were also calculated using the Kong and Cox linear model [21].

For all linkage analyses, we calculated nominal P values across the genome. For STR markers giving suggestive evidence of linkage (P < .01, for both quantitative trait locus [QTL] regression and variance components linkage analyses), we calculated chromosome-specific empirical P values by use of gene-dropping simulations in the Merlin program. Under this simulation approach, random genotypes are generated for each marker conditional on estimated allele frequencies, observed patterns of missing data, and the specified genetic map under the null hypothesis of no linkage or association with the phenotype [22]. For each chromosome with a region of interest, 10,000 replicates were simulated.

**RESULTS**

**Study families and subjects.** The study sample included 1254 subjects in 191 families with a mean size (± SD) of 5 ± 3 individuals. Population characteristics were similar to those described elsewhere, and some families were included in a previously published clinical report [7]. The study population consisted of members of 130 families with at least 1 member with VL and 61 non-VL families. There were 33 multiplex VL pedigrees, defined as families with 2 or more members with VL. These families were used in VL linkage analysis.
Figure 1. Summary of results of a genomewide scan for symptomatic visceral leishmaniasis (VL) and the size of the delayed-type hypersensitivity (DTH, or Montenegro) skin test response. The x-axis shows the distance (in centiMorgans) for each chromosome, on the basis of 385 autosomal markers across the human genome from the Marshfield Genetics screening set (version 8; Marshfield Clinic Research Foundation). Multipoint graphs of the logarithm of the odds ratio (LOD) score for VL and the DTH immune response are shown. The y-axis denotes the LOD score. The dashed red lines denote results of nonparametric linkage analysis of the VL phenotype. The solid blue lines denote results of variance components analysis of the size of the DTH skin test response, adjusted for age and sex. Dashed horizontal lines denote the Lander and Kruglyak [23] genomewide levels of suggestive ($P = .00074$) and significant ($P = .000022$) linkage.

Of the subjects with VL, 78 were symptomatic at the time of study entry. Other individuals with VL had a documented history of disease. A total of 42.5% of the relatives of subjects with VL were infected with *Leishmania* species, whereas 37.2% of subjects in non-VL families had infection, as documented by a positive DTH skin test response (induration, $\geq 5$ mm) or a positive result of serological testing for anti-Leishmania antibodies (table 1). Demographic information and the mean induration size, as determined by the DTH skin test, are shown in table 2.

We performed a genomewide linkage scan on autosomal chromosomes for both the VL phenotype, as a qualitative trait, and the size of DTH induration, as a quantitative trait (figure 1). The families that we studied included 21 concordant affected sibling pairs with VL and 161 sibling pairs who were discordant for VL (table 3). Despite the limited number of affected relative pairs, we identified 1 region of linkage to an unobserved gene controlling the risk for symptomatic VL on chromosome 9q near marker D9S1118 ($P = .003$; LOD score, 1.60; empirical $P = .0034$) (figure 1, dashed red lines).

For the quantitative DTH response, there were 440 sibling pairs with a correlation of 0.42, 212 grandparent-grandchild pairs with a correlation of 0.265, and 90 cousin pairs with a correlation of 0.13 (table 3). This is consistent with genetic control of the DTH response, where first-degree relatives have a stronger correlation than second- or third-degree relatives. Estimated heritability of the DTH immune response was 84%, suggesting a substantial genetic component to variation in induration size, as determined by the DTH skin test.

There were several regions of interest for the quantitative DTH immune response trait, which was measured by the (log-transformed) size of the induration (figure 1, solid blue lines). There were peak LOD scores on chromosome 15 (marker D15S657; variance components analysis [VCA] $P = .0003$; LOD score, 2.50; empirical $P = .008$) and chromosome 19 (marker D19S246; VCA $P = .0014$; LOD score, 1.93; empirical
In VL families only chromosomes 2 (marker D2S441; VCA; LOD score, 4). This analysis also identified regions of potential interest on p (marker D15S657; VCA; LOD score, 1.43) and 19 (VCA; LOD score, 2.47) in the DTH response in asymptomatic individuals, a marker that was previously shown to cause of VL in northeastern Brazil. We targeted as a phenotype of interest a family with at least 1 member with VL. In all families that exposure is uniformly higher among these families, the VCA linkage peak on chromosomes 13 (VCA; LOD score, 1.09). Interestingly, when we restricted our analysis to families with at least 1 member with VL, on the basis of the hypothesis that exposure is uniformly higher among these families, the VCA linkage peak on chromosomes 13 (VCA; LOD score, 1.09) and 19 (VCA; LOD score, 2.47) increased despite the smaller number of families analyzed (table 4). Selected candidate genes lying within or near the linkage peaks are presented in table 5.

**DISCUSSION**

The outcome of *Leishmania* infection depends on a complex interaction among the parasite, the sand fly vector, and the human host, with individual biological characteristics of each contributing to disease manifestations. The present study focuses on genetic factors of the human host controlling the outcome of infection with *Leishmania chagasi*, which is the cause of VL in northeastern Brazil. We targeted as a phenotype the DTH response in asymptomatic individuals, a marker that reflects an effective type-1 immune response to *L. chagasi*. The size of the DTH reaction may estimate the magnitude of acquired protective immunity. The study population was drawn from the site of a recent resurgence of VL in peri-urban Natal, the largest city in the northeastern state of Rio Grande do Norte, Brazil.

Thirty-six percent of the study population had asymptomatic infection (DTH+ phenotype), 11% had symptomatic VL, and ~50% of the subjects had no immunological evidence of *L. chagasi* infection (DTH−). Markers of “infection” were a positive serological test result during acute infection and a positive DTH skin test result, the latter of which is a marker of cured asymptomatic or symptomatic infection [6, 13]. DTH− subjects in this study had lived for 3 or more years in a household that either included a member with VL or was near the dwelling of a family with a member with VL, suggesting that they had a high chance of exposure to the parasite. However, because a positive DTH skin test response can revert to negative if there is no reexposure [24] (S.M.B.J., unpublished data), it is conceivable that some subjects in this group previously had a DTH+ phenotype. Because of this uncertainty, we considered only the VL phenotype and the size of DTH reaction in this linkage analysis.

We hypothesized that different genes control the development of symptomatic versus asymptomatic infection and the immune-response *Leishmania* antigens. Our cohort included a large group of DTH+ individuals without prior disease; this group had controlled previous *L. chagasi* infection with an effective immune response. As such, to our knowledge, this study represents the first genetic linkage study of asymptomatic *Leishmania* infection performed to date. Four regions of interest were identified. The strongest evidence for linkage to the DTH response occurred on chromosomes 15 and 19, with additional smaller peaks occurring on chromosomes 2 and 13. A sub-

**Table 4. Findings of genomewide linkage analysis of the delayed-type hypersensitivity (DTH) skin test (i.e., Montenegro test) immune response trait, with corresponding analyses for symptomatic visceral leishmaniasis (VL) in the same chromosomal regions, by analysis type.**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Nearest marker</th>
<th>Location of nearest marker, cM</th>
<th>VL, by NLA (n = 34)</th>
<th>DTH immune response trait</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In all families</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>By QRA (n = 98)</td>
<td>By VCA (n = 119)</td>
</tr>
<tr>
<td>15</td>
<td>D15S657</td>
<td>105</td>
<td>.2</td>
<td>.012</td>
</tr>
<tr>
<td>19</td>
<td>D19S246</td>
<td>78 (73)b</td>
<td>.3</td>
<td>.0012</td>
</tr>
</tbody>
</table>

**NOTE.** The n values denote the nos. of families that were informative for linkage analysis. Both nominal P values and logarithm of the odds ratio (LOD) scores are presented. NLA, nonparametric linkage analysis; QRA, quantitative regression analysis; VCA, variance components analysis; VL, families, families with at least 1 member with VL.

a Empirical P = .008.
b There were 2 estimates of the location of this marker.
c Empirical P = .029.
Table 5. Visceral leishmaniasis (VL) and delayed-type hypersensitivity (DTH) immune response linkage peaks, as determined by a genomewide scan.

<table>
<thead>
<tr>
<th>Chromosome, gene or marker</th>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 CDKN2A</td>
<td>9p21</td>
<td>Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)</td>
</tr>
<tr>
<td>9 CDKN2B</td>
<td>9p21</td>
<td>Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)</td>
</tr>
<tr>
<td>9 IFNk</td>
<td>9p21.2</td>
<td>Interferon-κ</td>
</tr>
<tr>
<td>9 D9S1118a</td>
<td>9p22–p13</td>
<td>Aconitase-1, soluble</td>
</tr>
<tr>
<td>9 TRBV20OR9-2 through TRBV29OR9-2</td>
<td>9p21</td>
<td>T cell receptor β-chain variable region genes 20–29 and A (9 genes)</td>
</tr>
<tr>
<td>9 IL11RA</td>
<td>9p13</td>
<td>Interleukin-11 receptor–α</td>
</tr>
<tr>
<td>9 CCL27, CCL19, CCL21</td>
<td>9p13</td>
<td>Chemokine (C-C motif) ligands 27, 19, and 21 (3 genes)</td>
</tr>
<tr>
<td>9 CD72</td>
<td>9p13.3</td>
<td>CD72 antigen (B cell marker)</td>
</tr>
<tr>
<td>15 CIB1</td>
<td>15q25.3–q26</td>
<td>Calcium and integrin binding–1 (calmyrin)</td>
</tr>
<tr>
<td>15 MAN2A2</td>
<td>15q26.1</td>
<td>Mannosidase</td>
</tr>
<tr>
<td>15 SLC03A1</td>
<td>15q26</td>
<td>Solute carrier organic anion transporter family member 3A1</td>
</tr>
<tr>
<td>15 LOC643806</td>
<td>15q26.1</td>
<td>Ferritin light chain–1 homologue</td>
</tr>
<tr>
<td>15 D15S657b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 IGFL1R</td>
<td>15q26.3</td>
<td>Insulin-like growth factor–1 receptor</td>
</tr>
<tr>
<td>19 LGALS family</td>
<td>19q13.2–q13.1</td>
<td>Galectins 7, 4, 13, 14</td>
</tr>
<tr>
<td>19 IL28B-IL28A-IL29</td>
<td>19q13.1</td>
<td>Interleukins 28B, 28A, 29</td>
</tr>
<tr>
<td>19 FCGBP</td>
<td>19q13.1</td>
<td>Fc γ receptor</td>
</tr>
<tr>
<td>19 TGFBI</td>
<td>19q13.1</td>
<td>Transforming growth factor–β1</td>
</tr>
<tr>
<td>19 APOE</td>
<td>19q13.2</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>19 PTGIR</td>
<td>19q13.3</td>
<td>Prostaglandin-I2 (prostacyclin) receptor</td>
</tr>
<tr>
<td>19 CD37</td>
<td>19p13–q13.4</td>
<td>CD37</td>
</tr>
<tr>
<td>19 IRF3</td>
<td>19q13.3-q13.4</td>
<td>Interferon regulatory factor 3</td>
</tr>
<tr>
<td>19 D19S246c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 CD33</td>
<td>19q13.3</td>
<td>CD33 (gp67)</td>
</tr>
<tr>
<td>19 CNOT3</td>
<td>19q13.4</td>
<td>CCR4 subunit 3</td>
</tr>
<tr>
<td>19 FCAR</td>
<td>19q13.2-q13.4</td>
<td>Fc α receptor</td>
</tr>
<tr>
<td>19 IL11</td>
<td>19q13.3-q13.4</td>
<td>Interleukin-11</td>
</tr>
</tbody>
</table>

NOTE. Shown are selected genes within the 1-LOD drop interval of the VL linkage peak on chromosome 9 and the DTH linkage peaks on chromosomes 15 and 19. Data are based on Homo sapiens Genome Build 36.1. Genes are presented in order, with the marker position listed at the mid-position of the chromosome entries.

a VL peak at marker D9S1118 (surrounding region in table: 21,900K–41,900K).
b DTH peak at marker D15S657 (surrounding region in table: 79,600,000K to the telomere).
c DTH peak at marker D19S246 (surrounding region in table: 43,650K to the telomere).

Analysis of VL families yielded higher LOD scores. This finding could reflect either unique susceptibility factors influencing DTH skin test responses in some families or the fact that exposure was more uniform in this subset of individuals. Although none of our regions were found to have significant linkage by the use of critical values defined by Lander and Krugylak [23], we had evidence of suggestive linkage on chromosomes 15 and 19 (P = .00074), which warrants further study. Two additional regions had interesting peaks (chromosome 2 and 13). Some good candidate genes within these linkage regions include solute anion carriers on chromosomes 15 and 19. The peak marker on chromosome 19 lies close to genes encoding galectins, the Fc receptor, transforming growth factor (TGF)–β1, latent TGF-β-binding protein 4, interleukin (IL)–28 subunits, and apolipoprotein E and the prostacyclin receptor (table 5). Finally, the gene for TGF-α lies near the peak of potential interest on chromosome 2.

Examination of the symptomatic VL phenotype in a subset of multiplex VL families yielded a small linkage peak on chromosome 9. This peak lies near the IL-11 receptor–α subunit, a cluster of T cell receptor β-chain variable region genes, and 3 chemokine receptor ligands (table 5). Further fine mapping studies are under way to test whether any of these candidate genes or other genes in these linkage regions control either the immune response to Leishmania species or the development of VL.
Three genomewide linkage scans of VL have been reported to date. The first scan focuses on residents of a Sudanese village exposed to *L. donovani*. This study reported significant evidence for linkage of symptomatic disease to a region of chromosome 22q12 (LOD score, 3.50) in a genomewide linkage scan of VL in multiplex families from a single village in the Sudan. A minor locus (2q22–q23) was also suggestive of linkage in families with negative LOD scores for the 22q12 locus (LOD score, 2.29) [25]. A second genomewide scan of 2 ethnic groups in a nearby region of Sudan not only failed to reproduce the 22q12 linkage peak, but it also described 2 regions with strong linkage to VL at loci 1p22 (LOD score, 5.65) and 6q27 (LOD score, 3.74) that were village-specific and Y chromosome–specific, suggesting significant contributions from distinct founders [26]. A genome scan of VL in multiplex pedigrees from northern Brazil, which included some of our VL families, confirmed the peak at locus 6q27 (LOD score, 1.08) and identified 2 additional regions of interest (7q11.22 and 17q11.2–q21.3; LOD scores, 1.34 and 1.14, respectively). Fine mapping in the chromosome 17 region suggested allelic association with 2 chemokine genes [27]. The current study of the subset of multiplex VL families did not replicate previous findings of linkage to the VL phenotype in the Sudanese or Brazilian populations. The relatively small number of multiplex VL families in our study undoubtedly contributed to this lack of replication, but one must also consider genetic differences between populations. Differences between our study and those performed in Sudan are perhaps not surprising, because a different *Leishmania* species is endemic (*L. donovani* in Sudan vs. *L. chagasi* in Brazil) and because of genetic differences between the Sudanese and Brazilian populations. Just as there were differences in susceptibility genes between close villages in the Sudan and even between populations from different founders within villages, the different outcome of our study versus the other genome scan of multiplex VL families in Brazil could reflect underlying genetic heterogeneity of the population and the VL trait. Indeed, Brazilians are a genetically admixed population, and regional differences have already been documented between residents of different Brazilian states [28, 29].

In summary, a genomewide scan of a Brazilian population exposed to *L. chagasi* infection identified regions of interest on chromosomes 2, 13, 15, and 19, with the latter 2 chromosomes potentially linked to an immune response to the parasite, as measured by the size of the DTH skin test reaction. This immune response was likely responsible for an asymptomatic outcome after infection with the parasite. The data also identified a novel region potentially linked to susceptibility to symptomatic VL on chromosome 9. Further fine mapping studies are needed to identify the specific genes that control the host immune response in leishmaniasis. A better understanding of the genetic factors determining whether an individual will develop symptomatic or asymptomatic infection on exposure to *L. chagasi* will provide a better picture of immune protection against disease caused by this parasite and has the potential to direct future efforts at immunotherapy or prevention.

References