Am. J. Hum. Genet. 61:968–979, 1997

Evidence for a Major Gene Controlling Susceptibility to Tegumentary Leishmaniasis in a Recently Exposed Bolivian Population

A. Alcaïs,[1] L. Abel,[1] C. David,[2] M. E. Torrez,[2] P. Flandre,[3] and J. P. Dedet[4]

[1]INSERM U 436, CHU Pitié Salpétrière, Paris; [2]IBBA, La Paz, Bolivia; [3]INSERM SC 10, Villejuif, France; and [4]Laboratoire d'Ecologie Médicale et Pathologie Parasitaire, Université de Montpellier 1, Montpellier

Summary

Tegumentary leishmaniasis due to *Leishmania braziliensis* is a parasitic disease that occurs in two stages after the infected sandfly bite: (1) a primary cutaneous lesion followed by (2) a secondary mucosal involvement generally resulting in severe facial deformities. In order to investigate the genetic and environmental factors involved in the development of the cutaneous lesion, a familial study was performed in a region of Bolivia in which the disease is endemic. Complete selection of 118 nuclear families (703 subjects, with 241 patients), each with at least one cutaneous affected subject, was achieved; 41 families were of native origin, and 77 (herein designated "migrant") recently had settled in the area. For the analysis, the trait under study was the time to onset of the primary cutaneous lesion. The start of the follow-up was birth, for native population, or date of arrival in the endemic area, for migrant population. Segregation analysis was performed by use of a model based on survival analysis methods that allows joint estimation of genetic and environmental effects and accounts for gene × covariate interactions. A significant effect of gender, home-forest distance, and forest-related activity was found. In the 77 migrant families there was evidence for a recessive major gene controlling the onset of the primary cutaneous lesion, with residual familial dependences and age × genotype interaction. Penetrance estimations show that young subjects are genetically more susceptible than older subjects, suggesting that this genetic component could concern mechanisms involved in the development of individual protection during childhood. There was also a significant genetic heterogeneity of the sample according to the native/migrant origin of the families, and no major-gene effect was found in the native subsample.

Received November 22, 1996; accepted for publication July 11, 1997.

Address for correspondence and reprints: Dr. A. Alcaïs, INSERM U 436, Hôpital Pitié-Salpètrière, 91 Boulevard de l'Hôpital, 75013 Paris, France. E-mail: aal@biomath.jussieu.fr

0002-9297/97/6104-0028$02.00

Introduction

Leishmaniasis is a parasitic disease that affects ~12 million people worldwide. New World leishmaniasis (NWL) is a complex of clinical entities caused by several leishmania subspecies. All NWL forms are zoonoses, and man acquires infection after an infected sandfly bite. The tegumentary forms, cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis, constitute the majority of cases of NWL. However, MCL, due to *Leishmania braziliensis braziliensis* (Lainson and Shaw 1987), also denoted as *Leishmania* (*Viannia*) *braziliensis* (Rioux et al. 1990), is responsible for primary cutaneous lesions, generally self-healing, which can be followed by mucosal involvement in a proportion of cases, which varies according to the geographical foci. This secondary mucosal involvement develops years after the original lesion affecting mucosae of the face (nasal, buccal, pharyngeal, and even laryngeal) and can result in severe facial mutilations or even in superinfections that could lead to death (Marsden 1986).

The expression of leishmaniasis results from the interactions between the parasite and the host response, with a central role being played by macrophages. Leishmania amastigotes live and multiply in macrophages within parasitophorous vacuoles (Chang and Dwyer 1978), and any mechanisms modifying either its penetration into the macrophage or its condition of life and multiplication could influence the natural resistance of the infected host. On the other hand, the macrophage presents the amastigote to mediating T cells (Kaye et al. 1988), probably via HLA class II antigens (Antoine 1995). These T lymphocytes will then initiate interleukin-4 and interferon-γ synthesis (Pirmez et al. 1993), which intervene in the acquired resistance as well as in the progression of the disease. This variation of host resistance has been shown to be genetically controlled in mice, and there is also accumulating evidence of genetic factors involved in human leishmaniasis.

Experimental studies in inbred mice have shown that the single autosomal gene located on mouse chromosome 1, which controls the natural resistance to different intracellular pathogens (Skamene et al. 1982), also regu-

lates resistance of mice to infection with *Leishmania donovani* (Bradley et al. 1979), which is responsible for visceral leishmaniasis (VL). This gene, called "*Lsh*/*Ity*/*Bcg*," has been shown to belong to a mouse-man conserved linkage group that is located in the telomeric region of human chromosome 2q (Schurr et al. 1989). A candidate for *Lsh*/*Ity*/*Bcg* has recently been identified in mice (Vidal et al. 1993) and has been denoted as *Nramp1*. In CL, two mouse genes, called "*Scl-1*" and "*Scl-2*," have been shown to influence early response to infection with *L. major* (Howard et al. 1980) and *L. mexicana* (Blackwell et al. 1985), respectively. The genetic control of lesion growth in *L. major* infection has been linked to the proximal end of chromosome 11 (Roberts et al. 1993), containing several candidate genes, such as the interferon-regulating factor 1 (IRF-1). Güler et al. (1996) recently suggested the role of IRF-1 in susceptibility to *L. major* infection and proposed a model of resistance, which is based on the maintenance of the IL-12 signaling pathway. The later phases of the immune response seem to depend on more-complex genetic mechanisms, among which the H2 complex has been shown to play a role in visceral (Bradley 1974; Blackwell et al. 1980) and cutaneous (Howard et al. 1980; Roberts et al. 1989) leishmaniasis.

In humans, classical epidemiological studies have shown familial aggregation of visceral (Fine 1981; Cabello et al. 1995) and cutaneous (Jones et al. 1987) leishmaniasis, as well as ethnic differences in both initial severity and progression of cutaneous lesions due to *L. braziliensis* (Walton and Valverde 1979). Studies of association between CL and HLA have provided controversial results with regard to the possible role of genetic factors. At the population level, the most consistent results were obtained with the HLA class II antigens, mainly HLA-DQ3, which was found to be associated with *L. braziliensis* infections (Lara et al. 1991; Petz-Erler et al. 1991). However, at the familial level, linkage analysis using LOD-score (Lara et al. 1991) or sib-pair (Barbier et al. 1987) methods failed to show an HLA-linked susceptibility locus for CL. A recent case-control study performed in Venezuela (Cabrera et al. 1995) has found evidence for association between MCL and the tumor necrosis factor (TNF). Homozygosity for allele 2 of a polymorphism in the TNF-β gene corresponded to a 7.5 relative risk (RR) of developing MCL, and homozygosity or heterozygosity for allele 2 in the TNF-α gene corresponded to an RR of 3.5. Recently, a complex segregation analysis performed in Peru in an *L. peruviana*–endemic area (Shaw et al. 1995) showed the role of genetic components controlling susceptibility to clinical CL and influencing severity of the disease, defined mainly on the basis of age at onset. In particular, individuals with early onset were found to be genetically more susceptible than those affected at a later age. In the same paper, the authors mentioned that no significant linkage was found between VL and 2q35 markers, where is located a human homologue to the murine *Nramp1,* a homologue denoted as *NRAMP1* (Cellier et al. 1994).

The aim of the present study was to investigate genetic factors involved in CL caused by *L. braziliensis,* by means of complex segregation analysis. As mentioned above, *L. braziliensis* can induce severe mucosal forms, which presents a major public-health problem in Bolivia because it is the predominant form of leishmaniasis (Dedet et al. 1995). In that country, the disease extends to the majority of the tropical Amazonian lowlands, including the high-endemicity areas of Alto Beni and the neighboring part of Beni (David et al. 1993). In addition to the native population that has been living there for several centuries, these areas are also colonization zones where high-altitude populations regularly migrate from the Andean highlands, an area free of leishmaniasis, for economic reasons (i.e., the end of mining activities, following the fall in metal prices) and because of a government policy of relocation. We performed segregation analysis using a model developed by Abel and Bonney (1990), which takes into account age-at-onset information and allows joint estimations of genetic parameters and covariates known to influence risk of CL. These two latter points were expected to increase the power of our analysis. Furthermore, taking advantage of the presence of two populations in the study area, we were able to address the question of a possible genetic heterogeneity according to the native/migrant status.

Subjects and Methods

Family Study and Subjects

From May 1990 to July 1991, active-case detection of CL and MCL was performed in the study area by the Instituto Boliviano de Biologia de Altura (IBBA), during a rural campaign for diagnosis and treatment of MCL in Bolivia, practical details of which have been provided elsewhere (Dedet et al. 1995). Within the study area, six zones have been chosen, with a total of 12,000 inhabitants who have all been clinically examined. Four hundred eighty patients with cutaneous lesions have been diagnosed, 34 of whom also had a mucosal form. All nuclear families with data on at least two first-degree relatives of a patient were retained. According to this ascertainment scheme, familial data could not been collected for 239 cases, because of unknown parents or sibs or because relatives were living far away. The remaining 241 patients (some of whom were first-degree relatives) belonged to 118 nuclear families (703 subjects). Forty-one of these families were of native origin (270 subjects), and 77 were migrants (433 subjects).

Table 1

Distribution of Families, by Number of Affected Children and Number of Affected Parents and According to Native/Migrant Status

No. of Affected Children	No. of Affected Parents						
	Migrants			Natives			
	None	One	Two	None	One	Two	Total
One	33	20	1	9	10	3	76
Two	7	5	1	7	1	3	24
Three	4	3	2	4	2	0	15
Four	1	0	0	1	1	0	3
Total	45	28	4	21	14	6	118

In the native sample 92 subjects were affected (6 with mucosal lesions), whereas in the migrant sample 149 subjects were affected (10 with mucosal lesions). Distribution of families, according to both number of affected children and parents' affection status, is shown in table 1.

The family members were visited by experienced French and Bolivian specialists in tegumentary leishmaniasis. The primary diagnosis was exclusively clinical, since this diagnosis is not difficult to make in these regions, as long as patients are examined by trained clinicians (Jones et al. 1987). However, bacterial and fungal infections, trauma, and neoplasm must be excluded. The typical active cutaneous lesion is a deep, rounded, well-circumscribed ulcer with raised borders, which is not cured by antibiotics (Llanos-Cuentas et al. 1984). When such a lesion was present, the patient was sent to the nearest health center, for further investigation, final diagnosis, and treatment. A history of previous cutaneous lesion was accepted in patients with characteristic scars and without a history of trauma to the site of the scars. Since the data collection was cross-sectional, active cases only represented 20% of the sample, whereas the remaining 80% were diagnosed retrospectively. Patients for whom diagnosis was doubtful were excluded from the study.

The trait under study was the time to onset of the first leishmanial cutaneous lesion. The start of the follow-up was either birth, for natives, or date of the arrival in the endemic area, for migrants. The endpoint was either date at onset of the cutaneous lesion (i.e., failure time), for affected subjects, or date of last clinical examination (i.e., censored time), for unaffecteds. For affected patients, figure 1 shows distribution of failure times for natives (fig. 1*a*), migrants born in the study area (fig. 1*b*), and all migrants (fig. 1*c*). For each subject, the recorded time-independent covariate was gender, and the time-dependent covariate were current and previous home-to-forest distances (since the sandfly lives in the forest), when subjects had moved during their follow-up, and current and previous activities (when subjects had changed their activity during the follow-up).

Study Area

The study area, already described elsewhere (Dedet et al. 1995), will be briefly summarized here. The region of Alto Beni (Department of La Paz) and the regions of Rurrenabaque, Yucumo, and San Borja (Department of Beni) were covered by the study. Alto Beni is situated at the foot of the Andes (altitude 400—700 m), whereas Beni lies in the plain. This zone, covered by tropical

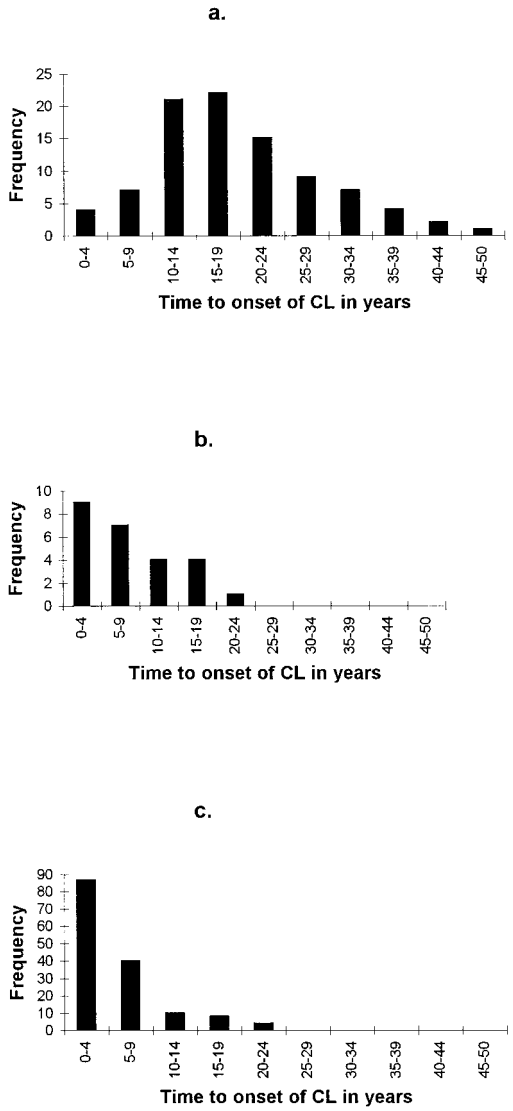

Figure 1 Distribution of time of onset of cutaneous leishmaniasis among (*a*) natives, (*b*) migrants born in the endemic area, and (*c*) all migrants.

rainforest, extends between Sararia and Covendo (west to east) and between Rurrenabaque and Caravani (north to south) and encompasses ~2,400 km^2. As has been mentioned in the Introduction, the population consists of two main groups. The first one is the native group, comprising two tribes of Amazonian Indians—Chimanes (3,000 persons) and Mosetenes (1,200 persons). The second is the migrant group, which consists of highland Indians (Quechua and Aymara), comprising ~50,000 persons who, after clearing initially forested land, have engaged in agriculture. In addition to being suitable because of the presence of two distinct ethnic populations, this area has been chosen because the prevalence of tegumentary leishmaniasis is supposed to be the highest in the country (David et al. 1993) and because the infecting parasite, *L. braziliensis,* is unique to it (Revello et al. 1992).

Segregation Analysis

Segregation analysis was performed by use of the model developed by Abel and Bonney (1990), which allows for variable age at onset, censoring, time-dependent covariates, and genotype × covariates interaction. In this model, based on the inclusion into the regressive logistic model (Bonney 1986) of survival analysis concepts, the event of interest is the time to onset of the disease. Therefore, age at onset is regarded as a failure time, and age at examination (for unaffecteds) is regarded as a censored failure time, where the scale for measuring time is age. The period of follow-up is partitioned into K mutually exclusive intervals, and it is assumed that the interval in which the onset of the disease occurs, rather than its exact time, is observable. In this discrete formulation, the conditional probability of being affected, for individual i within the kth interval ($k = 1, \ldots, K$), given that the individual is not affected before the interval, is the hazard function, denoted as $\lambda_i(k)$. From the hazard function are derived $f_i(k)$ the probability of being affected at an age of onset included within interval k,

$$f_i(k) = \lambda_i(k) \prod_{j=1}^{k-1} [1 - \lambda_i(j)] ,$$

and $S_i(k)$ the probability of not being affected by an age at examination included within interval k,

$$S_i(k) = \prod_{j=1}^{k-1} [1 - \lambda_i(j)] ,$$

which are used as the penetrance functions for the likelihood formulation (Abel and Bonney 1990). The present model differs from previous segregation-analysis models in terms of penetrance functions, which are specified by use of survival-analysis methods.

A logistic model is used to specify a regression relationship between this hazard function and a set of explanatory variables including genotype, phenotypes of preceding individuals (or antecedents), and other covariates which can be time dependent. Furthermore, covariates x genotype interactions can be taken into account. More specifically, let g_i be the genotype of the ith individual, Z_j be the phenotype of the jth antecedent of i, and $X_i(k)$ be the column vector of covariate values for the ith individual in the kth interval. The hazard function for the ith individual in the kth interval is written as $\lambda_i(k) = \exp[\theta_i(k)]/\{1 + \exp[\theta_i(k)]\}$, where $\theta_i(k)$, the so-called logit of the hazard function, has the following general form

$$\theta_i(k) = \alpha_{g_i} + \sum_{j=1}^{i-1} \Gamma_{ij} Z_j + \beta_{g_i} X_i(k) ,$$

where α_{g_i}, Γ_{ji}, and β_{g_i} are the parameters of the model defined in the following paragraphs.

α_{g_i} is, on the logit scale, the baseline hazard, which depends on the genotype of the ith subject, and is assumed to be constant along the intervals. In the present case, of a locus with two alleles, A and a (a being the susceptibility allele), α_{g_i} can take three values: α_{aa}, α_{Aa}, and α_{AA}.

$\Gamma_{ij} Z_j$ accounts for the effect of antecedents' phenotypes, denoted as Z_j, via regression coefficients Γ_{ij}. As proposed by Demenais (1991), Z_j is a column vector coded as $Z_j = (1\ 0)'$ if j is affected by age at examination, as $Z_j = (0\ 1)'$ if j is unaffected by age at examination, and as $Z_j = (0\ 0)'$ if j is unobserved, where the prime symbol denotes transposes. The main reason for this trichotomous coding is to take into account unknown phenotypes. If all phenotypes are known, a dichotomous coding can be used.

Each Γ_{ij} parameter is a vector of two coefficients (γ_{ij1} and γ_{ij2}), accounting for the regression relationship between individual i and the phenotype of his antecedent j. With the coding scheme described above, the logit of the hazard for the ith individual is modified by γ_{ij1} if antecedent j is affected, by γ_{ij2} if j is unaffected, and remains unchanged if j is not observed. We used in this analysis the class D pattern of familial dependence (Bonney 1986), which considers four types of phenotype dependences: spouse-spouse, mother-offspring, father-offspring, and sib-sib. Since, in the different analyses, we never observed a significant difference between father-offspring and mother-offspring dependence, a global parent-offspring dependence was considered. Moreover, the phenotypes of all children were known, and the dis-

tinction between unaffected and unknown was no longer necessary; therefore, four types of Γ_{ij} parameters have to be defined: $\Gamma_{ij} = \Gamma_S = (\gamma_{S1}, \gamma_{S2})$ if j is the preceding spouse of i, $\Gamma_{ij} = \Gamma_P = (\gamma_{P1}, \gamma_{P2})$ if j is the parent of i, $\Gamma_{ij} = \Gamma_C = (\gamma_{C1}, 0)$ if j is the preceding sib of i, and $\Gamma_{ij} = (0, 0)$ otherwise. The interpretation of the Γ_{ij} raises some difficulties that have been considered in detailed by Abel and Bonney (1990), since they are usually not directly interpretable in terms of odds ratios, because the phenotypes are both dependent and explanatory variables.

$\beta_{g_i} X_i(k)$ accounts for the effect of covariates within interval k, denoted as $X_i(k)$, via regression coefficient β_{gi}. The length of the interval used was 1 year; that is, in the specification of the hazard function, k denotes the kth year. β_{gi} is the row vector of covariate parameters, and g_i means that these regression coefficients can differ with the genotype level. In this analysis different covariates were used. One was time independent, gender coded 0 for male and 1 for female, and associated with regression coefficient β_{sex}. The others were time dependent—for example, the logarithm of home-to-forest distance, in meters, associated with β_{dist} and activity partitioned into three classes of exposure according to daytime spent in the forest. The high-risk class corresponded to >4 h/d spent in the forest (hunter, fisher, lumberman, or farmer) and was associated with the β_{high} regression coefficient; the medium-risk class corresponded to 2–4 h/d (carpenter or half-time farmer) and was associated with β_{med}; and the low-risk class corresponded to <2 h/d (schoolchild, nurse, teacher, or housewife) and represented the baseline class. Hazard was shown to vary with age (Alcaïs et al. 1997), and different functions of age were tested, to account for this dependence: polynomial in age, as suggested by prior results showing the quadratic evolution of the annual incidence with age (Jones et al. 1987; Davies et al. 1995, Alcaïs et al. 1997), and logarithm of age, since the major increase in annual incidences was observed for younger ages. Results based either on the likelihood-ratio test, for the nested models, or on Akaike's (1974) information criterion (AIC), for the other models, confirmed that the best-fitting function was quadratic on age. Furthermore, to account for the major influence of native/migrant status on the hazard function (Alcaïs et al. 1997), this age function was allowed to vary with native/migrant origin of the subjects. As mentioned above, the model can also accommodate for covariate × genotype interaction; to avoid a dramatic increase of parameters, we considered only age x genotype interaction. The regression coefficients on age and age^2 will be noted as $\delta_g(s)$ and $\omega_g(s)$, respectively, where s is an indicative variable taking value 1 for natives and 2 for migrants.

If we take as an example a native female of genotype AA who has one affected parent, one unaffected parent, and one affected preceding sib, and if we also assume that, within the given interval, she is living 50 m from the forest and has only medium exposure, then the logit of her hazard function at age 20 years—that is, within the 21st 1-year interval (since, in a native, age is confounded with follow-up)—is

$$\theta_i(21) = \alpha_{AA} + \gamma_{P1} + \gamma_{P2} + \gamma_{C1} + \beta_{sex} + \beta_{med} + \beta^*_{dist}(\ln 50) + \delta_{AA}(1)^*(21) + \omega_{AA}(1)^*(21)^2 .$$

For migrants, the current age within a given interval is equal to the age of arrival in the endemic area, denoted as y, added to the duration of the follow-up, which is equal to k. For a migrant father with genotype aa, with no preceding relative, and 20 years of follow-up, if we assume that he had high exposure and lived 500 m from the forest during the first 5 years after his arrival in the endemic area and then moved to a house located 10 m from the forest, then the logit of his hazard function is

$$\theta_i(k) = \alpha_{aa} + \beta_{high} + \beta^*_{dist}(\ln 500) + \delta_{aa}(2)^*(y + k) + \omega_{aa}(2)^*(y + k)^2 \quad \text{for } k \leq 5$$

$$\theta_i(k) = \alpha_{aa} + \beta_{high} + \beta^*_{dist}(\ln 10) + \delta_{aa}(2)^*(y + k) + \omega_{aa}(2)^*(y + k)^2 \quad \text{for } 5 < k \leq 20 .$$

Finally, to take account of the ascertainment procedure in the likelihood formulation, we used the correction based on the report by Elston and Sobel (1979) and decribed in detail by Abel and Bonney (1990). Given the ascertainment scheme (complete selection of affected patients with at least two first-degree relatives living in the study zone), the probability of ascertainment, which in the present case can be defined as the probability that a living affected child leads to the selection of his nuclear family, was fixed at 1.

Estimation of parameters and the strategy of the test are based on maximum-likelihood methods. Nested models were compared by means of the likelihood-ratio test. Models were also compared by use of the AIC, which gives the best-fitting model as the one with minimum AIC, defined as AIC = −2log(likelihood) + 2(no. of estimated parameters). For example, evidence for a major-gene effect is obtained by rejecting a model with familial dependences alone when it is compared with a model with both a major-gene effect and residual familial dependences. Furthermore, the parent-offspring transmission of the major effect is parameterized in terms of the three classical transmission probabilities as defined by Elston and Stewart (1971): τ_{aaa}, τ_{Aaa}, τ_{AAa}, which denote the probabilities of transmitting a, for individuals aa, Aa, and AA, respectively. Mendelian transmission is obtained by setting $\tau_{aaa} = 1$, $\tau_{Aaa} = .5$, and

$\tau_{AAa} = 0$; in this case, the major effect is actually a major gene. Two additional models including a major effect are considered: (1) a model in which three types of individuals—aa, Aa, and AA—are specified but in which absence of parent-offspring transmission is obtained by setting $\tau_{aaa} = \tau_{Aaa} = \tau_{AAa}$, and (2) the more general transmission model of the major effect, in which the three τ's are free parameters between 0 and 1. Segregation of a major gene can be inferred if the two consecutive tests lead to the following results (Demenais et al. 1986, 1992; Demenais and Abel 1989): (1) failure to reject the Mendelian transmission of the major effect when compared with the general transmission model and (2) rejection of the nontransmission hypothesis when compared with the general transmission model.

All computations were performed by use of a FORTRAN program that we developed, which was linked to the MAXFUN optimization subroutine of the software package SAGE (Elston et al. 1986). The optimization process was performed under the following constraints concerning the α_g and the Γ_{ij} parameters: $\alpha_{AA} \leqslant \alpha_{Aa} \leqslant \alpha_{aa}$ (where a is the deleterious allele) and $\gamma_{ij2} \leqslant 0 \leqslant \gamma_{ij1}$.

Three successive segregation analyses were conducted, one on the whole sample, one on the migrant sample, and one on the native sample. A test of the homogeneity of the sample was performed under a given fitting model by calculating twice the logarithm of the difference between the likelihood of the overall data and the summed likelihood of the two subsamples.

Results

Segregation Analysis of the Migrant Sample

Since the most interesting results were observed in the migrant sample, the analysis of the 77 migrant families will be presented first (table 2). There was strong evidence for familial dependences (model I vs. II, $\chi^2(3\text{ df}) = 32.6$, $P < 10^{-6}$), and, in presence of these familial dependences, a codominant major-gene effect was significant (model II vs. IIIb, $\chi^2(3\text{ df}) = 12.6$, $P < .006$), with genetic parameters converging toward recessive values (model IIIb = IIIc). The hypothesis of a dominant major gene (IIIa vs. IIIb) was rejected ($\chi^2(1\text{ df}) = 9$, $P = .003$). In the presence of a recessive major gene and residual familial dependences, there was evidence for an age × genotype interaction (IIIc vs. IIId, $\chi^2(2\text{ df}) = 1.3$, $P < 10^{-3}$). The hypothesis of no residual familial dependences (model IIId vs. IIIe) for the recessive major gene was rejected. In the presence of familial dependences, the Mendelian transmission of the recessive major effect (model IIId vs. V) was compatible with the data ($\chi^2(3\text{ df}) = 4.6$, $P > .2$), even with a conservative test, when the use of 2 df as one of the estimated parameters reached a bound, and the hypothesis of nontransmission of the recessive major effect (model IV vs. V) was rejected ($\chi^2(2\text{ df}) = 13.4$, $P < .004$). In conclusion, a recessive major gene with age × genotype interaction and residual familial dependences accounted for the distribution of ages at onset of cutaneous lesions among migrant families. The frequency of the susceptibility allele, a, was estimated as .13, indicating that ~2% of individuals are predisposed to tegumentary leishmaniasis. Estimation of parameters accounting for covariate effects is shown in table 3. Exponential of these parameters corresponds to the odds ratio (OR) of the hazard function between different levels of the covariate, given all the explanatory variables. The OR is .61 for females versus males, 1.78 for subjects living ⩽10 m from the forest versus subjects living ⩾100 m from the forest, and 2.77 for subjects with high exposure versus subjects with low exposure. The cumulative penetrances with age and different levels of covariates are shown in figure 2*a,* for aa subjects, and in 2*b,* for aA/AA individuals. The cumulative penetrances with age according to different ages of arrival in the endemic area are shown in figure 3*a,* for aa subjects, and in 3*b,* for aA/AA individuals.

Segregation Analysis of the Native and of the Whole Sample

In the whole sample (table 4), there was evidence for two distinct age functions, when age among natives and migrants was taken into account (model Ib vs. Ia, $\chi^2(2\text{ df}) = 26.1$, $P < 10^{-5}$). A recessive major effect with age × genotype interaction and residual sib-sib dependences was found to be significant. However, the Mendelian transmission of this recessive major effect was rejected (model IIIc vs. IV, $\chi^2(3\text{ df}) = 11.8$, $P = .01$). Similar results were obtained with the native sample (table 5), with evidence for a recessive major effect with age × genotype interaction and residual sib-sib dependences, but the Mendelian transmission of this recessive major effect was rejected (model IIIa vs. V, $\chi^2(3\text{ df}) = 8.1$, $P = .04$). Finally, under the recessive-major-gene model shown in table 3, the hypothesis of homogeneity of the sample according to ethnic origin of the families was rejected ($\chi^2(10\text{ df}) = 24.6$, $P = .006$).

Discussion

The results of our segregation analysis of the migrant sample showed a recessive major gene controlling the onset of the primary cutaneous lesion in MCL due to *L. braziliensis*. Figure 2*a* indicates that for susceptible (i.e., aa) migrant subjects born in the endemic area (mainly children of migrants), the penetrance for this cutaneous lesion is complete by age 15–25 years,

Table 2

Results of Segregation Analysis of 77 Migrant Families

Model and Hypothesis	α_{aa}	α_{Aa}	α_{AA}	q	τ_{aaa}	τ_{Aaa}	τ_{AAa}	γ_{P2}	γ_{P1}	γ_{C1}	$\delta_{aa}(2)$	$\omega_{aa}(2)$	$\delta_{AA}(2)$[a]	$\omega_{AA}(2)$[a]	$-2\ln L + c$[b]
I. Sporadic	−3.47	[=α_{aa}]	[=α_{aa}]	[0]	. . .	. . .	. . .	[0]	[0]	[0]	.083	−.0014	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	6.1
II. Familial dependences	−5.78	[=α_{aa}]	[=α_{aa}]	[0]	. . .	. . .	. . .	−.05	1.17	.67	.155	−.0022	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	27.5
III. Mendelian major gene:															
a. Dominant and residual familial dependences	−1.35	[=α_{aa}]	−5.39	.00	[1]	[.5]	[0]	−.04	1.48	.67	.177	−.0025	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	23.9
b. Codominant and residual familial dependences	−1.96	−7.16	−7.16	.09	[1]	[.5]	[0]	−.07	2.01	.86	.241	−.0032	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	14.9
c. Recessive and residual familial dependencs	−1.96	−7.16	[=α_{Aa}]	.09	[1]	[.5]	[0]	−.07	2.01	.86	.241	−.0032	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	14.9
d. Recessive and residual familial dependences and genotype × age	−6.10	−6.81	[=α_{Aa}]	.13	[1]	[.5]	[0]	−.04	1.90	.81	.775	−.0191	.194	−.0025	4.6
e. Recessive and genotype × age	−2.47	−4.79	[=α_{Aa}]	.45	[1]	[.5]	[0]	[0]	[0]	[0]	.272	−.0053	.111	−.0008	28.3
IV. Nontransmitted recessive major effect with genotype × age and residual familial dependences	−4.04	−4.33	[=α_{Aa}]	.00	.71	[=τ_{aaa}]	[=τ_{aaa}]	−1.03	.55	.98	.171	−.0000	.077	−.0011	13.4
V. General transmitted recessive major effect with genotype × age and residual familial dependences	−1.69	−4.34	[=α_{Aa}]	.18	.76	.85	→0	−1.25	→0	.61	.363	−.0067	.137	−.0013	0

NOTE.—All models include four additional parameters that take into account covariate effects that are not shown. An arrow (→) indicates that the parameter reached a bound; and an ellipsis (. . .) indicates that the parameter is useless in the model.

[a] AA = AA or Aa subjects.

[b] c = 961.1.

according to environmental-factors level. Under this model, all cases occurring in persons born in the endemic area at age >25 years of age should therefore be considered sporadic. Figure 3*a* and figure 3*b* show that the cumulative penetrance remains higher in aa subjects than in Aa/AA subjects, for different ages of arrival in the endemic area. However, for an arrival at age 30 years, this penetrance becomes incomplete, and the difference between aa subjects and Aa/AA subjects is less important (.26 vs. .13 at 50 years of age). These results are consistent with the prior segregation analysis (Shaw et al. 1995), which found that individuals affected at an early age were genetically more susceptible than those affected at a later age, and they suggest that the genetic component controlling susceptibility to tegumentary leishmaniasis could concern mechanisms involved in the development of individual protection during childhood. Furthermore, the genetic

Table 3

Data Used for Homogeneity Test: Parameter Estimates (Standard Error) and Likelihood under Recessive-Major-Gene Model with Genotype × Age Interaction and Residual Familial Dependences, in Whole, Migrants, and Native Samples

	α_{aa}	$\alpha_{Aa/AA}$	q	γ_{P2}	γ_{P1}	γ_{C1}	β_{sex}	β_{dist}	β_{med}	β_{high}	$\delta_{aa}(1)$	$\omega_{aa}(1)$	$\delta_{aa}(2)$	$\omega_{aa}(2)$	$\delta_{AA}(1)$[a]	$\omega_{AA}(1)$[a]	$\delta_{AA}(2)$[a]	$\omega_{AA}(2)$[a]	2lnL
Global	−4.9	−6.9	.48	−.12	.39	.61	−.78	−.28	.15	.92	.061	−.0003	.330	−.0059	.387	−.0090	.137	−.0008	−1,819.3
	(.13)	(.28)	(.016)	(.022)	(.04)	(.071)	(.041)	(.011)	(.051)	(.003)	(.014)	(.0001)	(.019)	(.0005)	(.032)	(.001)	(.021)	(.0003)	
Migrants	−6.1	−6.8	.13	−.04	1.90	.81	−.49	−.29	.02	1.02	. . .	. . .	.775	−.0191	. . .	. . .	.194	−.0025	−965.7
	(.46)	(.19)	(.031)	(.013)	(.11)	(.057)	(.018)	(.012)	(.045)	(.052)			(.049)	(.0031)			(.004)	(.0004)	
Natives	−5.8	−9.0	.59	→0	.04	.42	−.78	−.28	.52	.61	.148	−.0013	. . .	. . .	.721	−.0202	. . .	. . .	−829
	(.29)	(.20)	(.021)		(.01)	(.014)	(.042)	(.026)	(.026)	(.049)	(.012)	(.0002)			(.024)	(.001)			

NOTE—An arrow (→) indicates that the parameter reached a bound; and an ellipsis (. . .) indicates that the parameter is useless in the model.

[a] AA = AA or Aa subjects.

cases could explain, at least in part, the excess of risk observed, in a previous study (Alcaïs et al. 1997), among migrants versus natives, since this excess mainly occurred at younger ages. In this earlier study, the evolution of the hazard function with age was estimated by a nonparametric method and displayed a quadratic shape with a peak around adolescence. This evolution is consistent with that predicted in the present study, which uses a parametric quadratic function. In addition to a recessive major gene, residual familial dependences were found to be significant and could indicate either the presence of unmeasured family-shared environmental factors or other genetic factors, as suggested by a two-locus model used in Shaw et al. (1995). Figure 2 also underlines the importance of covariate effects, which were estimated simultaneously with the genetic parameters. It is interesting to note that the present estimations are similar to those obtained with a family-stratified Cox model, which does not account for genetic factors (Alcaïs et al. 1997).

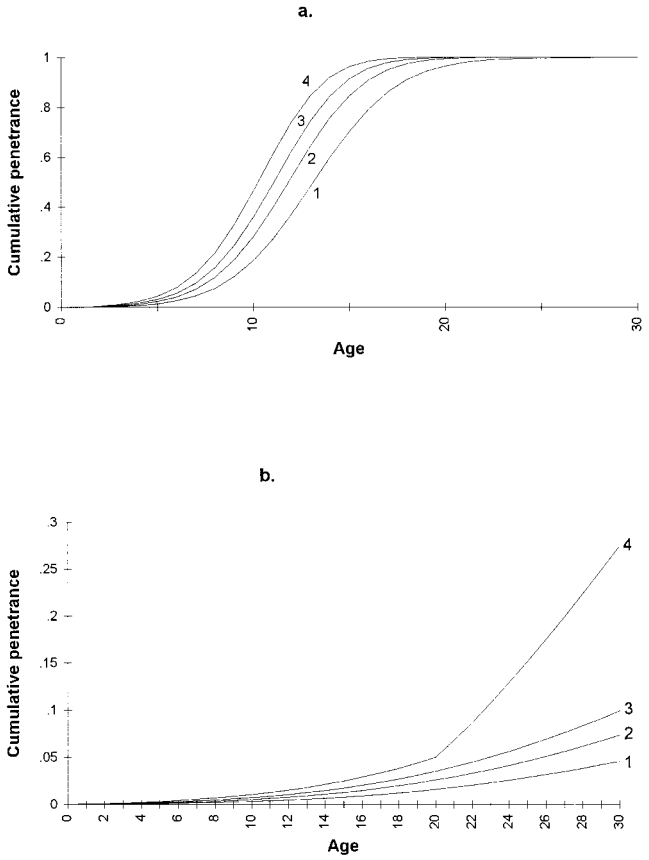

Figure 2 Evolution of cumulative penetrance, with age, for (*a*) aa subjects and (*b*) Aa/AA subjects. Curve 1 refers to a female with low exposure who is living 500 m from the forest and who has no affected relatives; curve 2 refers to a male with low exposure who is living 500 m from the forest and who has no affected relatives; curve 3 refers to a female with low exposure who is living 500 m from the forest and who has one affected older sib; and curve 4 refers to a male living 50 m from the forest who has had low exposure until 10 years of age, has had medium exposure at age 10–20 years, and who thereafter has had high exposure.

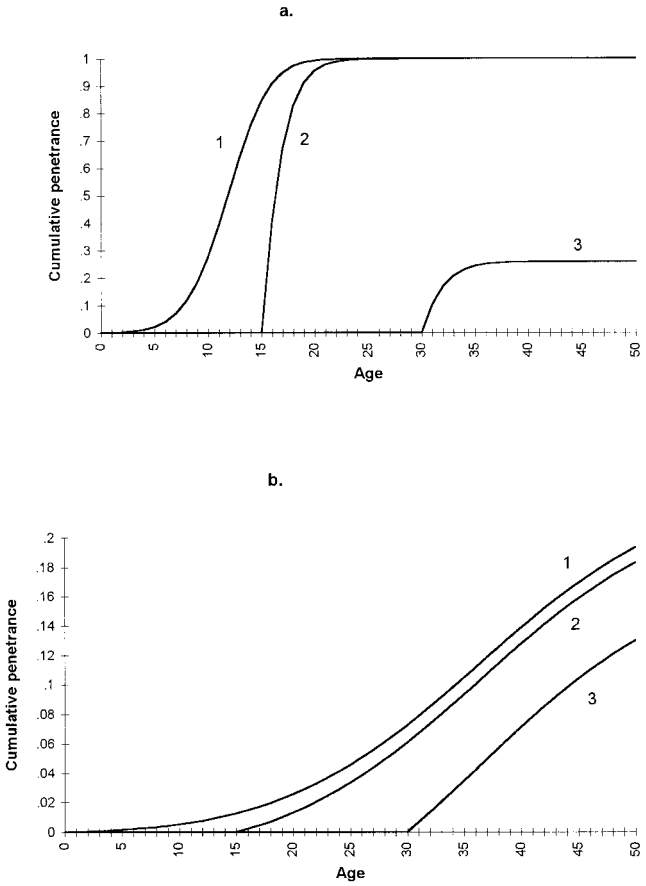

Figure 3 Evolution of the cumulative penetrance, with age, for (*a*) an aa male with low exposure and (*b*) an Aa/AA male with low exposure who are living 500 m from the forest and have been in the endemic area since birth (*curve 1*), since age 15 years (*curve 2*), and since age 30 years (*curve 3*).

Our segregation analyses of the native sample showed a recessive major effect with residual familial dependences, which is not compatible with Mendelian transmission. Moreover, under the hypothesis of a recessive major gene, homogeneity among natives and migrants was rejected. Figure 1 shows important differences, between natives and migrants, in the distribution of times of onset of CL, particularly for times of onset that are directly comparable for natives and for migrants born in the study zone. There is a great proportion of short times to onset (<10 years) in this latter subsample, which is not observed among natives, and these early-affected individuals could correspond to genetic cases. In addition to the genetic component

Table 4

Results of Segregation Analysis of Whole Sample

Model and Hypothesis	α_{aa}	$\alpha_{Aa/AA}$	q	τ_{aaa}	τ_{Aaa}	τ_{AAa}	γ_{P2}	γ_{P1}	γ_{S1}	$\delta_{aa}(1)$	$\omega_{aa}(1)$	$\delta_{aa}(2)$	$\omega_{aa}(2)$	$\delta_{AA}(1)$[a]	$\omega_{AA}(1)$[a]	$\delta_{AA}(2)$[a]	$\omega_{AA}(2)$[a]	$-2\ln L + c_b$
I. Sporadic:																		
a. $(\delta_1, \omega_1) \neq (\delta_2, \omega_2)$	−4.63	[=α_{aa}]	[0]	...	...	...	[0]	[0]	[0]	.047	−.0021	.12	−.0020	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	82.9
b. $(\delta_1, \omega_1) = (\delta_2, \omega_2)$	−5.37	[=α_{aa}]	[0]	...	...	...	[0]	[0]	[0]	.080	−.0010	[.08]	[−.001]	[=$\delta_{aa}(1)$]	[=$\delta_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\delta_{aa}(2)$]	109
II. Familial dependences	−5.28	[=α_{aa}]	[0]	[1]	[.5]	[0]	−.07	.43	.62	.067	−.0008	.16	−.0020	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	54.8
III. Mendelian major gene:																		
a. Recessive and γ_{P01}, γ_{P02}, and γ_{EE1}	−3.32	−5.70	.25	[1]	[.5]	[0]	−.02	.32	.63	.096	−.0011	.17	−.0023	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	46
b. Recessive and γ_{EE1}	−3.84	−6.38	.31	[1]	[.5]	[0]	[0]	[0]	.69	.121	−.0015	.18	−.0024	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	48.2
c. Recessive and γ_{EE1} and genotype × age	−4.52	−6.90	.44	[1]	[.5]	[0]	[0]	[0]	.52	.030	−.0000	.31	−.0056	.364	−.0079	.145	−.001	11.8
d. Recessive	−3.37	−5.92	.33	[1]	[.5]	[0]	[0]	[0]	[0]	.101	−.0013	.15	−.0022	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	[=$\delta_{aa}(2)$]	[=$\omega_{aa}(2)$]	66.5
IV. General transmitted recessive major effect with genotype × age and residual familial dependences	−4.26	−6.15	.85	.21	.92	→0	[0]	[0]	.46	.013	−.0000	.42	−.0140	.26	−.0051	.198	−.002	0

NOTE.—All models include four additional parameters that take into account covariate effects that are not shown. An arrow (→) indicates that the parameter reached a bound; and an ellipsis (. . .) indicates that the parameter is useless in the model.

[a] AA = AA or Aa subjects.

[b] c = 1,812.7.

Table 5

Results of Segregation Analyais of 41 Native Families

Model and Hypothesis	α_{aa}	α_{Aa}	α_{AA}	q	τ_{aaa}	τ_{Aaa}	τ_{AAa}	γ_{P2}	γ_{P1}	γ_{C1}	$\delta_{aa}(1)$	$\omega_{aa}(1)$	$\delta_{AA}(1)$[a]	$\omega_{AA}(1)$[a]	$-2\ln L + c$[b]
I. Sporadic	−5.21	[=α_{aa}]	[=α_{aa}]	[0]	. . .	. . .	. . .	[0]	[0]	[0]	.078	−.0011	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	42
II. Familial dependences	−5.54	[=α_{aa}]	[=α_{aa}]	[0]	. . .	. . .	. . .	→0	.01	.48	.088	−.0012	[=$\delta_{aa}(1)$]	[=$\omega_{aa}(1)$]	33.7
III. Mendelian major gene															
a. Recessive and residual familial dependences (sib-sib) and genotype × age	−5.84	−9.04	[=α_{Aa}]	.59	[1]	[.5]	[0]	[0]	[0]	.42	.148	−.0013	.721	−.0202	8.1
b. Recessive and residual familial dependences (parents-offspring and sib-sib) and genotype × age	−5.84	−9.04	[=α_{Aa}]	.59	[1]	[.5]	[0]	→0	.04	.42	.148	−.0013	.721	−.0202	7.6
IV. Nontransmitted recessive major effect with genotype × age and residual familial dependences	−6.31	−9.04	[=α_{Aa}]	.52	.62	[=τ_{aaa}]	[=τ_{aaa}]	[0]	[0]	.42	.200	−.0019	.773	−.0238	6.7
V. General transmitted recessive major effect with genotype × age and residual familial dependences	−5.94	−1.99	[=α_{Aa}]	.70	.40	.89	→0	[0]	[0]	.43	.294	−.0049	.837	−.0408	0

NOTE.—All models include four additional parameters that take into account covariate effects that are not shown. An arrow (→) indicates that the parameter reached a bound; and an ellipsis (. . .) indicates that the parameter is useless in the model.

[a] AA = AA or Aa subjects.

[b] c = 821.4.

mentioned above, environmental and behavioral factors should contribute to the excess of risk in the migrant sample. As an example, migrants engage in significant deforestation activity, whether during their installation or during their agricultural activities, whereas natives have a less aggressive activity toward the forest. Furthermore, natives generally light a smoky fire by burning plants during the night, to keep blood-sucking insects away, whereas this habit is unknown among migrants. Results in the whole sample can be explained by merging the results obtained in the two subsamples. The inclusion of natives in the whole sample may have created a background noise that made difficult the detection of a major-gene effect among migrants.

The present study, showing a recessive major gene controlling susceptibility to tegumentary leishmaniasis among the migrant sample, is a contribution toward an understanding of the complex mechanisms regulating expression of the disease. In particular, it is important that further genetic studies of CL and MCL—in particular, linkage analyses—focus on recently settled families and younger subjects. Several candidate regions should be investigated, such as the HLA and TNF-α/TNF-β gene region; the human 17q chromosomal region, which is homologous to the mouse region containing *Scl-1;* the human gene *NRAMP1,* located on 2q35; and the 5q21-q33 region, which is the human homologue of the proximal end of mouse chromosome 11 known to control the later phases of lesion growth in *L. major* infection (Shaw et al. 1995). This latter human region could be of particular interest, since it recently has been linked to a locus that controls intensity of infection by the parasite *Schistosoma mansoni* (Marquet et al. 1996).

Acknowledgments

Computations performed for this work used the subroutine MAXFUN of the program package S.A.G.E., which is supported by U.S. Public Health Service resource grant 1 P41 RR03655 from the Division of Research Resources.

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