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Genetic Control of Susceptibility to Leprosy in French Polynesia; No Evidence for Linkage with Markers on Telomeric Human Chromosome 2[1]

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Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and afflicts an estimated 5.5 million people in the world ([24]). This disease can cause permanent and extensive deformities of the skin and peripheral nerves and, thus, has serious social and economic consequences. Most individuals infected with *M. leprae* develop effective immunity without clinically evident forms of the disease; whereas those people who develop leprosy present different spectral manifestations, ranging from the tuberculoïd to the lepromatous pole accompanied by a gradual decrease of cell mediated immunity ([34]).

The possible role of the host genetic background in resistance and susceptibility to leprosy is supported by several lines of epidemiological evidence. These include the

variable incidence rate of leprosy among certain ethnic groups ([3, 43]) and the restriction of the disease to certain familial clusters living in endemic areas ([3, 31, 40]). In addition, the concordance rate for leprosy was shown to be significantly higher among monozygotic twins (60%–85%) than among dizygotic twins (15%–20%) ([6, 23]). Segregation analyses have provided more conclusive evidence regarding the influence of genetic factors and the mode of inheritance of the disease trait in the etiology of leprosy ([1, 17, 42, 48]). The mode of inheritance has been found to vary among populations. For all forms of leprosy (leprosy *per se*) a nearly dominant mode of inheritance was found in Thailand ([48]), while a recessive or codominant mode of inheritance was found in Desirade Island ([1]). In The Philippines, segregation of a recessive susceptibility gene was found for lepromatous leprosy ([42]), while recessive inheritance of nonlepromatous leprosy was concluded in India ([17]). The complex segregation analysis performed in the Desirade study also revealed the presence of incomplete penetrance and phenocopies. Overall, predisposition to leprosy appears to be of a multifactorial nature with at least two groups of genes participating in the control of the

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TABLE 1. *Characteristics of DNA polymorphisms for chromosome 2q markers studied.*

Location	Locus	Probe	Poly-morphism	Allele sizes (frequencies)		References
2q33-q35	CRYGP1	P5G1	TaqI	3.5/3.3	(0.88/0.12)	[41]
				2.3/1.1	(0.12/0.79)	
				2.0/1.4	(0.41/0.49)	
		P16G3	HindIII	13.0/11.5/8.4		[41]
				(0.26/0.46/0.28)		
2q34-q35	FN	FN61039	MspI	2.7/0.35	(0.79/0.21)	[14]
			HaeIII	2.0/1.5	(0.8/0.92)	[7]
2q35-q36	TNP1	Tp1	TaqI	12.6/8.0	(0.57/0.43)	[19] and this study
				7.7/6.2	(0.03/0.97)	
			MspI	3.9/3.6	(0.34/0.66)	[19] and this study
2q35-q36	VIL	E84	TaqI	1.9/1.0	(0.26/0.74)	[46] and this study
		E62	BglII	18.0/5.0	(0.64/0.36)	[46] and this study
		pEx2	MspI	9.4/6.5	(0.88/0.12)	[30]
		pJL	BglII	9.4/6.0	(0.88/0.12)	This study
2q35	DES	Des	EcoRV	12.0/8.0	(0.50/0.50)	[38]
2q33-qter	INH	Inhb	TaqI	2.6/1.5	(0.778/0.23)	This study
2q37	PAX3		[CA]n			[50]
2q37	UGT1A1	UGT1A1	MspI	4.0/2.7	(0.94/0.06)	[45] and this study
				2.6/1.8	(0.71/0.29)	

disease: non-HLA (human leukocyte antigen)-linked genes which determine susceptibility to leprosy *per se* and HLA-linked genes which are implicated in the spectral clinical manifestations of the disease ([1]). Population and family studies have shown that tuberculoid leprosy was associated with HLA-DR2 in India ([8]), in Japan ([22]) and in Thailand ([35]) or with HLA-DR3 in Surinam ([28]), while lepromatous leprosy was associated with HLA-DQw1 in Venezuela ([29, 44]).

In the mouse, innate resistance and susceptibility to several pathogens including mycobacteria is controlled by a single autosomal gene termed *Bcg* which is expressed in two allelic forms, the dominant resistance allele and the recessive susceptibility allele ([16]). A high resolution genetic map of the *Bcg* region on proximal mouse chromosome 1 has revealed the gene order: centromere-*Col3a1-Cryg-Fn-1-Tp-1-Vil/Bcg-Des-Inha-Pax3-Akp3-Acrg-Sag-Col6a3* ([11, 20, 21, 36-38]). A 35-cM fragment around the murine *Bcg* locus (from *Col3a1* to *Col6a3*) has been conserved between the murine chromosome 1 and the telomeric end of human chromosome 2q, region 2q32-2q37 ([38]). Moreover, the gene order appears to be conserved among this group of syntenic loci ([38, 39]). Thus, a human homolog for the *Bcg* locus could be located on chromosome 2q, region q32-37, which defines the main target area for the search of human *Bcg* homolog.

In French Polynesia, a monitoring and prevention policy for leprosy control has been implemented since the end of the 1940s. The prevalence rate decreased from 2.40/1000 in 1946 to 0.48/1000 in 1987. Despite this decline, leprosy is still endemic and considered as a public health problem ([5]). Furthermore, French Polynesia represents a geographic area where the history of leprosy families is well documented.

The objective of this study was to investigate, in French Polynesian families, the presence of a gene that could determine susceptibility to leprosy and be located on human chromosome 2q using a sib-pair method and the logarithm of odds (LOD) score method.

MATERIALS AND METHODS

Patients and families

Seven multicase pedigrees with two (4 families) or three (3 families) generations and with at least one affected parent were identified in 1990. Families were identified from the records of the Institut Territorial Louis Malardé in Tahiti, French Polynesia, where a central register has kept all information on leprosy patients since 1902. For each leprosy patient, a file is kept indicating

civil status, date and place of leprosy detection as well as the results of clinical, microbiological and pathological examination and the nature and duration of treatment. Leprosy patients are classified according to the Ridley-Jopling classification ([32]). Since 1982, multidrug therapy (MDT) has been implemented with daily administration of dapsone and rifampin for paucibacillary patients and a daily supplement of clofazimine during the first 12 months and of ethionamide for the first 2 months for multibacillary cases of leprosy.

All families were examined for possible nonpaternity by showing the segregation of the blood groups, the highly polymorphic genetic markers D2S53 ([25]) and D2S104 ([10, 49]), and other markers used in this study. Inconsistencies were found for four individuals, who were excluded from the study.

The entire study group of 84 individuals comprised a total of 39 leprosy patients, including 19 paucibacillary (indeterminate, BT, TT forms) and 20 multibacillary (BL and LL forms) patients. The leprosy patients comprised 24 males and 15 females with a mean age at onset of 27 years.

DNA preparation

A 10-ml, heparinized peripheral blood sample was collected from each patient following informed consent. Lymphocytes were immortalized by transformation with Epstein-Barr virus ([2]). Genomic DNA was prepared according to standard protocols using SDS lysis, proteinase K digestion, phenol/chloroform extraction and ethanol precipitation ([33]).

Typing

Individual family members were typed at 8 polymorphic loci (18 allelic systems) located on human chromosome 2q (Table 1). These marker loci were previously mapped on mouse chromosome 1 ([11, 20, 36-38, 45]).

Restriction fragment length polymorphism (RFLP) analysis. Five μg samples of DNA were digested to completion with 50 U of the appropriate restriction enzyme according to the recommendations of the manufacturer (Pharmacia; New England Biolabs, Beverly, Massachusetts, U.S.A.). The DNA fragments were separated in a 1% agarose gel, except for *Hae*III digested frag-

ments which were separated in a 1.4% agarose gel. Gels were transferred to N-Hybond membranes (Amersham, U.K.) as previously described ([33]). Southern blots were prehybridized at 65°C in hybridization solution (6× SSC, 5× Denardt's, 0.1% SDS and 100 mg/ml boiled salmon sperm DNA). For mouse exon probes E84 or E62, prehybridization and hybridization were at 54°C using the hybridization solution described above. The probes described in Table 1 were labelled to a high specific activity (5×10^8 cpm/μg DNA) with [α-^{32}P-dATP] (Dupont, NEN Research Products, Boston, Massachusetts, U.S.A.) using the random hexanucleotide method ([12]). Membranes were washed as follows: twice with 2× SSC and 0.1% SDS at room temperature for 15 min and twice with 0.5× SSC and 0.1% SDS at the hybridization temperature for 15 min. Membranes were exposed for 2 days at −70°C, using Kodak XAR films and Dupont Lightning-Plus intensifying screens.

Polymorphism analysis of *PAX3*. [CA] dinucleotide repeat polymorphisms were detected for the *PAX3* gene after PCR amplification ([50]). Amplification was performed in a total volume of 15 μl containing 250 ng of genomic DNA, 9 pmol of each primer, 0.05 mM TrisHCl pH8, 1 mM MgCl$_2$, 0.05% Nonidet P40, 0.005% Tween 20, 200 μM each of dCTP, dGTP, and dTTP, 30 μM dATP, 0.6 μCi ^{35}S dATP and 3.5 units of *Taq* polymerase. Samples were processed through one step of denaturation (94°C for 5 min), followed by 40 cycles of denaturation (94°C for 1 min), annealing (54°C for 1 min), elongation (72°C for 1 min) and a last step of elongation (72°C for 7 min). Amplification products were electrophoresed for 3–4 hr on a 6% polyacrylamide denaturing gel. Gels were dried and exposed for 2 days at room temperature on Kodak XAR-film.

All genotypes were interpreted from the autoradiographs by two independent observers.

Linkage analyses

Linkage analyses were carried out using a sib-pair method and the LOD score method. For the analyses, susceptibility to leprosy *per se* (any form of leprosy) was used as the phenotype to be analyzed.

TABLE 2. *Estimated liability classes used for linkage analysis.*

Age (yr)	aa or Aa[a]	AA[a]	
		BCG vaccinated	Not BCG vaccinated
0–9	0.00	0.091	0.152
10–19	0.00	0.181	0.302
20–29	0.00	0.278	0.463
30–39	0.00	0.524	0.582
40–49	0.00	0.584	0.649
50–59	0.00	0.646	0.719
> 60	0.00	0.675	0.750

[a] A represents the deleterious allele.

Sib-pair method. The nonparametric statistical method described by de Vries, *et al.* ([9]) was used to detect the segregation of parental haplotypes in sibships with at least two affected siblings. The observed difference (D) between the number of affected siblings with one and those with the other parental haplotype was compared with the expected difference (d) for random segregation using the statistic test: $(|\Sigma D - \Sigma d| - \frac{1}{2})^2/\Sigma \sigma_d^2$ (σ_d^2 is the variance of d and Σ d and Σ D the summations of d and D values for all sibships). This test has a chi-squared distribution of 1 df. Two different haplotypes were tested: the haplotype including *CRYGP1* and *FN* and the haplotype including *VIL* and *DES*. These haplotypes were chosen because of the absence of recombination events, except for one patient who was excluded from the analysis with the *CRYGP1-FN* haplotype and another for the analysis with the *VIL-DES* haplotype. For these analyses, the pedigrees were divided into nuclear families. Nuclear families with at least one heterozygous parent were tested: the *CRYGP1-FN* haplotype was assessed in 7 and the *VIL-DES* haplotype in 8 multiple-case nuclear families. All possible sib pairs were considered when more than two affected siblings were present.

LOD score method. Linkage analysis was carried out using the LINKAGE 5.1 computer program package ([18]). Two point LOD scores were calculated with the MLINK program using marker haplotypes for each locus, except for the *CRYGP1* locus. For this locus, recombination events have been detected between *CRYGP1* (P5G1) and *CRYGP1* (P16G3) (E. Schurr, unpublished

data). LOD scores obtained at different recombination fractions ($\Theta = 0$ to $\Theta = 0.5$) were added for all families. A LOD score of 3.0 is considered as significant evidence in favor of linkage, and a LOD score of -2.0 as evidence against linkage between the markers and the putative disease susceptibility locus at a given Θ value. From the Central Register, we determined the number of leprosy cases (affected or having been affected) in the French Polynesian population (2.22/1000). Under the assumption of Hardy-Weinberg equilibrium, we defined the disease gene frequency for each mode of inheritance: 0.0471 for the recessive, 0.0022 for the codominant, and 0.0011 for the dominant mode of inheritance.

Successive linkage analyses were performed: a) The three modes of inheritance with a range of assumed penetrance values from 100% to 40% for the susceptible allele as well as with affecteds only were compared. b) In addition, two-point analysis of the data was carried out using a complex model. The hypothesis was that a human homolog of the *Bcg* gene was located on chromosome 2q and that recessive alleles of this locus conferred susceptibility to leprosy with incomplete penetrance as suggested by Abel and Demenais ([1]). For heterozygotes, we assumed that the susceptibility allele was not expressed. The penetrance was considered to vary with age and BCG vaccination. Since leprosy has a variable age at onset, 10-year liability classes (frequencies of infection) were defined from the general population and the data recorded in the Central Register. Individuals were grouped by attained age in 1990 or at death. Different studies have shown a variation in the protective efficacity of BCG vaccination against leprosy ranging from 20% to 80% ([13]). A study carried out in New Guinea indicated a protection rate of about 40% for people under 30 years old ([4]). Since the New Guinea population and the French Polynesian population could be racially related, we assumed a 40% BCG protection rate for people under 30 years old and 10% for those older. Individuals were examined for evidence of BCG vaccination. For deceased people, the presence or absence of BCG vaccination was determined by medical records or interviewing the family members. Table 2 presents the parameters used for this anal-

TABLE 3. *Analysis of parental* CRYGP1-FN *or* VIL-DES *haplotype segregations observed in leprosy affected siblings.*

Parental haplotype	Observed (ΣD)	Expected (Σd)	$\Sigma \sigma^2_d$	χ^2	p Value
CRYGP1-FN	32	37	20.5	0.99	NS[a] (< 0.5)
VIL-DES	44	39	22.5	0.9	NS (< 0.5)

[a] NS = Not significant.

ysis. c) By considering all leprosy cases without affected relatives as nongenetic causes, we defined a 0.48 phenocopy rate. This parameter was introduced in the complex model described in b above. Allele frequencies for the RFLP markers listed in Table 1 were determined in 38 chromosomes from unrelated Polynesian individuals. For the *PAX3* marker, we considered 10 alleles with equal frequencies. Recombination frequencies were assumed to be equal in males and females.

RESULTS

In the mouse, the *Bcg* gene controls innate resistance to mycobacterial infections and is located on a syntenic linkage group which is conserved on human chromosome 2q ([38]). Therefore, we assumed that the human homolog of the *Bcg* gene was located on human chromosome 2q, and we evaluated 8 loci (18 allelic systems) for possible linkage with susceptibility to leprosy *per se*. The DNA of 84 patients from 7 multicase families (The Figure) was typed for polymorphic chromosome 2 markers using $[CA]_n$ repeats and RFLP analysis. The allele frequencies for the marker loci are presented in Table 1. Since the gene order on the syntenic fragment appears conserved between mouse and man ([38]), we constructed haplotypes using the following order: *CRYGP1-FN-TNP1-VIL-DES-INH-PAX3-UGT1A1*. For deceased individuals, haplotypes were deduced from the haplotypes of their offsprings and/or their parents.

Results with nonparametric method

The affected sib-pair method used in this study is based on the comparison of expected and observed identical by descent distribution. Two pairs and 11 trios were available for the *CRYGP1-FN* haplotype and 3 pairs and 7 trios for the *VIL-DES* haplotype. The probability of random pa-

rental haplotype segregation was calculated in Table 3 according to the statistical test described by de Vries, *et al.* ([9]). No significant deviation from random segregation was observed for the two haplotypes tested (p < 0.5).

Results with LOD score method

Although no evidence for the presence of a gene located on chromosome 2q conferring susceptibility to leprosy *per se* was suggested with the sib-pair method, we carried out LOD score analyses in order to confirm this result.

We first tested the three genetic models assuming a penetrance of 100%, 80% and 40% for the susceptible alleles. In addition, we used a penetrance-free model with affecteds only. Tables 4, 5 and 6 compare the LOD score values obtained for linkage between the putative leprosy susceptibility gene and all the gene markers. The LOD scores are predominantly negative regardless of the mode of inheritance and penetrance values. Under the recessive model, whatever the penetrance values assumed (100%, 80% and 40%), data provide evidence against the existence of a putative gene falling within 10 cM to 20 cM of VIL (LOD score value of −2 obtained at a recombination fraction between 0.1–0.2). Under the dominant model, there is also evidence against a putative gene falling within 5 cM to 20 cM of TNP1 (LOD score value of −2 obtained at a recombination fraction of 0.05–0.2). Considering the codominant model, data provide evidence against a putative gene falling within 5 cM to 10 cM of TNP1 with penetrance values of 100% and 80% (LOD score value of −2 obtained at a recombination fraction of 0.05–0.1). Results obtained with affecteds only are not conclusive assuming a recessive mode of inheritance of susceptibility to leprosy *per se* (LOD score values between −2 and +3).

TABLE 4. *LOD scores for linkage between chromosome 2q markers and susceptibility to leprosy* per se *assuming a recessive mode of inheritance of the disease trait.*

Locus	Recombination fraction (Θ)						
	0	0.01	0.05	0.1	0.2	0.3	0.4
CRYGP1(P5G1)							
100%	$-\infty$	−16.32	−8.21	−4.95	−2.09	−0.80	−0.18
80%	−11.58	−5.84	−3.57	−2.36	−1.08	−0.43	−0.1
40%	−4.55	−3.41	−1.99	−1.26	−0.55	−0.21	−0.05
Affecteds	−2.09	−1.85	−1.23	−0.80	−0.34	−0.13	−0.03
CRYGP1(P16G3)							
100%	$-\infty$	−11.11	−5.69	−3.45	−1.41	−0.50	−0.11
80%	−8.01	−4.12	−2.66	−1.80	−0.83	−0.32	−0.07
40%	−3.48	−2.63	−1.70	−1.18	−0.58	−0.24	−0.06
Affecteds	−1.78	−1.63	−1.21	−0.87	−0.45	−0.18	−0.05
FN							
100%	$-\infty$	−5.40	−2.67	−1.57	−0.62	−0.22	−0.05
80%	−2.98	−1.67	−0.92	−0.57	−0.24	−0.09	−0.02
40%	−1.55	−1.21	−0.71	−0.45	−0.20	−0.08	−0.02
Affecteds	−1.05	−0.90	−0.58	−0.38	−0.17	−0.07	−0.02
TNP1							
100%	$-\infty$	−8.49	−3.85	−2.06	−0.61	−0.09	0.03
80%	−7.63	−3.06	−1.68	−0.97	−0.30	−0.04	0.01
40%	−3.15	−2.11	−1.18	−0.71	−0.29	−0.10	−0.02
Affecteds	−1.53	−1.37	−0.95	−0.64	−0.30	−0.12	−0.03
VIL							
100%	$-\infty$	−17.08	−8.52	−5.07	−2.06	−0.75	−0.16
80%	−11.23	−8.95	−5.58	−3.65	−1.67	−0.67	−0.16
40%	−4.98	−4.49	−3.35	−2.35	−1.12	−0.45	−0.11
Affecteds	−2.81	−2.59	−1.99	−1.48	−0.76	−0.32	−0.08
DES							
100%	$-\infty$	−8.43	−3.81	−2.05	−0.65	−0.15	−0.01
80%	−9.72	−3.45	−1.87	−1.13	−0.43	−0.13	−0.02
40%	−3.37	−2.40	−1.34	−0.84	−0.36	−0.14	−0.03
Affecteds	−1.31	−1.19	−0.85	−0.58	−0.28	−0.11	−0.03
INH							
100%	$-\infty$	−4.26	−2.21	−1.37	−0.60	−0.24	−0.06
80%	−7.89	−3.26	−1.83	−1.18	−0.54	−0.22	−0.05
40%	−3.42	−2.48	−1.41	−0.89	−0.40	−0.16	−0.04
Affecteds	−1.82	−1.60	−1.06	−0.71	−0.33	−0.13	−0.03
PAX3							
100%	$-\infty$	−14.29	−6.39	−3.41	−1.08	−0.27	−0.03
80%	−15.77	−7.15	−3.56	−2.00	−0.66	−0.17	−0.02
40%	−5.97	−4.48	−2.66	−1.62	−0.62	−0.20	−0.04
Affecteds	−2.61	−2.32	−1.59	−1.05	−0.44	−0.15	−0.03
UGT1A1							
100%	$-\infty$	−7.82	−3.80	−2.22	−0.89	−0.32	−0.07
80%	−3.88	−3.31	−1.91	−1.20	−0.51	−0.19	−0.04
40%	−0.99	−0.99	−0.81	−0.54	−0.22	−0.07	−0.02
Affecteds	−0.20	−0.21	−0.22	−0.18	−0.09	−0.03	−0.01

Under a co-dominant or dominant model, there is no convincing evidence for the presence of a putative disease gene falling within 5 cM to 20 cM of the distal markers *CRYGP1* and *PAX3*. The reduction of the penetrance for the susceptible allele or analyses with affecteds only reduce the LOD score values, but these values remain predominantly negative and provide consistent evidence against linkage.

Although the results with simple models provide evidence against the presence of a
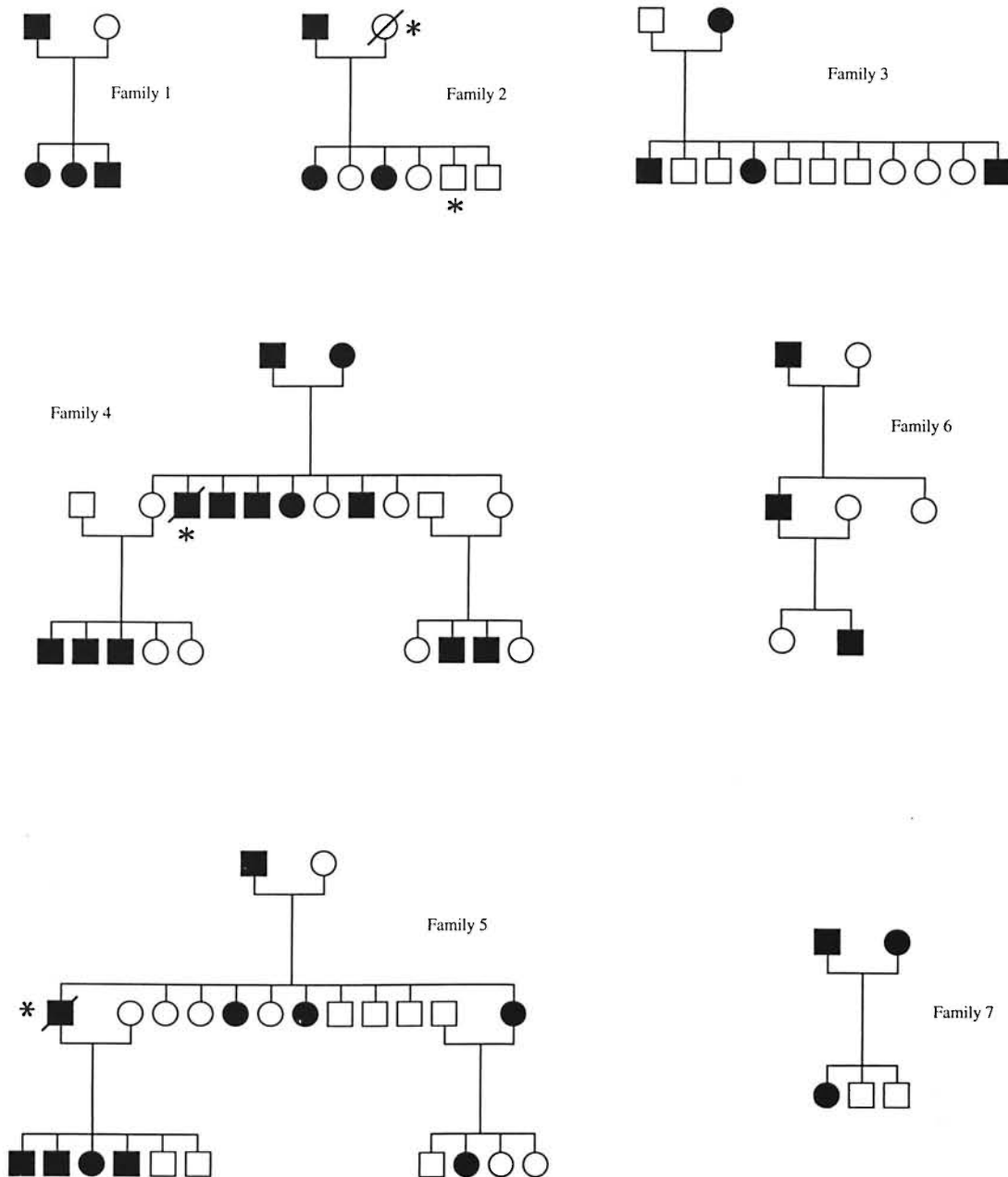
THE FIGURE.   Pedigrees of the seven families analyzed in this study. Solid symbol = affected subject; slash = subject deceased; * = subject not typed.

gene controlling the susceptibility to leprosy *per se* on human chromosome 2q, we tested a complex model. The assumptions for this model were a recessive mode of inheritance of the disease trait and an age-dependent and BCG-vaccination-dependent penetrance, inferred from previous studies ([1, 4]). The LOD score values obtained under these assumptions are presented in Table 6. Again, the LOD score values are predominantly negative. Evidence against the co-segregation of *VIL* with a gene controlling the susceptibility to leprosy *per se* within this region was provided (LOD score of −2 obtained at a recombination fraction between 0.1 and 0.2). The introduction of a

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TABLE 5. *LOD scores for linkage between chromosome 2q markers and susceptibility to leprosy* per se *assuming a co-dominant mode of inheritance of the disease trait.*

Locus	Recombination fraction (Θ)						
	0	0.01	0.05	0.1	0.2	0.3	0.4
CRYGP1(P5G1)							
100%	−15.40	−7.00	−4.57	−3.50	−2.10	−1.10	−0.43
80%	−7.08	−5.36	−3.66	−2.86	−1.88	−1.04	−0.42
40%	−5.42	−4.36	−2.08	−2.12	−1.36	−0.83	−0.36
Affecteds	−4.72	−3.91	−2.50	−1.80	−1.05	−0.61	−0.27
CRYGP1(P16G3)							
100%	−5.95	−4.28	−2.42	−1.48	−0.62	−0.24	−0.07
80%	−4.13	−3.22	−1.92	−1.20	−0.51	−0.20	−0.06
40%	−3.14	−2.64	−1.65	−1.05	−0.46	−0.19	−0.06
Affecteds	−2.79	−2.33	−1.49	−0.97	−0.44	−0.18	−0.05
FN							
100%	−2.42	−1.11	−0.51	−0.31	−0.17	−0.09	−0.02
80%	−1.84	−1.25	−0.69	−0.44	−0.22	−0.10	−0.02
40%	−1.55	−1.32	−0.85	−0.56	−0.26	−0.11	−0.03
Affecteds	−1.55	−1.38	−0.94	−0.63	−0.29	−0.12	−0.03
TNP1							
100%	−5.41	−4.14	−3.31	−2.80	−1.76	−0.92	−0.36
80%	−2.53	−2.46	−2.23	−1.98	−1.41	−0.80	−0.33
40%	−0.99	−0.98	−0.95	−0.90	−0.75	−0.51	−0.24
Affecteds	−0.35	−0.36	−0.37	−0.37	−0.34	−0.26	−0.14
VIL							
100%	−∞	−2.74	−1.14	−0.83	−0.33	−0.13	−0.05
80%	−2.25	−1.65	−0.96	−0.60	−0.27	−0.13	−0.06
40%	−1.69	−1.27	−0.73	−0.47	−0.24	−0.14	−0.07
Affecteds	−1.51	−1.19	−0.72	−0.48	−0.26	−0.15	−0.08
DES							
100%	−3.44	−2.42	−1.46	−0.95	−0.43	−0.16	−0.04
80%	−2.96	−2.21	−1.33	−0.85	−0.38	−0.14	−0.03
40%	−2.45	−1.94	−1.17	−0.74	−0.32	−0.12	−0.03
Affecteds	−2.10	−1.74	−1.06	−0.66	−0.28	−0.10	−0.02
INH							
100%	−2.74	−1.21	−0.60	−0.39	−0.23	−0.12	−0.03
80%	−2.66	−1.52	−0.88	−0.61	−0.32	−0.14	−0.04
40%	−2.45	−1.82	−1.16	−0.80	−0.39	−0.16	−0.04
Affecteds	−2.26	−1.90	−1.28	−0.88	−0.42	−0.17	−0.04
PAX3							
100%	−∞	−7.68	−4.83	−3.41	−1.84	−0.95	−0.39
80%	−9.51	−5.77	−3.75	−2.79	−1.63	−0.89	−0.38
40%	−6.74	−4.71	−2.91	−2.08	−1.24	−0.72	−0.32
Affecteds	−5.34	−4.15	−2.57	−1.76	−0.97	−0.54	−0.24
UGT1A1							
100%	−4.74	−3.24	−2.32	−1.68	−0.94	−0.53	−0.25
80%	−3.65	−2.56	−1.83	−1.39	−0.82	−0.47	−0.22
40%	−2.31	−1.76	−1.19	−0.90	−0.55	−0.39	−0.16
Affecteds	−1.59	−1.32	−0.88	−0.63	−0.36	−0.20	−0.09

phenocopy rate of 0.48 did not significantly modify these results (data not shown).

DISCUSSION

To test whether an innate susceptibility locus controlling susceptibility to leprosy is located on human chromosome 2q, the putative site of the human *Bcg* homolog, we have performed linkage analyses in multiple affected families of French Polynesia, a leprosy-endemic region. The results of our study do not provide evidence for the as-

TABLE 6. *LOD scores for linkage between chromosome 2q markers and susceptibility to leprosy* per se *assuming a dominant mode of inheritance of the disease trait.*

Locus	Recombination fraction (Θ)						
	0	0.01	0.05	0.1	0.2	0.3	0.4
CRYGPI(P5G1)							
100%	$-\infty$	-23.91	-12.41	-7.75	-3.56	-1.57	-0.52
80%	-14.12	-9.51	-6.90	-4.83	-2.45	-1.19	-0.45
40%	-8.79	-5.97	-4.09	-3.27	-2.06	-1.08	-0.43
Affecteds	-7.02	-4.89	-3.13	-2.39	-1.64	-0.99	-0.41
CRYGPI(P16G3)							
100%	$-\infty$	-8.99	-4.33	-2.48	-0.93	-0.31	-0.07
80%	-7.21	-3.77	-2.19	-1.38	-0.57	-0.20	-0.06
40%	-4.74	-3.14	-1.72	-1.05	-0.44	-0.18	-0.06
Affecteds	-3.82	-2.94	-1.75	-1.11	-0.50	-0.22	-0.07
FN							
100%	$-\infty$	-6.83	-3.47	-2.13	-0.94	-0.37	-0.09
80%	-2.77	-1.29	-0.62	-0.36	-0.17	-0.08	-0.02
40%	-2.32	-1.27	-0.65	-0.41	-0.20	-0.09	-0.02
Affecteds	-2.37	-1.42	-0.76	-0.48	-0.23	-0.10	-0.03
TNPI							
100%	$-\infty$	-19.74	-10.21	-6.37	-2.95	-1.33	-0.47
80%	-7.48	-7.16	-6.05	-4.43	-2.28	-1.10	-0.41
40%	-3.19	-3.08	-2.74	-2.40	-1.53	-0.81	-0.33
Affecteds	-1.85	-1.76	-1.52	-1.33	-1.02	-0.63	-0.28
VIL							
100%	$-\infty$	-8.42	-4.33	-2.67	-1.16	-0.45	-0.10
80%	-4.41	-2.80	-1.81	-1.23	-0.58	-0.24	-0.06
40%	-2.36	-1.46	-0.81	-0.52	-0.26	-0.14	-0.06
Affecteds	-1.78	-1.19	-0.62	-0.39	-0.20	-0.13	-0.07
DES							
100%	$-\infty$	-7.04	-3.64	-2.24	-0.99	-0.39	-0.09
80%	-5.04	-2.89	-1.82	-1.21	-0.57	-0.23	-0.05
40%	-3.16	-2.14	-1.26	-0.81	-0.36	-0.14	-0.03
Affecteds	-2.52	-1.91	-1.11	-0.69	-0.30	-0.11	-0.02
INH							
100%	$-\infty$	-9.63	-4.91	-3.01	-1.33	-0.52	-0.12
80%	-4.78	-1.93	-1.16	-0.79	-0.41	-0.19	-0.05
40%	-3.01	-1.61	-0.94	-0.64	-0.34	-0.15	-0.04
Affecteds	-2.43	-1.67	-1.00	-0.68	-0.35	-0.15	-0.04
PAX3							
100%	$-\infty$	-26.36	-13.47	-8.27	-3.66	-1.56	-0.51
80%	-17.12	-9.36	-6.29	-4.31	-2.20	-1.08	-0.42
40%	-10.04	-6.05	-4.03	-3.03	-1.73	-0.92	-0.39
Affecteds	-7.46	-5.14	-3.27	-2.41	-1.47	-0.84	-0.37
UGT1A1							
100%	$-\infty$	-18.07	-9.27	-5.74	-2.63	-1.20	-0.46
80%	-7.91	-5.47	-4.11	-2.96	-1.57	-0.81	-0.35
40%	-4.04	-2.77	-2.05	-1.56	-0.91	-0.51	-0.24
Affecteds	-2.68	-2.03	-1.44	-1.10	-0.66	-0.38	-0.17

sociation between leprosy *per se* and markers located on telomeric human chromosome 2q in these families.

Leprosy is a complex trait and, thus, linkage analysis of the disease is complicated by many factors including reduced pene-trance, genetic heterogeneity of the trait, gene interactions, and presence of phenocopies ([26]). To circumvent some of these difficulties, we tested a nonparametric sib-pair method, simple LOD score analysis models under different inheritance modes, and car-

TABLE 7. *Two point analysis between leprosy and markers on human chromosome 2q: LOD score results.*

Locus	Recombination fraction (Θ)						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
CRYGP1 (P5G1)	−5.31	−3.81	−2.17	−1.37	−0.60	−0.24	−0.06
CRYGP1 (P16G3)	−3.78	−2.71	−1.69	−1.15	−0.56	−0.23	−0.05
FN	−1.68	−1.33	−0.81	−0.53	−0.24	−0.1	−0.02
TNP1	−3.30	−2.11	−1.11	−0.65	−0.25	−0.09	−0.02
VIL	−5.66	−5.03	−3.50	−2.36	−1.10	−0.44	−0.10
DES	−4.07	−2.66	−1.44	−0.89	−0.38	−0.15	−0.04
INH	−3.13	−2.48	−1.38	−0.87	−0.38	−0.15	−0.04
PAX3	−6.75	−5.06	−2.89	−1.71	−0.65	−0.21	−0.04
UGT1A1	−1.60	−1.17	−1.07	−0.64	−0.24	−0.08	−0.02

ried out linkage analyses using affecteds only. The nonparametric affected sib-pair methods do not require specification of the genetic model if both parents are available for analysis ([27]), and they are robust to detect disease susceptibility loci which may have major or minor effects. However, the sample in our study was not of sufficient size to detect modifier effects of chromosome 2q loci. Our results with the affected sib-pair method described by de Vries, *et al.* ([9]), uses identity by descent and did not demonstrate linkage between *CRYGP1, FN, VIL* and *DES* and the disease phenotype. A second strategy we employed to detect linkage, provided the disease gene has a strong enough effect to be detected despite a possibly erroneous assumption made in the analysis, was to carry out LOD score analysis under simple modes of inheritance. We have tested three modes of inheritance with full and reduced penetrance. However, evidence remains consistently against the presence of a gene controlling leprosy *per se* on the region studied. Another strategy was to perform LOD score analysis using affecteds only to avoid the problem of reduced penetrance. Our results using this approach still did not provide any influence of a putative disease susceptibility locus in the region 2q33-q37. Finally, a complex model of linkage analysis was performed. In this model, we assumed a recessive mode of inheritance of susceptibility to leprosy *per se* as well as an age- and BCG-vaccination-dependent penetrance. Again, the results provide evidence against the presence in this region of a gene controlling susceptibility to leprosy *per se*.

A previous linkage analysis has been performed with 17 multicase Pakistani and Brazilian families typed for 9 markers in the 2q33-q37 region, including *FN, TNP1, VIL* and *DES* ([39]). The results of this study also excluded the presence of a putative disease susceptibility gene on the 2q33-q37 region, assuming a recessive mode of inheritance of susceptibility to leprosy *per se* and 100% penetrance. With reduced penetrance (80% and 60%) for the susceptibility allele, the presence of such a gene was excluded within the *TNP1-DES* region. Our data are in agreement with these observations.

Although our results did not reveal the influence of a putative gene in the chromosome 2q region on susceptibility to leprosy *per se* in the French Polynesian population, leprosy is a complex trait which could have a genetically different etiology in different families. With the low number of families included in this study, no genetic heterogeneity could be detected. Thus, it is possible that genetic linkage could have been masked by undetected heterogeneity ([27]). If only a proportion of the families (10% to 30%) are linked to a marker, the families that are unlinked will give negative evidence against linkage ([15]). One way to circumvent heterogeneity is to use large, multigenerational pedigrees so that only one form of the disease is likely to segregate.

Recently, a candidate gene for the murine *Bcg* gene, the *Nramp* gene, has been cloned ([46]). This gene encodes a putative integral membrane protein. Susceptibility to infection with *M. bovis* (BCG) seems to be associated with a substitution in the predicted second transmembrane domain. Additional linkage analyses between leprosy and the human homolog of this candidate gene in the French Polynesian population should

confirm or refute the results of our linkage analysis.

SUMMARY

Several lines of evidence have suggested a role of genetic factors in susceptibility to leprosy. In the mouse, natural susceptibility to infection with mycobacteria is controlled by the chromosome 1 *Bcg* locus, a region which is syntenic with a fragment of the human chromosome 2q, region q31-q37. It has been postulated that a human homolog of the *Bcg* gene controls susceptibility to leprosy *per se*, and may be located on chromosome 2q. In order to test the influence of this putative gene on leprosy *per se*, we performed linkage analyses in a set of seven multicase French Polynesian pedigrees, using an affected sib pair method and the LOD score method employing different modes of inheritance. Family members were typed for eight polymorphic loci on chromosome 2q: *CRYGP1, FN, TNP1, VIL, DES, INH, PAX3*, and *UGT1A1*. Our data provide evidence against the presence of a gene controlling susceptibility to leprosy *per se* on human chromosome 2q in the French Polynesian population.

RESUMEN

Hay varias líneas de evidencia que sugieren la participación de factores genéticos en la susceptibilidad a la lepra. En el ratón, la susceptibilidad natural a la infección por micobacterias está controlada por el locus *Bcg* del cromosoma 1, una región análoga a la región q31-q37 del cromosoma 2q humano. Se ha propuesto que un gene análogo al gene *Bcg* controla la susceptibilidad a la lepra *per se*, y que este gene podría estar localizado en el cromosoma 2q. Para probar la influencia de este gene putativo sobre la lepra *per se*, se hizo un análisis de ligamiento en 7 familias de la Polinesia francesa con casos múltiples de lepra, utilizando los métodos de análisis genético apropiados. Los miembros familiares se tipificaron en función de 8 loci polimórficos del cromosoma 2q: *CRYGP1, FN, TNP1, VIL, DES, INH, PAX3*, y *UGT1A1*. Los resultados no dieron evidencias de la presencia de genes, en el cromosoma 2q, que controlen la susceptibilidad a la lepra en la población de la Polinesia francesa.

RÉSUMÉ

Divers indices ont suggéré un role de facteurs génétiques dans la susceptibilité à la lèpre. Chez la souris, la susceptibilité naturelle à l'infection par des mycobactéries est sous le controle du locus *Bcg* du chromosome 1, une région synténique avec un fragment du chromosome humain 2q, région q31-q37. On a émis l'hypothèse qu'un homologue humain du gène *Bcg* controle la susceptibilité à la lèpre *per se*, et pourrait être localisé sur le chromosome 2q. Afin de tester l'influence de ce gène supposé sur la lèpre *per se*, nous avons réalisé des analyses de lien dans un ensemble de sept généalogies à cas multiples de Polynésie française, sur base d'une méthode des paires jumelles et de la méthode du score LOD employant différents modes d'héritage. Les membres des familles ont été typés en fonction de huit loci polymorphiques sur le chromosome 2q: *CRYGP1, FN, TNP1, VIL, DES, INH, PAX3* et *UGT1A1*. Nos données plaident contre la présence d'un gène controlant la susceptibilité à la lèpre *per se* sur le chromosome humain 2q dans la population de Polynésie française.

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