European Journal of Human Genetics (1999) 7, 88–97

http://www.stockton-press.co.uk/ejhg

ARTICLES

Full results of the genome-wide scan which localises a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31–q33

Sandrine Marquet[1], Laurent Abel[2], Dominique Hillaire[1] and Alain Dessein[1]

[1]*Immunology and Genetic of Parasitic Diseases/Laboratory Parasitology-Mycology, Faculty of Medicine, Marseille, France*
[2]*Mathematical and Statistical Modelling in Biology and Medicine, Hospital Pitié-Salpêtrière, Paris, France*

Three hundred million individuals are at risk of infection by schistosomes, and thousands die each year of severe hepatic disease. Previous studies have shown that the intensity of infection by *Schistosoma mansoni* in a Brazilian population is controlled by a major gene, denoted as *SM1*. We report here the full results of a genome-wide search that was performed on this population to localise *SM1*. Two hundred and forty-six microsatellites were used for the primary map, and only one region in 5q31–q33 provided significant evidence of linkage. *SM1* was subsequently mapped to this region, which contains several genes encoding cytokines or cytokine receptors which are involved in protection against schistosomes. Three additional regions, 1p22.2, 7q36 and 21q22–22-qter, yielded promising, although not significant, lod-score values. These regions contain candidate genes encoding cytokines or molecules relevant to anti-schistosome immunity.

Keywords: genome-wide scan; schistosomiasis; susceptibility gene; intensity of infection

Introduction

Schistosomiasis affects 300 million people throughout the world, and is a major public health problem in many developing countries.[1] The disease is spreading with the implementation of irrigation schemes in developing areas, and is caused by eggs laid by schistosome worms which live for years in the mesenteric and portal veins of their human host. Humans become infected when they bathe in waters infested by free-swimming schistosome larvae released by the molluscan vector. Eggs trigger an intense and chronic inflammatory reaction which causes fibrotic disease. In certain individuals, extended periportal fibrosis leads to severe portal hypertension and these subjects may die of haematemesis, co-infections or heart failure.

Severe clinical disease in schistosomiasis is often due to long-standing severe infections. Infection levels were shown to depend to a large extent on the resistance/susceptibility status of exposed subjects, and epidemiological studies performed in a Brazilian population indicated that individuals with the highest susceptibility were grouped within certain families, rather than

Correspondence: Professor Alain Dessein, Laboratoire d'Immunologie et Génétique des Maladies Parasitaires, INSERM U.399, Faculté de Médecine, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France. Tel: 33 4 91 32 44 52/53; Fax: 33 4 91 79 60 63; E-mail: Alain.Dessein-@medecine.univ-mrs.fr
Received 17 March 1998; revised 2 June 1998; accepted 19 June 1998

randomly distributed. These observations suggested that human susceptibility/resistance to infection by *S. mansoni* was genetically determined.[2–5] To investigate whether a major gene controls human levels of infection by *S. mansoni*, segregation analysis of infection intensities, adjusted for water contact, age and sex was performed on 20 Brazilian pedigrees comprising 269 individuals,[6] and led to the detection of a co-dominant major gene controlling the infection by *S. mansoni*. The frequency of the allele predisposing to high infection levels was 0.20–0.25, indicating that about 5% of the population was predisposed to high infection, 60% was resistant, and 35% had intermediate levels of resistance. To confirm the existence of this gene and to localise it, a genome-wide scan was performed on 142 Brazilian subjects belonging to 11 informative families. The parametric linkage analysis indicated the existence of this major locus and allowed us to map the gene to chromosome 5.[7] This linkage analysis was the first successful mapping of a gene involved in infectious diseases using a genome-wide search strategy, and demonstrates the feasibility of such an approach. For this reason, we report here the full results of this analysis indicating the other genetic regions of interest in addition to the 5q31–q33 region which was published previously in a short report.[7]

Materials and Methods

Study Area and Subjects

Study subjects live in a small village, Caatinga do Moura, in the north-east of Brazil where *S. mansoni* is endemic. All individuals in the population were infected because most agricultural and domestic activities require contact with an infested river. The measurements of individual exposure to infection and the determination of the phenotypes measuring susceptibility to *S. mansoni* infection have been extensively described in previous papers.[8,9] Infection intensities were measured by individual foecal egg counts prior to any treatment, expressed in eggs/gram and noted as E1 values.

Preparation of DNA

Blood samples were collected from 142 subjects either on ethylenediaminetetra acetic acid (EDTA) or on Na-heparine and frozen immediately at –80°C. DNA was extracted from human blood samples according to the following protocol: 6 ml of blood were thawed, and incubated for 30 min with 30 ml of blood lysis buffer pH 7.4 (7.75 mM NH_4Cl, 0.5 mM $KHCO_3$ 0.05 mM EDTA pH 7.4) at room temperature. Then samples were centrifugated at 3000 rpm for 10 min at 4°C, pellets were washed three times with 30 ml of the same buffer, and finally resuspended in 3 ml of lysing buffer 10 mM Tris-HCL, 400 mM NaCl, 2 mM Na_2 EDTA pH 8.2, and 1% SDS was added. Samples were then digested overnight with 100 µl proteinase K (20 mg/ml) at 37°C. 1 ml of NaCl 6 M was added followed by centrifugation at 3600 rpm, 20 min at room temperature. DNA was precipitated in the supernatant using standard methods[10] and kept at –20°C until use.

Genotyping using Microsatellites

Genomic DNA was diluted to a concentration of 4 µg/ml for amplification. Microsatellites were amplified by the PCR in 96-well Falcon flexi plates as described.[11] PCR was performed in 50 µl containing 40 ng of genomic DNA, 10 mM Tris-HCL, pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton, 1 µM of each primer, 31 µM of each dNTP (Boehringer Mannheim, Meylan, France) and 1 U of Taq DNA polymerase (Perkin-Elmer). Cycle conditions were 96°C for 5 min, 94°C for 10 min 'hot start', followed by 35 cycles of 92°C for 40 s, 55°C for 30 s, 72°C for 10 s and finally 72°C for 10 min. The amplification reaction products for 16 microsatellites were pooled and aliquots were precipitated and resuspended in 5 µl TE 10:1. 5 µl of loading buffer (80% formamide, 20% bromophenol and xylen blue) was added and samples were heated at 96°C for 2 min. A 4 µl aliquot of each sample was separated on a 6% denaturing polyacrylamide gel. Gels were transferred by capillary action to a nylon membrane overnight and hybridised with peroxydase labelled specific primers (using ECL direct nucleic acid labelling and detection systems RPN 3001 (Amersham, Les ulis, France) and added at a 15 ng/ml concentration.[11] Separated PCR products were visualised by autoradiography (Amersham RPN7 films). Genotypes were determined by two independent observers for each autoradiograph.

Eleven additional markers were analysed in the 5q31–q33 region including 8 Généthon markers (Figure 1). The three remaining markers were intragenic, using the following primers (annealing temperatures in parentheses): interleukin-4 (IL4-RP1) (Third intron), 5'- CTCAAAGTGCTGGGATTAGC-3' and 5'-AGCCATCTCGGTTGGATGGA-3' (57°C);[12] interleukin-9 (IL9) (fourth intron) 5'-AGGCTTTCTCTAATGCAGAG-3' and 5'-GGTGGTTGACCTCAAATTGG-3' (53°C);[13] colony stimulating factor 1 receptor (CSF1R) (second intron) 5'-TGTGTCCAGCCTTAGTGTGCA-3' and 5'-TCATCACTTCCAGAATGTGC-3' (53°C).[14]

Segregation Analysis

Evidence for the segregation of a major gene controlling *S. mansoni* infection levels was obtained in this Brazilian population in 1991;[6] in this study, phenotypes were adjusted on the covariates before the segregation analysis. Since this early publication, more information has been obtained on familial relationships, and a refined segregation analysis was performed by taking into account the covariates (water contact, age, sex) that had a significant effect on infection intensity, simultaneously with the major gene effect. Moreover, the phenotypes of the subjects with the lower water contact exposure were considered as unknown, since a significant proportion of these subjects did not have any contact with the river and provided meaningless null E1 value.

Segregation analysis was performed using the regressive models for continuous phenotypes developed by Bonney.[15] These regressive models are constructed by specifying a regression relationship between the phenotype (logarithmic transformation of E1 values noted as E2) and a major gene effect, the phenotype of relatives and covariates. The parameters of the major gene effect are q, the frequency of A, the allele predisposing to high infection levels, and the three

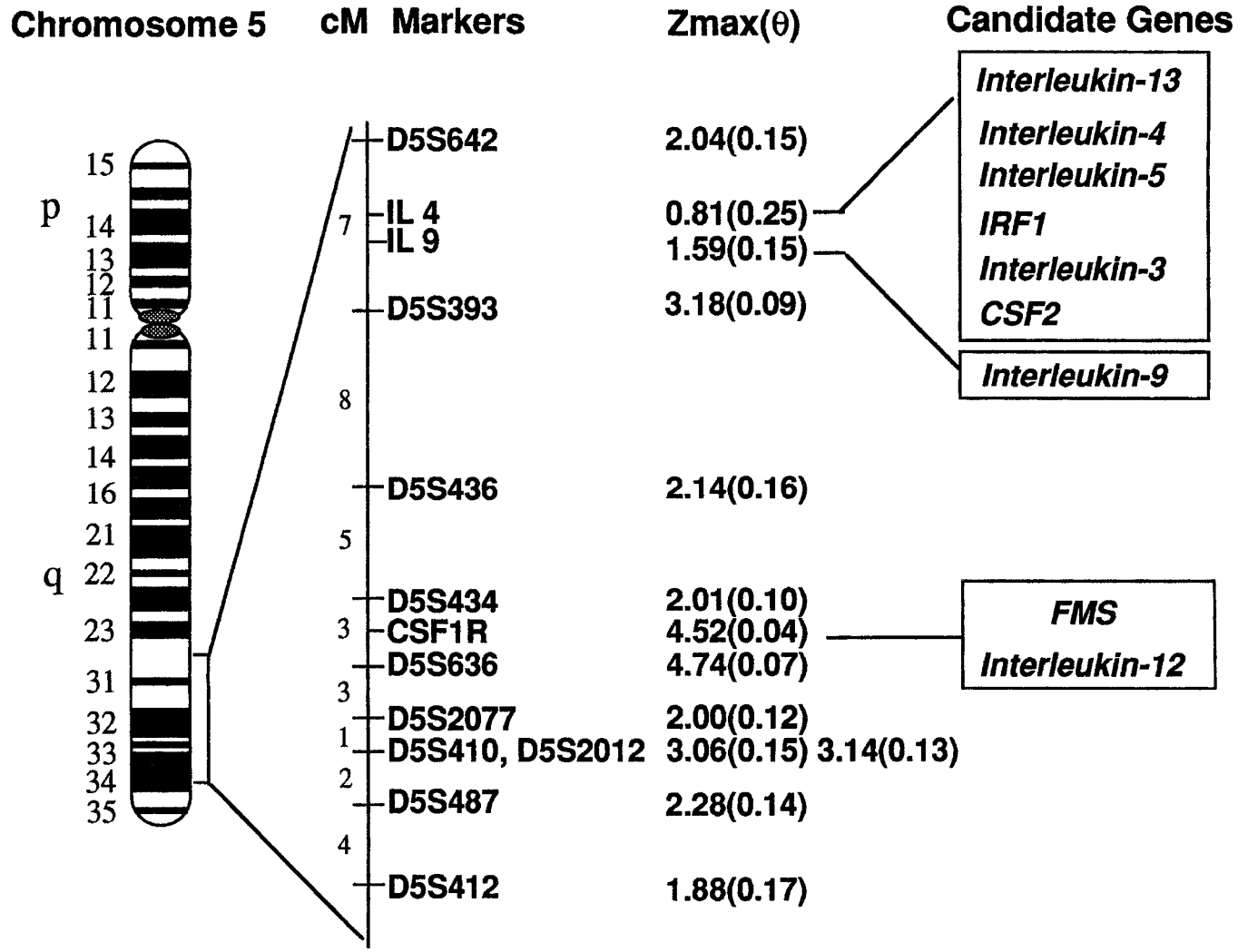


Figure 1 *Map of chromosome 5 showing the relative order of and distance between the polymorphic genetic markers used and the approximate location of the candidate genes. The order and distance (cM) between these genetic markers in 5q31–q33 region are based on genetic map[18,19] except for the intragenic markers interleukin-4 (IL4) and IL9.[13] CSF1R was placed on the basis of our multipoint analysis. Maximum two-point lod scores and recombination fractions (θ) are indicated for each marker. The map includes the following genes:* IL13, IL4, IL5, *interferon regulatory factor-1* (IRF1), IL13, *granulocyte-macrophage colony stimulating factor* (CSF2), IL9, IL12 *and* FMS *gene which encodes a colony stimulating factor-1 receptor (CSF1R).*

means of the phenotype adjusted for covariates, μAA, μAa and μaa, corresponding to the three genotypes, AA, Aa and aa; given the genotype, the distribution of the phenotype is assumed to be normal. The dependence on relative phenotypes is expressed in terms of phenotypic correlations, and the effects of covariates are parametrised using regression coefficients. The present analysis was performed using the program REGRESS,[16] which incorporates the regressive approach into the LINKAGE package[17] and allows simultaneously the effect of covariates (water contact, age, sex) with the major gene to be estimated.

Linkage Analysis

The linkage study strategy was a genome-wide search using microsatellites markers. The genome-wide search was performed on 142 subjects belonging to two large pedigrees (pedigrees 1 and 8 comprising 50 and 20 individuals, respectively), five smaller pedigrees (pedigrees 2, 3, 4, 5, and 7, including 12, 9, 14, 6 and 7 individuals, respectively), and four nuclear families (families 30, 40, 120, and 131 with 7, 5, 6, 6 subjects, respectively). These families are shown in Figure 2. The primary map consisted of 246 markers from the Généthon panel[18,19] with an average interval spanned by adjacent markers estimated to be 15 cM; no interval was greater than 35 cM, and 5 intervals were between 25 and 35 cM.

As segregation analysis provided evidence for a major gene model in the control of *S. mansoni* infection levels, we used a parametric lod-score approach to test for linkage.[20] For this analysis, E2 values were adjusted on the basis of covariates

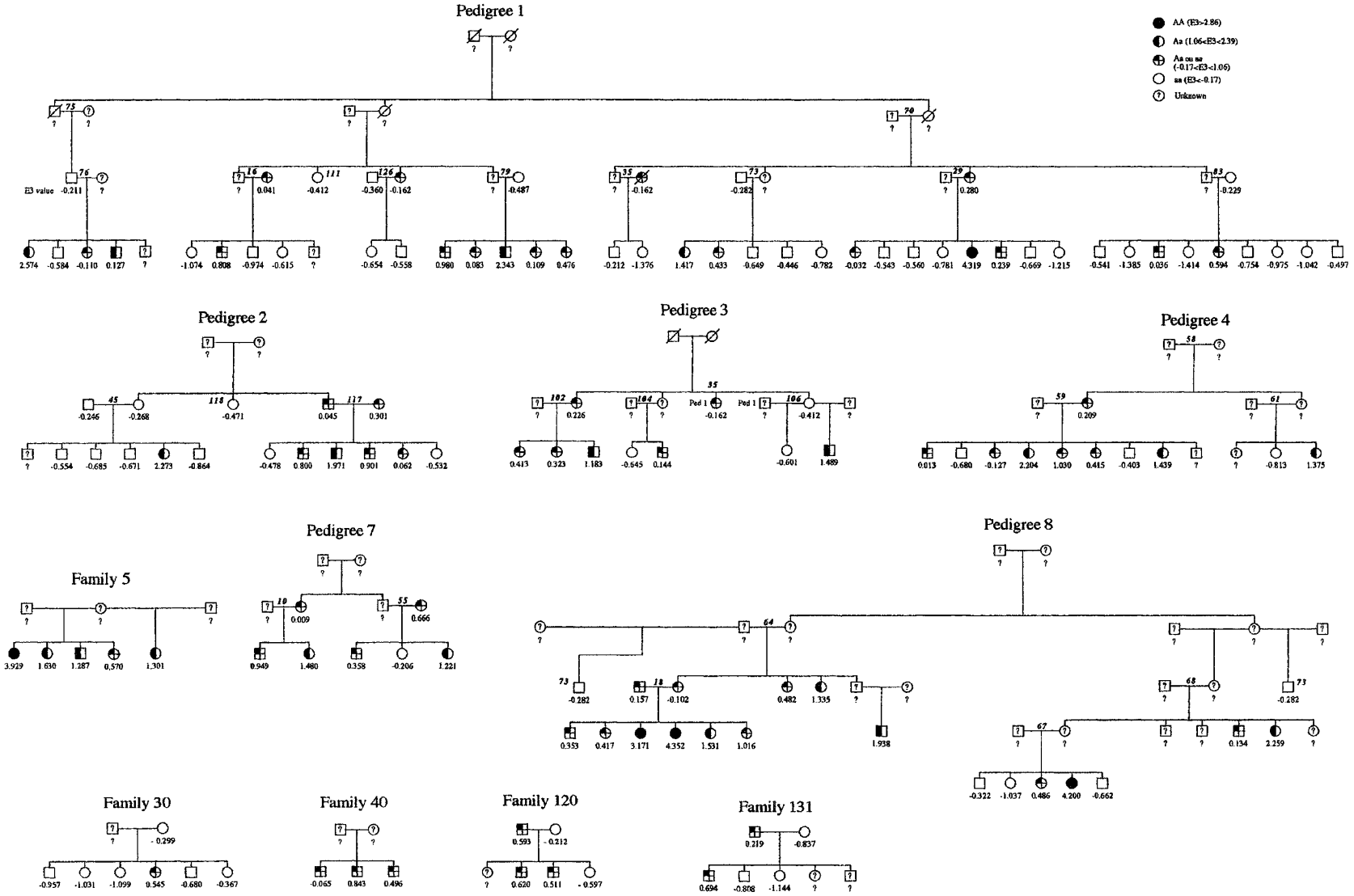


Figure 2 *Familial structure for two large pedigrees (numbers 1, 8), five smaller pedigrees (numbers 2, 3, 4, 5, 7) and four nuclear families (numbers 30, 40, 120, 131) used for linkage analysis. In the pedigree the number of the nuclear family is indicated in bold italic. For each subject the adjusted standardised infection intensity (E3 value) is specified and a genotype is assigned when its probability is greater than 0.9 based on E3 values. Subjects with ? for E3 value and genotype are classified as unknown, they correspond to subjects without E1 value expressed in eggs/gram or subjects with the lowest water contact level.*

that had a significant effect (sex, age and water contact) using the regression coefficients obtained under the major gene model of best fit; the standardised residuals were denoted as E3. Two point lod-score analyses were computed by means of the LINKAGE package[17] using the maximum likelihood major gene parameters obtained from the segregation analysis. The frequency of allele A predisposing to high E3 values was 0.16, and the three E3 means were 3.96, 0.78 and –0.43, for AA, Aa and aa individuals, respectively, with a residual variance equal to 0.33. The marker allele frequencies were assumed to be equal as some alleles found in this Brazilian population were not typed in the CEPH reference families. For the 5q31–q33 markers, the analysis was also performed considering allele frequencies estimated from our data. Multipoint analysis in the 5q31–q33 region was performed using the VITESSE program.[21]

Results

Segregation Analysis

Results confirmed the presence of a codominant major gene controlling infection intensities with parameters close to those of the previous analysis.[6] This gene, referred to as *SM1*, accounted for 66% (50% in the first study) of the infection intensity variance residual from covariate effects. The frequency of the allele A predisposing to high infection was 0.16 (around 0.2 in the first study). Then, under this genetic model, about 3% of the study population were homozygous for the deleterious allele and predisposed to high infections, 70% were resistant, and 27% of the study population were heterozygous and had an intermediate, although fairly strong, level of resistance.

Linkage Analysis

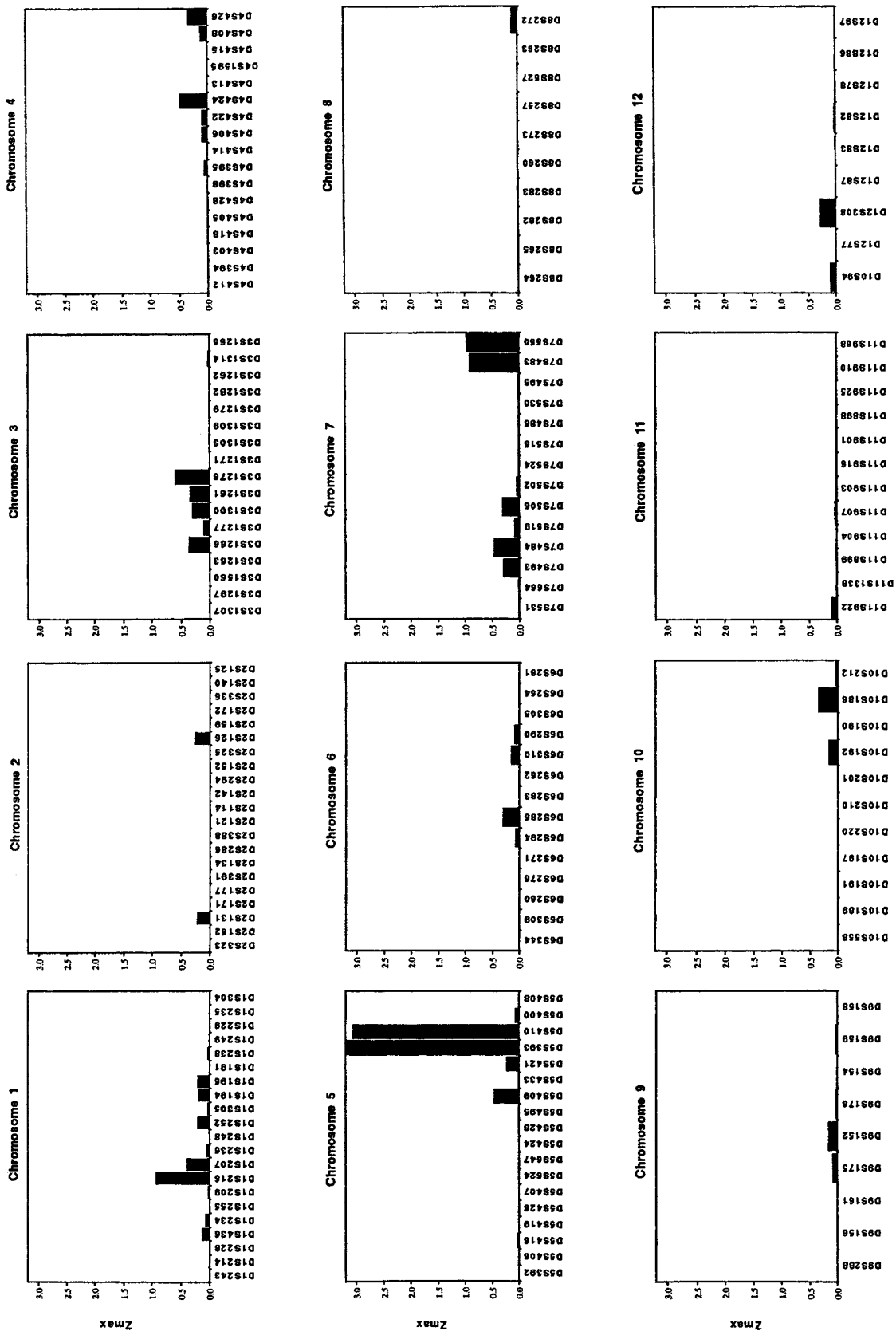
The maximum lod-score values obtained with the various microsatellites are presented in Figure 3 for each chromosome. Maximum lod scores greater than 0.1 were observed with 54 markers (Figure 4), and were distributed on almost all chromosomes (Figure 3). Six markers located in four regions on chromosomes 1, 5, 7, 21 yielded maximum lod scores greater than 0.83 ($P < 0.025$). It was striking that only two adjacent microsatellites in region 5q31–q33, D5S393 and D5S410, gave a maximum lod score (Z_{max}) above 3.0, while all other markers produced Z_{max} below 1.1 (Figure 4). The 5q31–q33 chromosomal interval was further analysed by genotyping 11 additional markers (Figure 1), and significant evidence of linkage (considered as a lod-score value above 3.3 according to Lander and Kruglyak[22] in this context of genome-wide search) was obtained with two closely linked markers: D5S636 ($Z_{max} = +4.74$, $\theta = 0.07$) and CSF1R ($Z_{max} = +4.52$, $\theta = 0.04$) using estimated marker allele frequencies.[7] For most of the markers the linkage information came from the two large pedigrees numbered 1 and 8, and haplotype co-segregation with high infection levels within these two pedigrees was given in our first report.[7] Here we present the detailed two-point lod-score results for D5S636 and CSF1R within each family (Table 1). Multipoint linkage analysis confirmed the mapping to 5q31–q33 with a multipoint lod-score value above +5.5 and a most likely location of *SM1* in close proximity to CSF1R.[7]

Whereas significant linkage of *SM1* was obtained with only one region, four additional markers in three other regions provided lod-score values above 0.83: 1p22.2 with D1S216 ($Z_{max} = +0.91$, $\theta = 0.20$), 21q22-qter with D21S1259 ($Z_{max} = +1.09$, $\theta = 0.19$), and the two adjacent markers D7S483 ($Z_{max} = +0.91$, $\theta = 0.20$) and D7S550 ($Z_{max} = +1.02$, $\theta = 0.22$) in the 7q36 region. These results could be due to the large number of tests performed in a genome-wide search, but it is interesting to note that some genes involved in the immune response against schistosomes are located in these regions. The beta T cell receptor (*TCR*β) is assigned close to the 7q36 region,[23] and chromosome 21 includes the human interferon α receptor gene (*IFNαR*) in 21q22.1[24] and the human interferon β receptor gene (*IFNβR*) in 21q22.2.[23] Finally, the interleukin-12 receptor β2 chain was recently localised to chromosome 1p31.3–p31.2 by *in situ* hybridisation.[25]

Discussion

The results presented here map the codominant major gene controlling human susceptibility/resistance to *S. mansoni*, *SM1*, and are the first example of a successful genome-wide scan in the study of susceptibility/resistance to infectious diseases. A new segregation analysis allowed us to refine the model for *SM1* which was used in the parametric linkage analysis. With this parametric approach, a single region on chromosome 5q31–q33 was found to be significantly linked to disease. Recently, Müller-Myhsok *et al*[26] confirmed this localisation by means of a nonparametric linkage method in a Senegalese population infected with *S. mansoni* and exposed for no longer than 7 years.[26] These two studies emphasise the importance of the locus *SM1* which influences the intensity of infection by *S. mansoni*.

The 5q31–q33 region where *SM1* is located contains several candidate loci involved in the regulation of the immune response to pathogens, in particular genes coding for interleukin-4 (IL4),[13,27] IL5,[13,27] IL12,[28] the


Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Chromosome 11
Chromosome 12
Zmax

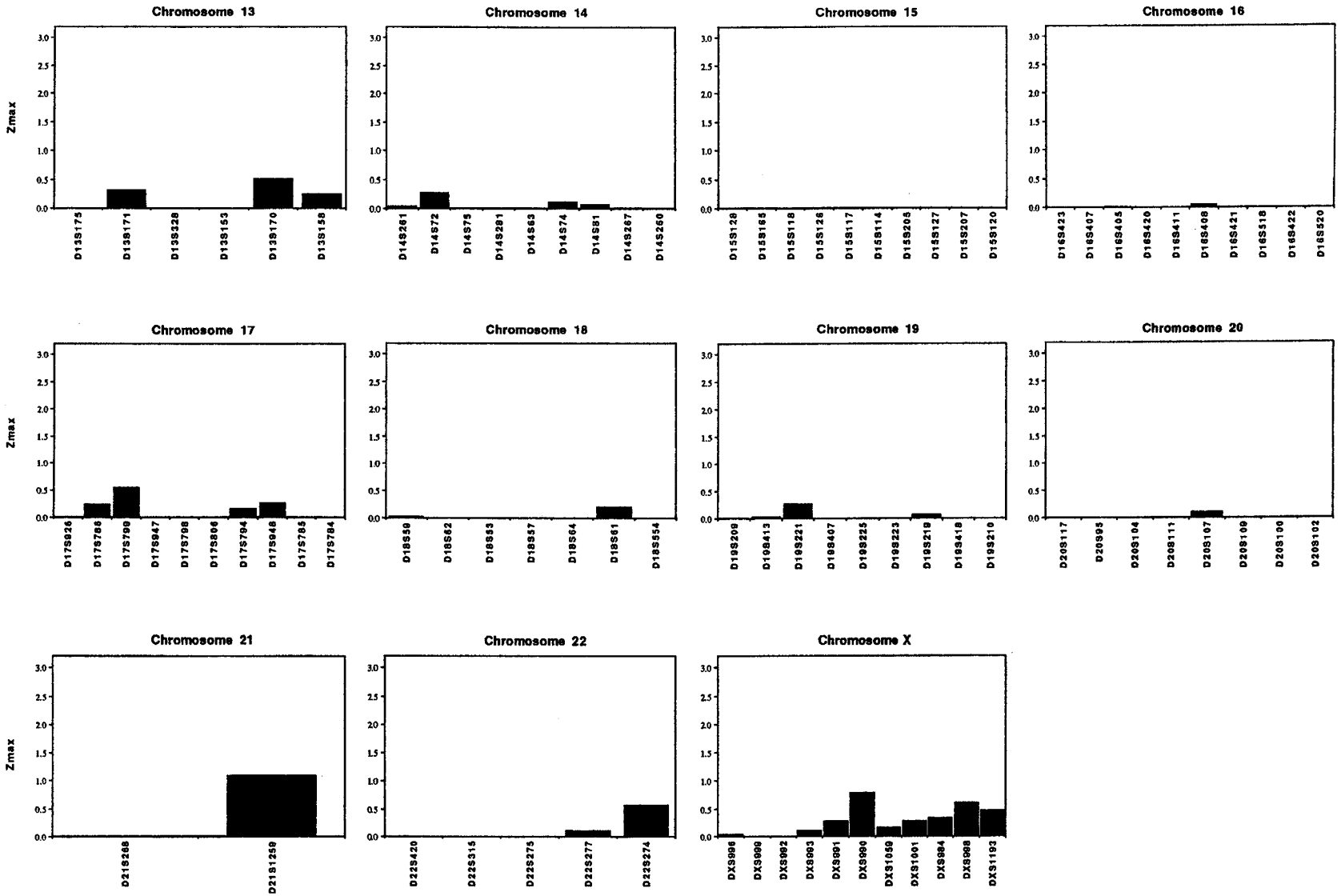


Figure 3 *Maximum lod scores (Z_{max}) by chromosome for the genomic screen of infection levels by* Schistosoma mansoni. *Lod-score values for 246 markers analysed are on the vertical axis; some of the markers used for the primary map are listed below the histogram.*

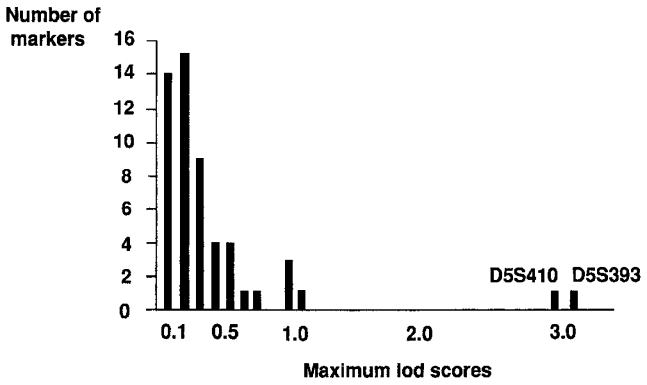


Figure 4 *Histogram of 54 markers with $Z_{max} > 0.1$.*

Table 1 Two-point lod scores for chromosome 5q31–33 markers in families affected by schistosomiasis showing evidence against linkage to *SM1*. These results were obtained using estimated marker allele frequencies

	Two-point lod scores at recombination fractions of 0.00 and 0.05			
	D5S636		*CSF1R*	
Family	*0.00*	*0.05*	*0.00*	*0.05*
1	2.63	3.10	1.57	2.36
2	–0.44	0.04	0.98	0.78
3	0.20	0.17	0.17	0.14
4	0.06	0.08	–0.04	–0.04
5	–0.49	–0.33	–0.49	–0.33
7	–0.20	–0.14	0.16	0.14
8	2.09	1.90	1.83	1.58
30	–0.02	–0.01	–0.02	–0.01
40	0.07	0.06	0.07	0.06
120	–0.08	–0.06	–0.08	–0.06
131	–0.13	–0.10	–0.14	–0.11
Total	3.69	4.71	4.03	4.52

interferon regulatory factor 1 (IRF1)[27] which encodes a transcriptional activator of interferon-alpha (IFNα), interferon-beta (IFNβ) and other IFN-inducibles genes, and finally CSF1R.[27] Furthermore, this region has been linked with loci related to IgE and/or eosinophilia production, ie a locus regulating IgE levels,[13,29] a locus controlling bronchial hyper-responsiveness in asthma,[30] and a locus involved in familial hypereosinophilia.[31] This localisation of *SM1* is consistent with the immunological analyses carried out on both blood lymphocytes and T-cell clones derived from susceptible (AA) subjects and resistant (aa) subjects. The larval and worm-specific T-lymphocytes from aa subjects produced larger amounts of IL4 and IL5 than T-cells from AA subjects. In contrast T-lymphocytes from AA subjects produced larger amounts of interferon-gamma (IFNγ) than T-cells from aa subjects. These results indicate that the parasite specific T-lymphocytes of resistant subjects are Th0/2-like,[32] while susceptible subjects exhibit a Th0/1-like response (Rodrigues V, Dessein A unpublished data 1998), and are consistent with the previous results indicating that resistance to infection by *S. mansoni* is associated with a high parasite specific IgE antibody response[33–35] that is dependent on IL4 and downregulated by IFNγ. The development of Th1 or Th2 cells is mainly regulated by certain cytokines, especially IL12 and IL4, when present during primary T-cell activation. At this time, we cannot exclude any of these candidate genes localised in the 5q31–q33 region.

Three other chromosomal regions, 1p22.2, 7q36 and 21q22–qter provided lod-score values above 0.83. Although these values are not significant in the context of a genome-wide search, it is interesting to note that some candidate genes are located within these regions. The IL12 receptor β2 chain is located close to the 1p22.2 region,[25] and plays an important role in the immune response, since the interaction between IL12 and its receptor induces the secretion of IFNγ and stimulates differentiation of T helper 1 (Th1) cells. The IL12 receptor β2 subunit is expressed on human Th1 but not Th2 clones, and is induced during differentiation of human naive cells along Th1 but not Th2 pathways.[36] Another potential region of interest is 21q22–qter, where the interferon-alpha receptor (IFNαR) and interferon-beta receptor (IFNβR) are located. It was recently demonstrated that the production of the cytokines IL12 and IFNγ is inhibited by interferon α and β (IFNα/β).[37] Finally the 7q36 region contains the beta T cell receptor (TCRβ), and T cells were shown to play a central role in the development of acquired immunity against schistosomes.

The main interest of the present genome-wide screen strategy compared with a candidate gene approach is to ensure that all major loci involved in the control of infection by *S. mansoni* are identified, and to provide the opportunity to discover new major genes, and consequently physiopathological pathways, that were not previously suspected of contributing to this control. However, since we used a parametric linkage method to localise *SM1*, it is possible that the major gene model employed in the present analysis was not appropriate for investigating the role of loci in addition to the one identified in 5q31–q33. Therefore, the identification of additional putative loci that could be involved in the control of intensity of infection by *S. mansoni* will require the use of nonparametric linkage methods

which do not need to specify the major gene model. The weighted pairwise correlation method,[38,39] which is a nonparametric linkage method for general pedigrees, appears to be the method of choice for the Brazilian population studied here, and we could use a recent two-locus extension of this method[40] which will allow us to test for the presence of a second locus taking into account the 5q31–q33 locus. The role of these regions will also be assessed by replication studies performed in other populations.

In conclusion, these data present the first successful genome-wide scan to map a major gene of susceptibility/resistance to infectious diseases. The full results show that only one region on chromosome 5q31–q33 was significantly linked to schistosomiasis. Further nonparametric two-locus linkage studies will assess the role of additional putative genes.

Acknowledgements

We thank S Fauré and the genotyping laboratory from Généthon for helpful assistance, F Demenais for providing the REGRESS software, and M Morton for critical reading of the manuscript. This work was supported in part by grants 492NS3 from INSERM, TS3-CT94-0296 from EC, 51/93 from GREG, and by INSERM/CNPQ exchange grants.

References

1 UNDP/World Bank/WHO: Schistosomiasis. In: *Tropical Diseases: Progress in Research*. :WHO: Geneva, 1990, pp 41–48.
2 Butterworth AE, Capron M, Cordingley JS *et al*: Immunity after treatment of human schistosomiasis. II. Identification of resistant individuals and analysis of their immune responses. *Trans R Soc Trop Med Hyg* 1985; **79**: 393–408.
3 Hagan P, Blumenthal UJ, Chaudri M *et al*: Resistance to reinfection with *Schistosoma haematobium* in Gambian children: analysis of their immune response. *Trans R Soc Trop Med Hyg* 1987; **81**: 938–946.
4 Wilkins HA, Blumenthal UJ, Hagan P, Tulloch S, Hayes RJ: Resistance to reinfection after treatment of urinary schistosomiasis. *Trans R Soc Trop Med Hyg* 1987; **81**: 29–35.
5 Dessein AJ, Begley M, Demeure C *et al*: Human resistance to *Schistosoma mansoni* is associated with IgG reactivity to a 37-kDa larval surface antigen. *J Immunol* 1988; **140**: 2727–2736.
6 Abel L, Demenais F, Prata A, Souza AE, Dessein A: Evidence for the segregation of a major gene in human susceptibility/resistance to infection by *Schistosoma mansoni*. *Am J Hum Genet* 1991; **48**: 959–970.
7 Marquet S, Abel L, Hillaire D *et al*: Genetic localization of a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31–q33. *Nat Genet* 1996; **14**: 181–184.
8 Dessein AJ, Abel L, Coussinier P *et al*: Environmental, genetic and immunological factors in human resistance to *Schistosoma mansoni*. *Immunol Invest* 1992; **21**: 423–453.
9 Demeure CE, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ: Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J Infect Dis* 1993; **168**: 1000–1008.
10 Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning*. Cold Spring Harbor Laboratory Press: New York, 1989.
11 Vignal A, Gyapay G, Hazan J *et al*: A non-radioactive multiplex procedure for genotyping of microsatellites markers. In: Adolph KW (ed.). *Methods in Molecular Genetics 1: Gene and Chromosome Analysis*. Academic Press: San Diego, 1993, pp 211–221.
12 Mout R, Willemze R, Landegent JE: Repeat polymorphisms in the interleukin-4 gene (IL4). *Nucleic Acids Res* 1991; **19**: 3763.
13 Marsh DG, Neely JD, Breazeale DR, *et al*: Linkage analysis of IL4 and other chromosome 5q31.1 markers and totral serum immunoglobulin E concentrations. *Science* 1994; **264**: 1152–1156.
14 Polymeropoulos MH, Xiao H, Rath DS, Merril CR: Dinucleotide repeat polymorphism at the human c-fms proto-oncogene for the CSF-1 receptor (CSF1R). *Nucleic Acids Res* 1991; **19**: 1160.
15 Bonney GE: On the statistical determination of major gene mechanisms in continuous human traits: regressive models. *Am J Med Genet* 1984; **18**: 731–749.
16 Demenais FM, Lathrop M: REGRESS: A computer program including the regression approach into the LINKAGE package. *Genet Epidemiol* 1994; **11**: 291.
17 Lathrop GM, Lalouel JM, Julier C, Ott J: Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984; **81**: 3443–3446.
18 Gyapay G, Morisette J, Vignal A, *et al*: The 1993–94 Généthon human genetic linkage map. *Nat Genet* 1994; **7**: 246–339.
19 Dib C, Fauré S, Fizames C *et al*: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152–154.
20 Morton NE: Sequential tests for the detection of linkage. *Am J Hum Genet* 1955; **7**: 277–318.
21 O'Connell JR, Weeks DE: The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. *Nat Genet* 1995; **11**: 402–408.
22 Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; **11**: 241–247.
23 Frezal J (ed.): *Genome Interactive Databases*. John Libbey Eurotext: 1996; vol 3(2). France.
24 Lutfalla G, Gardiner K, Proudhon D, Vielh E, Uze G: The structure of the human interferon α/β receptor gene. *J Biol Chem* 1992; **267**: 2802–2809.
25 Morton SM, Bocaccio I, Depetris D, Mattei M, Dessein A: Assignment of IL12Rβ2 to human chromosome 1p31.3-p31.2 between D1S230 and D1S198. *Cytogenet Cell Genet* 1997; **79**: 282–283.

26 Müller-Myhsok B, Stelma FF, Guissé-Sow F *et al*: Further evidence suggesting the presence of a locus on human chromosome 5q31-q33 influencing the intensity of infection with *Schistosoma mansoni*. *Am J Hum Genet* 1997; **61**: 452–454.
27 Saltman DL, Dolganov GM, Warrington JA, Wasmuth JJ, Lovett M: A physical map of 15 loci on human chromosome 5q23-q33 by two-color fluorescence *in situ* hybridization. *Genomics* 1990; **16**: 726–732.
28 Boultwood J, Fidle C, Lewis S *et al*: Molecular mapping of uncharacteristically small 5q deletions in two patients with 5q-syndrome: Delineation of the critical region on 5q and identification of a 5q-breakpoint. *Genomics* 1994; **19**: 425–432.
29 Meyers DA, Postma DS, Panhuysen CIM *et al*: Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994; **23**: 464–470.
30 Postma DS, Bleecker ER, Amelung PJ *et al*: Genetic susceptibility to asthma – Bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995; **333**: 894–900.
31 Lin AY, Rioux JD, Nutman T *et al*: A gene for familial hypereosinophilia maps to chromosome 5q31–q33. *Am J Hum Genet* 1997; Suppl 61: A283.
32 Couissinier-Paris P, Dessein AJ: Schistostoma-specific helper T cell clones from subjects resistant to infection by *Schistosoma mansoni* are Th0/2. *Eur J Immunol* 1995; **25**: 2295–2302.
33 Rihet P, Demeure C, Bourgois A, Dessein AJ: Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 1991; **21**: 2679–2686.
34 Hagan P, Blumenthal UJ, Dunne D, Simpson AJG, Wilkins HA: Human IgE, IgG4, and resistance to reinfection with *Schistosoma haematobium*. *Nature* 1991; **349**: 243–245.
35 Dunne DW, Butterworth AE, Fulford AJC *et al*: Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 1992; **22**: 1483–1494.
36 Rogge L, Barberis-Maino L, Biffi M *et al*: Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* 1997; **185**: 825–831.
37 Cousens LP, Orange JS, Su HC, Biron CA: Interferon-α/β inhibition of interleukin-12 and interferon-γ production *in vitro* and endogenously during viral infection. *Proc Natl Acad Sci USA* 1997; **94**: 634–639.
38 Commenges D: Robust genetic linkage analysis based on a score test of homogeneity: the Weighted Pairwise Correlation statistic. *Genet Epidemiol* 1994; **11**: 189–200.
39 Commenges D, Abel L: Improving the robustness of the WPC test for linkage analysis. *Genet Epidemiol* 1996; **13**: 559–573.
40 Zinn-Justin A, Abel L: Two-locus developments of the Weighted Pairwise Correlation method for linkage analysis. *Genet Epidemiol* (in press).