Am. J. Hum. Genet. 65:709–721, 1999

Severe Hepatic Fibrosis in *Schistosoma mansoni* Infection Is Controlled by a Major Locus That Is Closely Linked to the Interferon-γ Receptor Gene

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Summary

Lethal disease due to hepatic periportal fibrosis occurs in 2%–10% of subjects infected by *Schistosoma mansoni* in endemic regions such as Sudan. It is unknown why few infected individuals present with severe disease, and inherited factors may play a role in fibrosis development. *Schistosoma mansoni* infection levels have been shown to be controlled by a locus that maps to chromosome 5q31-q33. To investigate the genetic control of severe hepatic fibrosis (assessed by ultrasound examination) causing portal hypertension, a segregation analysis was performed in 65 Sudanese pedigrees from the same village. Results provide evidence for a codominant major gene, with .16 as the estimated allele A frequency predisposing to advanced periportal fibrosis. For AA males, AA females, and Aa males a 50% penetrance is reached after, respectively, 9, 14, and 19 years of residency in the area, whereas for other subjects the penetrance remains <.02 after 20 years of exposure. Linkage analysis performed in four candidate regions shows that this major locus maps to chromosome 6q22-q23 and that it is closely linked (multipoint LOD score 3.12) to the *IFN-γR1* gene encoding the receptor of the strongly antifibrogenic cytokine interferon-γ. These results show that infection levels and advanced hepatic fibrosis in human schistosomiasis are controlled by distinct loci; they suggest that polymorphisms within the *IFN-γR1* gene could determine severe hepatic disease due to *S. mansoni* infection and that the *IFN-γR1* gene is a strong candidate for the control of abnormal fibrosis observed in other diseases.

Received August 12, 1998; accepted for publication June 28, 1999; electronically published August 9, 1999.

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0002-9297/1999/6503-0016$02.00

Introduction

Schistosomiasis affects 200 million people and is a major public health problem in many southern countries. The larvae of *Schistosoma mansoni*, the most common schistosome species, penetrate the skin of humans in contact with infested waters. Within a few weeks, the parasites migrate to the portal and mesenteric veins of their host, where they mature into either male or egg-laying female worms. Although most eggs pass into the intestine, a number of them are taken, by the portal-vein blood flow, to the liver, where they are lodged in small vessels, causing inflammatory granuloma. The granuloma may subside, succeeded by tissue repair, which, in certain subjects, leads to extended and irreversible fibrosis denoted as "Symmers fibrosis" (Symmers 1904; Bogliolo 1954). Mortality in *S. mansoni* infections is mainly the consequence of portal-blood hypertension that is caused by hepatic periportal fibrosis. It is not known why severe disease occurs only in certain subjects, although it has been suggested that advanced disease might be more frequent in subjects with the highest infections (Kloetzel 1962; Cheever 1968; Cook et al. 1974; Arap Siongok et al. 1976).

Animal studies have indicated that disease development is affected by the interleukins (IL), which regulate the granulomatous response—especially IL-10 (Wynn et al. 1998), IL-12 (Wynn et al. 1995), and tissue-necrosis factor TNF-α (Leptak and McKerrow 1997). Fibrosis following granulomatous inflammation has also been shown to be dependent on the fibrogenic action of cytokines such as IL-4 (Cheever et al. 1994) and transforming growth factor TGF-β1 (Czaja et al. 1998*a*) and on the antifibrogenic effect of interferon-γ (IFN-γ) (Czaja et al. 1989*b*). Interestingly, animal studies indicate that IFN-γ is probably not involved in the acute inflammatory phase of the granuloma but regulates fibrosis in the healing phase of the periovular reaction. The hypothesis that either IFN-γ, IL-4, IL-10, IL-12, TNF-α, or TGF-β plays a similar role in human schis-

tosomiasis has never been tested, because immunological studies have not been performed on well-defined fibrosis phenotypes. Various reports, however, point to IFN-γ as a major antifibrogenic cytokine in tissue fibrosis (Jimenez et al. 1984; Duncan and Berman 1985; Tamai et al. 1995), including hepatic fibrosis (Mallat et al. 1995).

In previous studies we have demonstrated that levels of infection by *S. mansoni* in a Brazilian population were controlled by a major gene referred to as "*SM1*" (MIM 181460) (Abel et al. 1991), which we mapped to chromosome 5q31-q33 (Marquet et al. 1996). Since severe disease in this Brazilian population had been prevented by repeated chemotherapy, the present study was performed in a Sudanese population that inhabits an endemic region and that had never been treated for schistosomiasis, in order to analyze the factors that determine severe disease in *S. mansoni* infections. Phenotypes were assessed by liver and spleen ultrasonography, which has been shown to be a highly reliable tool for the diagnosis of Symmers fibrosis (Cerri et al. 1984; Homeida et al. 1988*a,* 1988*b;* Abdel-Wahab et al. 1989, 1990; The Cairo Working Group 1992). The following observations have suggested that inherited factors might play a role in advanced fibrosis in this Sudanese population (Mohamed-Ali et al. 1999): (1) advanced fibrosis was more frequent in certain families, despite similar epidemiological conditions for the whole study population; and (2) fibrosis grades between parents and children showed correlation but those among spouses did not. To test for genetic control of fibrosis, a segregation analysis was performed on 781 individuals belonging to 65 pedigrees. This analysis provides evidence for the presence of a major gene referred to as "*SM2*." Linkage analysis was then performed in the following four candidate regions:

1. **Chromosome region 5q31-q33.**—This region contains *SM1* and several interleukin genes—including *IL-4, IL-5,* and *IL-12*—that have been shown to play a role in granuloma formation and/or in fibrosis in experimental animals; this region also has been linked to loci controlling phenotypes—such as total serum IgE levels (Marsh et al. 1994; Meyers et al. 1994), familial hypereosinophilia (Rioux et al. 1998), and bronchial hyperresponsiveness in asthma (Postma et al. 1995)—that may be relevant to immunity in schistosomiasis.
2. **HLA-TNF region 6p21.**—Associations have been reported between some HLA alleles and hepatosplenomegaly in schistosomiasis (Salam et al. 1979; Abaza et al. 1985; Abdel-Salam et al. 1986; Cabello et al. 1991; Secor et al. 1996), and TNF-α is an essential cytokine in the development of schistosome granuloma in mice (Leptak and McKerrow 1997); furthermore, polymorphisms in the promoter of TNF-α have been associated with both aggravation of immunopathological lesions in mucocutaneous leishmaniasis (Cabrera et al. 1995) and an increased risk for cerebral malaria in children infected by *Plasmodium falciparum* (McGuire et al. 1994).
3. **Chromosome region 12q15.**—This region includes the gene coding for IFN-γ, which has also been linked to both asthma control and serum total IgE levels (Barnes et al. 1996; Nickel et al. 1997).
4. **Chromosome region 6q22-q23.**—This region contains the gene, *IFN-γR1,* coding for the IFN-γ receptor ligand-binding chain, mutations in which are responsible for major alterations in the granulomatous reaction to nontuberculous mycobacteria causing severe disseminated infections (Jouanguy et al. 1996; Newport et al. 1996).

Linkage analysis performed with these four genetic regions showed that *SM2* maps to chromosome 6q22-q23, suggesting that the *IFN-γR1* gene itself may be involved in the control of severe hepatic fibrosis due to *S. mansoni.*

Subjects and Methods

Family Data

This study was conducted in Al Taweel, a small Sudanese village of the Managil area at the southwestern extension of the Gezira's irrigated scheme, 300 km south of Khartoum. All study subjects are migrants who settled in the village ~20 years ago, coming from the same region of western Sudan, where schistosomiasis is not endemic. Two tribes are present in the village: the Tama-Messeria tribe (two-thirds of the population) and the Rawashda tribe (one-third of the population), which are closely related ethnic groups. The village was initially a refugee camp that had been built in the middle of fields where the villagers were employed. Various canals encircle the village, within a short distance (10–100 m) from the houses, and the village is also crossed by a small canal that is full 6 mo/year. These canals are populated with snails infected by *S. mansoni* and are the only source of water for drinking, domestic use (bathing and washing), and agricultural use (irrigation of fields). The socioeconomic status of Al Taweel's families, as assessed on the basis of dwellings, furniture, nutrition, and domestic animals, is homogeneous. Information on familial relationships was obtained on the basis of several interviews with family heads and their wives. Subjects were also interviewed on the causes of death of their recently deceased relatives, mostly parents. The 781 subjects on whom ultrasound data were available belonged to 65 families: 5 large pedigrees (30 members), 29 smaller pedigrees (6–29 members), and 31 nuclear families. Of the 65 families, 54 were from the Tama-Messeria tribe, and 11 were from the Rawashda ethnic group. This study was approved by medical-ethics committees of the University of Gezira and of Sudan's Na-

tional Ministry of Health. Informed consent was obtained from the study subjects, who were free to leave or to enter the study at any time.

Ultrasound Methods and Definition of the Severe Fibrosis Phenotype

Study subjects were evaluated by ultrasound examination with a portable Aloka SSD 500 Echo camera and a 3.5-MHz convex probe. Liver size, portal-vein diameter (PVD), degree of periportal fibrosis, thickness of the walls of peripheral portal branches (PPB), spleen size, and splenic vein diameter were assessed as described elsewhere (Homeida et al. 1988*a,* 1988*b;* The Cairo Working Group 1992). PVD was measured at the point of the portal vein's entrance into the porta hepatis, at the lower end of the caudete lobe. Periportal fibrosis was graded as 0–3 (F0–FIII). Grade 0 (F0) corresponds to normal liver, with no thickening of the wall of the PPB. In grade 1 (FI), there is a patchy fibrosis, which causes a "fishes in the pond" appearance. The thickness of second-order PPBs is ~1 mm. Grade 2 (FII) corresponds to continuous, in addition to patchy thickening of PPB branches. Most second-order branches appear as long segments of fibrosis that have ~1-mm lumen and ~2-mm wall thickness. Gall-bladder wall thickness may be increased above normal (2–4 mm). Grade 3 (FIII) shows wall thickening of almost all PPBs. The fibrosis reaches the surface of the liver, and, in some branches, the lumen is occluded with second-order branches that have a wall thickness >2 mm. Gall-bladder wall thickness is always increased to >4 mm.

On the basis of ultrasound measurements, a binary phenotype was used to classify the subjects as either affected or unaffected. The criteria used for the classification were fibrosis grade *and* evidence of portal-blood hypertension. Subjects with FIII were assigned to the affected group, since previous studies have shown that FIII patients have the most severe clinical manifestations—that is, bleeding (Homeida et al. 1988*a,* 1988*b;* Richter et al. 1992*a,* 1992*b;* Domingues et al. 1993). Four subjects who, during recent years, died from severe schistosomiasis (diagnosed on the basis of hematemesis, ascites, and lower-limb edema) also were included in the affected group. Subjects with either F0 or FI were classified as unaffected because various clinical studies have confirmed that these grades correspond to either no fibrosis or limited periportal fibrosis associated with the mildest clinical forms of schistosomiasis. For the classification of the subjects with FII, additional echographic informations was used, for two reasons: (1) FII has not been systematically associated with severe clinical manifestations (Richter et al. 1992*a,* 1992*b;* Domingues et al. 1993), and (2) a second echographic evaluation performed on 172 patients showed that FII subjects could be misclassified whereas the classification of all FIII subjects was confirmed by the second evaluation. For these reasons, FII patients were assigned to the affected group when they exhibited a PVD above a given threshold, since increased PVD in schistosomiasis patients is a direct indicator of portal-blood hypertension (Abdel-Latif et al. 1981) and correlates with the occurrence of gastrointestinal bleeding (Richter et al. 1992*a*); this PVD threshold was defined as the 95th percentile of the PVD values observed in subjects with either F0 or FI. Since PVD varies with age, five age-dependent thresholds were defined: >10 mm (age 0–5 years), >11 mm (age 6–10 years), >12 mm (age 11–15 years), >14 mm (age 16–20 years), and >14 mm (age >20 years). FII patients with a PVD lower than the age-specific threshold were classified as having an unknown phenotype.

Values for splenic-vein diameter and spleen volume were comparable in the FII high-PVD group and in the FIII group, for subjects >20 years of age (table 1). Splenic-vein diameter and spleen size in these affected subjects were increased compared with the values observed in the FII group with normal PVD, whose values themselves were close to those of groups F0 and FI (table 1). These observations further indicate portal-blood hypertension in the subjects with an enlarged portal vein. This classification is consistent with observations, by others, that have indicated that the most frequent ultrasound findings in severe schistosomiasis are extended periportal fibrosis, widening of portal and splenic veins, and, eventually, splenomegaly (Cerri et al. 1984; Richter et al. 1992*a,* 1992*b*). In contrast, liver volume was smaller in affected subjects than in unaffected subjects; this finding is explained by the shrinkage of the left lobe in subjects with extended Symmers fibrosis.

Segregation Analysis

Segregation analysis was performed by the regressive logistic model (Bonney 1986). This model specifies a regression relationship between the probability of a person being affected (i.e., having a severe fibrosis) and a set of explanatory variables including major genotype, phenotypes of antecedent individuals in the pedigree, and other covariates.

The parameters of the major gene are (1) q, the frequency of allele A predisposing to severe fibrosis, and (2) α_{AA}, α_{Aa}, and α_{aa}, the three baseline risks of being affected, on the logit scale corresponding to the three genotypes, AA, Aa, and aa. To ensure that the parent-offspring transmission of the major gene is Mendelian, three additional parameters—τ_{AA}, τ_{Aa}, and τ_{aa}—have been defined, which denote the probabilities of transmission of A, for individuals with genotypes AA, Aa, and aa, respectively. These parameters are used to test the hypothesis of Mendelian transmission ($\tau_{AA} = 1$,

$\tau_{Aa} = .5$, and $\tau_{aa} = 0$) against alternative hypotheses such as general transmission (free τ's) and no parent-offspring transmission (equal τ's).

The dependence on relative phenotypes is parametrized through regression coefficients accounting for the relationship between the phenotype of an individual and the phenotype of his antecedent relatives. In the class D pattern of familial dependence, which is used in the present analysis, four types of phenotypic familial dependencies are considered: spouse-spouse, father-offspring, mother-offspring, and sib-sib. Since neither spouse-spouse dependence nor difference between father-offspring and mother-offspring dependence was found to be significant, results are presented with a global parent-offspring parameter and a sib-sib regression parameter, which are denoted as "Γ_{PO}" and "Γ_{SS}," respectively. To account for unknown phenotypes, each of the Γ parameters is a vector of two coefficients (Demenais 1991)—that is, $\Gamma_{PO} = (\gamma_{PO1}\ \gamma_{PO2})$ and $\Gamma_{SS} = (\gamma_{SS1}\ \gamma_{SS2})$; for example, for an individual, the logit of being affected either is modified by either γ_{PO1} or γ_{PO2} or remains unchanged when his or her father is affected, unaffected, or unobserved, respectively.

The effects of covariates are specified through regression coefficients. Of the covariates having effects on the phenotype, those evaluated were gender (coded as "0" for males and as "1" for females), age (in years), and duration of exposure (in years). Since study subjects began to settle in this village 20 years ago, the duration of exposure was fixed at either 20 years, for subjects $\geqslant$20 years of age, or the subject's age, for individuals $<$20 years of age. Therefore, age and duration of exposure were two highly correlated covariates, and the best-fitting function was a simple regression on the duration of exposure. Furthermore, the genotype × covariate interactions were never found to be significant, and, finally, the results of the present analysis are presented with two parameters, β_{sex} and β_{dur}, which are the regression coefficients associated with sex and duration of exposure, respectively.

All calculations were performed by the computer program REGRESS (Demenais and Lathrop 1994), which incorporates the regressive approach into the LINKAGE package (Lathrop et al. 1984) and allows combined segregation-linkage analysis (Bonney et al. 1988). No ascertainment correction was needed for likelihood computations, since all families of the village were included in the analysis. Nested models were compared by means of the likelihood-ratio test.

Linkage Analysis

Two-point LOD scores for various values of the recombination fraction (θ) were computed by means of the LINKAGE package and by use of the major-gene model obtained from segregation analysis (20 liability classes were defined according to gender and duration of exposure considered in terms of 2-years intervals). Marker-allele frequencies were estimated from our data, since some alleles found in this Sudanese population were not referred in the CEPH reference families. The analysis was also performed under the assumption that the marker-allele frequencies were equal. For marker D6S310, a combined segregation-linkage analysis using the REGRESS program was also performed. In this case, linkage is tested by comparison of the maximum likelihood obtained under a genetic model assuming no linkage ($\theta = .5$) versus that obtained under the same genetic model accounting for linkage (θ estimated). This combined analysis also allows a new estimation of major-gene parameters in the presence of linkage. Multipoint analysis was conducted by the VITESSE program (O'Connell and Weeks 1995), which needed to simplify two pedigrees and to break one loop. Because of both the large size of some families and the number of marker alleles (i.e., 5–13), calculations were confined to a five-point analysis including the following four markers: D6S1009, FA1, D6S310, and D6S308.

Genotyping

The potential role of four regions containing genes that could be involved in the control of severe hepatic fibrosis was investigated by linkage analysis with the polymorphic markers indicated in table 2. Markers for the 5q31-q33 region were as described elsewhere (Marquet et al. 1996). Markers D12S92 and D6S1009 were from the Généthon panel (Dib et al. 1996), and the other "D" markers were from the ABI PRISM Linkage Mapping Panel, version 1 (Perkin-Elmer). The FA1 marker (GenBank) has been described by Altare et al. (1998), and the TNF markers have been described by Cabrera et al. (1995). Amplifications and PCR-product analysis were performed as described by Marquet et al. (1996).

Results

Determining the Severe Hepatic Fibrosis Phenotype in the Sudanese Study Subjects

The study was conducted in Al Taweel, a Sudanese village highly endemic for *S. mansoni,* as are many villages in Gezira region (Omer et al. 1976). Detailed demographic, epidemiological, and clinical observations of this village have been reported elsewhere (Mohamed-Ali et al. 1999). The entire population of the village, ~900 people, was studied, except for those subjects (~13%) who, because they were then traveling to remote areas, were not available at the time of either the first or second echographic evaluation. Of 781 subjects (361 males and 420 females) evaluated by ultrasound, 217, 460, 85, and

Table 1

Mean ± Standard Error of the Mean, of Ultrasound Measurements in 337 Subjects >20 Years of Age, According to Affected Status

	Status of Subjects				
	Unaffected: FO or FI[a]	Affected			Unknown: FII[c]
		FIII	FII[b]	All	
No. of subjects	256	17	21	38	43
PVD (mm)	11.9 ± .09	15.1 ± .27	15.3 ± .17	15.2 ± .15	12.9 ± .18
Left-liver-lobe volume (cm^3)	114.4 ± 1.7	92.2 ± 4.2	111.7 ± 5.3	102.9 ± 3.8	106.7 ± 4.2
Splenic-vein diameter (mm)	7.6 ± .13	11.0 ± .51	10.4 ± .59	10.71 ± .40	8.2 ± .34
Spleen volume (cm^3)	247.0 ± 11.3	617.8 ± 112.9	513.7 ± 99.1	560.2 ± 74.0	269.2 ± 21.0

[a] Subjects with grade 0 or 1.
[b] Grade 2 subjects with PVD >14 mm.
[c] Grade 2 subjects with PVD ⩽14 mm.

19 exhibited grades 0, 1, 2, and 3, respectively; 24 FII subjects had a PVD above their age-specific threshold and were classified as affected, whereas the remaining 61 were considered as having an unknown phenotype (table 1). Therefore, the total number of affected subjects was 47 (19 FIII, 24 FII with high PVD, and 4 who died of severe schistosomiasis), corresponding to 6% of the evaluated individuals. This proportion was much higher in males (41/361 [11.3%]) than in females (6/420 [1.4%]). The distribution of affected subjects, stratified by age class, is shown in table 2; only five affected subjects were <20 years of age. Of the five large pedigrees, four included more than three affected subjects each, whereas the remaining pedigree had no affected members.

Detecting a Major Gene for Severe Fibrosis (SM2)*, by Segregation Analysis*

The results of segregation analysis are presented in table 3. The hypothesis of no familial dependencies was rejected (model I vs. model IIa; $\chi^2 = 13.3$, 4 df, $P < .02$), and the sib-sib dependence was not significant. The most parsimonious model (model IIb) included only the γ_{P1} parameter, which, in this case, is directly interpretable in terms of log odds ratio (Abel et al. 1993)—that is, the odds ratio that compares the likelihood that a child with an affected parent will be affected versus the likelihood that a child with an unaffected or an unknown parent will be affected—$\exp(\gamma_{P1}) = 4.26$. In the presence of parent-offspring dependence, there was evidence for a codominant major gene (model IIb vs. model IIIa; $\chi^2 = 12.1$, 3 df, $P < .01$). Both the recessive (model IIIc vs. model IIIa) and the dominant (model IIIb vs. model IIIa) hypotheses for the major-gene effect were rejected. Parent-offspring dependence residual from the codominant major gene (model IIId vs. model IIIa) was not significant ($\chi^2 = 0.3$, 1 df, $P > .5$). The Mendelian transmission of the codominant major effect (Model IIId vs. model Vb) was compatible with the data ($\chi^2 = 6.9$, 3 df, $P > .07$), whereas the estimate of τ_{AA} was far from its Mendelian value (i.e., it was .0 instead of 1); however, it should be noted that τ_{AA} is not a critical parameter in this analysis, since the expected proportion of AA individuals is very low (.008) under model Vb and fixing τ_{AA} at 1 only increased the value of minus twice the log likelihood (−2logL) of model Vb by .8 (not shown in table 3). Finally, the non–parent-offspring transmission hypothesis (model Va vs. model Vb) was rejected ($\chi^2 = 14.6$, 2 df, $P < .007$). In conclusion, a codominant major gene referred to as "*SM2*" accounts for the familial distribution of the phenotype defined as hepatic fibrosis and portal hypertension in schistosomiasis. The frequency of the deleterious allele A was estimated as .162; consequently, the respective proportions of AA, Aa, and aa subjects were .03, .27, and .70.

Mapping SM2 *to Chromosome 6q22-q23*

To localize *SM2*, all informative families with multiple cases of severe fibrosis (i.e., eight families including 112 individuals) were genotyped, and linkage analysis was

Table 2

Distribution of 781 Subjects Who Had Ultrasound Measurements, by Age and Affection Status

Age Group (years)	No. (%) of Subjects			
	Unaffected[a]	Affected[b]	Unknown[c]	Total
<10	217 (96.9)	0	7 (3.1)	224
10–14	138 (93.9)	4 (2.7)	5 (3.4)	147
15–19	72 (92.3)	1 (1.3)	5 (6.4)	78
20–29	80 (71.4)	11 (9.8)	21 (18.8)	112
30–39	72 (82.7)	8 (9.2)	7 (8.1)	87
40–49	44 (71.0)	8 (12.9)	10 (16.1)	62
>50	54 (76.1)	11 (15.5)	6 (8.4)	71
Total	677 (86.7)	43 (5.5)	61 (7.8)	781

[a] Subjects with grade 0 or 1.
[b] Grade 3 subjects and grade 2 subjects with PVD above age-specific threshold.
[c] Grade 2 subjects with PVD below age-specific threshold.

Table 3

Results of Combined Segregation-linkage Analysis

Model and Hypothesis	Value of Parameter													
	α_{AA}	α_{Aa}	α_{aa}	β_{sex}	β_{dur}	q	θ	τ_{AA}	τ_{Aa}	τ_{aa}	γ_{Po1}	γ_{Po2}	γ_{SS1}	γ_{SS2}	$-2\ln L+c$[a]
I. Sporadic	−7.26	(=α_{AA})	(=α_{AA})	−2.39	.31	(0)	…	…	…	…	(0)	(0)	(0)	(0)	37.0
II. Familial dependences:															
a. All familial dependencies	−9.02	(=α_{AA})	(=α_{AA})	−2.32	.39	(0)	…	…	…	…	1.38	−.12	.20	.20	23.7
b. Parent-offspring (γ_{PO2} = 0)	−8.73	(=α_{AA})	(=α_{AA})	−2.32	.37	(0)	…	…	…	…	1.48	(0)	(0)	(0)	25.1
IV-1. Mendelian major gene, under the assumption of no linkage with D6S310:															
a. Codominant, residual familial dependencies	−15.15	−31.69	−34.99	−10.62	1.61	.165	(.5)	(1)	(.5)	(0)	.73	(0)	(0)	(0)	13.0
b. Dominant, residual familial dependencies	−7.90	(=α_{AA})	−10.96	−3.01	.45	.075	(.5)	(1)	(.5)	(0)	1.23	(0)	(0)	(0)	21.3
c. Recessive, residual familial dependencies	−15.69	−34.99	(=α_{Aa})	−12.14	1.68	.186	(.5)	(1)	(.5)	(0)	1.19	(0)	(0)	(0)	18.5
d. Codominant, no residual familial dependencies	−14.71	−30.91	−34.99	−10.00	1.58	.162	(.5)	(1)	(.5)	(0)	(0)	(0)	(0)	(0)	13.3
IV-2. Mendelian major gene accounting for linkage with D6S310, codominant, no residual familial dependencies	−13.94	−29.03	−33.82	−7.67	1.50	.153	.0	(1)	(.5)	(0)	(0)	(0)	(0)	(0)	.0
V. Non-Mendelian codominant major effect (under the assumption of no linkage):															
a. No vertical transmission	−15.83	−31.52	−34.99	−8.74	1.62	.084	(.5)	.23	(=τ_{AA})	(=τ_{AA})	(0)	(0)	(0)	(0)	21.0
b. General transmission	−14.53	−31.01	−34.99	−10.65	1.60	.087	(.5)	.0	.68	.08	(0)	(0)	(0)	(0)	6.4

[a] L = likelihood, and c = 2lnL of model IV-2.

Table 4

LOD Scores at Various θ Values for Linkage of *SM2* to Four Candidate Regions

Region and Marker (Distance[a])	LOD Score at θ = .0	.01	.05	.10	.20	.30	.40
5q31-q33:							
D5S471	−11.20	−6.57	−3.90	−2.52	−1.12	−.42	−.09
IL4 (4[a])	−4.92	−2.86	−1.65	−1.02	−.42	−.14	−.03
D5S393 (10)	−4.64	−3.04	−1.75	−1.08	−.44	−.17	−.05
D5S436 (17)	−.25	−.20	−.06	.03	.07	.03	−.01
D5S636 (23)	−5.84	−3.37	−2.21	−1.50	−.72	−.32	−.10
D5S2077 (26)	−1.95	−1.63	−.89	−.50	−.20	−.09	−.04
D5S673 (27)	−4.60	−1.55	−.81	−.49	−.23	−.12	−.05
D5S410 (27)	−1.16	−.68	−.17	.02	.10	.06	.02
6q22-q23:							
D6S292	−1.40	−1.27	−.88	−.57	−.22	−.07	−.01
D6S1009 (2)	1.26	1.26	1.23	1.14	.84	.47	.15
FA1 (3)	1.80	1.76	1.58	1.35	.89	.47	.14
D6S310 (4)	2.81	2.74	2.46	2.10	1.37	.70	.20
D6S308 (5)	−.56	−.50	−.34	−.21	−.07	−.02	−.00
D6S441 (16)	.80	.83	.91	.89	.66	.33	.07
6p21:							
TNF	−5.60	−2.09	−1.13	−.67	−.25	−.09	−.02
D6S276 (1)	−2.50	−2.35	−1.81	−1.29	−.62	−.24	−.05
12q15:							
D12S83	.34	.37	.43	.44	.33	.17	.05
D12S92 (8)	−1.23	−5.64	−3.33	−2.12	−.92	−.34	−.07
D12S326 (11)	−6.61	−3.99	−2.58	−1.75	−.84	−.35	−.09

[a] Approximate genetic distance (in cM) from the first marker listed for each region.

conducted in four candidate regions: (1) the 5q31-q33 region, where *SM1* and several candidate genes—such as those coding for the granulocyte-macrophage colony–stimulating factor (*CSF2*), several interleukins (*IL-13, IL-3, IL-4, IL-5, IL-9,* and *IL-12*), the interferon regulatory factor 1 (*IRF1*), the colony-stimulating factor-1 receptor (*CSF-1R*), and the gene(s) controlling total serum IgE levels and familial hypereosinophilia—are located; (2) the HLA-TNF region (6p21), containing the HLA locus and the *TNF-α* and *TNF-β* genes; (3) the 12q15 region, including the *IFN-γ*gene and a gene controlling total serum IgE levels; and (4) the 6q22-q23 region, containing the *IFN-γR1* gene. Two-point LOD scores are shown in table 4. No maximum LOD-score (Z_{max}) values >.1 were observed with any marker from regions 5q31-q33 and 6p21. In the 12q15 region, D12S83 provided a Z_{max} of .44 at θ = .08, but multipoint analysis showed a LOD score <−4 at a location corresponding to the gene coding for IFN-γ. In contrast, significant Z_{max} values were observed in region 6q22-q23, with both D6S310 (Z_{max} = 2.81 at θ = .0) and the FA1 intragenic marker (Z_{max} = 1.80 at θ = .0). The D6S308 marker was poorly informative, with a heterozygosity of <60%. The use of equal marker-allele frequencies did not strongly influence the results for D6S310 and *FA1,* which had Z_{max} = 2.77 at θ = .0 and Z_{max} = 2.14 at θ = .0, respectively. The segregation of haplotypes for the six markers in this region in two families is presented in figure 1.

To further investigate the linkage with the 6q22-q23 region, we performed a combined segregation-linkage analysis with the D6S310 marker (table 3). Results showed that the hypothesis of no linkage with D6S310 was highly rejected (model IIIa vs. model IV; χ^2 = 13.3, 1 df, $P < .0003$). Under the model accounting for linkage with D6S310, the maximum-likelihood estimate of θ was .0, and the LOD score was 3.11. The parameters of *SM2* (i.e., penetrance and allele frequency) estimated in this combined segregation-linkage analysis are very close to those obtained under the assumption of no linkage, and figure 2 shows the penetrance, with duration of exposure, for AA subjects and Aa males, that is predicted by the model accounting for linkage. For AA subjects the penetrance is almost complete after 12 and 17 years of exposure, for males and females, respectively, whereas for Aa males the penetrance is .73 after 20 years of exposure. For aa males the penetrance reaches .02 after 20 years of exposure and is <.01 before then; for Aa and aa females the penetrance remains lower <.001 after 20 years of exposure.

We also conducted a five-point analysis using the VITESSE program (fig. 3), which needed to simplify two large pedigrees. The multipoint Z_{max} of 3.12 was obtained with D6S310, and a LOD of 2.49 was observed

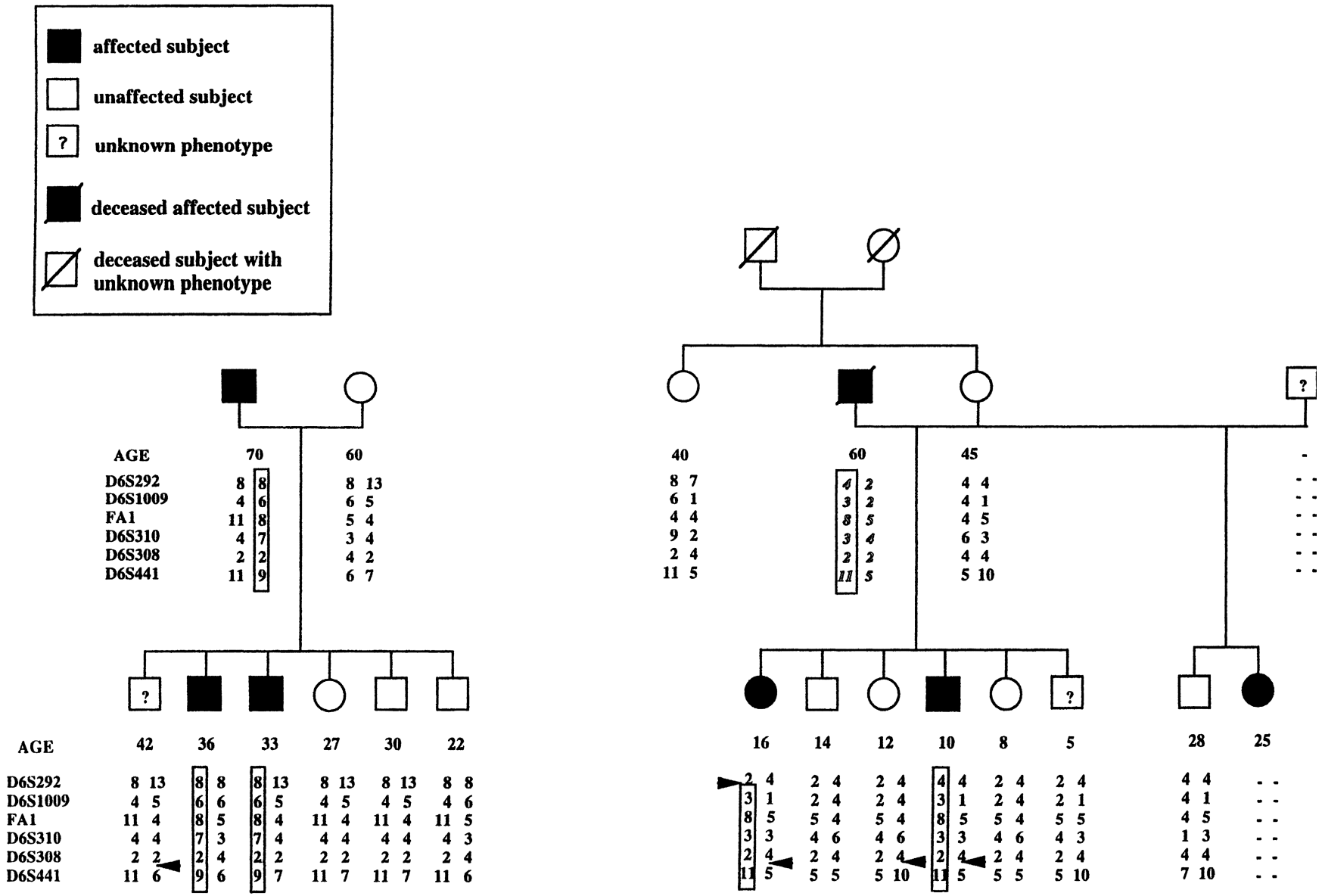

Figure 1 Segregation of the six markers of the 6q22-q23 region in two families, indicating, for each subject, the phenotype, age, and the haplotype minimizing the number of recombinants for the six markers. Alleles inferred from other relatives are displayed in outline italic. The likely susceptibility haplotypes are boxed. Recombinations are indicated by arrowheads.

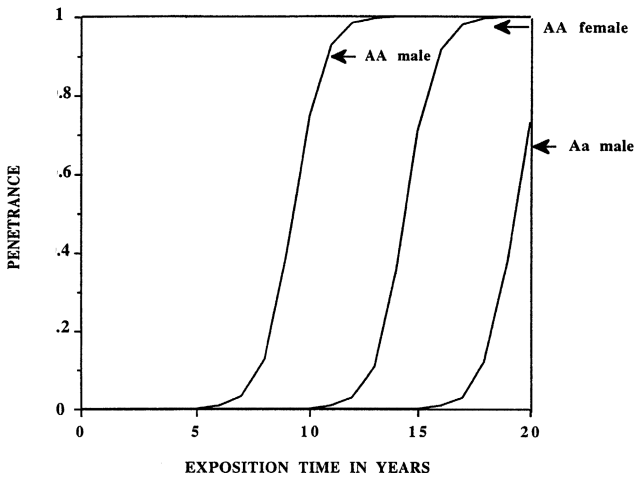

Figure 2 Penetrance, according to exposure time, predicted by model IV of table 3, for AA subjects and Aa males.

with the FA1 intragenic marker. This precise location of *SM2* should be interpreted in light of the linkage results that were obtained with *SM2* parameters estimated under the hypothesis of a single gene, whereas the true genetic model could be more complex. In the latter case, *SM2* parameters could be partly misspecified, leading to bias in the estimation of θ (Clerget-Darpoux et al. 1986); however, this possible misspecification does not influence the robustness of the analysis (i.e., it does not lead to a false conclusion with regard to linkage) and consequently does not affect the validity of our findings showing a linkage within the region 6q22-q23.

Discussion

The report of a major locus controlling susceptibility to Symmers fibrosis brings considerable progress to our understanding of why only a fraction of subjects infected by *S. mansoni* develop severe fibrosis. Since hepatic fibrosis is the consequence of the inflammation induced by eggs and worm products in the portal spaces, it has been thought that disease development is dependent on a patient's worm load. This view has been supported by reports indicating that hepatosplenomegaly is both more frequent in areas of high endemicity (Kloetzel 1962; Arap Siongok et al. 1976) and is correlated with infection levels (Cheever 1968; Cook et al. 1974) in endemic populations; however, ultrasound examination have revealed that high fibrosis grades are associated with high infection only in children (Homeida et al. 1988*a;* Abdel-Wahab et al. 1990; Doehring-Schwerdtfeger et al. 1990; Domingues et al. 1993) and that hepatomegaly is not necessarily associated with severe fibrosis (Homeida et al. 1988*a;* Doehring-Schwerdtfeger et al. 1992; Richter et al. 1992*a*). In the present Sudanese study, severe fibrosis (with hypertension) was associated with the duration of infection (Mohamed-Ali et al. 1999). Taken together, these observations indicate either that infection intensity is probably not a major factor in disease development or that its effects are hidden by the action of more-important factors. We made no attempt to evaluate exposure to infection in this Sudanese population, because advanced fibrosis developed 10–15 years after infection, indicating that measurement (to be meaningful) would have to be performed over a very long period—and this was, in practice, impossible. Furthermore, we have previously shown that differences in exposure account for no more than 20%–30% of infection-intensity variance in a population whose members have similar levels of water contact (Abel et al. 1991). This, together with the observation that infection intensity is probably not a major factor in disease development, makes it unlikely that exposure differences between subjects in this population could be an important determinant of severe fibrosis.

A characteristic of this study is that the present population migrated into this area 20 years ago, coming from a nonendemic area. Therefore, it is unlikely that study subjects had developed schistosomiasis disease or immunity to disease previously and that selective pressures have been exerted by *S. mansoni* infections on this population. The genetic model indicates that a 50% penetrance is reached after 9, 14, and 19 years of residency in the area, for AA males, AA females, and Aa males, respectively, whereas for other subjects the penetrance remains <.02 after 20 years of exposure. Nevertheless,

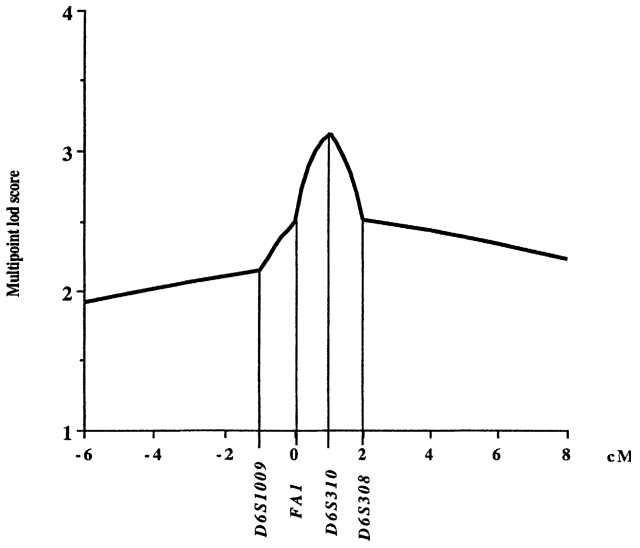

Figure 3 Multipoint analysis showing the location of *SM2* in relation to the following four markers: D6S1009, FA1, D6S310, and D6S308. The horizontal axis represents the genetic distance (in cM), with the intragenic *IFN-γR1* marker FA1 chosen as the origin.

with a time of residence sufficiently long, all heterozygote males are likely to present with the disease. Consequently, in this population, 30% (i.e., 3% of homozygotes and 27% of heterozygotes) of males could potentially develop severe schistosomiasis if left untreated. It would now be interesting to determine whether the same proportions will be recorded in populations that have been living in endemic areas for longer time periods. The estimated penetrance of *SM2* strongly depends on gender, accounting for the lower prevalence of fibrosis in females than in males. Such gender differences in the prevalence of fibrosis cases have been reported by other groups working either in Sudan (Homeida et al. 1988*a,* 1988*b*) or in Egypt (Abdel-Wahab et al. 1990). Elsewhere, we have shown that the low prevalence of FII and FIII in females is likely due to a sharp decrease in the prevalence of infection in females $\geqslant$16 of age (Mohamed-Ali et al. 1999). This decrease might be due either to a reduction in exposure after puberty (after puberty, females in Muslim countries might be less exposed to infection than are males) or to differences, in the development of immunity, between males and females.

Previously, we investigated the causes of high infection levels by *S. mansoni* in an endemic Brazilian population, and we found that, to a large extent, they depended on a major gene, *SM1* (Abel et al. 1991), which we mapped to the 5q31-33 region (Marquet et al. 1996). This result subsequently was confirmed in a Senegalese population (Müller-Myhsok et al. 1997). Ongoing work is attempting to characterize this gene. The present report that the major locus controlling fibrosis is not linked to chromosome 5q31-33 and that it maps to another region demonstrates that antidisease immunity and anti-infection immunity are under distinct major-gene control. Obviously our results do not rule out an interaction between *SM1* and *SM2*. It is reasonable to postulate that disease development is accelerated in *SM2* subjects predisposed to high infections. Unfortunately, the low and rather uniform levels of infection in our study population did not allow us either to search for *SM1* or to evaluate possible interactions between *SM1* and *SM2*. Our linkage results obtained with two microsatellite markers of the HLA-TNF region also indicate that the major locus *SM2* is very unlikely to be located within this region, whereas associations have been reported between (*a*) some HLA class I alleles (A1 and B5) and hepatosplenomegaly in Egypt (Salam et al. 1979; Abaza et al. 1985; Abdel-Salam et al. 1986) and (*b*) an HLA class II allele (DQB1*0201) and biopsy-confirmed hepatic schistosomiasis in Brazil (Secor et al. 1996). However, it should be stressed that the present results do not exclude the role of additional polymorphisms, such as specific HLA antigens, which could be detected by an appropriate association study in this population.

It is too early to speculate on the mechanism of action of *SM2;* however, given the results of multipoint linkage analysis, which maps the susceptibility locus close to the *IFN-γR1* gene, polymorphism(s) in this IFN-γ receptor may account for increased susceptibility to severe fibrosis. This hypothesis is consistent with reports in experimental models showing that IFN-γ is the major antifibrogenic cytokine in the schistosome granuloma (Czaja et al. 1989*a*). In addition, human IFN-γ has been shown to inhibit proliferation of human fibroblasts (Duncan and Berman 1985), to down-regulate both collagen (Duncan and Berman 1985) and prolyl 4-hydroxylase biosynthesis (Kawaguchi et al. 1992), and to stimulate the synthesis of metalloproteases (collagenases) (Tamai et al. 1995). More specifically, IFN-γ inhibits proliferation and down-regulates collagen biosynthesis by Ito cells (Mallat et al. 1995), which are the major source of collagen in the liver of schistosome-infected subjects. Various works have also shown that tissue fibrosis can be reduced by injections of IFN-γ (Granstein et al. 1990; Hein et al. 1992). Finally, recent works have shown that mutations in the *IFN-γR1* gene can dramatically alter susceptibility to infectious diseases such as nontuberculous mycobacterial infections (Jouanguy et al. 1996; Newport et al. 1996)

In conclusion, this work shows that severe fibrosis in subjects infected by *S. mansoni* is determined by a major locus that maps close to the *IFN-γR1* gene. This finding opens the way to both the identification of the gene and the evaluation of its role in the determination of abnormal fibrosis of other etiological origins. Finally, our results will stimulate new strategies in drug and vaccine development.

Acknowledgments

We thank Jean-Laurent Casanova for providing information about the FA1 marker, and we thank Michelle Morton and Christophe Chevillard for helpful assistance. This work received financial assistance from INSERM, Centre National de la Recherche Scientifique, the European Community/STD3 program, the WHO special program for research and training in tropical diseases, and the Conseil Regional Provence Alpes Côte d'Azur.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Perkin-Elmer, http://www.perkin-elmer.com (for ABI-PRISM)
GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for FA1 [U84721])
Généthon, http://www.genethon.fr
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for *SM1* [MIM 181460])

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