Infection and disease in human schistosomiasis mansoni are under distinct major gene control

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1. Introduction

Considerable evidence has accumulated in the past few years indicating that infection levels and prevalence of schistosome infections in endemic populations depend principally on the resistance/susceptibility of the human host. Several studies have associated the control of infection and reinfection after treatment with antilarval IgE antibodies, eosinophils, and with a specific Th0/2-dependent immune response. In endemic areas, several authors have reported that high infection intensities and severe disease occur more frequently in certain families; moreover, disease and heavy worm burden are more frequent in children of affected parents. Such observations suggest that some inherited factors may have a major effect on human resistance/susceptibility to infection and disease caused by schistosomes. Obviously, other explanations, such as familial resemblance in behavior or in nutrition, could also account for these observations. Nevertheless, the facts were interesting enough to stimulate further research and we decided to evaluate the importance of genetic factors in schistosomiasis. If some genetic polymorphisms had a major effect on parasitological and clinical phenotypes, then they would be very valuable tools for understanding pathogenesis mechanisms; they would also enable us to identify critical steps in the pathogenesis process.

2. Genetic control of infection levels in the population of Caatinga do Moura

Our first major gene, *SM1*, was discovered in Caatinga do Moura, a small village in the state of Bahia in Brazil; the village is located in a semi-desert area that is irrigated by tiny canals and a small river. The river as well as the canals are densely populated by *Biomphalaria glabrata* snails infected by *Schistosoma mansoni*. The region is hyperendemic for schistosomiasis in spite of major efforts to control parasite transmission. The population selected for that study comprised all subjects living on the left bank of the river, without exception. In later studies in other endemic areas, we came to realize that epidemiological conditions in Caatinga were ideal, since all subjects lived within 100 to 500 m of the river and had water contact at only a few sites. This made possible an accurate evaluation of exposure for all subjects. How this epidemiological study was carried out in this population was previously described [1]. These studies showed that exposure, age, and gender accounted for 25 to 30% of the variance of infection levels in Caatinga's population.

Infection levels adjusted for water contacts, age, and gender were markedly variable among study subjects, and elevated infections were concentrated in certain families, suggesting inherited factors in resistance/susceptibility to infection [1]. For this reason, we tested for the presence of a major genetic effect controlling infection levels using segregation analysis performed on all families from the study area.

In a recent article, we discussed the advantage of using family-based studies in genetic analysis [2]. These methods do not require a hypothesis as to the identity of the gene. As a result, new loci in pathogenesis can be uncovered. Segregation analysis is basically mathematical modelling. It tests whether the segregation of the studied phenotype (infection levels) in pedigrees follows mendelian expectations. The probability (likelihood) of observing the sample population under different hypothetical conditions (models) of transmission are computed by maximum like-

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lihood methods, and likelihood ratio tests are performed to determine which models provide the best fit to explain the observed familial distributions of the phenotype (infection levels). The models which are tested range from a nongenetic model to a sophisticated major gene model which specifies the frequency of the susceptibility allele and the average mean of infection levels associated with the genotypes. Other covariates such as exposure or gender can be included in the analysis, and the method also tests for the presence of residual familial correlations.

Segregation analysis applied to infection levels in the population study provided strong evidence for a major codominant gene controlling infection levels [3]. Based on this model, the susceptibility allele was predicted to have a frequency of 0.18; thus homozygous susceptible subjects represented about 4%, heterozygous 35%, and homozygous resistant 61% of the population. The susceptible phenotype was clearly distinct from that of heterozygous individuals, indicating that the gene had a profound effect on susceptibility to infection.

3. A 'genome-wide search' performed on infection intensity showed linkage of the major gene with the genetic region 5q31q33

The major locus detected by segregation analysis must be confirmed by mapping it in the subject's genome. This can be done by linkage analysis. This analysis tests whether any genetic loci segregate with the predicted major gene in the study population. Or to put it differently, whether the major gene remains linked to a known genetic locus after extended chromosomal exchanges during meiosis. We used an approach that tests the linkage of the major gene to any regions of the human genome. This approach termed 'genome-wide search' has the enormous advantage of screening the whole genome and therefore detecting the susceptibility locus wherever it is located in the genome. This procedure is carried out with microsatellite markers (CA repeats) that are evenly distributed in the human genome [4]. The different markers are identified by the specific sequence of the regions flanking the repeat. Alleles differ by one or several base(s); since the size of CA repeats is highly variable (size polymorphism), parent genotypes are often heterozygous (in size) and different for each parent. Microsatellites are amplified by PCR using DNA sequences complementary to the sequences of the repeat flanking region. PCR products are subsequently analyzed by acrylamide gel electrophoresis to characterize both alleles present at the locus. Around 300 markers are required for a 20-centimorgan map, representing around 45 000 PCR reactions (for the genotyping of 145 subjects).

Linkage is tested by computing the lod score which is the ratio of the likelihood of observing the marker distribution in the sample population using the hypothesis of linkage to the likelihood of observing this same marker distribution using the hypothesis of no linkage. This statistical function is computed for various recombination ratios (genetic distance between the marker and the gene) ranging from 0.50 (no linkage) to 0 (the gene and the marker are entirely linked).

Maximum lod scores greater than 0.1 were observed with 54 markers and were distributed on almost all chromosomes [5]. Six markers located in four regions on chromosomes 1, 5, 7, and 21 yielded maximum lod scores greater than 0.83 ($P < 0.05$). 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 others markers produced Z_{max} below 1.1. The 5q31-q33 chromosomal interval was further analyzed by genotyping 11 additional markers, and significant evidence of linkage (considered as a lod score value above 3.3 according to Lander and Kruglyak [6] in this context of genome-wide search) was obtained with two closely linked markers: D5S636 (Z_{max} = + 4.74, q = 0.07) and CSF1R (Z_{max} = + 4.52, q = 0.04) using estimated marker allele frequencies [5] 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 [5].

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: D1S216 (Z_{max} = + 0.91, q = 0.20), D21S1259 (Z_{max} = + 1.09, q = 0.19), and the two adjacent markers D7S483 (Z_{max} = + 0.91, q = 0.20) and D7S550 (Z_{max} = + 1.02, q = 0.22).

Thus this parametric linkage analysis maps the codominant major gene controlling human susceptibility/resistance to *S. mansoni,* SM1, and is the first example of a successful genome-wide scan in infectious diseases. More recently, Müller-Myhsok et al. [7] confirmed this localization by means of a nonparametric linkage method in a Senegalese population infected with *S. mansoni* and exposed for no longer than seven years. These two studies emphasize the importance of the locus *SM1* which influences the intensity of infection by *S. mansoni*.

The 5q31-q33 region where *SM1* was mapped contains several candidate loci involved in the regulation of the immune response to pathogens, in particular genes coding for interleukin-4 (IL-4), IL-5, IL-12, IL-13, and CSF-1R, the interferon regulatory factor-1 (IRF-1) which encodes a transcriptional activator involved in the regulation of interferon-alpha (IFN-α), IFN-β and other IFN-inducibles genes. Furthermore, this region has been linked to loci related to IgE and/or eosinophilia production, i.e., a locus regulating IgE levels [8, 9], and a locus controlling bronchial hyperresponsiveness in asthma [10].

This localization of *SM1* is consistent with the immunological analyses carried out on both blood lymphocytes and T-cell clones derived from susceptible subjects and resistant subjects. The larva-specific T lymphocytes from homozygous resistant individuals produced higher amounts of IL-4 and IL-5 than T cells in homozygous susceptible subjects. Parasite-specific T lymphocytes isolated from resistant subjects were found to be Th0/2-like [11], while clones from susceptible subjects exhibited a Th0/1-like response (Rodrigues V, Dessein, A, unpublished data). Furthermore, previous results indicated that the resistance to infection by *S. mansoni* was associated

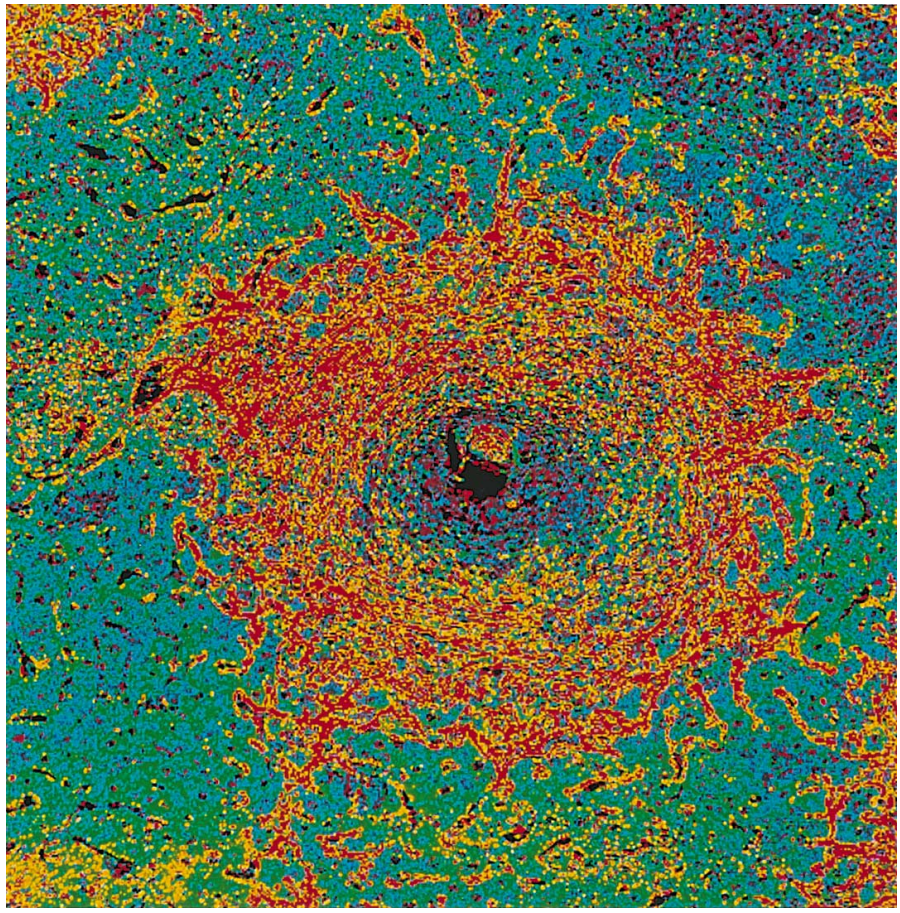

Figure 1. Collagen deposition around an *S. mansoni* egg in mouse liver (false-color image: collagen is red).

with a high parasite-specific IgE antibody response [12–14] and with increased eosinophilia.

4. Severe hepatic disease in *S. mansoni*-infected subjects

Chronic periportal inflammation due to the immunological reactions triggered by schistosome eggs *(figure 1)* and by antigens of the worms that lodge in hepatic and mesenteric vessels may lead to severe clinical manifestations. These manifestations are for the most part the consequence of portal hypertension caused by extended periportal fibrosis which is part of the repair process that follows tissue damage caused by inflammation. Ultimately, some patients with elevated portal hypertension may die of internal bleeding, superinfections, or heart or kidney failure. The management of patients with advanced disease is difficult in countries where hospitals and doctors may be far from the infected populations; as a consequence, advanced schistosomiasis is often fatal.

The reasons why severe disease occurs only in a fraction of infected subjects are not known and are difficult to study since there are no experimental models reproducing Symmer's fibrosis which can be easily studied in the laboratory. In addition, the onset of the hepatic disease, which is the development of advanced periportal fibrosis, has been, until recently, difficult to study in populations of endemic areas. Most studies had to rely on palpation of the liver and of the spleen to detect abnormal fibrosis. Unfortunately, splenomegaly may have other etiologies (leishmaniasis, malaria etc.) in these populations; in addition, it became clear with the use of ultrasound that hepatomegaly is not a good indicator of the degree of hepatic fibrosis, since hepatomegaly occurs in subjects with different fibrosis grades; recent work, including our study, showed that the volume of the liver tends to decrease in advanced fibrosis. Consistency (soft or hard, nodular etc.) of the organ may give some indication as to the extent and/or severity of Symmer's fibrosis; however, these measurements are too imprecise to base studies on the physiological and immunological manifestations associated with the appearance of advanced fibrosis. A better evaluation of hepatic fibrosis can be performed on hospitalized patients; however, the latter are often at a late stage of disease development and it is difficult to determine whether the observations made on them are causes or consequences of hepatic fibrosis. Fortunately, recent work has shown that hepatic fibrosis can be staged by echography using portable ultrasound machines that can be taken

into the field, allowing the screening of 50 to 60 patients per day. A report by specialists who met in Cairo [15] has set forth some relatively simple criteria that allow any good echographist to stage the hepatic disease. It has been our experience, however, that great care should be taken to associate qualitative and quantitative measurements in this evaluation, since intermediate fibrosis grades are sometimes difficult to classify on the basis of qualitative observations only. Intermediate fibrosis grades must be carefully determined because they are critical steps in the progression of disease from mild to severe fibrosis.

5. Which factors determine disease progression?

It has long been felt that severe schistosomiasis develops in subjects with the most severe infections [16, 17]. Early reports clearly associated high disease prevalence with a high rate of transmission, and several studies reported the association of advanced disease with severe infections. However, no general consensus exists concerning this question [18, 19], possibly due to several reasons. First, severe fibrosis likely causes a reduction in egg excretion leading to an underestimate of parasite load in such patients; second, the disease takes several years to develop and may occur years after the peak infection intensity; third, the demonstration of the association between infection levels and disease requires more than just a rapid statistical analysis and some studies may have wrongly concluded in the negative; fourth, different studies analyzed different clinical phenotypes (hepatosplenomegaly or fibrosis evaluated by ultrasound). Nevertheless, a number of reports indicate that severe infections are not necessarily associated with disease. A well known study performed in Brazil by Prata and Bina [20] reported that subjects from Bahia with black characteristics did not develop severe disease in spite of high infections.

Our opinion is that these data are not really conflicting; they simply indicate that infection levels, although important in disease progression, are not the only critical factors and that in different individuals, different factors may be playing the most critical role. We received confirmation of this view in a study performed with our Sudanese colleagues from the Gezira University and from the Faculty of Medicine of Omdurman. This work could not be performed in Brazil in the population that allowed the discovery of *SM1* because that population received chemotherapy several times during the study period. Various reports have shown that chemotherapy, especially repeated chemotherapy, causes regression of hepatic disease in a large number of treated subjects.

6. Determining the severe hepatic fibrosis phenotype in Sudanese study subjects

This study on hepatic fibrosis was carried out in Al Taweel, a Sudanese village that is highly endemic for *S. mansoni*, as are many villages in the Gezira region. Detailed demographic, epidemiological and clinical observations on this village will be reported in another manuscript (Qurashi et al., unpublished). The study population settled in the village 15 to 20 years ago after arriving from a nonendemic region.

Fibrosis in study subjects was evaluated by ultrasound. Liver size, peripheral portal vein branches (PPBs), the degree of periportal fibrosis, spleen size, and splenic and portal vein diameter were assessed. The size of the liver and spleen were measured. Periportal fibrosis was graded 0 to 3 as suggested in [15]. Grade 0 (F0) corresponded to normal liver with no thickening of the wall of the PPBs. The PPB diameter was around 2 to 3 mm; grade 1 (FI) corresponded to a pattern of small stretches of fibrosis around secondary portal branches; this patchy fibrosis usually yielded a 'fish in the pond' appearance *(figure 2)*. The PPB diameter was around 4 mm; grade 2 (FII) corresponded to continuous, in addition to patchy, thickening

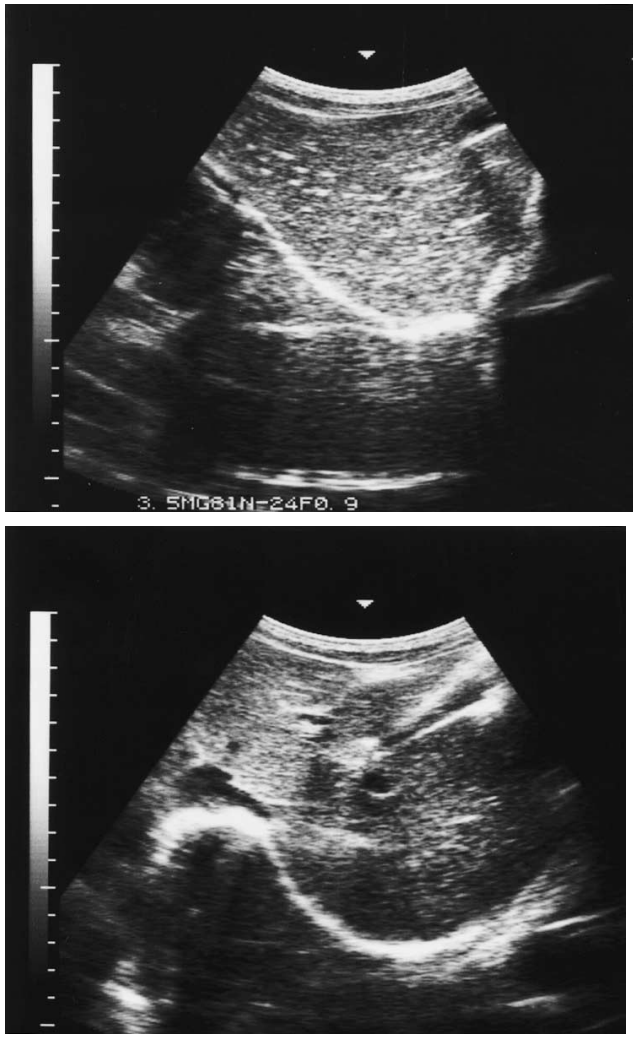

Figure 2. Grade I periportal fibrosis showing short stretches of fibrosis around second- or third-order branches. The patchy fibrosis has a 'fish in the pond' appearance. Top, longitudinal section; bottom, cross-section.

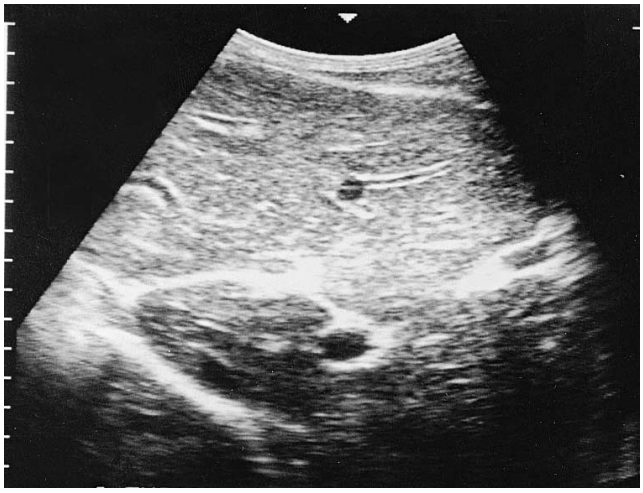

Figure 3. Grade II periportal fibrosis showing longer stretches of patchy or continuous fibrosis. The fibrous vessels may approach the surface of the liver, but are almost always patent.

of PPBs. Most second-order branches appeared as long segments of fibrosis *(figure 3)*; the PPB diameter was around 5 to 6 mm. The gall bladder wall thickness could increase to over 4 mm; grade 3 (FIII) showed wall thickening of almost all PPBs; fibrosis attained the surface of the liver, and in some branches the lumen was occluded *(figure 4)*. The gall bladder wall thickness was usually increased to above normal.

The entire population of the village was studied, except for subjects (around 13%) who were not available at the time of the first or second ultrasound evaluation, as they were traveling to remote areas. Out of 781 subjects (361 males, 420 females) evaluated by ultrasound, 217, 460, 85, and 19 exhibited F0, FI, FII, and FIII, respectively. A fraction of FII subjects (and all FIIIs) exhibited a portal vein diameter (PVD) above normal values, providing evidence of portal hypertension in these individuals. These subjects also had an increased splenic vein diameter and, for most FIIIs, an enlarged spleen. The data taken together indicated that these subjects had developed a more severe disease than other subjects. The total number of FIII and FII subjects with evidence of portal hypertension was 47 (19 FIIIs, 24 FIIs with high PVD, and 4 subjects who died of severe schistosomiasis), corresponding to 6% of evaluated individuals. This proportion was much higher in males (41/361 = 11.3%) than in females (6/420 = 1.4%) and only five affected subjects were under 20 years of age. As for the infection levels, subjects with grade 2 (with or without portal blood hypertension) or grade 3 fibrosis were concentrated in certain families as illustrated in the pedigree shown in *figure 5*. Correlations between parent and offspring clinical phenotypes were also observed. These findings suggested some inherited factors in the control of disease progression.

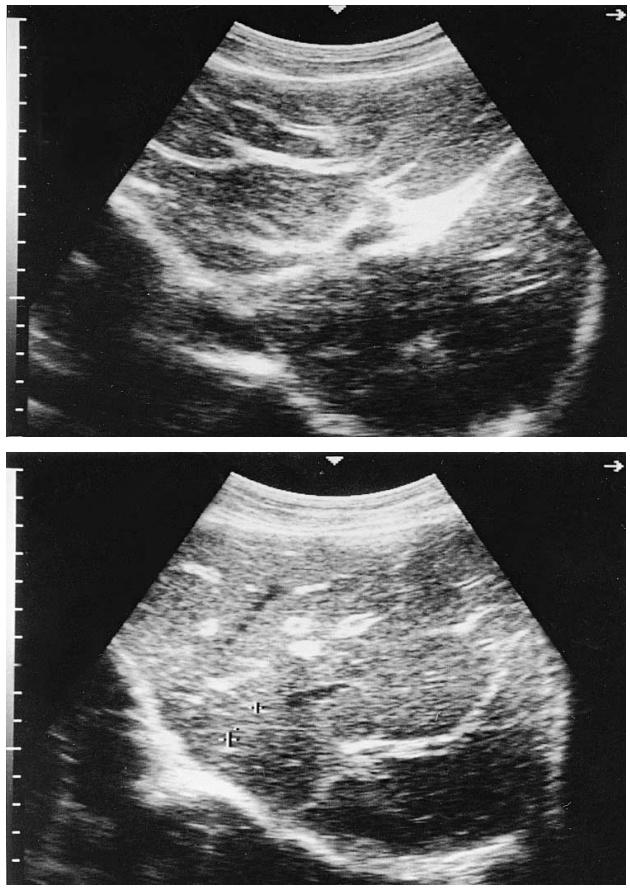

Figure 4. Grade III periportal fibrosis showing markedly thickened peripheral branches (top, longitudinal section). The fibrous vessels reach the surface of the liver and show occluded segments (bottom, cross-section).

7. A major gene controls disease progression

Segregation analysis was used to test for major genetic control of disease development. Subjects with FII and FIII fibrosis and evidence of portal hypertension were classified as affected. The results of segregation analysis have been detailed in Dessein et al. (unpublished). Briefly, the hypothesis of no familial dependence was rejected, and sib-sib dependence was not significant. In the presence of parent-offspring dependence, there was evidence for a codominant major gene. Both the recessive and dominant hypotheses for the major gene effect were rejected. Mendelian transmission of the codominant major effect was compatible with the data, and non-parent-offspring-transmission was rejected. In conclusion, a codominant major gene referred to as *SM2* accounted for familial distribution of the affected phenotype. The frequency of the deleterious allele B was estimated at 0.16; consequently, the respective proportions of BB, Bb, and bb subjects were 0.03, 0.27, and 0.70.

To determine whether gene *SM2* controlling disease development and *SM1* that controls infection levels were

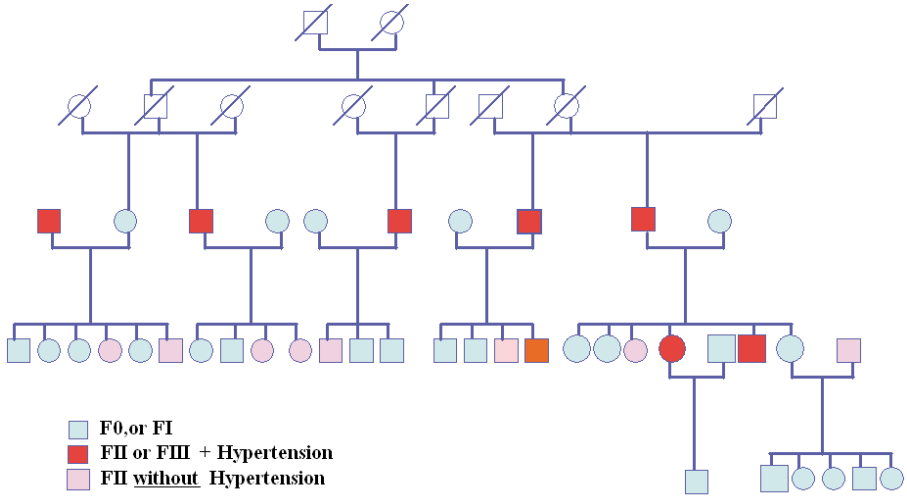

Figure 5. Distribution of fibrosis grades II and III in a Sudanese pedigree.

distinct, we tested whether *SM2* was linked to the 5q31-q33 genetic region. Eight informative families (112 individuals) with multiple cases of severe fibrosis were genotyped, and linkage analysis was conducted with markers of the 5q31-q33 region. No maximum lod score (Z_{max}) values greater than + 0.1 were observed with any marker of region 5q31-q33. Thus, the major gene controlling disease progression does not map in that region, indicating that *SM1* and *SM2* are distinct.

8. Conclusion

These studies show that two distinct genetic loci control human susceptibility to *S. mansoni*: *SM1* located in 5q31-q33 controls infection levels, probably by acting on the production of certain cytokines, and *SM2* controls disease progression.

The observation that the major locus controlling fibrosis is not linked to chromosome 5q31-33 demonstrates that anti-disease immunity and anti-infection immunity are under distinct major gene controls. Obviously, our result does not rule out an interaction between *SM1* and *SM2*. It is reasonable to postulate that disease development is accelerated in *SM2*-predisposed subjects by heavy infections. Unfortunately, the low levels of infection in Cambo Taweela's population did not allow us to search for *SM1* in that population or to evaluate possible interactions between *SM1* and *SM2*.

A characteristic of this study is that the present population migrated to this area 20 years ago from a nonendemic area. Therefore, it is unlikely that study subjects had developed schistosomiasis disease or immunity to disease previously, or that selective pressure had been exerted by *S.mansoni* infections on this population. The genetic model indicates that 50% penetrance was reached after 9, 14, and 19 years of residency in the area for BB males, BB females and Bb males, respectively, whereas penetrance remained lower than 0.02 after 20 years of exposure for other subjects. Nevertheless, with a long enough time of residence, all heterozygous males were likely to present with the disease. Consequently, in this population, 30% of males (3% of homozygous and 27% of heterozygous) could potentially develop severe schistosomiasis if left untreated. It would now be interesting to determine whether the same proportions will be recorded in populations who have been living in endemic areas for longer 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 were reported by other groups working in Sudan. A reasonable explanation is that adult females in Moslem countries are less exposed to infection than males. This explanation is consistent with the observation that the prevalence of infection is markedly lower in females than in males above 20 years of age. However, the possibility should also be considered that gender- related factors may affect schistosome infections.

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