Determination of candidate genes for Schistosomal fibrosis

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ABSTRACT

The specific aims of this study are to characterize genetic patterns associated with schistosome-induced hepatic fibrosis and to link the development of hepatic fibrosis to polymorphic markers located near potential disease susceptibility genes. Approximately 70% of those age>11in the community of Shamarka, in the Nile Delta were surveyed (n=2,038) for parasites and ultrasound evidence of fibrosis using the latest WHO criteria. The potential role of eleven regions containing genes that could be involved in the control of severe hepatic fibrosis was investigated by linkage analysis with the polymorphic markers. Segregation analysis was performed by the regressive logistic model. Tow – point LOD scores for various values of the recombination fraction (θ) were computed by means of the LINKAGE package and by the use of the major-gene model obtained from segregation analysis. A candidate gene search was carried out on 90 affected sibling pairs from 40 pedigrees in this community. From 2-4 single nucleotide polymorphisms (SNPs) were used per locus.Using the most conservative correction for multiple comparisons and the WHO ultrasound pattern, he INFGR1 locus demonstrated a significant linkage for 1 of 4 SNPs ($p=0.0014298$). The analysis further suggests that TGF beta 1 and the IL 13-4 region might also contribute to the development of fibrosis, but their scores did not reach significance after correction.The present work shows that severe fibrosis in subjects infected by S. mansoni is determined by a major locus that maps close to the IFN-γ R1 gene. This finding opens that 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.

Key words: *Schistosomal Fibrosis, Genetics.*

INTRODUCTION

Despite an effective and cheap therapy for schistosome infections, schistosomiasis remains the most important helminth infection in the world. It affects 200 million people around the world and is a major public health problem in many countries. Around 200,000 die each year of this disease (UNDP/ World Bank / WHO 1990). In Egypt despite the drop of schistosome infection rate, still many patients suffer from its complications. The most serious consequence of infection with the

species *Schistosoma mansoni* is hepatic fibrosis leading to portal hypertension and esophogeal bleeding. While hundreds of millions of people are infected, only a small proportion develops severe disease (Dessein *et al.,* 1999).This is common for infectious diseases, and the gap between infection and disease is usually explained by differences in exposure or intensity of infection, environmental conditions, or host genetics. In general there is a poor correlation between intensity and disease. Severe clinical disease is determined largely by the susceptibility / resistance of individuals (Marquet *et al.,* 1996).

Animal studies have indicated that disease development in schistosomal infection 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, *et al.,* 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.,* 1989a) 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.

At the population level we carried a comparative study on two infected groups, one from Egypt and the other from kenya. We found that while the Kenyan population had higher intensity and prevalence of infection than the Egyptian population on the Nile Delta, the prevalence of fibrosis in the Egyptian population was 10 fold higher (Blanton *et al.,* 2002). Environmental factors in the form of connection with hepatitis C, which was much higher in the Egyptian population, might explain these differences, however, hepatitis C infection in no way correlated with intensity of schistosomal fibrosis, we therefore, investigated the role of host genetics in a linkage study of 11 cadidate genes.

The specific aims of this study are to characterize genetic patterns associated with schistome-induced hepatic fibrosis and to link the development of hepatic fibrosis to polymorphic markers located near potential disease susceptibility genes.

MATERIALS AND METHODS

Since hepatic fibrosis in Egypt is more prevalent in the Nile Delta subjects infected with *schistosoma mansoni*; the community of Shamarka, on the Nile Delta was chosen for a study of the genetics of severe fibrosis.

The socioeconomic status of the families, as assessed on the basis of jobs, 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.

Approximately 70% of those age>11 in the central community and the nearby hamlets (ezbas) were surveyed (n=2,038) for parasites and ultrasound evidence of fibrosis using the latest WHO criteria. Measurements were also made of portal branch wall thickness and portal vein diameter. Blood was collected from patients with grade C fibrosis (ultrasonic) and from first-degree relatives in families with more than one sibling with grade C or higher fibrosis. In addition, 35 blood samples were collected at random from the whole population database.

Genotyping

The potential role of eleven regions containing genes that could be involved in the control of severe hepatic fibrosis was investigated by linkage analysis with the polymorphic markers.

Ultrasound methods and infection grades

Study subjects were evaluated by ultrasound examination with a portable Shimadzu SDU-350A 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. PVD was measured at the point of the portal vein's entrace into the porta hepatis, at the lower end of the caudate lobe. Periportal fibrosis was graded as A, B, C, D, and E. 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 C, D & E were assigned to the affected group.

Segregation analysis

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

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). Multipoint analysis was conducted by the VITESSE program, which needed to simplify two pedigrees and to break one loop.

RESULTS

Detailed demographic, epidemiological, clinical observation and ultrasound examination results have been previously reported (King *et al.,* 2003). In the total population, 32.7% of individuals 11 years and older had grade C fibrosis by WHO ultrasound criteria. Table 1 compares intensity of infection with prevalence of fibrosis in Egypt and kenya. A candidate gene search was carried out on 90 affected sibling pairs from 40 pedigrees in this community. From 2-4 single nucleotide polymorphisms (SNPs) were used per locus. SNP analysis was carried out using single nucleotide extension. Based on both qualitative and quantitative phenotypes. Linkage was tested to those genes that initiate the process and stimulate or synthesize collagen or that control collagen degradation. The 11 candidates were: (1) Immune modifiers genes: IL 10, Interferon γ receptor 1 (INFGR1) & IL 13-IL4 (2) Connective tissue synthesis genes : tissue growth factor beta 1, (TGFβ) connective tissue growth factor, (CTGF), Collagen 1 α 1 and Collagen 3 α 1 (3) Connective tissue modifiers genes: Lysyl oxidase, metalloproteinase 1 & 2 (MMP1 & 2) & tissue inhibitor of metalloproteinase 1 (TIMP1). In all, 189 individuals were genotyped at 48 markers. Table (2) represents the pedigree characteristics. Table (3) illustrates the potential factors leading to fibrosis.

Table (1): An infection / disease paradox.

Characteristics	Egypt n=2038	Kenya n=2120
Sex Ratio (%males)	55.8*	42.0
Mean Age	31.7±14.0	30.7±18.4
S. mansoni prevalence	20.0%*	63.6%
Intensity of infection	76.9±1.5*	105.7±3.9
Fobrosis prevalence	36.8%*	2.5%
HCV prevalence	39.7%*	11.3%

*P < 0.05

Table (2): Pedigree characteristics.

% male	62.7%
Mean age	39.7+14.3
Pedigrees (mean size)	40(5.70 + 1.65)
Nuclear Families	36
Extended Families	4
Sibships (mean size)	46 (3.09 +1.30)
Affected Sibpairs	90

Table (3): Potential factors in fibrosis.

Exposure	Social / environmental
Infection	SMI and others
Granuloma	IFNγ, IL4, IL10, IL12, CCLs and co-infections
Collagen deposition	IL13,TGFβ, Col 1α1, Col3 α1 and others
Collagen maturation	Lysyl oxidase, MMPs, TIMPs and others
Portal hypertension	Develompmental genes, angiogenesis, elastin

Preliminary model- based analysis failed to yield any significant lod scores. Model- free modified Haseman- Elston analysis was performed using qualitative and quantitative definitions of the phenotype. Using the most conservative correction for multiple comparisons and the WHO ultrasound pattern, the INFGR1 locus demonstrated a significant linkage for 1 of 4 SNPs (P=0.0014298). The analysis further suggests that *TGFbeta 1* and the *IL 13-IL4* region might also contribute to the development of fibrosis, but their scores did not reach significance after correction. Since the SNP markers are not independent, a less conservative correction may be appropriate. Association studies of the IFNGRI may reveal the basis for population differences in fibrosis.

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 (Arap sionglk *et al.*, 1976) and is correlated with infection levels in endemic populations; (Cook *et al.*, 1974). However, ultrasound examination have

revealed that high fibrosis grades are associated with high infection only in children (Domingues *et al.,* 1993) and that hepatomegaly is not necessarily associated with severe fibrosis (Doehring-Schwerdtfeger, *et al.,* 1992). In the present study, severe fibrosis (with hypertension) was associated with the duration of infection. 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. Previously, we have reported associations between some HLA class I alleles (A1 and B5) and hepatosplenomegaly in Egypt (Abdel-Salam *et al.,* 1979) and also between HLA class II allele (DQB1*0201) and familial hepatosplenomegaly (Abdel-Salam *et al.,* 1986) .Various works have also shown that tissue fibrosis can be reduced by injections of IFNγ (Granstein *et al.,* 1990; Hein *et al.,* 1992) Recent works have also shown that mutations in the *IFN-γR1* gene can dramatically alter susceptibility to infectious diseases such as nontuberculous mycobacterium infections (Jouanguy *et al.,* 1996; Newport *et al.,* 1996).

The present work shows that severe fibrosis in subjects infected by *S.mansomin* 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.

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الملخص العربي

العوامل الوراثية المتسببة في تليف الكبد نتيجة الإصابة بالبلهارسيا المانسوني

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بالرغم من وجود علاج فعال للإصابة بالبلهارسيا إلا أن مرض البلهارسيا لازال من أكثر الأمراض الناتجة عن الطفيليات انتشارا في العالم . ويعتبر تليف الكبد نتيجة الإصابة بالبلهارسيا من ضمن أهم المشكلات الصحية القومية خاصة في الريف وقد لوحظ زيادة نسبة تليف الكبد بالرغم من انخفاض نسبة الإصابة في مصر عنها في كينيا .

ويهدف البحث إلى تحديد الجينات المتسببة في زيادة نسبة تليف الكبد في المصريين وذلك بدراسة إحدى عشر جينى قد تكون متسببة في ظهور هذا التليف .وقد تم اختيار قرية في الدلتا (الشمارقة) نظراً لوجود نسبة عالية من تليف الكبد بها . وتمت الدراسة على حوالي ٧٠% من الأشخاص الذين تجاوز عمرهم إحدى عشر سنة (٢٠٣٨ شخص) وذلك بتحليل البراز للبلهارسيا المانسوني ودراسة تليف الكبد بواسطة الموجات فوق الصوتية وتم أخذ عينات دم من المرضى الذين ثبت عندهم تليف كبد بدرجة (ج) وكذلك من أقاربهم من الدرجة الأولى . وكذلك تم تجميع عينات دم عشوائية من ٣٥ شخصا من نفس القرية كعينة ضابطة .

وقد أثبتت النتائج أن نسبة الإصابة بالبلهارسيا في هذه القرية هي ٢٠% مقارنة بـ ٦٣,٦% في كينيا بينما كانت نسبة التليف ٣٦,٨% من المصريين المصابين بالبلهارسيا مقارنة بـ ٢,٥% في كينيا .

وبدراسة العوامل الوراثية المتسببة في زيادة نسبة التليف في المصريين وتحليلها إحصائياً إستنتجنا أن وجود بعض الجينات مثل (*INFGRI, TGF beta1& IL13-IL14*) يساعد على زيادة التليف عند الإصابة بالبلهارسيا . وهذه الدراسة تساعد على تفهم أسباب زيادة التليف وتجنبها كما أنها تساعد في تطوير علاج البلهارسيا وإنتاج المصل المضاد لها .