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IL-13 Polymorphism (IL-13 rs1800925 (-1055) (C/T) is Associated with Severe Hepatic Fibrosis in Human Schistosomiasis

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Abstract: An association study of a cohort of 177 Sudanese patients infected with *Schistosoma mansoni* {82 (46%) males and 95 (54%) females} was conducted to investigate the correlation of four polymorphisms {IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), rs1327474 (A/G), and IL-13 rs1800925 (-1055) (C/T)} to the regression and progression of liver fibrosis 39 months after treatment with praziquantel (PZQ). Regression and progression phenotypes were evaluated by ultrasound. DNA from patients infected with *S. mansoni* was extracted, purified and amplified by PCR. Allelic typing was done using RFLP, primer extension reaction, DHPLC, and allelic discrimination assays (TAQ-MAN). SDS (Sequence Detection Systems) software was used for genotyping. There was an association between IL-13 rs1800925 T allele and the low grades of periportal fibrosis (PPF) ($P = 0.02$). No significant association was found between three polymorphisms (IFN-γ rs2069705 (C/T) $P = 0.5$, rs1327474 (A/G) $P = 0.3$ and IFN-γ R1 rs11914 (G/T), and PPF as response to PZQ. We conclude that IL-13 rs1800925 T allele is protective against PPF ($P = 0.02$).

Keywords: Periportal fibrosis (PPF), Regression, Progression, Praziquantel (PZQ), Polymorphisms.

1. Introduction

Many parasites cause chronic infections in human with mild clinical symptoms, while others cause severe disease (Dessein *et al,* 2001). Periportal fibrosis of the liver (PPF) is a serious consequence of *S. mansoni* infection that involves remodelling of the extra-cellular matrix (ECM) and excessive deposition of collagen along the branches of the portal tract (Booth *et al,* 2004). PPF is formed due to the granulomatous reactions around the schistosome eggs in the liver, and it represents one of the main mechanisms of tissue repair (Grimaud and Lortat-Jacob, 1994).

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Genetic factors explain, at least in part, why some individuals resist infection in general more successfully than others do, although they are living in the same environment with the same living conditions. Other factors such as health condition, acquired immunity and the variability of infectious agent have contributory effect (Kwiatkowski, 2000).

Studies in animal models indicated that disease development is affected by interleukin 10 and 12 (*IL* 10, 12) and tissue-necrosis factor (*TNF-α*) which regulate the granulomatous response (Wynn *et al*, 1998, 1995, Leptak and McKerrow, 1997). It was found that, fibrosis following granulomatous inflamation was dependent on the fibrogenic action of cytokines such as *IL*-4 (Cheever *et al*, 1994), transforming growth factor *TGF-β1* and on the antifibrogenic effect of interferon-γ (Czaja *et al*, 1989a, 1989 b).

In human *schistosomiasis* many reports mentioned the antifibrogenic effect of interferon-γ *(IFN-γ*) in hepatic fibrosis (Duncan and Berman, 1985, Tamai *et al*, 1995, Mallat *et al*, 1995 and Marquet *et al*, 1999). Polymorphisms such as *IFN-γ* +2109 and *IFN-γ* +3810 were associated with severe hepatic fibrosis in human (Chevillard *et al* , 2003). Recent studies had shown that human susceptibility to *S. mansoni* infection is controlled by genetic loci: *SM1* located in chromosome 5q31-q33 which controls the infection levels in Brazilian population (Dessein *et al*, 1999b) and we have shown that susceptibility to PPF is controlled by *SM2* which located in chromosome 6q22-q23 and that is closely linked to *IFNGR1* (gene encoding the alpha chain of the *IFN-γ* receptor) in a Sudanese population (Henri *et al*, 2002). In addition to other factors which include gender, age, duration and intensity of infection (Mohamed-Ali *et al,* 1999), we have shown in the same cohort of patients that severe PPF is associated with an increase in *TNF-α* production and the progression to severe PPF in Schistosomiasis was not associated with polymorphisms in the *TNF-α* gene (Moukoko *et al*, 2003).

Based on the above findings, and since *SM2* locus was reported to control the progression of the disease (Dessein *et al,* 2001), we suggest that, the regression of PPF also could be, at least in part, under genetic control. The aim of this study is to investigate the correlation of four polymorphisms {IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), rs1327474 (A/G), and IL-13 rs1800925 (-1055) (C/T)} to the regression and progression of liver fibrosis in *S. mansoni* infected subjects after PZQ therapy.

2. Material and Methods

Four polymorphisms (IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), rs1327474 (A/G), and IL-13 rs1800925 (-1055) (C/T) were identified in this study (Table1) using three genotyping methods. Study area and population, parasitological examination, clinical and ultrasound evaluations, fibrosis grading, regression and progression phenotypes (Fig: 1, 3 & 4) were evaluated as reported by Rahoud *et al*. (2010). DNA from patients infected with *S. mansoni* was extracted using standard salting-out method (Sambrook *et al*, 1989), purified and amplified by PCR (Model MBS 0.25 Hybaid,) in a 30µl reaction.

PCR Conditions

Briefly, PCR conditions were initial denaturation step at 94 °C for 5 min, second denaturation 35 cycles at 94 °C for 1 min, annealing temperature at 53 °C for 45 seconds, first elongation at 72 °C for 45 seconds and final elongation at 72 °C for 10 min. The annealing temperature is variable according to the type of polymorphism.

Allelic Typing by Restriction Fragment Length Polymorphism (RFLP)

RFLP method was used to screen interferon gamma (IFN-γ) 12q14 rs2069705 polymorphism (C/T). Briefly, PCR fragment of 303bp length including the IFN-γ rs2069705 polymorphism was generated with use of a forward primer (Seq.# rs2069705 F) TCCAATGTGCCAAAATAATAATAAA and a reverse one (Seq.# rs2069705 R) AAGCCCTCCACTCTTTGGTT (All form Operon Biotechnologies, GmbH, 50829, Germany). The PCR product was digested overnight at 37 °C in water path with restriction enzyme *Alu*I (Roche Diagnostics GmbH, Mannheim, Germany).

The digested PCR products (5 µl) with 5 µl Loading dye (Bromophenole blue) in acrylamide gel, run in TBE 1% at 110 volts for 2 hours, stained with ethidium bromide (0.5 µg/ml) for 10 minutes, and then visualized under the photo-documentation system (Baby Imager, Appligene). *AluI* enzyme (from Arthrobacter luteus bacteria) cuts whenever the AG ↓ CT or TC ↓ GA sites were found within the PCR fragment. The *Alu1* analysis of the PCR product obtained from subjects bearing the IFN-γ rs2069705 homozygous T/T alleles gives three bands (193, 110 and 19 bp) on acrylamide gel (Sigma). Subjects bearing the IFN-γ rs2069705 homozygous C/C alleles also give three bands (174, 110 and 19 bp). Whereas the same analysis performed on subjects bearing the IFN-γ rs2069705 heterozygous T/C alleles gives four bands (193, 174, 110 and 19 bp). Bands 110 and 19 bp are not relevant in the genotyping (Fig: 2).

Allelic typing by primer extension reaction, DHPLC, and allelic discrimination assays (TAQ-MAN) was performed following the procedures described by Syvanen, A.C.(2001), ODonovan, M.C. *et al*(1998) and Mc Guigan, F.E. and Ralston, S.H.(2002) respectively. SPSS (Statistical Package for Social Science) software was used for statistical analysis and SDS (Sequence Detection Systems) software for genotyping.

3. Results

This study was conducted in Um-Zukra, a Sudanese village endemic for *S. mansoni.* The role of four polymorphisms (IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), rs1327474 (A/G), and IL-13 rs1800925 (-1055) (C/T) in controlling the regression of liver fibrosis in *Schistosoma mansoni* infected subjects after PZQ therapy has been investigated (Table 1).

Screening for Polymorphism in the Promoter Region of IFN-γ Gene.

Table 2 shows the allele frequency of polymorphism IFN-γ rs2069705 (C/T) in 136 study subjects of Um-Zukra population. The percentage of patients having genotype C/T heterozygous alleles, C/C and T/T homozygous alleles was (40, 29.4 %, 2, 1.5 % and 94, 69.1 %) respectively. The allele frequency of the wild type allele (allele C) was 16.2 %, and that of mutant allele (allele T) was 83.8 %. When the different genotypes of SNP IFN-G rs2069705 (C/T) were crossed tabulated with fibrosis grades before treatment, the genotype frequency of C/C homozygous was (1, 0.74 %) in patients with fibrosis grade one (F I) and grade three (F III), while the genotype frequency of C/T heterozygous and T/T homozygous together was (94, 69.1 %) in patients with fibrosis grade one (F I) and was (40, 29.4 %) in patients with fibrosis grade three (F III), (table 3). No association was reported between SNP IFN-G rs2069705 (C/T) and fibrosis grade ($P = 0.5$). Table 4 shows the different genotypes of SNP IFN-G rs2069705 (C/T) when crossed tabulated with disease prognosis (regression and progression phenotypes). No difference in genotype frequency of C/C homozygous in patients with regression phenotype and those with progression phenotype (1, 0.74 %) in both cases. The genotype frequency of C/T heterozygous and T/T homozygous together was (46, 33.8 %) in patients with regression phenotype and was (88, 64.7 %) in those with progression phenotype. No association was reported between SNP IFN-G rs2069705 (C/T) and disease prognosis ($P = 0.5$).

Screening for Polymorphism in the Coding Sequence (Exon1) of IFN-GR1 Gene

Table 5 shows the allele frequency of polymorphism IFN-GR1 rs11914 (G/T) in 103 study subjects of Um-Zukra population. The percentage of patients having genotype G/T heterozygous and T/T homozygous alleles was (30, 29.1% and 73, 70.9 %) respectively. No patient with genotype G/G homozygous was found in this study subjects. The allele frequency of the wild type allele (allele G) was 17 %, and that of mutant allele (allele T) was 83 %. When the different genotypes of polymorphism IFN-GR1 rs11914 (G/T) were crossed tabulated with fibrosis grades before treatment, the genotype frequency of G/T heterozygous and T/T homozygous together was (65, 63.1 %) in patients with fibrosis grade one (F I) and was (38, 36.9 %) in patients with fibrosis grade three (F III) see table 6. Table 7 shows the different genotypes of SNP IFN-GR1 rs11914 (G/T) when crossed tabulated with disease prognosis (regression and progression phenotypes). The genotype frequency of G/T heterozygous and T/T homozygous together was (39, 37.9 %) in patients with regression phenotype and was (64, 62.1 %) in those with progression phenotype.

Screening for Polymorphism in the Promoter Region of IL-13 rs1800925 Gene

Table 8 shows the allele frequency of polymorphism IL-13 rs1800925 (C/T) in 138 study subjects of Um-Zukra population. The percentage of patients having genotype C/T heterozygous alleles, C/C and T/T homozygous alleles was (62, 44.9 %, 53, 38.4 % and 23, 16.7 %) respectively. The allele frequency of the wild type allele (allele C) was 61 %, and that of mutant allele (allele T) was 39 %. When the different genotypes of SNP IL-13 rs1800925 (C/T) were crossed tabulated with fibrosis grades before treatment, the genotype frequency of C/C homozygous was (30, 21.7 %) in patients with fibrosis grade one (F I) and was (23, 16.7 %) in patients with fibrosis grade three (F III), while the genotype frequency of C/T heterozygous and T/T homozygous together was (64, 46.4 %) in patients with fibrosis grade one (F I) and was (21, 15.2 %) in patients with fibrosis grade three (F III), see table 9. There was an association between SNP IL-13 rs1800925 (C/T) and fibrosis grade ($P = 0.02$).

Table 10 shows the different genotypes of SNP IL-13 rs1800925 (C/T) when crossed tabulated with disease prognosis (regression and progression phenotypes). The genotype frequency of C/C homozygous in patients with regression phenotype was (15, 10.9 %) and was (38, 27.5 %) in those with progression phenotype. The genotype frequency of C/T heterozygous and T/T homozygous together was (35, 25.4 %) in patients with regression phenotype and was (50, 36.2 %) in those with progression phenotype. There was no association between SNP IL-13 rs1800925 (C/T) and disease prognosis ($P = 0.08$).

Screening for Polymorphism in the Un-translated (UTR) Region of IFN-GR1 Gene

Table 11 shows the allele frequency of polymorphism IFN-GR1 rs1327474 (A/G) in 99 study subjects of Um-Zukra population. The percentage of patients having genotype A/G heterozygous alleles, A/A and G/G homozygous alleles was (30, 30.3 %, 61, 61.6 % and 8, 8.1 %) respectively. The allele frequency of the wild type allele (allele A) was 77 %, and that of mutant allele (allele G) was 23 %. When the different genotypes of SNP IFN-GR1 rs1327474 (A/G) were crossed tabulated with fibrosis grades before treatment, the genotype frequency of A/A homozygous was (33, 33.3 %) in patients with fibrosis grade one (F I) and was (23, 28.3 %) in patients with fibrosis grade three (F III), while the genotype frequency of A/G heterozygous and G/G homozygous together was (26, 26.3 %) in patients with fibrosis grade one (F I) and was (12, 12.1 %) in patients with fibrosis grade three (F III) (Table 12). There was no association between SNP IFN-GR1 rs1327474 (A/G) and fibrosis grade ($P = 0.1$). Table 13 shows the different genotypes of SNP IFN-GR1 rs1327474 (A/G) when crossed tabulated with disease prognosis (regression and progression phenotypes). The genotype frequency of A/A homozygous in patients with regression phenotype was (20, 20.2%) and was (41, 41.4%) in those with progression phenotype. The genotype frequency of A/G heterozygous and G/G homozygous

together was (15, 15.2 %) in patients with regression phenotype and was (23, 23.2 %) in those with progression phenotype. There was no association between SNP IFN-GR1 rs1327474 (A/G) and disease prognosis (*P = 0.3*).

4. Discussion

Genotyping of Single Nucleotide Polymorphisms (SNP)

Polymorphisms are heritable genetic markers. The majority of human DNA sequence variation is attributed to the single nucleotide polymorphism (SNP), in which there is a change in a single nucleotide, while insertions or deletions of one or more bases, and repeat length polymorphisms (microsatellites) are also found. In normal individual SNP occurs once in every 1000-2000 nucleotides, suggesting that about 10 million SNP are present across the whole genome (Cargill et al, 1999; Knight, 2001). Only a small proportion of these polymorphisms are of functional relevance by causing structural alteration of the protein encoded by a gene or by altering neighbouring regions of DNA that control gene regulation. All these changes are of potential value as genetic markers for mapping regions of DNA that determine disease susceptibility (Kwiatkowski, 2000).

The pathologies of many infectious diseases are influenced by the profiles of cytokine production in pro-inflammatory (Th1) and anti-inflammatory (Th2) T cells. Differences in cytokine profiles among individuals appear to be at least in part, due to allelic polymorphism within regulatory regions of cytokine gene (Bidwell et al, 1999). Many studies have examined the relationship between cytokine gene polymorphism and susceptibility to severity of disease (Henri et al, 2002b; Chevillard et al, 2002; Jahromi et al, 2000; Koch et al, 2002).

In a study done in Sudanese population of endemic area for *S. mansoni*, that two polymorphisms located in the third intron of the IFN-γ gene are associated with PPF. The IFN-γ +2109 (A/G) polymorphism is associated with higher risk for developing PPF, whereas the IFN-γ +3810 (G/A) polymorphism is associated with less risk for developing PPF. These polymorphisms result in changes in nuclear protein interactions with the intronic regions of the gene, suggesting that they may modify IFN-γ mRNA expression (Chevillard et al, 2003). In a different study on the same population, Moukoko et al, (2003) found no evidence of association between four polymorphisms of TNF-γ gene (TNF-γ -376 G/A, -308 G/A, -238 G/A, and +488 G/A) and PPF.

In this study, four polymorphisms (IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), rs1327474 (A/G), and IL-13 rs1800925 (-1055) (C/T) were screened for possible association with the PPF response to PZQ treatment (Table 2.1).

The selection of the genes of (IFN-γ, IFN-γ R1, and IL-13) cytokines is based on their Role in the regulation of granuloma formation as reported by many authors (Henri *et al,* 2002b; Chevillard *et al*, 2002; Jahromi *et al*, 2000; Koch *et al,* 2002; Chevillard *et al*, 2003; Moukoko *et al*, 2003).

IFN-γ rs2069705 (C/T), IFN-γ R1 rs11914 (G/T), and rs1327474 (A/G) Polymorphisms and PPF

The localization of SM2 in the 6q22-23 chromosomal region close to IFN-γR1 gene (Dessein *et al*, 1999) identifies this gene as a candidate to SM2 (El-Wali, 2002). IFN-γ is a key regulator of the development and functions of the immune system. It plays a major role in immune defence against infections by various human pathogens (Henri et al, 2002a; 2002b). IFN-γ is a strong antifibrogenic cytokine that inhibits the production of extracellular matrix proteins (ECMP) by stellate cells and increases the collagenase activity of the liver by stimulating metaloprotease (MP) synthesis and by inhibiting the synthesis of tissue inhibitors of MP (TIMP) (Doncan *et al,* 1985; Tamai *et al,* 1995).

Polymorphisms in the IFN-γ gene (12q14), including the transcription regulatory region might affect host resistance to infectious agents including schistosomes. Many authors had reported that, polymorphism in the IFN-γ R1 gene (6q23-q24) has been associated with susceptibility to non-pathogenic mycobacterial infection (New port *et al*, 1996;

Jouanguy *et al*, 1997). El-Wali, *et al.* (2002), were reported a polymorphism (+95 T/C) in IFN-γ R1 gene that affects disease progression.

In this study, we investigated whether polymorphisms in IFN-γ (rs2069705 C/T) and IFN-γ R1 (rs11914 G/T, rs1327474 A/G) genes could contribute to disease prognosis in schistosomiasis. Although, the allele frequencies of the mutant alleles (allele T) for SNPs IFN-γ rs2069705 (C/T) and (allele G) for IFN-γR1 rs1327474 (A/G) were 83.3 % and 23 % respectively in the study subjects (Tables 3.7,3.16), but our findings indicated that, neither IFN-γ (rs2069705 C/T) nor IFN-γ R1 (rs1327474 A/G) polymorphisms have an association with PPF (*P = 0.5, P = 0.1*) respectively (Tables 3.8, 3.17). No statistically significant association of IFN-γ R1 rs11914 (G/T) and PPF was found (Table 3.11). Since all study subjects were graded before PZQ treatment (having FI, FII, and FIII), the presence of high frequencies of mutant allele (allele T) 83.3 % for SNP IFN-γ rs2069705 (C/T) and 83 % for IFN-γ R1 rs11914 (G/T) in the study subjects (Tables 3.8. 3.10), could not be explained unless the allele frequencies in the control group (subjects with F0) were performed.

IL-13 rs1800925 (-1055) (C/T) Polymorphism and PPF

In the present study, we were evaluated whether the polymorphism (IL-13 rs1800925 (-1055) C/T) has an association to PPF induced by *S. mansoni* infection. Tables (3.14, 3.15) show the frequency of different genotypes of SNP IL-13 rs1800925 (C/T) when cross-tabulated with the grade of fibrosis before treatment and disease prognosis respectively, in 138 study subjects. We found that IL-13 rs1800925 T/T homozygous and C/T heterozygous genotypes were more frequent (46.4 %, n = 64, *P = 0.02*) in subjects with low grade of fibrosis (F I) (Table 3.14), and on the other hand, were more frequent (36.2 %, n = 50, *P = 0.08*) in subjects with progression phenotype (Table 3-15). IL-13 rs1800925 T/T and C/T genotypes were reported to associate with low grades of PPF. The risk for PPF to reverse to lower grades is increased by IL-13 rs1800925 T allele.

The pathologies of many infectious diseases including schistosomiasis, are influenced by the profiles of cytokine production in pro-inflammatory (Th1) and anti-inflammatory (Th2) T- cells, and the differences of these profiles in any individual appear to be due, at least in part, to allelic polymorphism within regulatory regions of cytokine gene (Bidwell *et al*, 1999). Th2-mediated immunity is critical to human defences against schistosomiasis, since schistosomes are vulnerable to immune attack when in the skin and lungs (Phillips and colley, 1978; Sher, 1977).

Repeated antigenic challenge through the skin by molecules released from schistosomula biases the immune response to Th2, and this response is in particular eosinophils and IgE, which has been reported to be associated with protection against infection (Dessein *et al*, 1988; Rihet *et al*, 1991; Hagan *et al*, 1991; Demeure *et al*, 1993).

IL-13 gene is located on chromosome 5q31, and it was reported to play a unique role in skin and lung immunity against schistosome (Hirrick and Bottomly, 2003; Kouriba *et al*, 2005). Biochemical and molecular studies have shown that IL-13 is the major stimulus for the development of egg-induced liver fibrosis (Chiaramonte *et al,* 1999). In experimental animals, when the activity of IL-13 is blocked (IL-13 $^{-/-}$ animals), the mortality is delayed indicating the major contribution of IL-13 to the progression of the chronic murine schistosomiasis (Fallon *et al,* 2000). In vitro studies demonstrated the ability of IL-13 to stimulate collagen deposition by fibroblasts (Chiaramonte *et al,* 1999). In human schistosomiasis, IL-13 has been reported to exhibits chemotactic activity for human eosinophils and may play a role in eosinophil survival by stimulating the production of granulocyte-macrophsge colony stimulating factor (GM-CSF) (Mentink-Kane and Wynn, 2004).

Previous studies on 5q31-q33 locus had showed an association between IL-13 rs1800925 (-1055 C/T) polymorphism and *Schistosoma haematobium* infection and protection against schistosomes is increased by the IL-13 rs1800925 T/T genotype (Kouriba *et al*, 2005). In other studies on asthma, IL-13 rs1800925 T allele was reported to increase gene

transcription (Wills-karp *et al*, 1998). This is in agreement with the fact that IL-13 cytokine enhances resistance to infection by schistosome in humans (Kouriba *et al*, 2005). In this study, IL-13 rs1800925 T/T and C/T genotypes were reported to be associated with low grades of PPF due to *S. mansoni* infection. The risk for PPF to reverse to lower grades of fibrosis is increased by IL-13 rs1800925 T allele.

5. Conclusion

There was an association between IL-13 rs1800925 T allele and the low grades of PPF ($P = 0.02$). No significant association was found between three polymorphisms (IFN-γ rs2069705 (C/T) $P = 0.5$, rs1327474 (A/G) $P = 0.3$ and IFN-γ R1 rs11914 (G/T), and PPF as response to PZQ. IL-13 rs1800925 T allele is protective against PPF.

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Tables & Figures

Table 1: Selected single nucleotide polymorphisms (SNPs), their chromosomal and structural location.

Polymorphism (SNP)	Gene	Chromosomal location	Gene Bank ID	Structural location
C/T	IFN-γ	12q14	rs2069705	Promoter
G/T	IFN-γ R1	6q23-q24	Rs11914	Exon 1
A/G	IFN-γ R1	6q23-q24	rs1327474	UTR
C/T	IL-13	5q31	rs1800925 (-1055)	Promoter

Table 2: The frequency of different genotypes of IFN-γ rs2069705 (C/T) in 136 study subjects.

Genotype	Frequency	(%)	Allele frequencies
C/T	40	(29.4)	Allele C 16.2 %
C/C	2	(1.5)	Allele T 83.8 %
T/T	94	(69.1)	
Total	136	(100)	

Table 3: The frequency of different genotypes of SNP (IFN-GR1 rs2069705 C/T) when cross-tabulated with the grade of fibrosis before treatment in 136 study subjects.

Fibrosis grade	C/C (%)	C/T or T/T (%)	Total (%)
FI	1 (0.74)	94 (69.1)	95 (69.9)
FIII	1 (0.74)	40 (29.4)	41 (30.1)
Total	2 (1.5)	134 (98.5)	136 (100)

($P = 0.5$)

Table 4: The frequency of different genotypes of SNP IFN-GR1 rs2069705 (C/T) when cross-tabulated with the disease prognosis in 136 study subjects.

Disease prognosis	C/C (%)	C/T or T/T (%)	Total
Regression	1 (0.74)	46 (33.8)	47 (34.6)
Progression	1 (0.74)	88 (64.7)	89 (65.4)
Total	2 (1.5)	134 (98.5)	136 (100)

($P = 0.5$)

Table 5: The frequency of different genotype IFN-GR1 rs11914 (G/T) in 103 study subjects.

Genotype	Frequency	(%)	Allele frequencies
G/T	30	(29.1)	Allele G 17 %
T/T	73	(70.9)	Allele T 83 %
G/G	0	(0)	
Total	103	(100)	

Table 6: The frequency of different genotypes of SNP IFN-GR1 rs11914 (G/T) when cross-tabulated with the disease prognosis in 103 study subjects.

Fibrosis grade	G/G (%)	G/T or T/T (%)	Total (%)
FI	0 (0)	65 (63.1)	65 (63.1)
FIII	0 (0)	38 (36.9)	38 (36.9)
Total	(0)	103 (100)	(100)

($P = 0.5$)

Table 7: The frequency of different genotypes of SNP IFN-GR1 rs11914 (G/T) when cross-tabulated with the disease *prognosis in 103 study subjects.*

Disease prognosis	G/G (%)	G/T or T/T (%)	Total (%)
Regression	0 (0)	39 (37.9)	39 (37.9)
Progression	0 (0)	64(62.1)	64 (62.1)
Total	0 (0)	103 (100)	103 (100)

Table 8: The frequency of different genotypes of SNP IL-13 rs1800925(C/T) in 138 study subjects.

Genotype	Frequency	(%)	Allele frequencies
C/T	62	(44.9)	Allele C 61 %
C/C	53	(38.4)	Allele T 39 %
T/T	23	(16.7)	
Total	138	(100)	

Table 9: The frequency of different genotypes of SNP IL-13 rs1800925 (C/T) when cross-tabulated with the grade of fibrosis before treatment in 138 study subjects.

Fibrosis grade	C/C (%)	C/T or T/T (%)	Total (%)
FI	30 (21.7)	64 (46.4)	94 (68.1)
FIII	23 (16.7)	21 (15.2)	44 (31.9)
Total	53 (38.4)	85 (61.6)	138 (100)

(P = 0.02)

Table 10: The frequency of different genotypes of SNP IL-13 rs1800925 (C/T) when cross-tabulated with the disease prognosis in 138 study subjects.

Disease prognosis	C/C (%)	C/T or T/T (%)	Total (%)
Regression	15 (10.9)	35 (25.4)	50 (36.2)
Progression	38 (27.5)	50 (36.2)	88 (63.8)
Total	53 (38.4)	85 (61.6)	138 (100)

(P = 0.08)

Table 11: The frequency of different genotypes of SNP IFN-GR1 rs1327474 (A/G) in 99 study subjects.

Genotype	Frequency	(%)	Allele frequencies	
A/G	30	(30.3)	Allele A	77 %
A/A	61	(61.6)	Allele G	23 %
G/G	8	(8.1)		
Total	99	(100)		

Table 12: The frequency of different genotypes of SNP IFN-GR1 rs1327474 (A/G) when cross-tabulated with the grade of fibrosis before treatment in 99 study subjects.

Fibrosis grade	A/A (%)	A/G or G/G (%)	Total (%)
FI	33 (33.3)	26 (26.3)	59 (59.6)
FIII	28 (28.3)	12 (12.1)	40 (40.4)
Total	61 (61.6)	85	99 (100)

(P = 0.1)

Table 13: The frequency of different genotypes of SNP IFN-GR1 rs1327474 (A/G) when cross-tabulated with the disease prognosis in 138 study subjects.

Disease prognosis	A/A (%)	A/G or G/G (%)	Total (%)
Regression	20 (20.2)	15 (15.2)	35 (35.4)
Progression	41 (41.4)	23 (23.2)	64 (64.6)
Total	61 (61.6)	38 (38.4)	99 (100)

(P = 0.3)

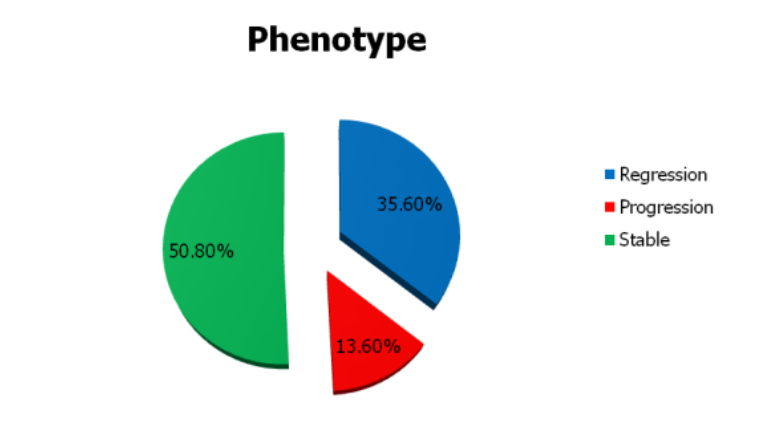

Figure 1: State of PPF 39 months post- treatment reported as phenotypes.

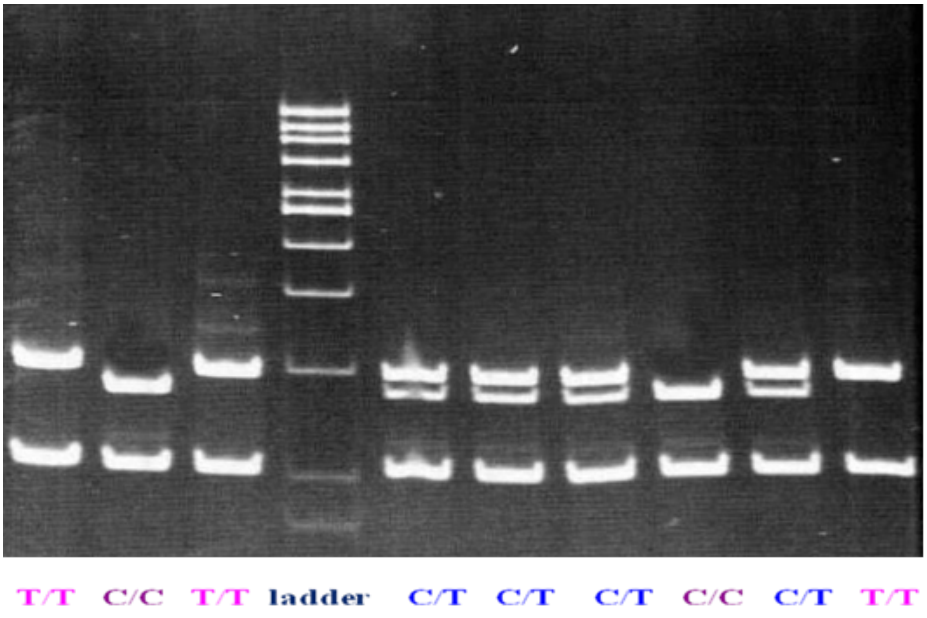

Figure 2: Genotyping of IFN-γ rs2069705 polymorphism (C/T) in DNA samples of Sudanese patients infected with *S. mansoni* showing T/T, C/C homozygous and T/C heterozygous.

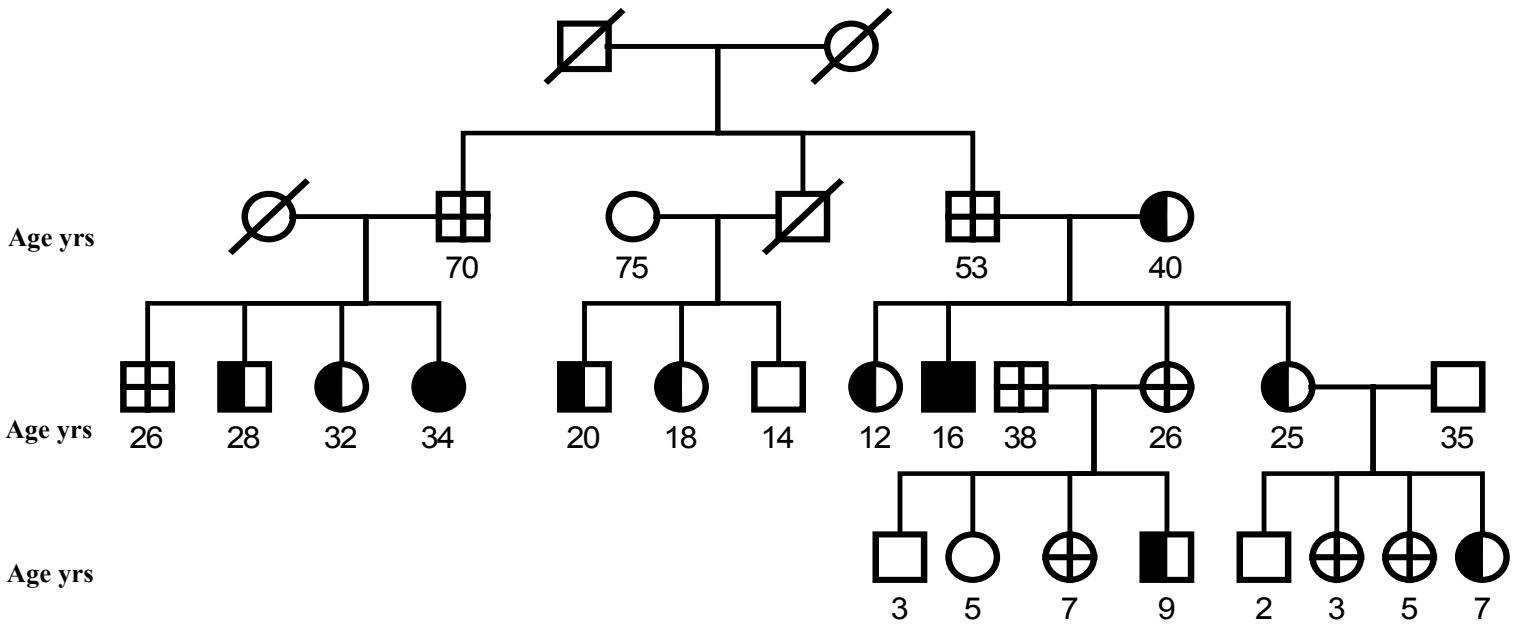

Figure 3: The clustering of regression phenotype (Half-dark symbols) in certain families, stable phenotype (Crossed symbols) and not evaluated person (Open symbols).

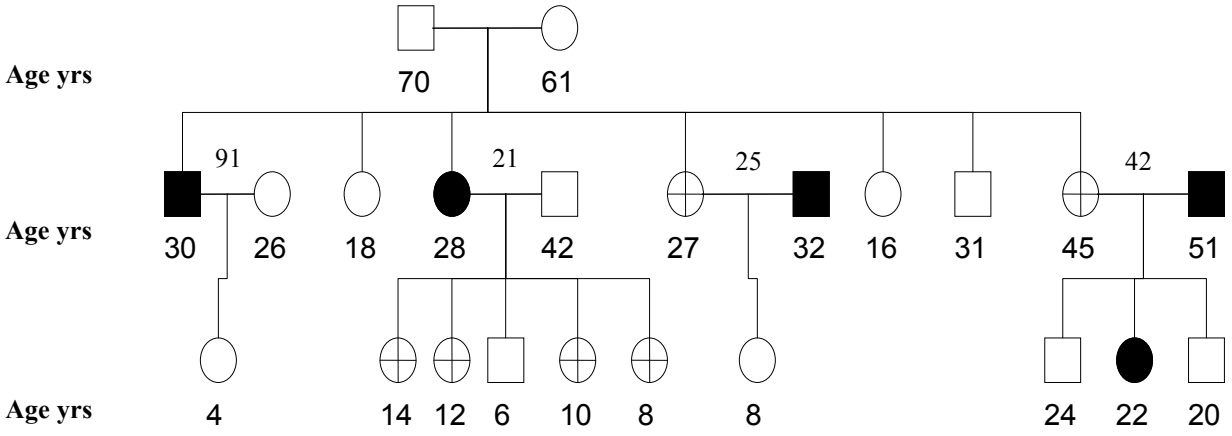

Figure 4: The clustering of progression phenotype (Dark symbols) in certain families, stable phenotype (Crossed symbols) and not evaluated person (Open symbols).