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Description of three new polymorphisms in the intronic and 3′UTR regions of the human interferon gamma gene

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Interferon-gamma (IFN-γ) is a key regulator of the development and functions of the immune system. In particular, this cytokine plays a major role in immune defense against infections by various human pathogens and polymorphisms in the IFN-γ gene, including the transcription regulatory region, and might affect host resistance to infectious agents such as schistosomes. In this study on the genetics of human schistosomiasis we uncovered three new single nucleotide polymorphisms in the IFN-γ genes. Two polymorphisms are located in the third intron and the third is in the 3′UTR region of this gene: an A to G transition at position +2109 from the transcription start and two G to A transitions at positions +3810 and +5134. In a SUDANESE population living in an endemic area of malaria and schistosomiasis, the allelic frequencies are: 0.85 (+2109A), 0.15 (+2109G), 0.92 (+3810G), 0.08 (+3810A), (+5134G) and 0.04 (+5134A).
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Introduction

Interferons are a small group of cytokines that include alpha, beta and gamma interferons (IFN-α, β, γ). IFN-γ is a pleiotropic cytokine that is a key regulator of the development and functions of the immune system. The principal sources of IFN-γ are helper T cells and natural killer cells, although many other cells can produce it. The human *IFN*-γ gene, consists of four exons and is located near D12S335 and D12S313 microsatellites on 12q15.[1,2] It encodes a polypeptide of 166 amino acids with a 20-amino acid signal peptide.

Deletion analysis studies have defined the core *IFN*-γ promoter[3,4] which covers the –100 to –30 region and is much conserved through evolution. Furthermore enhancer sites have been located in the first intron and several NFκB binding sites have been described in introns 1, 2 and 3.[5,6] Genomic DNA constructs lacking most intronic sequences have consistently exhibited a decrease of transcription activity. It is not clear, however, whether NFκB/NFAT complexes have positive or negative effects on transcription. These complexes interact with CD28 and CD28 responsive elements in the promoter and in the third intron. These data indicate that several transcription regulatory regions are distributed along the gene. We have searched for polymorphisms in the *IFN*-γ gene because of its important role in regulating inflammation and fibrosis in human schistosome infection. We describe here three new polymorphisms in the intronic and 3′UTR regions of the *IFN*-γ gene.

Results and discussion

We searched for IFN-γ polymorphisms in SUDANESE subjects from a region of endemic schistosomiasis. We sought single-strand conformational polymorphisms (SSCP) with sets of primers covering the full gene (see primers in Table 1). Polymerase chain reaction (PCR) products were electrophoresed at 4°C and at room temperature. PCR products obtained from the DNA of patient 27 amplified with the 10a/b primers exhibited an additional band on SSCP gel compared to the products obtained from DNA of patient 33 (Figure 1a). Two other polymorphisms were detected using the 16a/b and 21a/b primer sets: PCR products obtained from the DNA of patient 38 and 26 with primers 16a/b and 21a/b respectively exhibited SSCP patterns different from other subjects (subjects 18 and 6 respectively) (Figures 2a and 3a).

Then, PCR products from subjects 27, 33 (10a/b) 18, 38 (16a/b) 6, 26 (21a/b) were sequenced in both directions. Chromatograms are shown in Figures 1b, 2b and 3b. Sequence alignments are provided on Figures 1c, 2c and 3c. Figure 1b and 1c identified an A to G transition at position +2109 from the transcription start in the third intron. Subject 33 was found to be homozygous (A/A) whereas subject 27 was heterozygous (G/T). Likewise, a G to A transition was identified at position +3810 (third intron) in Figure 2b and 2c. Subject 18 was homozygous

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Table 1 Primers used in the analysis of the *IFN-γ* gene

Primer sets	*Primer sequences 5′–3′ (forward and reverse)*	*Position on the reference sequence*
1a/1b	AAT GTG CTT TGT GAA TGA CTC CTC TGG CTG CTG GTA	12/325
2a/2b	CCT ATC TGT CAC CAT CTC ATC T AGG TTT TCT GCT TCT TTT ACA T	251/554
3a/3b	TTG GGT TCT CTT GGC TGT TA ATG AGT TCC CAC CAC AAA AT	520/859
4a/4b	CCT TCT GCT CAG TTT GTA GAA ATC ACT TTT TGG AGA	766/1054
5a/5b	ATA ACT GAT AGG TGA TTT TC GAT TTG ATT TTG TGT TGT AA	1036/1328
6a/6b	ATT GAT TTT ATT CTT ACA AC GAC TAT TAT GTT CTT TTA GC	1315/1635
7a/7b	GGT TAT ATT GGG AAA TAA AAG CAA GCA ACA GGA AAA	1613/1924
8a/8b	GCG GAT AAT GGA ACT CTT TT TCT TTT GGA TGC TCG GTC	1849/2064
9a/9b	GAG TGA CAG AAA AAT AATGC GGA AAT AGT AAG GTA GAG TT	1993/2298
9a bis/9b bis	TCT TTC TTT GGT TTC ATT GC ACT CCC TGC TTC TTC CTC AC	2191/2446
10a/10b	AAT CGC TGA AGT ATG TAA T GCA TTG TAG AGT TTT GGA G	2354/2700
11a/11b	AAG GAA ACT GTT AGG TTC AC TGA AAG TTG ATA GAG AGT TG	2656/2909
12a/12b	AGA TTG TTC TAT CAA CTC TC CTG TTT CTG GGG GCT TAC	2897/3213
13a/13b	GCC TCT CAC TCC TCT CAT AAG AAA CCA CAG ATT TTT	3195/3452
14a/14b	GTA AAA TCT GTC ACT TGC ACC TTC CTC TTG GCT CTG	3372/3674
15a/15b	CCC AAG ATT AGA AAA ATG AAC GAG TGA AAA CTG TAA	3659/3923
16a/16b	GAT TGG GGG TGT TTA TTT TA AAG GAG GAT GAG ACT GTT TC	3852/4212
17a/17b	TAG AGA GGT CAT AGA AAC AGT C TGG TCA GTG AAA ATA AAA GTA T	4199/4507
18a/18b	TAA GGT AAT ACG GTC CAT GAT TGA TGA GTC TAA AAA TA	4478/4813
19a/19b	GAA GAG CAT CCC AGT AAT ATT GTA TCA TCA AGT GAA AT	4718/5038
19a bis/19b bis	TTT TTA GAC TCA TCA ATC AA CTG ACA CAT TCA AGT TCT GT	4816/5147
20a/20b	CAC TTG ATG ATA CAA TGA ACA C AAT TTT CAC AGC TAA GAA GAC T	5042/5396
21a/21b	GAC CTG GAG TGA AAG AAC TA GAT GAG GGA GAG GAA GAT TC	5350/5650
22a/22b	GCA CAC AGA GAT TTA TTT CT AAG GTC TAC AAC AGC ACC AG	5592/5907

The sets of primers used to amplify the human *IFN-γ* gene were designed with the Oligo.O.4 software. The sequence and the position of each primer are indicated (reference sequence used GenBank : J00219). Polymerase chain reactions were carried out on a robocycler gradient 96 (Stratagene, La Jolla, CA, USA) in 30-μl reactions containing 1 μM of each primer 100 ng DNA, 10 mM Tris-HCl pH = 9, 0.1% Triton X-100, 50 mM KCl, 0.2 mg/ml BSA, 1.5 mM $MgCl_2$, 1 mM of dNTP and 1.5 U Taq polymerase (Appligene, Illkirsch, F). Following the initial denaturation step (94°C, 5 min) the samples were subjected to 35 cycles consisting of 94°C for 1 min, annealing temperature for 45 s and 72°C for 45 s

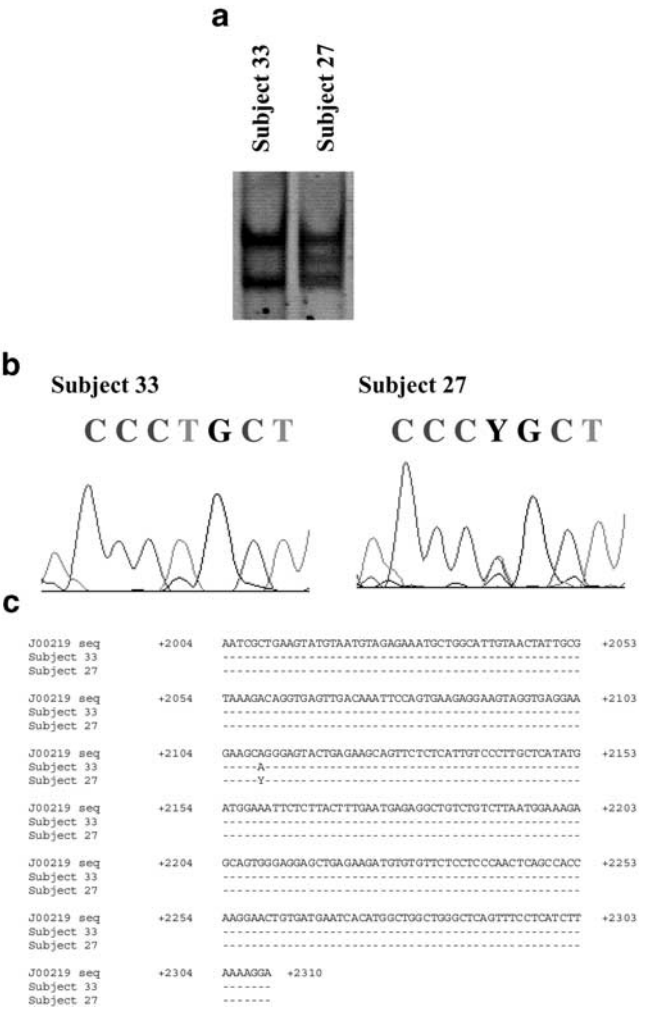

Figure 1 Analysis of the point mutation at position +2109. This polymorphism detection analysis was performed on subjects of a SUDANESE village of Arabic origin (Meisseria and Rawashda tribes). Venous blood was collected and DNA extractions were performed using the standard salting out method. (**a**) Analysis of PCR products by SSCP.[9] 20 μl of PCR reaction products were added to 20 μl of 0.2N NaOH solution and denatured (95°C for 5 min). 20 μl of loading buffer were added to the denatured products prior to electrophoresis on MDE gel (×0.5) in TBE ×1 at 7 mA (constant amperage) for 16 h at room temperature. Gel were stained for 10 min in ethidium bromide solution (1 μg/ml). (**b**) Purified PCR reaction products were sequenced using ABI Prism BigDye Terminator cycle sequencing system (PE Applied Biosystems, Foster City, CA, USA) on an ABI Prism 310 automatic sequencer. Sequencing reactions were performed on both strands. (**c**) The PCR reaction products obtained from subjects 27 and 33 were sequenced with primers 10a and 10b. Chromatograms confirmed the heterozygous status of subject 27. Sequences obtained from subjects 27 and 33 have been deposited in GenBank (numbers: AF408170 and AF408171 respectively). Sequence alignment located the mutation at position +2109 from the transcription start using the J00219 sequence as reference.

(G/G) whereas subject 38 was heterozygous (G/A). Finally a transition G to A was identified at position +5134 in the 3′UTR region (Figure 3b and 3c). Subject 6 was heterozygous (G/G) whereas subject 26 was heterozygous (G/A).

The transition +2109 (A to G) creates an *AciI* restriction site. Restriction site analysis of PCR products from

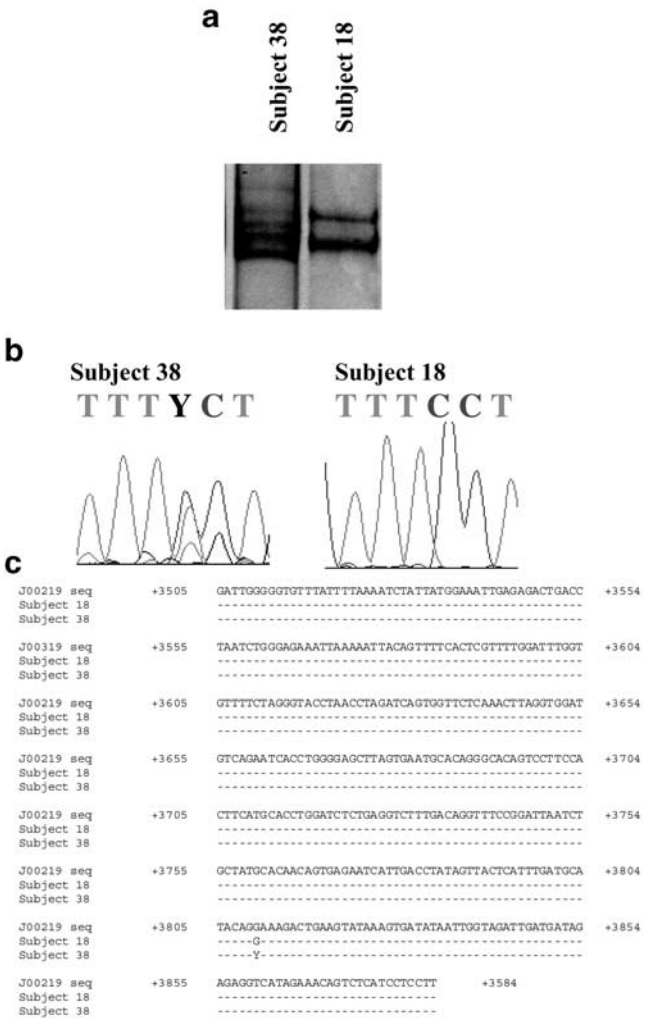

Figure 2 Analysis of the point mutation at position +3810. (**a**) SSCP analysis shows a polymorphism in the human interferon gamma gene. This SSCP electrophoresis was carried out at 4°C. (**b**) The PCR reaction products obtained from subjects 18 and 38 were sequenced with primers 16a and 16b. Chromatograms confirmed the heterozygous status of subject 38. Sequences obtained from subjects 18 and 38 have been deposited in GenBank (numbers: AF408172 andAF408173 respectively). (**c**) Sequence alignment located this mutation at position +3810 from the transcription start.

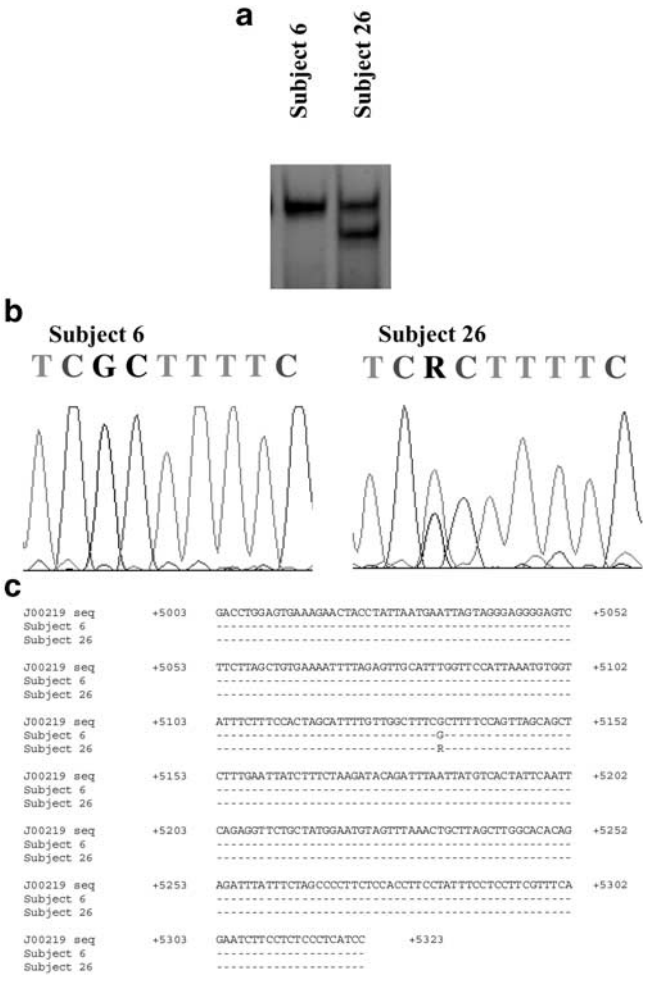

Figure 3 Analysis of the point mutation at position +5134. (**a**) SSCP analysis shows one polymorphism in the human interferon gamma gene. This SSCP electrophoresis was carried out at 4°C. (**b**) The PCR reaction products obtained from subjects 6 and 26 were sequenced with primers 21a and 21b. Chromatograms confirmed the heterozygous status of subject 26. Sequences obtained from subjects 6 and 26 have been deposited in GenBank (numbers: AF408174 and AF408175 respectively). (**c**) Sequence alignment located this mutation at position +5134 from the transcription start.

patients 27 and 33 obtained with 10a/b confirmed this polymorphism. Subject 33 PCR products were not digested (one 365-bp band) whereas subject 27 PCR products were partially digested as three fragments are visible on gel (106-bp, 259-bp and 365-bp fragments) (Figure 4).

The frequency of each polymorphism were determined in the population from which originate the subjects studied above. Out of 134 subjects, 95 and 39 individuals presented the +2109A/A and +2109A/G genotype respectively. Allelic frequencies were 85% (+2109A) and 15% (+2109G). Out of 99 subjects, 84 and 15 individuals had the +3810G/G and +3810G/A genotype respectively. Allelic frequencies were 92% (+3810G) and 8% (+3810A). Finally, out of 78 subjects, 72 and six individuals had the +5134G/G and +5134G/A genotype respectively. Allelic frequencies were 96% (+5134G) and 4% (+5135A).

The frequency of each polymorphism was also determined in the CEPH reference population. In 111 individuals studied, no polymorphism was detected at positions +3810 and +5134 and all the subjects were +3810 G/G and +5134 G/G. However out of 110 subjects, 51, 48 and 11 individuals presented the +2109A/A and +2109A/G and +2109G/G genotype respectively. Allelic frequencies were 68% (+2109A) and 32% (+2109G) (Table 2).

The three new polymorphisms identified in the present study were located in the intronic and 3'UTR regions which are involved in transcriptional regulation. Although none of these new polymorphisms are located in a sequence that was previously demonstrated to be involved in gene regulation. This analysis has been performed on a SUDANESE population living in a region where *Schistosoma mansoni* infection is endemic. Lethality in *Schistosoma mansoni* infections is the consequence of portal hypertension caused by hepatic periportal fibrosis. Development of hepatic periportal fibrosis is controlled

Table 2 Allelic frequencies of the three polymorphisms in the Sudanese and in the CEPH reference populations

	Position from the transcription start					
	+2109		*+3810*		*+5134*	
	Sudanese population	*CEPH population*	*Sudanese population*	*CEPH population*	*Sudanese population*	*CEPH population*
Genotype						
A/A	95/134 (71%)	51/110 (46%)	0/99 (0%)	0/110 (0%)	0/78 (0%)	0/100 (0%)
A/G	39/134 (29%)	48/110 (44%)	15/99 (15%)	0/110 (0%)	6/78 (8%)	0/110 (0%)
G/G	0/134 (0%)	11/110 (10%)	84/99 (85%)	110/110 (100%)	72/78 (92%)	110/110 (100%)
Allelic frequencies						
A	85	68	8	0	4	0
G	15	32	92	100	96	100

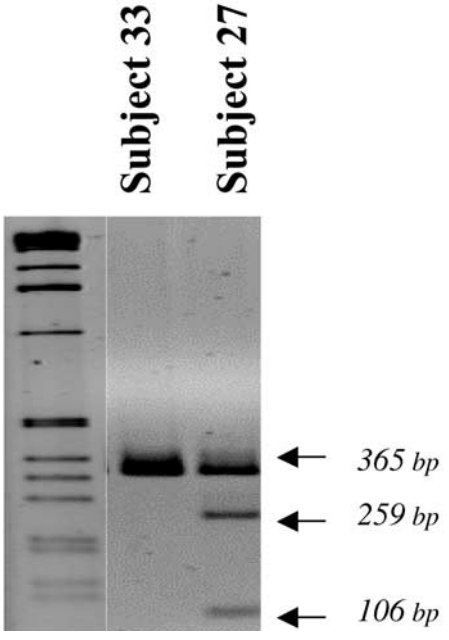

Figure 4 Restriction analysis of 10a/b PCR products by AciI. The +2109 transition creates a novel *AciI* restriction site. The search for restriction sites was conducted using the program (http://www.firstmarket.com/cutter). 5 μl of PCR product from subjects 27 and 33 were digested by the restriction enzyme AciI (New England Biolabs, Beverly, MA, USA) under the conditions described by the manufacturer. Digested products were separated on a 2% agarose gel at 100 V.

by a major locus on human chromosome 6 in q22-q23, closely linked to the gene coding for the α chain of the IFN-γ receptor.[7,8] This localization is consistent with the strongly antifibrogenic property of IFN-γ. We are currently investigating the importance of the three novel polymorphisms described here in the immune response and in the development of fibrosis.

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