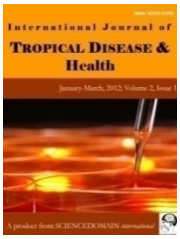

International Journal of TROPICAL DISEASE & Health
2(3): 182-197, 2012

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Association of *IL-10* & *IL-10RA* Polymorphisms with Lymphatic Filariasis in South Indian Population

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Authors' contributions

This work was carried out in collaboration between all authors. YS had a substantial role in acquisition of data, genotyping of IL-10 SNPs and drafting the manuscript. SFQ carried out the genotyping of IL-10RA. SN done the literature search, AV carried out the statistical analysis along with interpretation of data and BM helped in obtaining patients data and clinically assessing patients. PN had a vital role in designing the study, approval of protocols and critical evaluation of data & manuscript at every step. All authors had read and approved the final manuscript.

Research Article

Received 30th April 2012
Accepted 7th July 2012
Online Ready 16th July 2012

ABSTRACT

Aim: The filariasis infection is initiated by mosquito derived third stage larva (L3), which establishes itself in different immunocompetent niches by adopting different evasion and immunomodulatory mechanisms. Immunological and clinical outcomes can vary considerably at the individual and population levels during lymphatic filariasis infection. The protein product coded by the interleukin-10 (*IL-10*) gene has broad immunomodulatory function in filarial load and patency of the disease. The potential influence of altered IL-10 expression encoded by *IL-10* promoter single nucleotide polymorphisms (SNPs) and *IL-10RA* signaling pathway, in pathogenesis and clinical

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outcome of filarial infection was established in the present study
Study Design: Genetic association based on case-control study.
Place and Duration of Study: Lymphatic filariasis cases referred to National Filariasis Control Program (NFCP), Siddipet, Medak, Andhra Pradesh, India between Feb 2006 to Dec 2009.
Methodology: A total of 100 non-endemic, 50 endemic and 118 lymphatic filariasis patients were included in the present study based on clinical and diagnostic criteria. Genetic polymorphisms in the *IL-10* promoter region (-1082G/A, -819C/T and -592 A/C) and *IL-10 RA* coding region S138G were screened following PCR-RFLP and ARMS-PCR technique respectively.
Results: Patients with familial aggregation of lymphedema exhibited significant association with *IL-10* -1082 'A' allele (A vs G OR 2.68, CI - 1.12-6.37, *P*=0.02) coding for lower *IL-10* levels. Similarly the G variant of *IL-10RA* S138G SNP revealed a significant association with lymphatic filariasis in the endemic population studied (GG vs AA OR 2.50 CI-1.22-5.13, *P*= 0.021). The Haplotype analysis also revealed the low signaling ATA is significantly associated with the disease in this cohort (*P*=0.03). The Multifactor Dimensionality Reduction Analysis (MDR) for *IL-10* and *IL-10RA* SNPs interaction revealed the three locus model as the best model wherein the epistatic interactions of variant G allele of *IL-10RA* S138G, the A allele of the -1082G/A and the T allele of the -819C/T SNPs in *IL-10* were found to be a possible risk genotype for filarial infection. (TA = 0.5230, CV-10/10, *P*=0.001).
Conclusion: *IL-10* promoter haplotypes and *IL-10 RA* S138G polymorphisms are the possible genetic determinants of susceptibility to lymphatic filariasis. Further functional studies are warranted to validate these results.

Keywords: Lymphatic filariasis; genetic; interleukins; IL-10; IL-10RA; MDR.

1. INTRODUCTION

Human filarial parasites can survive for decades within normally immunocompetent host as they suppress and modulate the host protective immune response. The filariasis infection is initiated by mosquito derived third stage larva (L3), which, on development to adult parasite, establishes itself comfortably in different immunocompetent niches by adopting different evasion and immunomodulatory mechanisms.

Filarial nematodes exert profound down-regulatory effects on the immune systems of their hosts and IL-10 and TGF-β are the two cytokines most strongly implicated in down-regulation of such immune responses (Maloy et al., 2001). In general, the Th1 and Th2 balance is skewed in filarial infection wherein the parasite induces Th2 populations that suppress effector T cell responses, promoting its own survival (Maizels et al., 2003). IL-10 is implicated in striking a balance between Th1 and Th2 responses by involving a third class of T_{reg} cells (Belkaid et al., 2006). The T_{reg} activity, when diminished, increases induction of Th1 and Th17 in a chronic pathologic development state (Babu et al., 2009).

IL-10 is produced primarily by macrophages and T lymphocytes. It has important anti-inflammatory and immunosuppressive activities; including the ability to downregulate Th1 cytokine and macrophage costimulatory molecule expression. Increased production of IL-10

occurs in chronic human filarial infections and has been associated with filarial immunosuppression (King et al., 1993; Yazdanbaksh et al., 1993).

Gene encoding for *IL-10* is located on chromosome 1q31-1q32. Genetic variations in the *IL-10* promoter region determine the variation in IL-10 production (Westerndorp et al., 1997). The 5'-flanking region of *IL-10* is polymorphic, with three SNPs at -1082, -819, and -592 influencing the IL-10 expression has been extensively studied in various infectious and autoimmune diseases (Lazarus et al., 1997; Gallagher et al., 2003; Kang et al., 2010). Interaction of IL-10 with the IL-10R complex consisting of two subunits - IL-10RA involved in high–affinity ligand binding and signal transduction and IL-10RB required for signal transduction alone, activates the JAK-Stat pathway (Spencer et al., 1998).

The human *IL-10RA* gene has been mapped to chromosome 11q23.3. Among the several listed variations, the S138G SNP results from a A→G transition at nucleotide position 536, in exon 4 which is functionally significant as it has an altered ligand binding interaction and affects the downstream IL-10 signaling pathway (Christopher et al., 2003).

Host genotypes can affect the development and progression of certain diseases and disorders by encoding altered gene products, resulting in poor immune responses. Previous studies have implicated various innate immunity genes in lymphatic filariasis susceptibility (Choi et al., 2001; Junpee et al., 2010; Debrah et al., 2007). It has also been observed that in filaria-endemic regions. filarial disease tends to aggregate in families and the Microfilaria(Mf) levels observed are more attributable to genetic factors rather than shared environment or transmission intensity (Cuenco et al., 2004(a); Cuenco et al., 2004(b); Curenco et al., 2009) The influence of host genetics on nature of the responses to filarial infection and the development of lymphedema in lymphatic filariasis is still obscure and unraveling the genetic basis is necessary to understand the complex heterogenous outcome of the disease wherein only a small percentage of the infected individuals develop chronic manifestation (lymphedema). Given the prominent regulatory role of *IL-10* in lymphatic filariasis host response, the putative role of genetic variations in the *IL-10* gene promoter region and *IL-10 RA* coding region and their possible interactions in influencing host immunity was elucidated.

2. MATERIALS AND METHODS

2.1 Subjects

This study was evaluated and approved by the ethics committee of the Osmania University. The diagnosis of lymphatic filariasis was based on clinical evaluations and blood samples of 118 lymphatic filariasis cases referred by the National filariasis control program center (NFCP), Siddipet, Andhra Pradesh, were included in the present study. Pedigree data on study subjects and their relatives were also observed and the families with at least one family member affected with lymphedema in addition to the proband were considered to have familial clustering of the disease. Microfilaria (Mf) positivity was checked by microscopic examination of blood smears and circulating filarial antigen (CFA) detection was done by ICT (Binax Inc., USA) test. Patients having any one of the clinical symptoms like lymphedema, episodes of Adenolymphangitis (ADL) or hydrocoel or presenting diagnostic criteria were included in the study. The absence of any clinical symptoms and negative test for Mf or CFA was considered as exclusion criteria. Lymphedema grading was done according to Gyapong et al. (1994). For a comparative analysis, blood samples from age and sex matched controls which included 100 non-endemic healthy voluntary donors from

Osmania General Hospital, Hyderabad and Gandhi Hospital, Hyderabad, India and 50 endemic healthy donors from Siddipet region were used.

2.2 Genotyping

DNA was extracted from blood samples using the Rapid Non enzymatic method (Lahiri and Nurenberg, 1991). Three promoter SNPs of *IL-10* at position -1082, -819 and -592 were genotyped by PCR-RFLP whereas the coding region SNP of *IL-10RA* at position A536G was genotyped by allele specific PCR method as summarized in Table 1.

PCR assays were carried out in a 25µl volume with 100ng of genomic DNA, 10pM of each primer, 2.0mM dNTP (Merck, Germany), 1.5mM $MgCl_2$ and 10x PCR buffer [50mM KCl, 500mM Tris buffer, 160mM $(NH_4)2SO_4$, pH 8.8, and 0.1% Tween 20], 0.1% Triton X-100 and 0.5U *Taq* polymerase (Sigma Aldrich). The thermal cycling was carried out in Eppendorf Gradient Thermal cycler (Germany) with initial denaturation at 94ºC for 5 mins followed by n (No of cycles for each SNP is mentioned in Table 1) cycles of 30 sec at 94ºC, 45 sec at specific annealing temp, 1 min at 72ºC; and a final extension at 72ºC for 10 min.

For restriction digestion, 5µl of the PCR product was digested with 2U of restriction enzyme at 37ºC overnight and the samples were separated on a 2% agarose gel for genotyping of these polymorphisms.

ARMS PCR was carried out for detection of *IL-10RA* S138G genotypes. The primer sequences used are mentioned in Table 1. The PCR *a*ssay for a 25µl reaction mixture consisting of 100ng of genomic DNA, 10pM of each primer, 2.0 mM of dNTP, 1.5mM of $MgCl_2$, 10X PCR buffer and 0.5U of Taq polymerase, was carried in a thermocycler with initial denaturation at 95ºC for 5min, 35 cycles of 95ºC for 30sec, 55ºC for 30sec and 72ºC for 1 min and 72ºC for 7 min as final extension. The PCR products of the S138G reaction were 464 bp (control PQ-138), 337 bp (wild-type AQ-138) and 183 bp (variant PB-138) which were detected by genotyping on a 10% PAGE (Polyacrylamide Gel Electrophoresis) gel.

2.3 Statistical Analysis

Analyses of the associations between lymphatic filariasis cases and the control group were performed by Fisher's exact test and odds ratio. Disease association with individual and interacting SNPs was analyzed by means of logistic regression to determine statistical significance, odds ratios (OR), and 95% confidence intervals (CI) and haplotype frequencies were estimated using the implementation of the EM algorithm and linkage disequilibrium was inferred using SNPstat software (Sole et al., 2006). Difference between groups was statistically significant when the test value was $P < 0.05$ and Bonferrroni's adjustment was done for multiple testing corrections. The gene- gene interaction for *IL-10* promoter and *IL-10RA* coding SNPs were analyzed by non parametric Multi Dimensionality Reduction (MDR) analysis using the MDR version 2.0 (Moore et al., 2006). Furthermore, to delineate the effect of strong linkage disequilibrium between SNPs in the same gene on MDR analysis, when SNPs were in strong pair-wise LD, defined as $D' > 0.8$, one of the pair was randomly dropped as deleting the highly correlated SNPs may increase the chance of detecting a possible haplotype effect, or a cis effect of the two or more functional SNPs in a single gene (Xu et al., 2005).

Table 1. Genotyping methods used for detection of *IL-10* and *IL-10RA* polymorphisms

Polymor-phisms	Genotyping Method	Primer Sequence	Annealing Temp(ºC) n= no of cycles	Restriction Enzyme	Allele Size (in bp)	Ref
IL-10 -1082G/A	PCR-RFLP*	AACTGGCTCCCCTTACCTTC AGGAGGTCCCTTACTTTCCGC	42 n-30	*MnlI*	G:141 A: 92 & 49	Zheng et al., 2001
-819C/T	PCR-RFLP	GACAACACTACTAAGGCTCCTTTGGGA GTGAGCAAACTGAGGCACAGAAT	55 n-35	*SSpI*	C: 315 T : 291&24	Ma et al., 2005
-592C/A	PCR-RFLP	GGTGAGCACTACCTGACTAGC CCTAGGTCACAGTGACGTGG	53.2 n-35	*RsaI*	C: 419 A: 236 & 176	Ma et al., 2005
IL-10RA +536A/G	PCR-ARMS**	P-TCAGCCCTCAAGTCTCATGGTATTC Q-TTGCTTCATCTACAAGGGCTCTGG A-GGGCGGGGCGGCRAATGACACATATGAAA B-GGGGCGGGGCGAAGTGACTGAAGATGCC	55 n-35	-	A: 337 (with AQ) G: 183 (with PB)	Gasche et al., 2003

**Restriction Fragment Length Polymorphism, ** Amplified Refractory Mutation System*

3. RESULTS AND DISCUSSION

3.1 Clinical Profile

The demographic profile of the lymphatic filariasis cohort is presented in Table 2. The mean age at onset incase of infection was **32.77±15.4** yrs. The females were predominant (**66.1%**) in the present cohort. The microfilaria rate was found to be **4.3 %** whereas the antigenaemia rate was **88.9%**. Lymphedema was exhibited in **92.3%** of the cases of which **28.8%** individuals had severe lymphedema (Grade III/IV) and **70.2%** had milder form of lymphedema pathology. **33%** of the pedigrees of lymphatic filariasis have revealed a familial aggregation for the lymphedema.

Table 2. Demographic profile of lymphatic filariasis patients

Variable	Lymphatic Filariasis cases (n=118)	Controls	
		Endemic (n=50)	Non-Endemic (n=100)
Mean age at onset(yrs)	32.77±15.4	39.02±11.52	36.82±7.21
Gender			
Male(%)	33.9	30.0	35.0
Female(%)	66.1	70.0	65.0
Familial Aggregation			
Familial cases (%)	33.3	-	-
Non familial cases (%)	66.6	-	-
Parasitological parameters			
Mf+ve(%)	4.3	-	-
CFA+ve(%)	88.9	-	-
Lymphedema pathology			
Grade(I/II)(%)	70.2	-	-
Grade (III/IV)(%)	28.8	-	-

3.2 Genotype Distribution and Association Analysis of *IL-10* & *IL-10RA* Polymorphisms

The genotypic distribution, allelic frequencies and Odds risk estimates of *IL-10* and *IL-10RA* gene polymorphism in patients and controls are given in Table 3. In the case of *IL-10* -1082 G/A SNP, the homozygous AA genotypes were predominant in non-endemic controls (**65.0%**), endemic controls (**64.0%**) as well as lymphatic filariasis patients (**72.0**%), whereas the GG genotype frequency was low for non-endemic controls (**11.0%**), endemic controls (**14.0**%) and lymphatic filariasis group (**5.9%**) which is on par with other studies reported from Indian population (Malhotra et al., 2005).

Table 3. Distribution of genotypes and Odds risk estimates of *IL-10* and *IL-10RA* polymorphisms lymphatic filariasis groups compared to control group

Gene Loci	dbSNP	Controls				LF group		OR (95%CI)			
		NE n(100)		E n(50)		n(118)		LF vs NE	*P*	LF vs E	*P*
		n(%)	MAF	n(%)	MAF	n(%)	MAF				
IL-10 -1082G/A	rs1800896										
AA		65(65.0)	0.23	32(64.0)	0.25	85(72.0)	0.17	1.00		1.00	
AG		24(24.0)		12(24.0)		26(22.1)		0.83 (0.44-1.57)	0.34	0.93 (0.46-1.90)	0.16
GG		11(11.0)		7 (14.0)		7 (5.9)		0.49 (0.18-1.32)		0.32 (0.10-1.03)	
IL-10 -819C/T	rs1800871										
TT		26(26.0)	0.48	18(36.0)	0.43	39(33.0)	0.48	1.00		1.00	
CT		52(52.0)		22(44.0)		45(38.1)		0.59 (0.31-1.11)	0.16	0.94 (0.44-2.01)	0.65
CC		23(23.0)		11(22.0)		34(28.8)		0.99 (0.48-2.04)		1.38 (0.57-3.34)	
IL-10 -592C/A	rs1800872										
AA		26(26.0)	0.48	18(36.0)	0.43	39(33.0)	0.48	1.00		1.00	
CA		52(52.0)		22(44.0)		45(38.1)		0.59 (0.31-1.11)	0.16	0.94 (0.44-2.01)	0.65
CC		23(23.0)		11(22.0)		34(28.8)		0.99 (0.48-2.04)		1.38 (0.57-3.34)	
IL-10RA +536A/G	rs2512146										
AA		43(43.0)	0.42	9(18.0)	0.62	30(26.0)	0.43	1.00		1.00	
AG		30(30.0)		20(40.0)		38(33.0)		1.37 (0.66-2.85)	**0.021**	1.18 (0.52-2.66)	0.47
GG		**27(27.0)**		**21(41.0)**		**47(41.0)**		**2.50 (1.22-5.13)**		0.67 (0.246-1.81)	

For -819C/T polymorphism, **33.0**% TT, **38.1**% CT & **28.8**% CC genotypes were observed in lymphatic filariasis group when compared to **26.0**% TT, **52.0%** CT & **23.0%** CC in non-endemic controls and **36.0**% TT, **44.0**% CT & **22.0**% CC in endemic controls. Similarly for -592C/A polymorphism, **33.0**% AA, **38.1**% CA & **28.8**% CC genotypes were observed in lymphatic filariasis group when compared to **26.0**% AA, **52.0%** CA & **23.0%** CC in non-endemic controls and **36.0**% AA, **44.0**% CA & **22.0**% CC in endemic controls. No significant difference in the genotype and allelic frequencies distribution of −819 (T/C) and *IL-10* −592(A/C) was found between lymphatic filariasis patients and healthy controls. The genotypes were found to be consistent with Hardy-Weinberg equilibrium. Odds risk estimates of the three *IL-10* polymorphisms did not reveal any association with the disease.

In case of S138G substitution of *IL-10RA*, the predominance of GG genotype was observed in case subjects (**41%**) and endemic controls (**41%**) when compared to non-endemic controls (**27%**). **43%** of non-endemic, **18%** of endemic and **26%** lymphatic filariasis were homozygous for major allele (AA), whereas **30%** of non-endemic, **40%** of endemic and **33%** of lymphatic filariasis cases groups were carriers. A significant association of GG genotype was observed with lymphatic filariasis group (**GG vs AA OR 2.50, CI 1.22-5.13**), wherein G allele seems to have a risk predilection to lymphatic filariasis.

3.3 Haplotype Frequencies & Linkage Disequilibrium Analysis

The haplotype frequencies of all the allelic combinations between the three *IL-10* polymorphisms were analyzed for their possible association with lymphatic filariasis **(Table 4).** These haplotypes are associated with high (GCC), intermediate (ACC), and low (ATA) IL-10 production (Stewart et al., 2002). The frequency of **ACC** haplotype was highest in non-endemic (***0.*45**) and endemic (***0.*38**), whereas the **ATA** haplotype was predominant in lymphatic filariasis group (***0.*43**). There was a significant difference in the ATA haplotype frequency in lymphatic filariasis group (***P*=.03**) when compared to non-endemic controls. The increased significance of the association with the *IL-10* ATA haplotype may implicate the functional role of the compound haplotypes in disease susceptibility.

Table 4. Haplotype frequencies of *IL-10* polymorphisms observed in the study

Haplotype	Controls		LF group	*P*(NE)	*P(E)*
	NE	E			
ACC	0.4506	0.3855	0.4015	-	0.53
ATA	**0.3194**	0.3596	**0.4291**	**0.034**	-
GTA	0.1956	0.2090	0.0921	0.073	0.02
GCC	0.0344	0.0459	0.0774	0.059	0.66

A pair-wise comparison of the three *IL-10* polymorphisms, depicting the LD measures is represented in Fig. 1. A significant LD was observed for the -819 & -592 SNPs (**D' 0.99**) indicating a tight linkage between these two SNPs which justifies only four haplotype combinations found in our study.

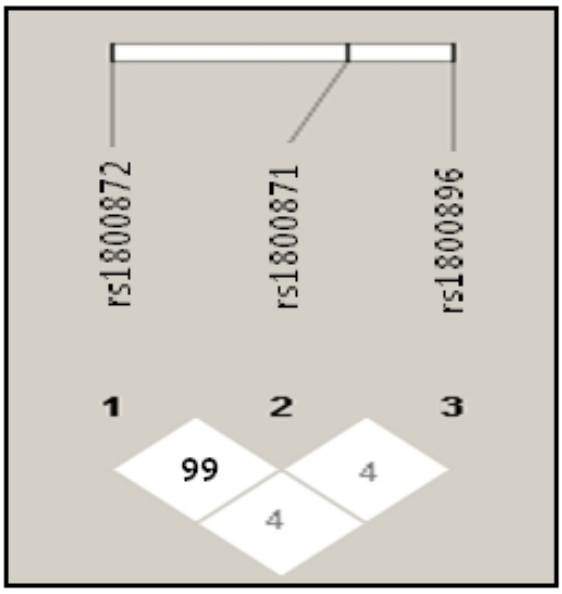

Fig. 1. Pairwise Linkage disequilibrium analysis of *IL-10* promoter SNPs. LD structures of the interval are shown quantified using D'(%). D' with value 1.00 refers to tight linkage and a value below 0.5 refers to no linkage between the SNP pairs

3.4 Association with Gender, Lymphedema Severity and Familial Aggregation Status

The intragroup genotype frequency comparison of the *IL-10 and IL-10 RA* polymorphisms is summarized in Table 5. For the *IL-10* -1082A/G, -819 C/T, -592C/A and *IL-10RA* S138G polymorphisms, there was no significant difference in genotype prevalence between the severe (Grade III/IV) and milder (Grade I/II) lymphedema groups. Similarly comparison of genotype frequencies of these polymorphisms between the disease group, with and without familial aggregation of lymphedema revealed no significant difference. However, -1082 A allele was significantly higher in familial aggregation cases than in non-familial aggregation group (**A vs G OR 2.68 CI 1.12-6.37, *P*=0.02**). The finding reports the familial clustering of cases to have a genetic basis wherein the *IL-10*-1082 A allele coding for low production of *IL-10* may be a risk allele for lymphatic filariasis.

In males, the G allele of *IL-10RA* S138G was found to be a risk allele **(G vs A OR 1.80 CI 1.01-3.17, *P*=0.04)** for lymphatic filariasis. The males were found to be at a risk of developing the disease when compared to the females.

Table 5. Distribution of genotypes and Odds risk estimates of *IL-10* and *IL-10RA* polymorphisms in lymphatic filariasis groups compared to control group

Alleles	Gender				Lymphedema grades				Familial Aggregation			
	M n(%)	F n(%)	OR (95% CI)	p	III/IV n(%)	I/II n(%)	OR(95% CI)	*P*	FA n(%)	N. FA n(%)	OR(95% CI)	P
IL-10												
-1082G/A												
AA	25(62.5)	60(76.9)	1.00		22(64.7)	60(76.0)	1.00		33(84.2)	52(65.8)	1.00	
AG	12(30.0)	14(17.9)	2.06 (0.84-5.07)	0.26	11(32.4)	14(17.7)	2.14 (0.85-5.42)	0.21	5(12.8)	21(26.6)	0.38 (0.13-1.09)	0.08
GG	3(7.5)	4(5.1)	1.80 (0.38-8.63)		22(64.7)	5(6.3)	0.55 (0.06-4.93)		1(2.6)	6(7.6)	0.26 (0.03-2.28)	
A vs G			0.56 (0.28-1.12)	0.10			0.76 (0.36-1.59)	0.46			**2.68 (1.12-6.37)**	**0.02**
-819 C/T												
TT	12(30.0)	27(34.6)	1.00		11(32.4)	27(34.2)	1.00		13(33.0)	26(32.9)	1.00	
CT	17(42.5)	28(35.9)	1.37 (0.55-3.39)	0.78	15(44.1)	29(36.7)	1.27 (0.50-3.24)	0.73	16(41.0)	29(36.7)	1.10 (0.45-2.72)	0.85
CC	11(27.5)	23(29.5)	1.08 (0.40-2.89)		8(23.5)	23(29.1)	0.85 (0.29-2.48)		10(25.6)	24(30.4)	0.83 (0.31-2.25)	
T vs C			1.05 (0.61-1.80)	0.84			1.08 (0.60-1.90)	0.79			0.90 (0.52- 1.55)	0.70
-592C/A												
AA	12(30.0)	23(29.5)	1.00		11(32.4)	27(34.2)	1.00		13(33.0)	26(32.9)	1.00	
CA	17(42.5)	28(35.9)	1.37 (0.55-3.39)	0.78	15(44.1)	29(36.7)	1.27 (0.50-3.24)	0.73	16(41.0)	29(36.7)	1.10 (0.45-2.72)	0.85
CC	11(27.5)	23(29.5)	1.08 (0.40-2.89)		8(23.5)	23(29.1)	1.27 (0.29-2.48)		10(25.6)	24(30.4)	0.83 (0.31-2.25)	
A vs C			1.05 (0.61-1.80)	0.84			1.08 (0.60-1.90)	0.79			0.90 (0.52- 1.55)	0.70
IL-10RA												
+536A/G												
GG	17(43.6)	30(39.5)	1.00		15(45.5)	30(38.5)	1.00		17(43.6)	30(39.5)	1.00	
AG	18(46.1)	20(26.3)	**0.27 (0.08-0.91)**	**0.008**	11(33.3)	26(33.3)	0.85 (0.33-2.16)	0.7	11(28.2)	27(35.5)	0.72 (0.29-1.80)	0.73
AA	4(10.3)	26(34.2)	1.59 (0.66-3.80)		7(21.2)	22(28.2)	0.64 (0.22-1.82)		11(28.2)	19(25.0)	1.02 (0.39-2.65)	
G vs A			**1.80 (1.01-3.17)**	**0.04**			1.01 (0.58-1.76)	0.06			1.33 (0.74- 2.40)	0.33

3.5 Gene-gene Interactions

Possible interactions between the SNPs of the *IL-10* receptor subunit and ligand were also analyzed. Table 6 summarizes, for each number of loci evaluated, the average CV consistency and training and testing accuracy obtained from MDR analysis of the data set of subjects with and without lymphatic filariasis. The three-locus model had a maximum testing accuracy (or least prediction error) of **0.64 (*P*=0.001)** and a maximum CV consistency of 10 out of 10. This **three-locus** model, which included the S138G polymorphism in exon 4 of *IL-10RA*; -1082G/A and -819C/T promoter polymorphism in the *IL-10* gene, was found to be the best model (Fig. 2).

The higher risk genotypes corresponded to the interaction between the variant G allele of *IL-10RA* S138G, the A allele of the -1082G/A SNP and the T allele of the -819C/T SNP in *IL-10* further supporting the epistatic interactions conferring susceptibility to the filarial pathology.

Table 6. Results from multifactor dimensionality reduction analysis

No. of loci	Genes included in best combination in each model	CVC	Testing accuracy	Training accuracy	*P*
1	SNP3	6/10	0.5604	0.4750	**0.05**
2	SNP2, SNP3	9/10	0.6041	0.5227	0.81
3	SNP1, SNP2, SNP3	10/10	**0.6453**	**0.5230**	**0.001**

SNP1 - IL-10 -1082G/A, SNP2 - IL-10 -819C/T, SNP3 - IL-10RA S138G

4. DISCUSSION

Recent studies have shown the association of *IL-10* genotypes in the helminth infections (Timman et al., 2004; Grant et al., 2011). IL-10 is considered a key mediator of immunosuppression and tolerance (Moore et al., 2001). It appears to be primarily produced by monocytes and T regulatory (Tr) lymphocytes and acts on a number of cells, including monocytes, helper, cytotoxic T cells and B cells. IL-10 has a broad immunomodulatory effect through down-regulating pro-inflammatory cytokines, co-stimulatory molecules, as well as major histocompatibility complex (MHC) class II proteins and hence suppressing cell mediated immunity (Shrestha et al., 2010).

The *IL-10* promoter SNPs have regulatory effects wherein −1082 G/A polymorphism lies in a putative ETS (E-twenty six specific) like recognition site while the -592 C/A polymorphism has been localized to a region of negatively regulatory function (Lazarus et al., 1997). Furthermore, the −1082G/A alleles physically interact with a nuclear protein identified as poly (ADP-ribose) polymerase 1 (PARP-1) which is a transcription repressor and apoptotic, in an allele-specific manner resulting in different levels of *IL-10* transcription (Kang et al., 2010). Experimental studies have demonstrated that the −819C/T SNP affects the estrogen receptor element and has a functional role in determining binding of nuclear transcription factors and levels of *IL-10* gene expression (Lazarus et al., 1997).

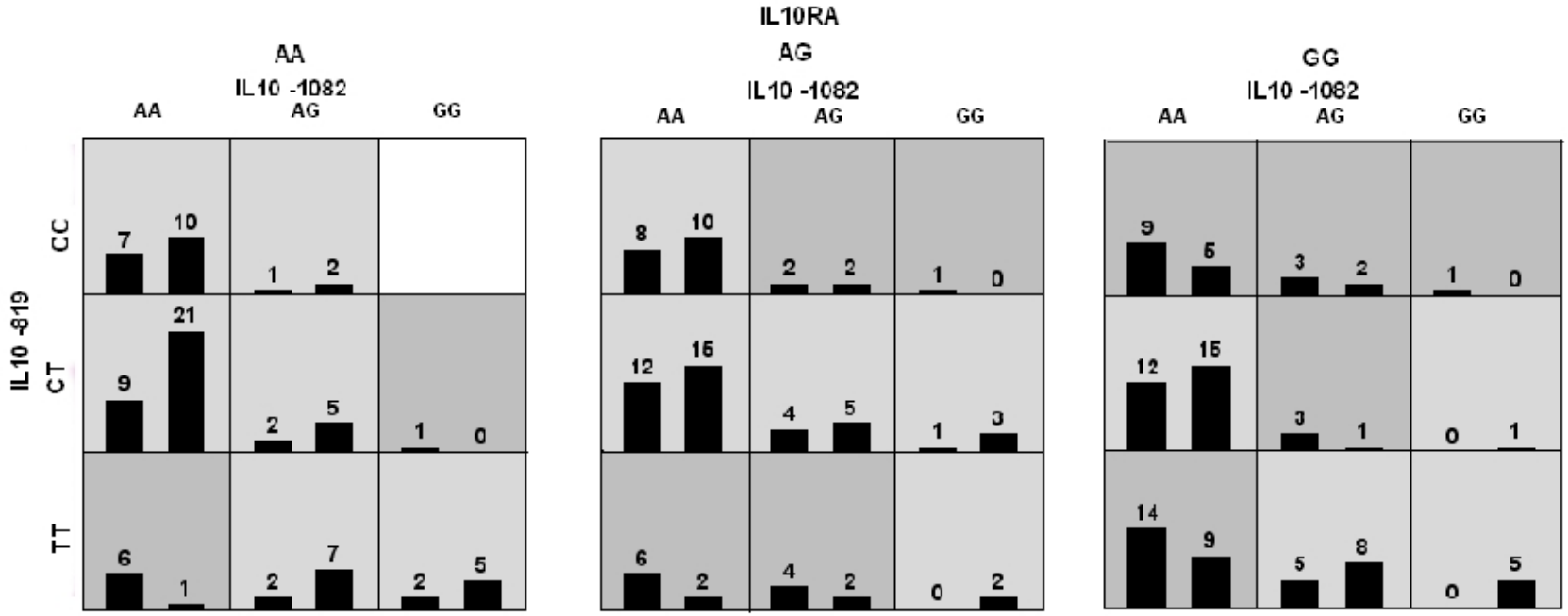

Fig. 2. The best multi-locus model showing the distribution of high risk and low risk genotypes of *IL-10* and *IL-10RA* SNPs in lymphatic filariasis

The dark gray cell indicates the high risk genotype and the gray cell indicates the low risk genotype, along with the corresponding distribution of cases (left bar) and of controls (right bar) for each combination. The white cell shows the genotype without any interactions.

The observations of the present study revealed a significant association of -1082 A allele with the cohort having familial clustering of lymphedema (**A vs G** OR **2.68 CI 1.12-6.37**). The -1082AA homozygous genotype is associated with diminished IL-10 production compared to 1082GA or GG genotypes (Turner et al., 1997; Márka et al., 2005). A tight linkage has been found between -819C/T and -592C/A polymorphisms which are in concordance with earlier reports wherein -819T/C polymorphism is in complete linkage disequilibrium with the -592C/A polymorphism (Kaur et al., 2007). Furthermore, the haplotype analysis revealed a significant association of ATA haplotypes responsible for low IL-10 secretion (***P*=.03**). Timman et al. (2004) have reported that the promoter haplotypes of *IL-10* influence *in vitro* proliferation of PBCs in response to filarial *O. volvulus* antigen (OvAg). Hoerauf et al. (2005) have suggested that the predisposition for patency in lymphatic filariasis may be genetically biased and there may be a correlation between low blood Mf levels and 'low-*IL-10*-signaling haplotype in lymphatic filariasis.

IL-10 production has long been associated with filarial infection wherein the parasites down-regulate the host immune response to establish infection by inducing IL-10-mediated T-cell suppression (Madhumathi et al., 2010). This down-regulation of IL-10 is achieved by inducing IgG4 secretion (Hoerauf and Brattig, 2002). The chronicity of the disease is associated with generation of regulatory cells secreting IL-10 which is correlated with high Mf loads and is intimately involved in the regulation of a microfilarial killing mechanism (Hoerauf and Brattig, 2002; Simons et al., 2010). *In vivo* studies on knockout mice with filariasis infection have shown that IL-4 and IL-10 functions as antagonist in an infection and *IL-10* suppresses the Th2 immune responses rendered by IL-4 (Specht et al., 2004). Therefore the genetically determined lower production of IL-10 may be a risk factor for lymphatic filariasis.

Association analysis of *IL10-RA* S138 SNP revealed a significant risk of the variant G allele with lymphatic filariasis (G vs A OR **1.57, CI 1.06-2.32**). The S138 is on the C-terminal domain which interacts with *IL-10* and the S to G exchange causes conformational rearrangements and hence affects the downstream signal of the IL-10RA complex through impaired IL-10 binding (Christoph et al., 2003). In the present study, the ATA haplotypes coding for a reduced IL-10 cytokine and the *IL-10RA* G allele responsible for impaired signal transduction seem to play a significant role in the predisposition to lymphatic filariasis. Furthermore, MDR analysis suggest that these SNPs interact synergistically influencing the downstream signaling impairment and hence effector mechanisms of IL-10 significantly leading to pathogenesis of chronic lymphatic filariasis.

5. CONCLUSION

In conclusion, our study established an association of *IL-10* -1082G/A SNP and *IL-10RA* S138G SNP with lymphatic filariasis. Haplotype reconstruction of the SNPs in *IL-10* promoter and the gene-gene interaction for *IL-10* and *IL-10RA* SNPs also revealed a significant association with filarial pathology which suggests that the *IL-10* promoter haplotypes and *IL-10RA* S138G polymorphisms are genetic determinants of susceptibility to lymphatic filariasis. However functional studies to further implicate the role of IL-10 pathway in the disease is necessitated.

ACKNOWLEDGEMENTS

We thank all the patients who participated in this study and the NFCP staff for their assistance in clinical diagnosis and sample collection of the patients. Y. S. is a recipient of

Senior Research Fellowship from University Grants Commission (UGC); S.F.Q is a recipient of Junior Research Fellowship under the MANF scheme of University Grants Commission (UGC), New Delhi and the study is funded by Indian Council of Medical Research (ICMR), New Delhi, India (2009-2012).

ETHICAL APPROVAL

All authors hereby declare that all human studies have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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