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. SFO 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.

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<sub>reg</sub> cells (Belkaid et al., 2006). The T<sub>reg</sub> 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); Cuenco 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 Nurenenberg, 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 25 l volume with 100ng of genomic DNA, 10pM of each primer, 2.0mM dNTP (Merck, Germany), 1.5mM MgCl₂ and 10x PCR buffer [50mM KCl, 500mM Tris buffer, 160mM (NH₄)₂SO₄, 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 94°C for 5 mins followed by n (No of cycles for each SNP is mentioned in Table 1) cycles of 30 sec at 94°C, 45 sec at specific annealing temp, 1 min at 72°C; and a final extension at 72°C for 10 min.

For restriction digestion, 5µl of the PCR product was digested with 2U of restriction enzyme at 37°C 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 assay for a 25 l reaction mixture consisting of 100ng of genomic DNA, 10pM of each primer, 2.0 mM of dNTP, 1.5mM of MgCl₂, 10X PCR buffer and 0.5U of Taq polymerase, was carried in a thermocycler with initial denaturation at 95°C for 5min, 35 cycles of 95°C for 30sec, 55°C for 30sec and 72°C for 1 min and 72°C 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 were 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 Bonferroni’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

| Polymorphisms | Genotyping Method | Primer Sequence | Annealing Temp(°C) n= no of cycles | Restriction Enzyme | Allele Size (in bp) | Ref                  |
|---------------|-------------------|-----------------|------------------------------------|-------------------|-------------------|---------------------|
| **IL-10**     |                   |                 |                                     |                   |                   |                     |
| -1082G/A      | PCR-RFLP*         | AACTGGCTCCCTACCTTC AGGAGGTTCCCTTACTTTCCGC | 42 n-30              | MnlI              | G:141 A: 92 & 49   | Zheng et al., 2001  |
| -819C/T       | PCR-RFLP          | GACAACACTAAGGCTCTTTGGGA GTGAGCAACTGAGGCACAGAAT | 55 n-35              | SSpI              | C: 315 T: 291 & 24 | Ma et al., 2005     |
| -592C/A       | PCR-RFLP          | GGTGAGCACTACTGACTGC CCTAGGTCACAGTGAGGTG | 53.2 n-35             | RsaI              | C: 419 A: 236 & 176| Ma et al., 2005     |
| **IL-10RA**   |                   |                 |                                     |                   |                   |                     |
| +536A/G       | PCR-ARMS**        | P-TCAGGCCCTCAAGTCTCATGGTATTC Q-TTGCTTCTACTCAAGGGCTCTGG A- GGCGGGGGCGGGCGCGRAATGACACATATGAAA B-GGGCGGGGGCGAAGTGACTGAGATGCCC | 55 n-35 | - | A: 337 (with AQ) G: 183 (with PB) | Gasche et al., 2003 |

*R 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 |
|---------------------------|------------------------------------|----------|
|                           | Mean age at onset (yrs)             | Endemic (n=50) | Non-Endemic (n=100) |
| 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       | LF vs NE |            |
|           |            | n(100)   | E        | LF vs E    |
|           |            | n(%)     | n(50)    | P          |
| IL-10 -1082G/A | rs1800896 |          |          |            |
|            |            | AA       |          |            |
|            |            | 65(65.0) | 0.23     | 85(72.0)   | 1.00       |
|            |            | AG       | 24(24.0) | 0.25       | 26(22.1)   | 0.83 (0.44-1.57) 0.34 |
|            |            | GG       | 11(11.0) | 7 (14.0)   | 7 (5.9)    | 0.49 (0.18-1.32) 0.32 |
| IL-10 -819C/T | rs1800871 |          |          |            |
|            |            | TT       |          |            |
|            |            | 26(26.0) | 0.48     | 39(33.0)   | 1.00       |
|            |            | CT       | 52(52.0) | 18(36.0)   | 35(28.8)   | 0.99 (0.48-2.04) 1.38 |
|            |            | CC       | 23(23.0) | 11(22.0)   | 34(28.8)   | 0.99 (0.48-2.04) 1.38 |
| IL-10 -592C/A | rs1800872 |          |          |            |
|            |            | AA       |          |            |
|            |            | 26(26.0) | 0.48     | 39(33.0)   | 1.00       |
|            |            | CA       | 52(52.0) | 18(36.0)   | 35(28.8)   | 0.99 (0.48-2.04) 1.38 |
|            |            | CC       | 23(23.0) | 11(22.0)   | 34(28.8)   | 0.99 (0.48-2.04) 1.38 |
| IL-10RA +536A/G | rs2512146 |          |          |            |
|            |            | AA       |          |            |
|            |            | 43(43.0) | 0.42     | 30(26.0)   | 1.00       |
|            |            | AG       | 30(30.0) | 0.62       | 38(33.0)   | 1.37 (0.66-2.85) 0.021 |
|            |            | GG       | 27(27.0) | 21(41.0)   | 47(41.0)   | 2.50 (1.22-5.13) 0.67 |
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=0.03) 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.

| 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.
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 |
|---------|--------|-------------------|----------------------|
|         |        | III/IV            | OR (95% CI) p        | FA | N. FA | OR (95% CI) P |
|         | M n(%) | F n(%)            | I/II n(%)            |     |       |               |
| 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               |
| -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               |
| -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)| 0.85 (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               |
| 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               |

Note: Bold values indicate statistical significance.
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**|

\(\text{SNP}1\) - \text{IL-10} -1082G/A, \(\text{SNP}2\) - \text{IL-10} -819C/T, \(\text{SNP}3\) - \text{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.

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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.

REFERENCES

Babu, S., Bhat, S.Q., Kumar, N.P., et al. (2009). Filarial lymphedema is characterized by antigen-specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl Trop Dis., 3(4), e420.
Belkaid, Y., Blank, R.B., Suffia, I. (2006). Natural regulatory T cells and parasites: a common quest for host homeostasis. Immunol. Rev., 212, 287–300.
Choi, E.H., Zimmerman, P.A., Foster, C.B., et al. (2001). Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India. Genes Immun., 2, 248-53.
Christoph, G., Paul, G., Petra, Z., et al. (2003). Novel variants of the IL-10 receptor 1 affect inhibition of monocyte TNF-α production. J Immunol., 170, 5578-5582.
Cuenco, K.T., Halloran, M.E., Lammie, P.J. (2004a). Assessment of families for excess risk of lymphedema of the leg in a lymphatic filariasis-endemic area. Am J Trop Med Hyg, 70, 185-190.
Cuenco, K.T., Halloran, M.E., Louis-Charles, J., Lammie, P.J. (2004b). A family study of lymphedema of the leg in a lymphatic filariasis-endemic area. Am J Trop Med Hyg, 70, 180-184.
Cuenco, K.T., Ottesen, E.A., Williams, S.A., Nutman, T.B., Steel, C. (2009). Heritable factors play a major role in determining host responses to Wuchereria bancrofti infection in an isolated South Pacific island population. J Infect Dis, 200, 1271-1278.
Debrah, A.Y., Mand, S., Toliat, M.R., et al. (2007). Plasma vascular endothelial growth Factor-A (VEGF-A) and VEGF-A gene polymorphism are associated with hydrocele development in lymphatic filariasis. Am J Trop Med Hyg, 77, 601–608.
Gallagher, P.M., Lowe, G., Fitzgerald, T., et al. (2003). Association of IL-10 polymorphism with severity of illness in community acquired pneumonia. Thorax., 58, 154-156.
Gasche, C., Grundtner, P., Zwirn, P., et al. (2003). Novel Variants of the IL-10 Receptor 1 Affect Inhibition of Monocyte TNF-alpha Production. The J of Immunol., 170, 5578–5582.
Grant, A.V., Araujo, M.I., Ponte, E.V., et al. (2011). Polymorphisms in IL-10 are associated with total Immunoglobulin E levels and Schistosoma mansoni infection intensity in a Brazilian population. Genes Immun., 2, 46-50.
Gyapong, J.O., Magnussen, P., Binka, F.N., (1994). Parasitological and clinical aspects of Bancroftian filariasis in Kassena-Nankana district, upper east region, Ghana. Transactions of the Royal Society of Tropical Medicine and Hygiene., 88, 555-557.

Hoerauf, A., Brattig, N. (2002). Resistance and susceptibility in human onchocerciasis: beyond Th1 vs. Th2. Trends Parasitol., 18, 25.

Hoerauf, A., Santoguina, J., Saefelt, M., Specht, S. (2005). Immunomodulation of filarial nematodes. Parasite Immunol., 27, 417–429.

Junpee, A., Tencomnao, T., Sanprasert, V., Nuchprayoon, S. (2010). Association between Toll-like receptor 2 (TLR2) polymorphisms and asymptomatic bancroftian filariasis. Parasitol Res., 107(4), 807-816.

Kang, X., Kim, H.J., Ramirez, M., Salameh, S., Ma, X. (2010). The septic shock-associated IL-10 -1082 A > G polymorphism mediates allele-specific transcription via poly (ADP-ribose) polymerase 1 in macrophages engulfing apoptotic cells. J. Immunol., 184(7), 3718 -3724.

Kaur, G., Rapthap, C.C., Kumar, N., Kumar, S., Neolia, S., Mehra, N.K. (2007). Frequency distribution of cytokine gene polymorphisms in the healthy North Indian population. Tissue Antigens., 69 , 113.

King, C.L., Mahanty, S., Kumaraswami, V., et al. (1993). Cytokine control of parasite-specific anergy in human lymphatic filariasis: preferential induction of a regulatory T helper type 2 lymphocyte subset. J. Clin. Invest., 92, 1667.

Lahiri, D.K., Nuremberg, J.I. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucl. Acids Res., 19, 5444.

Lazarus, M., Hageer, A.H., Turner, D., Sinnott, P., et al. (1997). Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. J Rheumatol., 24(12), 2314–2317.

Ma, S.L., Tang, N.L., Lam, L.C., Chiu, H.F. (2005). The association between promoter polymorphism of the interleukin-10 gene and Alzheimer’s disease. Neurobiol. Aging., 26, 1005–1010.

Madhumathi, J., Prince, P.R., Rao, D.N. and Kaliraj, P. (2010) Dominant T-cell epitopes of filarial BmALT-2 and their cytokine profile in BALB/c mice. Parasite Immunology., 32,760 –763.

Maizels, R.M., Yazdanbakhsh, M. (2006). Regulation of the immune response by helminth parasites: cellular and molecular mechanisms. Nat. Rev. Immunol., 3, 733.

Malhotra, D., Darvishi, K., Sood, S., et al. (2005). IL-10 promoter single nucleotide polymorphisms are significantly associated with resistance to leprosy. Human Genetics., 118(2), 295-300.

Maloy, K.J., Powrie, F. (2001). Regulatory T cells in the control of immune pathology. Nat. Immunol., 2, 816.

Márka, M., Bessenyei, B., Zeher, M., Semsei, I. (2005). IL-10 promoter -1082 polymorphism is associated with elevated IL-10 levels in control subjects but does not explain elevated plasma IL-10 observed in Sjögren’s syndrome in a Hungarian cohort. Scand J Immunol., 62(5), 474-480.

Moore, J.H., Gilbert, J.C., Tsai, C.T. et al. (2006). A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. J Theor Biol., 241, 252–261.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., O’Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol., 19, 683–765.

Shrestha, S., Wiener, H.W., Aissani, B., et al. (2010). Interleukin-10 (IL-10) pathway: genetic variants and outcomes of HIV-1 Infection in African American Adolescents. PLoS ONE., 5(10), e13384.
Simons, J.E., Gray, C.A., Lawrence, R.A. (2010). Absence of regulatory IL-10 enhances innate protection against filarial parasites by a neutrophil-independent mechanism. Parasite Immunology., 32, 473-478.

Sole, X., Guino, E., Valls, J., Iniesta, R., Moreno, V. (2006). SNPStats: a web tool for the analysis of association studies. Bioinformatics., 22,1928–1929.

Specht, S., Volkmann, L., Wynn, T., Hoerauf, A. (2004). Interleukin-10 (IL-10) counterregulates IL-4-dependent effector mechanisms in Murine Filariasis. Infect Immun., 72(11), 6287-6293.

Spencer, S.D., Di Marco, F., Hooley, J., et al. (1998). The orphan receptor CRF2–4 is an essential subunit of the interleukin 10 receptor. J. Exp. Med., 187, 571-578.

Stewart, D., Fulton, W.B., Wilson, C., et al. (2002). Genetic contribution to the septic response in a mouse model. Shock., 18, 342-347.

Timmann, C., Fuchs, S., Thoma, C., et al. (2004). Promoter haplotypes of the interleukin-10 gene influence proliferation of peripheral blood cells in response to helminth antigen. Genes Immun., 5, 256–260.

Turner, D.M., Williams, D.M., Sankaran, D., Lazarus, M., Sinnott, P.J., Hutchinson, I.V. (1997). An investigation of polymorphism in the interleukin-10 gene promoter. Eur. J. Immunogenet., 24, 1-8.

Westendorp, R.G., Langermans, J.A., Huizinga, T.W., et al. (1997). Genetic influence on cytokine production and fatal meningococcal disease. Lancet., 349,170-173.

Xu, J., Lowey, J., Wiklund, F., et al. (2005). The interaction of four genes in the inflammation pathway significantly predicts prostate cancer risk. Cancer Epidemiol. Biomarkers Prev., 14, 2563–2568.

Yazdanbakhsh, M., Paxton, W.A., Kruize, Y.C.M., et al. (1993). T cell responsiveness correlates differentially with antibody isotype levels in clinical and asymptomatic filariasis. J. Infect. Dis., 167, 925.

Zheng, F., Gabriel, C., Batavia, A., Zhou, X., Ye, S. (2001). A MnlI restriction site polymorphism in the interleukin-10 gene promoter. Biochem Genet., 39(9-10), 351-356.

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