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Association of a *PD-L2* Gene Polymorphism with Chronic Lymphatic Filariasis in a South Indian Cohort

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Abstract. Lymphatic filariasis (LF) is a parasitic infection, caused by three closely related nematodes, namely *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Previously, we have shown that lysate from *B. malayi* microfilariae induces the expression of interleukin *(IL)-10* and programmed death-ligand *(PD-L) 1* on monocytes, which lead to inhibition of $CD4^+$ T-cell responses. In this study, we investigated associations of *IL-10* and programmed cell death *(PD)-1* pathway gene polymorphisms with clinical manifestation in LF. We evaluated the frequency of alleles and genotypes of *IL-10* (rs3024496, rs1800872), *IL-10RA* (rs3135932), *IL-10RB* (rs2834167), *PD-1* (rs2227982, rs10204525), *PD-L1* (rs4143815), *PD-L2* (rs7854413), and single-nucleotide polymorphisms (SNPs) in 103 patients with chronic pathology (CP), such as elephantiasis or hydrocele and 106 endemic normal (EN) individuals from a South Indian population living in an area endemic for LF. Deviations from the Hardy–Weinberg equilibrium were tested, and we found a significant difference between the frequency of polymorphisms in *PD-L2* (rs7854413; $P < 0.001$) and *IL-10RB* (rs2834167; $P = 0.012$) between the CP and the EN group, whereas there were no significant differences found among *IL-10*, *IL-10RA*, *PD-1*, and *PD-L1* SNPs. A multivariate analysis showed that the existence of a CC genotype in *PD-L2* SNP rs7854413 is associated with a higher risk of developing CP (OR: 2.942; 95% confidence interval [CI]: 0.957–9.046; $P = 0.06$). Altogether, these data indicate that a genetically determined individual difference in a non-synonymous missense SNP of *PD-L2* might influence the susceptibility to CP.

INTRODUCTION

Lymphatic filariasis (LF) is a mosquito-borne, deforming and disabling parasitic disease that is widespread in tropical and subtropical regions, such as sub-Saharan Africa, Southeast Asia, and India. The life cycle of parasitic nematodes that cause LF involves both mosquito vectors and human hosts. Individuals infected with these filarial parasites present a wide spectrum of clinical manifestations ranging from clinically asymptomatic disease associated with microfilaraemia to chronic lymphatic pathology involving lymphoedema, elephantiasis, and hydrocele.[1] Globally, approximately 120 million people are subclinically infected and about 40 million people have chronic LF.[2] Moreover, India accounts for around 40% of the global LF burden (http://apps.who.int/neglected_diseases/ntddata/lf/lf.html). These numbers are static in the literature since decades, despite the strict implementation of mass drug administration (MDA) with anti-filarial drugs to eliminate LF in endemic areas. However, since 2000, only 20 of the 72 endemic countries are now under post-MDA surveillance to demonstrate that elimination has been achieved, and for the remaining countries effective MDA regimen is still in progress.[3] Importantly, a recent publication on the current perspectives of MDA to eliminate LF estimates a 59% fall in the prevalence with close to 100 million cases of LF being prevented or cured after the implementation of MDA.[4]

Chronic infection is characterized by immune dysregulation involving both innate and adaptive immune responses.[5] Asymptomatic infection is associated with a T helper (Th) 2-dependent immune response with a concurrent reduction of Th1 responses followed by a regulated response[6–9]; asymptomatic individuals have impaired monocyte and $CD4^+$ T-cell function as reflected by their inability to produce inflammatory cytokines in response to activating stimuli,[10,11] and elevated levels of interleukin (IL)-10.[12,13] Previously, we demonstrated that monocytes from asymptomatic individuals harboring microfilariae from *Brugia malayi* upregulate programmed death-ligands 1 and 2 (*PD-L1* and *PD-L2*, also known as B7-H1 and B7-DC, respectively), and *IL-10*, which is recapitulated by monocytes from healthy individuals from non-endemic regions when stimulated with *B. malayi* microfilarial lysate in vitro. Importantly, these regulatory markers were capable of inhibiting autologous $CD4^+$ T-cell functions effectively.[13]

The cellular receptor programmed cell death (PD)-1 is an immune-inhibitory receptor expressed by activated T cells, B cells, and myeloid cells. The ligands for *PD-1* (*PD-L1* and *PD-L2*) are type I trans-membrane proteins, structurally related to the B7 family. Programmed death-ligand 1 and *PD-L2* are expressed on a variety of cells; expression on dendritic cells (DCs) and on other cell types down-regulates T-cell immune responses.[14–17] The interaction of *PD-1* with *PD-L1* and *PD-L2* results in inhibition of T-cell receptor–mediated lymphocyte proliferation and cytokine secretion, and blockade of CD28-mediated co-stimulation.[15,18–21] In addition, the anti-inflammatory cytokine *IL-10* plays a major role in regulating inflammatory diseases, such as allergies and autoimmune disorders.[22] Interleukin-10 is secreted by myeloid cells, B cells, and lymphocytes, and suppresses the production of pro-inflammatory cytokines and the activation of Th cells.[23–25] The *IL-10* receptor complex is composed of *IL-10* receptor 1 (*IL-10RA*) and *IL-10* receptor 2 (*IL-10RB*). Interleukin-10 first binds to *IL-10RA* inducing a conformational change that enables *IL-10RB* to interact with the *IL-10/IL-10RA* complex.[25] The

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frequency of T cells expressing *IL-10*[26] and plasma cytokine levels of *IL-10*[27] has been shown to be elevated in asymptomatic cases of LF, as compared with uninfected individuals.[28]

Generally, LF is considered to be a complex, multifactorial disease with a broad spectrum of clinical phenotypes associated with both genetic and environmental factors, which might play a role in determining disease outcome.[29] The identification of potential genetic variants of regulatory molecules involved in mediating host immune responses during various outcomes of LF may inform and improve the development of future therapeutic strategies. A number of genetic studies have provided evidence of associations of *PD-1*–regulated and *IL-10* pathway gene variants with infectious diseases (e.g. extra-pulmonary tuberculosis, influenza, leprosy, and malaria); autoimmune diseases (e.g. ankylosing spondylitis, diabetes mellitus, systemic lupus erythematosus, and ulcerative colitis); as well as asthma and cancer. However, there are currently no known associations of polymorphisms in genes belonging to the *IL-10* and *PD-1* pathway with disease outcome in LF.

With regard to our previous findings, we hypothesized that single-nucleotide polymorphisms (SNPs) in the *PD-1* and *IL-10* pathway genes might be associated with disease susceptibility and/or clinical outcome. Association studies using a candidate-gene approach are the most frequently applied method to identify potential genetic variations modifying risk for disease outcome. In this study, we selected eight SNPs in six candidate genes (*IL-10*, *IL-10RA*, *IL-10RB*, *PD-1*, *PD-L1*, and *PD-L2*) to determine their potential involvement in susceptibility to chronic pathology (CP) in LF and analyzed the frequency of each SNP in a cohort of a South Indian population comprising endemic normal (EN) individuals and patients with CP.

MATERIALS AND METHODS

Study population from India. Samples used for this study were collected in South India, a region that is highly endemic for *Wuchereria bancrofti* infection. Study participants were recruited in cooperation with the National Institutes of Health—International Center for Excellence in Research—National Institute for Research in Tuberculosis (NIRT), Chennai, India, and the Blue Peter Health and Research Center (BPHRC), Hyderabad. We obtained ethical approval for the study from the Institutional Review Board of the NIRT, Chennai (NIRT2013001), and the BPHRC, Hyderabad (project number: 05/2009). Written, informed consent was obtained from all study participants.

DNA extraction. Genomic DNA was obtained from peripheral blood mononuclear cells of patients with CP and EN volunteers using a QIAamp DNA investigator kit (Qiagen, GmBH, Hilden, Germany) or from whole blood using an innuPREP Blood DNA master kit (Analytik Jena AG, Jena, Germany). The quantity and purity of the obtained DNA was confirmed by optical density 260/280 ratios using a Nanodrop spectrophotometer (ND1000; PeQLab Biotechnologie, GmbH, Erlangen, Germany).

Single-nucleotide polymorphism selection and genotyping. We selected two *IL-10* SNPs (rs3024496, rs1800872), one *IL-10RA* SNP (rs3135932), one *IL-10RB* SNP (rs2834167), two *PD-1* SNPs (rs2227982, rs10204525), one *PD-L1* SNP (rs4143815), and one *PD-L2* SNP (rs7854413). We used allele frequency data of a Gujarati Indian population living in Houston, Texas, from the International Haplotype Mapping Project for the selected SNPs in candidate genes (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_viewTable.cgi?pop=12159). The final list of SNPs located within the region of six candidate genes that were selected and analyzed further for a probable association with disease outcome are summarized in the result section. High-purity DNA was used to perform the genotyping analysis by using commercially available hybridization probe–based primers and probes (FastStart master DNA Hybprobe; TIB Molbiol, Berlin, Germany). The genotype results were obtained using a LightCycler 480 II detection system (Roche Diagnostics, Mannheim, Germany). Raw genotype data were uploaded to the statistics database server for association analysis.

Statistical analysis. We analyzed genetic data in patients with CP and EN individuals. The percentage of genotypes in EN individuals was examined for deviation from Hardy–Weinberg equilibrium (HWE) by using a chi-square test. Chi-square test was also used to determine the differences in genotypic and allelic frequencies between patients with CP and EN individuals. Multivariable logistic regression models were used to investigate the influence of SNPs, age, and gender. To choose the best model, co-dominant, dominant, and recessive models were fitted. The model with the lowest 2 log likelihood was selected for further modeling. The OR was calculated to compare the odds of expressing one SNP between the groups. Ninety-five percentage confidence interval [CI] values display the range in which the true OR is located with a probability of 95%. *P*-values smaller than 0.05 were considered statistically significant. The SPSS Statistics 24.0 (SPSS, Inc., Chicago, IL) software was used to analyze the data.

RESULTS

A polymorphism in the *PD-L2* gene is associated with CP in LF. We applied a candidate-gene approach in a south Indian population to evaluate whether SNPs in *IL-10* (rs3024496, rs1800872), *IL-10RA* (rs3135932), *IL-10RB* (rs2834167), *PD-1* (rs2227982, rs10204525), *PD-L1* (rs4143815), and *PD-L2* (rs7854413) genes were associated with susceptibility to LF. Single-nucleotide polymorphisms selected to examine disease association are summarized in Table 1. Inclusion criteria were the following: First, the SNP should be nonsynonymous or present in a putative regulatory region. Second, the allelic frequencies of the selected SNPs should have been registered in the HapMap database for the Gujarati Indian population and have a minor allele frequency of > 0.02.

Demographic characteristics of study population. The study population comprised a total of 209 individuals, including 103 patients with CP and 106 EN individuals. Clinically asymptomatic individuals were excluded from the study because of scarcity of samples ($n = 7$). Table 2 summarizes the basic demographics of the study population. No significant differences existed at baseline in relation to the gender ratio or mean age values between patients with CP and EN.

Allele frequency analysis. The allelic frequency in all 209 individuals (103 CP patients and 106 EN individuals) was calculated for each of the eight SNPs. Table 3 shows allelic frequencies for the selected SNPs in the CP and EN groups versus those derived from the HapMap database for Gujarati Indians are shown for the selected SNPs. This comparison analysis revealed a higher deviation in allele frequencies ($> 0.05\%$) in the

TABLE 1
Selected SNPs of the candidate genes and their association with diseases

Gene	SNP ID	Type of polymorphism	SNP influence
IL-10	rs3024496	3′ UTR	Associated with reduced *IL-10* production in *Ascaris lumbricoides*–stimulated peripheral blood mononuclear cells[30]; associated with *Helicobacter pylori* infection[31]
IL-10	rs1800872	Promoter	Associated with higher susceptibility and greater risk of developing type II diabetes mellitus[32]; correlated with asthma susceptibility[33]; increased risk for colorectal cancer development[34]; decreased risk of breast cancer[35]
IL-10RA	rs3135932	Missense (Ser159Gly)	Associated with extra-pulmonary tuberculosis[36]
IL-10RB	rs2834167	Missense (Lys175Glu)	Associated with systemic sclerosis[37]; systemic lupus erythematosus[38]; benign prostate hyperplasia[39]; ischemic stroke with hypertension[40]
PD-1	rs2227982	Missense (Val215Ala)	Associated with ankylosing spondylitis[41,42]; decreased the risk of breast cancer[43]
PD-1	rs10204525	3′ UTR	Increased risk of oesophageal squamous cell carcinoma development[44]
PD-L1	rs4143815	3′ UTR	Associated with the risk of gastric adenocarcinoma[45]; type 1 diabetes mellitus in Chilean population[46]; *PD-L1* overexpression in gastric cancer[47]; early stage non-small cell lung cancer after surgical resection[48]
PD-L2	rs7854413	Missense (Ile241Thr)	Not associated with systemic lupus erythematosus[49]

IL = interleukin; PD = programmed cell death; PD-L = programmed death-ligand; rs = reference SNP cluster ID number obtained from dbSNP; SNP = single-nucleotide polymorphism; UTR = untranslated region. The positive or negative association of the genetic variants from regulatory pathway genes with infectious diseases, inflammatory diseases, and cancer conditions are listed.

HapMap population versus our study population for *IL-10* (rs3024496, rs1800872), *IL-10RB* (rs2834167), and *PD-L2* (rs7854413) SNPs, whereas SNPs from *IL-10RA*, *PD-1*, and *PD-L1* did not vary substantially.

Case–control association analysis. Next, we investigated whether the allelic frequencies of the EN group were distributed equally with patients with CP using a HWE and the respective chi-square test. The frequencies of the major alleles, heterozygous and minor alleles, and the *P*-values for eight SNPs, are reported in Table 4. Statistically significant differences were found for the polymorphisms in *IL-10RB* and *PD-L2* between EN individuals and patients with CP ($P = 0.012$ and < 0.001, respectively). Regarding *IL-10RB*, AG and GG genotypes were observed more frequently in the EN group than expected, whereas the AA genotype was observed less frequently. For the *PD-L2* SNP, the genotypes CC and CT in EN individuals occurred more frequently than expected. The Cochran-Armitage test for trends in proportions also confirmed significant differences between the EN and CP groups for the *PD-L2* SNP. We failed to detect any significant differences between the EN and CP groups regarding the genotypes of any other SNPs except rs7854413, which revealed a *P*-value of 0.08 (Table 5).

Multivariable logistic regression models were fitted including SNP genotype as influence factor, and gender and age as confounding factors on the probability to be in group EN compared with group CP. Age significantly increased the probability to be in group CP ($P < 0.001$); however, gender did not (*P*-values between 0.154 and 0.221, Table 6). Although not formally statistically significant, an influence of a SNP (CC compared with either TT or CT) on the group was observed for *PD-L2* ($P = 0.06$). An adjusted odds ratio (aOR) of 2.942 (95% confidence interval: 0.957–9.046) showed that the odds for genotype CC was 2.942 times higher for the CP group than for the EN group (Table 6). These data reveal that this rare variant (CC) of rs7854413, which is a non-synonymous SNP located in the coding region of the *PD-L2* gene, appears to contribute to the risk of developing chronic lymphatic filariasis in a South Indian population.

DISCUSSION

The principle finding of this study demonstrates an association between the rs7854413 polymorphism of the *PD-L2* gene and increased risk of developing pathology in LF in a South Indian population. In the present study, we elucidated a possible association of immune components, which are considered to be vital elements in host immune responses during LF infection. To our knowledge, this is the first case–control study examining an association of SNPs in the *IL-10* and *PD-1* pathway genes with human filarial infections, as many studies have previously reported the association of SNPs from these pathway genes mostly with autoimmune diseases and various cancer conditions, but not with human filarial infections. On the other hand, some studies have reported on genetic variants in other immune-related genes suggesting a strong role in disease outcomes of filarial infection: A transforming growth factor-1 variant was associated with a lack of microfilariae in the blood of asymptomatic individuals,[50] a gene variant in chitotriosidase (*CHIT1*) with susceptibility to human filarial infection and a variant from C-type collectin, mannose-binding lectin (*MBL2*) was associated with protection against filarial infection in a South Indian population.[51] However, another study reported a non-correlation of SNPs in *CHIT1*, toll-like receptor (*TLR*) *2* and *TLR4* with infection status or LF disease phenotype in a Melanesian population.[52] Aside from this, SNPs in the cytotoxic T-lymphocyte antigen-4 promoter gene were associated

TABLE 2
Study population

	Endemic normal ($n = 103$)				Chronic pathology ($n = 103$)			
Cohorts	Male, *n* (%)	Female, *n* (%)	Age, years (mean ± SD)	Age range, years	Male, *n* (%)	Female, *n* (%)	Age, years (mean ± SD)	Age range, years
Hyderabad	22 (65)	12 (35)	40.9 ± 12.61	18–64	11 (32)	23 (68)	49.9 ± 12.01	28–74
Chennai	34 (49)	35 (51)	37.5 ± 11.67	20–64	38 (55)	31 (45)	46.6 ± 12.35	22–70
Total count	56 (54)	47 (46)	38.6 ± 12.09	18–64	49 (48)	54 (52)	47.7 ± 12.28	22–74

SD = standard deviation. Population demographics are shown for the control group of endemic normal individuals and patients with chronic pathology.

TABLE 3
Allele frequencies of selected polymorphisms from patients and control individuals

Gene	SNP ID	Alleles	Ancestral allele	AF (EN)	AF (CP)	AF (HapMap)	AF (EN + CP)
IL-10	rs3024496	C/T	C	C = 0.203	C = 0.209	C = 0.284	C = 0.206
				T = 0.797	T = 0.791	T = 0.716	T = 0.794
IL-10	rs1800872	A/C	C	A = 0.443	A = 0.485	A = 0.381	A = 0.464
				C = 0.557	C = 0.515	C = 0.619	C = 0.536
IL-10RA	rs3135932	A/G	A	A = 0.839	A = 0.855	A = 0.805	A = 0.847
				G = 0.161	G = 0.145	G = 0.195	G = 0.153
IL-10RB	rs2834167	A/G	A	A = 0.665	A = 0.597	A = 0.568	A = 0.631
				G = 0.335	G = 0.403	G = 0.432	G = 0.369
PD-1	rs2227982	C/T	C	C = 0.929	C = 0.927	C = 0.972	C = 0.928
				T = 0.071	T = 0.073	T = 0.028	T = 0.072
PD-1	rs10204525	A/G	A	A = 0.151	A = 0.189	A = 0.182	A = 0.170
				G = 0.849	G = 0.811	G = 0.818	G = 0.830
PD-L1	rs4143815	C/G	G	C = 0.151	C = 0.141	C = 0.148	C = 0.146
				G = 0.849	G = 0.859	G = 0.852	G = 0.854
PD-L2	rs7854413	C/T	T	C = 0.217	C = 0.310	C = 0.244	C = 0.264
				T = 0.783	T = 0.690	T = 0.756	T = 0.737

AF = allele frequency; CP = chronic pathology; EN = endemic normal; rs = reference SNP cluster ID number obtained from dbSNP; SNP = single-nucleotide polymorphism. Comparative analysis of AF between our study population and the HapMap database registered for Gujarati Indians for the selected polymorphisms in the *IL-10* and *PD-1* pathway genes.

with susceptibility to human LF in an east Malaysian population[53] and a positive association was noted between plasma vascular endothelial growth factor-A gene polymorphism and hydrocele development in patients with LF patients from Ghana.[54] In addition, the association of human leukocyte antigen (*HLA*) gene variants with elephantiasis has been demonstrated among an Asian population,[29] and significant differences in the frequency of the *HLAB15* antigen have been shown between patients with elephantiasis and endemic controls in Sri Lanka and South India.[55] Furthermore, an association has been reported between localized onchodermatitis and nsSNPs in the *IL-13* gene.[56]

Our study results reveal that selected SNPs in putative promoter regions of the human *IL-10* gene and variations that cause a missense mutation failed to show any association with LF. Notably, no significant associations with clinical outcomes for LF were found for the SNPs within the *IL-10RA* and *IL-10RB* regions. However, numerous other SNPs within the *IL-10* gene have shown a strong association with many diseases in different study populations. These include: benign prostate hyperplasia (BPH) in a Korean population[39]; leprosy[57]; susceptibility to tuberculosis in Indian human immunodeficiency virus–positive individuals[58]; malaria in young children from Southern Mozambique[59]; asthma susceptibility in an Asian population[33]; and risk of developing hepatitis C virus infection in a Chinese population.[60] An association exists between SNPs in the *IL-10RA* gene and risk of developing extra-pulmonary tuberculosis in Tunisian individuals.[36] There is also an association between *IL-10RA* gene SNPs and the risk of developing cervical adenocarcinoma cancer.[61] Interleukin-10 receptor 2 gene SNPs are associated with autoimmune diseases, such as systemic sclerosis,[37] systemic lupus erythematosus,[38] BPH[39] hypertension, and the risk of ischemic stroke.[39,40] The above-mentioned SNPs within the *IL-10* pathway genes and their possible disease associations largely vary not only between the different conditions, but also depend on the study populations involved.

In agreement with our primary objective, rs7854413, a nonsynonymous SNP within *PD-L2*, was associated with an increased risk of developing pathology in patients with LF compared with EN individuals (Table 6). As mentioned previously there are currently no published data studying the

TABLE 4
Association between SNPs of candidate genes and patients with CP in the study cohort

				Genotypic analysis			
Gene	SNP ID	Group	HWE	pp	pq	qq	*P*-value
IL-10	rs3024496	EN	0.122	68 (0.64)	33 (0.31)	5 (0.05)	0.886
	C/T	CP		67 (0.65)	29 (0.28)	7 (0.07)	
IL-10	rs1800872	EN	0.151	37 (0.35)	44 (0.42)	25 (0.23)	0.415
	A/C	CP		29 (0.28)	48 (0.47)	26 (0.25)	
IL-10RA	rs3135932	EN	0.399	77 (0.73)	24 (0.23)	5 (0.05)	0.692
	A/G	CP		76 (0.74)	24 (0.23)	3 (0.03)	
IL-10RB	rs2834167	EN	**0.012**	46 (0.43)	49 (0.46)	11 (0.10)	0.131
	A/G	CP		33 (0.32)	57 (0.55)	13 (0.13)	
PD-1	rs2227982	EN	0.488	93 (0.88)	11 (0.10)	2 (0.02)	0.939
	C/T	CP		89 (0.86)	13 (0.13)	1 (0.01)	
PD-1	rs10204525	EN	0.115	76 (0.72)	28 (0.26)	2 (0.02)	0.296
	A/G	CP		68 (0.66)	31 (0.30)	4 (0.04)	
PD-L1	rs4143815	EN	0.117	77 (0.73)	26 (0.24)	3 (0.03)	0.779
	C/G	CP		78 (0.76)	21 (0.20)	4 (0.04)	
PD-L2	rs7854413	EN	**< 0.001**	65 (0.61)	36 (0.34)	5 (0.05)	**0.037**
	C/T	CP		52 (0.50)	38 (0.37)	13 (0.13)	

CP = chronic pathology; EN = endemic normal; HWE = Hardy–Weinberg equilibrium; SNP = single-nucleotide polymorphism. Bold values represent statistic significance between the EN individuals and CP patients for IL-10RB and PD-L2 SNPs. Genotype and allelic frequencies for *IL-10* and *PD-1* pathway gene polymorphisms among chronic pathology and control groups were studied using the HWE and association between the SNP and CP was determined.

TABLE 5
Case–control genetic association

Gene	SNP ID	Chi-square statistic (χ^2)	*P*-value
IL-10	rs3024496	0.556	0.757
IL-10	rs1800872	1.120	0.571
IL-10RA	rs3135932	0.464	0.863
IL-10RB	rs2834167	2.867	0.238
PD-1	rs2227982	0.545	0.761
PD-1	rs10204525	1.221	0.543
PD-L1	rs4143815	0.638	0.727
PD-L2	rs7854413	5.012	**0.082**

CP = chronic pathology; EN = endemic normal; SNP = single-nucleotide polymorphism. Bold values represent the significant differences between EN individuals and CP patients for genotypes of PD-L2 SNP. Genotype differences in selected SNPs between EN individuals and patients with CP were tested using the χ^2 test statistic.

association of *PD-L2* gene variant with infectious diseases or in cancers, because most studies focused mainly on the associations of *PD-1* and *PD-L1* gene variants. This might be because of the previously well-demonstrated immune-inhibitory function of *PD-L1* on immune cells in both mice and humans. Besides this, the upregulation of *PD-L2* is mostly restricted to activated antigen-presenting cells including monocytes, macrophages, and DCs.[62] In recent years, the focus has increased considerably toward the understanding of the exact immune mechanisms of *PD-L2* and its influence on other immune cells. Most published results suggest that an engagement of *PD-1* by *PD-L2* dramatically inhibits T-cell receptor–mediated proliferation and cytokine production by $CD4^+$ T cells.[18,63,64] Also, the effect of *PD-L2* in modulating asthma severity by inhibiting allergen-driven IL-12 production in DCs has been described.[65] In addition, the role of *PD-L2*–expressing DCs was demonstrated in a chronic *Schistosoma mansoni* infection model in mice.[66] In parallel, a few studies with conflicting results have been published on the role of *PD-L2* and its specific function on other immune cells. Possible functional differences in the *PD-L2*/*PD-1* versus *PD-L1*/*PD-1* complexes are suggested by studies demonstrating differential upregulation of *PD-L1* and *PD-L2* by Th1/Th2 environments on inflammatory macrophages[67] and detailed insights into the complex interfaces using crystallography.[68] A recently published study on differences in the molecular mechanisms of *PD-L1* and *PD-L2* and their interactions with *PD-1*[69] suggests differential interactions between *PD-L2* and *PD-1* compared with *PD-L1* and *PD-1*. Moreover, a study has previously demonstrated that the interactions of *PD-L1*/*PD-1*, but not *PD-L2*/*PD-1* are essential in diminishing T-cell responses in experimental autoimmune encephalomyelitis (EAE) because cells from *PD-1* and *PD-L1* knockout mice produced higher levels of the pro-inflammatory cytokines interferon-γ, tumor necrosis factor, IL-6, and IL-17, and also developed severe EAE compared with wild-type and *PD-L2* knockout mice.[70] Furthermore, there was a study demonstrating the contribution of *PD-L2* on establishing parasite-specific $CD4^+$ T-cell responses in mice, which protects against lethal malaria. And in the same study, they showed that *PD-L2* expression was important for parasite control as higher frequencies of *PD-L2*–expressing DCs were associated with lower parasitemia in malaria-infected volunteers. This study suggests that *PD-L2* can out-compete *PD-L1* for *PD-1* binding and thus inhibit *PD-L1* functions that were reported to inhibit Th1 cell responses.[71]

In addition, there was a report suggesting an interaction of *PD-L2* with an alternative receptor on T cells—repulsive guidance molecule b (RGMb). Repulsive guidance molecule b induces respiratory tolerance in ovalbumin-exposed mice, as blockade of RGMb-PD-L2 interaction impairs the development of respiratory tolerance significantly.[72,73] Altogether, these results disclose multifaceted consequences of *PD-L2* expression and function, which is distinct from those of *PD-L1*. Finally, the precise mechanism of how *PD-L2* and different polymorphisms affect the function of T cells or possibly other immune cells expressing its receptors in the context of LF remains to be elucidated.

CONCLUSION

Based on our data, a significant association between nsSNP rs7854413 in *PD-L2* and CP with an OR value of 2.9 for the "CC" genotype indicate that a mutation in the *PD-1* pathway genes and its downstream effector mechanisms could play a role in the development of the various clinical manifestations of LF. Our data reveal that individuals differing in this genetic variant (CC genotype) in the *PD-L2* gene are three times more prone to develop pathology than EN individuals. Therefore, we further propose that an individual carrying the "CC" genotype in rs7854413 of *PD-L2* gene is unable to provide the appropriate signal to the *PD-1* receptor on T cells because of *PD-L2* incompetency and/or partial impairment in ligand-receptor interaction. Furthermore, this could eventually lead to a disruption of immune-inhibitory signals via the *PD-1* pathway resulting in active T-cell responses, which engender an inflammatory phenotype within the T cells to challenge the filarial parasite as commonly seen in patients with CP. However, the exact ligand–receptor interactions and the actual immune mechanism of the *PD-L2*/*PD-1*

TABLE 6
Multivariate logistic regression analysis including SNP, age, and gender

				SNP			Age	Gender
Gene	SNP ID	Genotype	Co-dominant analysis	Adjusted odds ratio	95% confidence interval	*P*-value	*P*-value	*P*-value
IL-10	rs3024496	CC	CT or TT	1.970	0.550–7.055	0.297	< 0.001	0.216
IL-10	rs1800872	CC	CA or AA	0.753	0.398–1.422	0.381	< 0.001	0.217
IL-10RA	rs3135932	GG	GA or AA	0.541	0.110–2.661	0.450	< 0.001	0.209
IL-10RB	rs2834167	AA	AG or GG	0.699	0.379–1.289	0.252	< 0.001	0.194
PD-1	rs2227982	TT	CT or CC	0.268	0.023–3.169	0.296	< 0.001	0.173
PD-1	rs10204525	AA	AG or GG	1.813	0.302–10.890	0.515	< 0.001	0.221
PD-L1	rs4143815	CC	CG or GG	0.802	0.398–1.614	0.536	< 0.001	0.220
PD-L2	rs7854413	CC	CT or TT	**2.942**	**0.957–9.046**	**0.060**	< 0.001	0.154

SNP = single-nucleotide polymorphism. Bold values represent the influence of a PD-L2 SNP (CC genotype compared with either TT or CT genotypes). Adjusted odds ratio for factor association with chronic pathology using multivariable analysis.

pathway in terms of disease severity in LF deserves further investigation involving more endemic donors.

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